Biological Forum – An International Journal **17(1): 107-112(2025)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Survey, Cultural Variation and Pathogenicity of *Macrophomina phaseolina* causing Stem and Root Rot of Cowpea (*Vigna unguiculata* (L.) Walp.) in Jaipur District of Rajasthan

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(Received: 18 October 2024; Revised: 30 November 2024; Accepted: 28 December 2024; Published online: 20 January 2025)
(Published by Research Trend)

ABSTRACT: The annual legume cowpea [*Vigna unguiculata* (L.) Walp.] is also known as black-eyed pea, southern pea, and so on. Stem and root rot of cowpea is caused by *M. phaseolina* also one of the major factor of decline in cowpea production. Hence, the present investigation was conducted with an objective to assess the prevalence and incidence of stem and root rot disease in different locations of Jaipur district of Rajasthan during 2019 and assess the cultural characters and pathogenic variability among the isolates of *M. phaseolina*. From the Agronomy Farm at the S.K.N. College of Agriculture in Jobner, Rajasthan, diseased samples were gathered. The hyphal tip approach was used to purify the pathogen after it was isolated from cowpea roots. Seed, soil, and seed + soil inoculation procedures were used to demonstrate the fungus pathogenicity; the seed + soil inoculation technique showed the highest illness incidence. It was determined that *Macrophomina phaseolina* was the pathogenic fungus. In *kharif* 2019, a disease survey on cowpea stem and root rot was conducted in the Jaipur district's surrounding areas. From 12.67% (Kalwar, Jhotwara) to 25.67% (Bhojpura Kalan, Jobner), the illness incidence was noted. The *M. phaseolina* isolates growth characteristics revealed comparable.

Keywords: Cowpea, disease survey, pathogens, disease incidence.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is grown in Uttar Pradesh, Punjab, Haryana, Rajasthan, and MP and are also known as crowder peas, southern peas, and black-eyed peas. It is one of the important cereal legumes and grass crops in many tropical and subtropical areas (Fang *et al.*, 2007). Cowpea is an important part of cropping system; it fits well in a variety of farming systems and is grown as a cover crop, mixed crop, catch crop or green manure crop in different states of India (Alexandre *et al.*, 2016). Cowpea is grown across the world on an estimated 23.4 mha with a production of 18.29 mt and productivity of 637 kg/ha. In India cowpea is grown in an area of 4.00 mha with a production of 2.70 mt and productivity of 567 kg/ha (FAO, 2020). The cowpea crop is affected by number of fungal, bacterial and nematode diseases. Among Stem and root rot incited by *Macrophomina phaseolina* (Tassi.) Goid has been rated as most devastating disease of cowpea which can cause yield loss up to 5 to 39 per cent (Mohanapriya *et al.*, 2017). The districts of Jaipur, Sikar, Jhun-jhunu, Nagaur, Churu, and Bikaner are the principal cowpea-growing regions in Rajasthan. Because of its short growing season, high yield potential, and rapid growth tendencies, as well as its high protein content and ability to function as a cover crop that aids in soil conservation, cowpeas are very important in Rajasthan. In dry land farming, it is cultivated as an alternative crop.

Cowpea is tropical grain legume crop which plays an important nutritional role in developing countries of the tropics and sub tropics, especially in Africa, Asia, Central and South America (Singh *et al.*, 1997). Cowpea has been referred to as "Poor man's meat" because of its high protein content (20-25%). Cowpea young leaves, pods and beans contain vitamins and minerals which have fuelled its usage for human consumption and animal feeding (Nielson *et al.*,1997) and considered as one of agriculture's oldest legume used as protein source for humans and livestock (Steele, 1972). It contains high amount of quality protein (23.4%), carbohydrate (60.3%), fat (1.8%) and sufficient amount of calcium (76mg/100gm), iron (57mg/100gm) and vitamins such as thiamine (0.92mg/100gm), riboflavin (0.18mg/100gm) and nicotinic acid (1.9mg/100gm) (Chatterjee and Bhattaacharya 1986).

The dried seeds and green pods are eaten as vegetables and used to make a variety of snacks and main courses. For milch cattle, its straw is an excellent source of feed. In addition, the cowpea crop completely covers the ground, preventing water loss from the field and soil erosion. Particularly in Rajasthan, these characteristics make it a perfect part of diverse cropping systems (Gupta and Saxena 2015). Cowpea's high (20–25%) protein content has earned it the moniker "poor man's meat."

