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# Isolation, purification, identification and pathogenicity of *Macrophomina phaseolina* (Tassi) goid caused dry root rot of chickpea

**Lalita Lakhran, RR Ahir, Meera Choudhary and Sangeeta Choudhary**

**Abstract**
Dry root rot of chickpea caused by *Macrophomina phaseolina* (Tassi) Goid is one of the more severe yield destabilizing factors causing serious yield losses each year. In recent years, *Macrophomina phaseolina* is becoming more prevalent in agricultural areas where climate change is leading to higher temperatures. We conducted a experiment in which, a series of fungal isolation, purification, identification and pathogenicity from the infected plant parts. The plant show typical symptoms like Drooping of petioles and leaflets and in advance stage scattered sclerotial bodies. The infected isolates may be seen on the affected tissues after reinoculation and produced mycelia growth and sclerotia both plants and culture plates. On re-isolation it was found that the fungus was identical to the original isolate. It was observed that the infection was much higher in inoculated plants as compared to control.

**Keywords:** chickpea, identification, *Macrophomina phaseolina*, pathogenicity, root rot

## Introduction
Gram (*Cicer arietinum* L.) popularly also known as Bengal gram is one of the most important winter season pulse crop grown in India. It is said to be one of the oldest pulse crop cultivated from ancient times over throughout the Asia. However, India has the distinction of being the world's single largest producer of pulses, the difference in production and population ratio is significant. Infection of chickpea by *Macrophomina phaseolina* occurs most frequently at the flowering and pod formation stage (Singh *et al*., 1990) [11] or seed development stage (Trapero-Casas and Jimenez – Diaz, 1985) [12]. The infected stems and leaves appear dry and become brittle and straw coloured. The characteristic symptom of root rot was yellowing of the leaves and within two of three days these leaves may drop off. The plant may wilt within a week, if the plants are pulled out from the soil and examined the basal stem and main roots show root rot symptoms. In advance stage scattered sclerotial bodies may be seen on the affected tissues (Singh and Srivastava, 1988) [10]. *Macrophomina phaseolina* survives in or on seed and persisted in the soil in the form of black sclerotia which are produced in large number on infected host tissues and are subsequently dispersed in soil during tillage operations (Sheikh and Ghaffar, 1978). In India Madhya Pradesh, Karnataka, Andhra Pradesh, Chhattisgarh or Central and Northern parts are affected by dry root rot of chickpea (Ghosh *et al.* 2013) [2]. Dry root rot chickpea caused 10-25% losses (Pal,1998) [7] but it become severe in most of chickpea growing areas and caused more than 50% losses (Massoud and Shiv kumar, 2001) [6]. So, the present investigation was conducted to isolate, purify, identify and pathogenicity test of dry root rot of chickpea.

## Material and Methods
### Collection, isolation and purification of the pathogen.
Diseased samples will be collected from Agronomy farm, S.K.N. College of Agriculture, Jobner and brought to the laboratory for further studies. Prior isolation and other laboratory experiments, all the glasswares were cleaned with Potassium dichromate sulphuric acid, solution washed with sterilized water, sterilized in hot air oven at 180 ˚C for two hours. Media (PDA) were sterilized by autoclaving at 1.045 kg $cm^2$ pressure for 20 minutes. Roots of chickpea plants were first washed under the tap water and then cut into small piece along with healthy portion. These pieces were surface sterilized by dipping in 0.1 per cent Sodium hypochlorite solution for 1-1½ minute after three consecutive washing with sterilized distilled water, the pieces were transfer to autoclaved Potato Dextrose Agar medium in petriplates incubated at 25±1ºC and plates are placed into BOD incubator for 7 days. The fungal colonies emanating from bits were examined on 7 days of incubation.

**Purification of pathogen**

Pure culture of the fungus was obtained by hyphal tip method (Singh, 1988) [9] on plain agar medium. For this, hypal tips were obtain from culture slants after 96 hours of incubation and were suspended in sterilized distilled water. The dilution of suspension was adjusted such that in one loopful, 5-10 spores could be counted under the low power objective of the microscope. One ml of above suspension was spread in Petriplates containing 20 ml sterilized plain agar medium. After 12-24 hours of inoculation, the germinating spores were located under the microscope and marked with the help of dummy objective and then transferred to PDA slant and kept in BOD for further growth. The culture was maintained by periodical transfer on PDA slants for further studies.

**Pathogenicity test**

To as-certain ability of the organism to cause root rot in chickpea plant. The isolated and purified fungus from diseased roots was tested for its pathogenicity. The pathogenicity of *Macrophomina phaseolina* was tested under pot conditions by seed and soil inoculation techniques suggested by (Kataria and Grover, 1976) [4]. Apparently healthy surface sterilized seeds of chickpea (var. L-550) were rolled on 7 days old culture of *Macrophomina phaseolina* thriving on PDA in petriplates to provide seed borne inoculums. Inoculated seeds were sown in 9 x 12 inches earthen pods containing sterilized soil. The pathogen grown on sorghum grain medium at 25±1ºC for one week was used as the soil inoculums. Prior to sowing, pots were sterilized with copper sulphate solution and filled with sterilized soil (soil: FYM = 3:1) sterilized at 1.045 kg $cm^2$ for one hour for three consecutive days. These pots were inoculated with fungus inoculum multiplied on sorghum grain medium. Ten apparently healthy and surface sterilized Chickpea seeds were sown in each pot and replicated thrice. Surface sterilized seed sown in un-inoculated sterilized soil served as check. These pots were kept in cage house and watered regularly as and when required and maintained under identical condition. Observation on seed germination and pre and post emergence mortality were record and per cent disease incidence was calculated as follows.

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

**Seed inoculation technique**

For this, seeds were rolled on 7 days old culture of fungus thiriving on PDA contained in Petri plates. The inoculated seeds were sown in cemented pots. The un–inoculated apparently healthy seeds served as check. These pots were kept in cage house and watered regularly as and when required.

**Soil inoculation technique**

Prior to sowing, pots (30 cm diameter) were sterilized with copper sulphate solution and filled with sterilized soil + FYM (Soil: FYM=3:1; sterilized at 1.045 kg/$cm^2$ for one hour for three consecutive days). These pots were inoculated with inoculum, multiplied on sorghum grains @ 20 g/pot. 10 apparently healthy and surface sterilized chickpea seeds (L-550) were sown in each pot with four replications. Surface sterilized seeds sown in un-inoculated sterilized soil, served as check. These pots were kept in cage house and watered regularly as and when required and maintained under identical conditions.

**Identification of the pathogen**

The isolated fungus was identified on the basis of morphological characters. The culture was also sent to ITCC, Division of Plant Pathology, IARI, New Delhi for further confirmation or identification of fungus. The fungus was identified as *Macrophomina phaseolina* with ID No. 6621.

**Results and Discussion**

**Collection, isolation and purification of *Macrophomina phaseolina*.**

Root rot infected plants of Chickpea were collected from Agronomy farm, S.K.N. College of Agriculture, Jobner where disease was prevalent and collected. Samples were brought to the laboratory for isolation and further studies. The fungus was isolated on PDA from infected roots of chickpea plants under aseptic conditions. the fungus emerging form root bits placed on PDA was observed to have profuse white aerial mycelium later turn brown to black on PDA. Black hard sclerotia were formed after 10-15 days at the periphery of the colony. The culture was purified by hyphal tip technique. El–Araby (2009) [1] observed symptoms including shrunken, unfilled pods and brown wilted attached to dead petioles and stems along with the stem cortex have been also report due to *Macrophomina phaseolina* causes charcoal rot in soybean. The fungus is mainly a soil dweller and spreads from plant to plant through irrigation water, food and implements and cultural operation. The sclerotia & pycniospore may also become air borne and cause further spread of the pathogen (Rangaswami and Mahadevan 2008) [8].

**Pathogenicity**

*Macrophomina phaseolina* (Tassi) Goid isolated from infected roots of chickpea plant found pathogenic when seed and soil was inoculated artificially to chickpea plant under pot house conditions. The characteristic symptoms of root rot appeared after 20 days. The most recognizable symptoms were sudden death of chickpea seedlings. First leaves start withering and drying and the root rot was followed gradually killing whole plant. Roots of the disease plant showed brown to blackish lesions. Isolation from artificially inoculated plant yielded *M. phaseolina* (Tassi) Goid which was identical to original once (Table 1 and Plate 1). Pathogenicity was proved by following two methods of inoculation i.e. seed and soil inoculation techniques. Among these, soil inoculation method was proved highly effective. This study is in conformity of earlier findings of Hinguera (1991) [3] who studied three different methods to establish pathogenicity of *M. phaseolina* in cowpea *viz*., toothpick inoculation, inoculation using cowpea seeds colonized by *M. phaseolina* and concluded that soil borne inoculum multiplied on rice seeds was more effective in disease development in cowpea seedlings. Lodha (1993) [5] also noticed the effect of sources of inoculum on population dynamics of *M. phaseolina* and disease intensity in cluster bean and suggested significant correlation between higher plant mortality and increased soil inoculum under moisture stress conditions. He also reported that *M. phaseolina* and *Fusarium solani* produce charcoal rot (dry root rot) and root rot of jojoba by inoculating the soil as well as by incorporation of inoculum of *M. phaseolina* through seed-cum-soil and drenching 15 days after sowing in cluster bean.