Numerous phytopathogenic diseases are known to affect the crop, including Bacterial blight caused by *Xanthomonas axonopodis pv. vignicola* (Nandini and Kulkarni 2014), rust from *Uromyces vignae* (Deshpand *et al.,* 2010), fusarium wilt from *Fusarium solani* (Joshi *et al*., 2011), anthracnose from *Colletotrichum lindemuthianum* (Sreeja and Girija 2016), root rot and charcoal rot from *Macrophomina phaseolina* (Yang and Navi 2005), anthracnose from *Erysiphe polygoni* (Surendra *et. al*., 2012), cercospora leaf spot from *Cercospora canescens* (Sarkar *et. al.*, 2017), and yellow mosaic caused by *Cowpea yellow mosaic virus* (Kumar *et al.*, 2018).

The findings underscore the significance of a well-balanced INM (Integrated Nutrient Management) strategy in optimizing cowpea productivity while potentially promoting soil health through enhanced organic matter incorporation (Patel *et al.*, 2023).

The most destructive cowpea disease is thought to be stem and root rot caused by *Macrophomina phaseolina* (Tassi.) Goid. During the *Kharif* season, the illness causes a great deal of damage. The disease strikes plants in Rajasthan while they are seedlings, just below the cotyledonary node. The disease's initial sign is yellowing of the leaves, which droop and then wither off during the next two or three days. Within a week following the onset of the first symptom, the plant can wilt. A thorough examination of the stem may reveal black lesions on the ground-level bark. The basal stem and main root may exhibit signs of dry rot if the plants are removed from the ground; the tissues are fragile and brittle.

Several of its natural hosts create sclerotial bodies, which produce pycnidia, and the fungus is a facultative parasite that can live saprophytically on decaying organic tissue. Since the fungus targets a variety of plant species, the pycniospores can survive for more than a year when the ambient temperature rises beyond 30˚C. The fungus primarily lives in soil and is transferred from one plant to another by irrigation water, tools, and cultural practices. Additionally, the sclerotia and pycniospore may fly into the air and disseminate the infection further (Rangaswami and Mahadevan 2008).

This disease has significantly reduced cowpea production in Jaipur, Rajasthan, in recent years. In order to determine the prevalence and incidence of dry root rot of cowpea in several blocks of Jaipur, Rajasthan, India, during *Kharif* 2019, as well as the cultural traits and pathogenic variability among the isolates of *M. phaseolina*, the current study was carried out. To successfully implement an efficient disease control strategy, this information is required.

MATERIALS AND METHODS

Survey. The *Kharif* season is when cowpeas are grown in Rajasthan. During the *Kharif* 2019, a disease survey on cowpea stem and root rot was conducted in the surrounding areas of four blocks in the Jaipur district of Rajasthan: Jobner, Phulera, Renwal, and Jhotwara. Each block was assessed at two different sites. A 2 × 2 m² patch was chosen at random from each of the four fields to examine disease signs and plant mortality in the surveyed areas. A conversation regarding the incidence of disease was conducted with farmers. The following formula was used to get the disease incidence percentage.

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

Collection of diseased sample. In order to isolate the pathogen, rhizosphere soil and infected plants exhibiting the characteristic symptoms of root rot caused by *M. phaseolina* infection were gathered. The samples were taken to the laboratory for further research work.

Isolation of the test pathogen. All glassware was cleaned with a solution of potassium dichromate and sulphuric acid, rinsed with sterile water, and sterilized in a hot air oven set at 180°C for two hours before isolation and other laboratory tests. The media (PDA) was autoclaved for 15 minutes at 121.6°C temperature and 15 psi pressure.

Cowpea plant roots and healthy portions were first cleaned with tap water before being chopped into little pieces. These parts were dipped in a 1% sodium hypochlorite solution to surface sterilize them and then washed it three times in distilled water. All the PDA media containing petri-plates were inoculated with the infected portion of the plant and incubated in BOD incubator at 25 ± 1°C for seven days.

Purification of test pathogen. The hyphal tip approach on PDA media produced a pure culture of the fungus. After 96 hours of incubation, hyphal tips were removed from culture slants and suspended in sterile distilled water for this purpose. The suspension was diluted so that, using the microscope's low-power objective, five to ten spores could be counted in one loopful. Twenty milliliters of sterile PDA medium were placed on Petri dishes with one milliliter of the aforementioned suspension. The germinating spores were found under a microscope and identified using a mock objective after 12 to 24 hours of inoculation. They were then moved to a PDA slant and maintained in BOD for additional growth. In order to preserve the culture for future research, it was periodically transferred on PDA slants.

Identification of the test pathogen. Morphological characteristics were used to identify the isolated fungus. It was determined that the fungus was *Macrophomina phaseolina* (Tassi.) Goid.