**Identification of the pathogen**

Identification of the isolated fungus was done on the basis of cultural characteristics and morphological characters of sclerotia. The young hyphae of the fungus was observed to be hyaline, thin walled light brown to dark brown in colour and having of more septa. Branches from the main hyphae are generally formed of right angle to parent hyphae with constriction of the point of origin. The colour of the sclerotia was light brown in the beginning which became darker with age and finally brown to black, sclerotia varied in shape irregular or spherical or oval or oblong, measuring form 90-120 x 60-150 µm with an average of 82.5 to 105 µm in size. Pycnidia were observed only on host surface. These were larger than sclerotia, dark brown to black, rough, globose, or irregular, beaked and ostiolate. On the basis of morphological character of this fungus has been identified as *Macrophomina phaseolina*. it was further confirmed from the Indian type culture collection (ITCC), Division of Plant Pathology, IARI, New Delhi as *Macrophomina phaseolina* (Tassi) Goid and the culture(s) has been deposited at ITCC under accession No. 6621.

**Table 1:** Pathogenicity of *Macrophomina phaseolina* with chickpea seeds

| Inoculation technique | Germination (%)* | Disease incidence (%)* |
|---|---|---|
| Seed inoculation | 50.30 | 50.35 |
| | (45.17) | (45.20) |
| Soil inoculation | 60.15 | 40.10 |
| | (50.86) | (39.29) |
| Control | 90.10 | 0.00 |
| | (71.66) | (0.00) |
| SEm± | 1.12 | 0.53 |
| CD (p = 0.05) | 3.44 | 1.63 |

Figures given in parentheses are angular transformed value

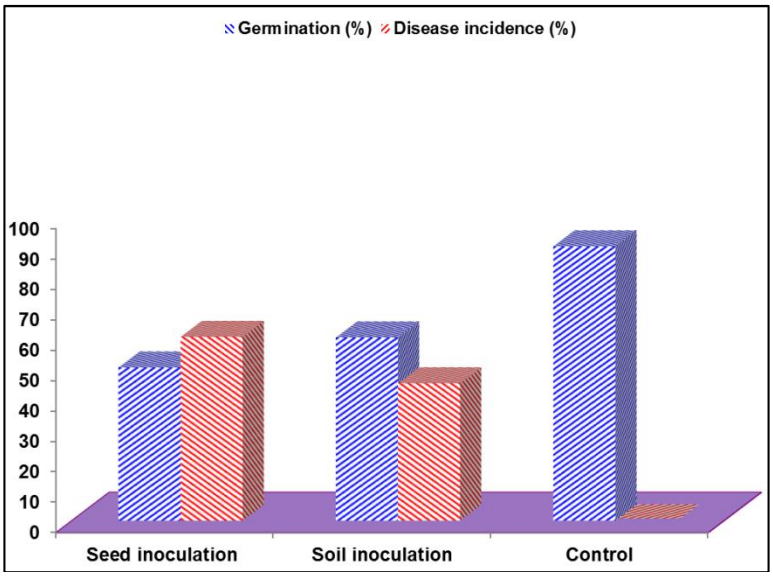


**Fig 4.1:** Pathogenicity of *Macrophomina phaseolina* with chickpea.

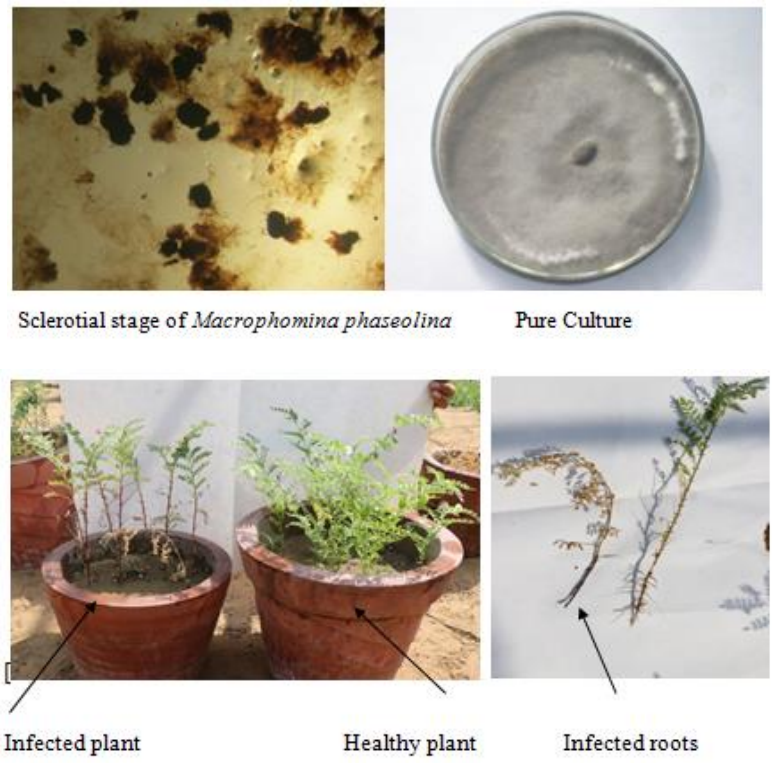


**Plate 1:** Pathogenicity test of *Macrophomina phaseolina* with chickpea plant

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