Pathogenicity of the pathogen. The pathogenicity of determines whether the bacteria can rot cowpea plants' stems and roots. The virulence of the isolated and purified fungus from damaged roots was examined. *Macrophomina phaseolina*'s pathogenicity was evaluated in pots using the seed and soil inoculation and seed + soil inoculation methods recommended by

Kataria and Grover (1976). To create seed-borne inoculums, cowpea (var. RC-19) seeds that appeared to be in good condition were surface sterilized and rolled on cultures of *Macrophomina phaseolina* that were growing on PDA in Petri plates after seven days. Sterilized soil was used to plant inoculated seeds in 9 × 12-inch earthen pots. The pathogen was cultured at 30ºC on sorghum grain medium for the soil inoculum was employed for 15 days. Before planting, pots were filled with sterile soil (soil: FYM = 3:1) that had been sterilized at 1.045 kg/cm² for an hour for three days in a row using copper sulphate solution. For every treatment, three replications were kept. To demonstrate pathogenicity, an inoculum was introduced at the time of planting using one of three inoculation techniques. Data on both healthy and diseased plants were tracked for 60 days, and the formula was used to determine the percentage of disease occurrence.

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

Seed inoculation technique. To do this, seeds were placed on Petri plates containing 7-day-old fungal cultures that were flourishing on PDA. Cemented containers were used to plant the inoculated seeds. As a check, the un-inoculated, seemingly healthy seeds were used. These pots were housed in a cage house and received regular watering as needed.

Soil inoculation technique. Pots (30 cm in diameter) were filled with sterilized soil + FYM (soil: FYM = 3:1; sterilized at 1.045 kg/cm² for one hour for three days in a row) after being sterilized with copper sulphate solution prior to sowing. The inoculum used to inoculate these pots multiplied on sorghum grains at a rate of 20 g/pot. Three replications of five surface-sterilized, seemingly healthy cowpea seeds (RC-19) were planted in each container. A check was provided by surface-sterilized seeds planted in un-inoculated sterilized soil. These pots were housed in a cage house, where they were maintained under the same circumstances and watered frequently as needed.

Seed-cum-soil inoculation technique. Pots were filled with sterilized soil + FYM (soil: FYM = 3:1; sterilized at 1.045 kg/cm² for one hour for three days in a row) after being sterilized with copper sulphate solution prior to sowing. The inoculum used to inoculate these pots multiplied on sorghum grains at a rate of 20 g/pot. Additionally, the seeds were rolled on cultures of fungus growing on PDA that were 7 days old and kept in Petri dishes. Infected pots were used to plant these infected seeds. As checks, inoculated pots were used to plant surface sterilized seeds that appeared to be healthy. To verify the fungus' authenticity, it was re-isolated from artificially inoculated plants and the resulting culture was compared to the original.

RESULTS AND DISCUSSION

A. Survey on the incidence of cowpea root rot disease in cowpea growing areas of Jaipur and Ajmer districts of Rajasthan

The data presented in Table 1 indicated that cow-pea stem and root rot incidence was found in the areas that were surveyed. The disease incidence ranged from 12.67 (Renwal, Renwal) to 25.67 (Bhojpura Kalan, Jobner) percent. The highest disease incidence (25.67%) was found in Bhojpura-Kalan of the Jobner block, followed by Hingonia (23.93%) and Dehra (19.17%) of the Phulera block, while the lowest incidence was found in both Jhotwara block locations (Kalwar and Lalpur, 12.67 and 13.33 percent, respectively). Overall, the Jobner block had a higher root rot disease incidence than the Jhotwara block in the Jaipur district. According to the survey, rainfed crops had a higher prevalence of disease than irrigated ones. The higher level of illness incidence may have been caused by the pathogen being favored by the dry circumstances that were common in the rainfed environments. Similar results were found in a survey of cultivated cowpea fields in northern Ghana in 2016 and 2017, which showed that rainfed environments had a higher incidence of root rot disease than irrigated conditions (Lamini *et al*., 2020). According to survey research by Vengadeshkumar *et al.* (2019), black soil had a greater incidence of root rot disease than clay or black cotton soil, and rainfed conditions had a higher prevalence of the disease than irrigated ones. There is a strong correlation between moisture and heat stress conditions on the occurrence, incidence, and severity of Macrophomina root rot infection (Gangopadhyay *et al*., 1982; Kaur *et al*., 2012).

Table 1: Survey of stem and root rot of cowpea in vicinity area of Jaipur district.

Isolate No.	District	Block	Village	Soil type	Situation	Percent Disease incidence (40 DAS)*
JP_1	Jaipur	Jobner	Bhojpura Kalan	Sandy	Rainfed	25.67_a
JP_2	Jaipur	Jobner	Hingonia	Sandy	Rainfed	23.93_b
JP_3	Jaipur	Phulera	Dehra	Sandy	Rainfed	19.17_c
JP_4	Jaipur	Phulera	Asalpur	Sandy	Rainfed	16.44_d
JP_5	Jaipur	Renwal	Renwal	Sandy	Rainfed	14.47_f
JP_6	Jaipur	Renwal	Bhadwa	Sandy	Rainfed	15.67_e
JP_7	Jaipur	Jhotwara	Kalwar	Sandy	Irrigated	12.67_h
JP_8	Jaipur	Jhotwara	Lalpura	Sandy	Irrigated	13.33_g

*Days after sowing

B. Growth and cultural characters of M. Phaseolina isolates

It was determined that *M. phaseolina* was the causative agent of cowpea root rot disease because all of the isolates had the same morph-cultural traits, including branching mycelium and black-grey, abundant aerial growth. Mycelial growth was highest in isolate JP1 (90.54 mm), followed by isolates JP2 (87.23 mm), JP5 (85.26 mm), and JP4 (84.62 mm). Additionally, JP1 was the isolate that produced the most sclerotia (175.11 and 102.66 µm), followed by JP2 (166.37 and 90.52 µm) and JP5 (165.61 and 86.80 µm). The isolate JP7 had the smallest sclerotia size, quantity, and growth (79.34 mm, 152.93, and 70.66 µm) (Table 2). These outcomes were consistent with the information provided by Tandel *et al*. (2012). In a similar vein, several previous workers have reported such variations in the mycelial growth among the isolates of *M. phaseolina* (Edraki and Banihashemi 2010; Ijaz *et al.*, 2013).

Table 2: Growth and cultural characters of *M. phaseolina* isolates.

Isolates	Mycelial growth (mm)	Mycelium characters	No. of sclerotia in (9 mm disc)	Sclerotial size (µ)
JP_1	90.54	Black grey profusely aerial growth	175.11_a	102.66_a
JP_2	87.23	Black grey profusely aerial growth	166.37_b	90.52_b
JP_3	82.33	Light grey scanty aerial growth	155.24_g	84.30_d
JP_4	84.62	Black grey profusely aerial growth	162.40_e	82.27_e
JP_5	85.26	White grey profusely aerial growth	165.61_c	86.80_c
JP_6	83.69	Black scanty aerial growth	164.30_d	81.10_f
JP_7	79.34	Grey profusely aerial growth	152.93_h	70.66_h
JP_8	81.57	Light grey scanty aerial growth	161.37_f	75.61_g

C. Pathogenicity of M. Phaseolina isolates

Cowpea was susceptible to the stem and root rot caused by isolates of *Macrophomina*. On the hypocotyl of cowpea plants, lesions resembling *M. phaseolina* were seen at seedling emergence and for up to 30 days following planting. However, the cowpeas that were not injected showed no symptoms. Table 3 displays the information regarding the pathogenicity of *M. phaseolina* isolates. With a maximum mean percent disease incidence (33.28%) on cowpea, isolate JP1 was the most virulent of the eight isolates tested. JP6 had a PDI of 28.06 percent, JP2 had a PDI of 27.11 percent, and JP4 had a PDI of 25.12 percent. Isolate JP7 had the lowest reported PDI (19.50%). The pathogen was re-isolated, and its characteristics were examined and contrasted with those of the original culture in order to verify its pathogenicity. The virulence of the *M. phaseolina* isolates that are common in each place can be directly linked to the differences in root rot incidence in those areas. In a related investigation, Ratnoo *et al.* (1997) found that the isolates of *M. phaseolina* from various Udaipur cowpea-growing regions varied. Previous researchers have also documented the variation in virulence across *M. phaseolina* isolates (Su *et al.*, 2001; Rayatpanah and Dalili 2012). The aforementioned reports concur with the current inquiry. Besides, an increase in the root rot incidence was observed with an increase in the age of the crop.

Table 3: Pathogenicity of *M. phaseolina* isolates.

Sr. No.	Isolates	Root rot incidence (%)			
		30 DAS	60 DAS	90 DAS	Mean (%)
1.	JP_1	18.02	31.65	50.16	33.28_a
2.	JP_2	16.29	25.18	39.86	27.11_c
3.	JP_3	13.40	24.41	36.05	24.62_e
4.	JP_4	14.18	23.28	37.91	25.12_d
5.	JP_5	12.44	20.78	35.27	22.83_g
6.	JP_6	15.22	27.35	41.61	28.06_b
7.	JP_7	10.62	18.40	29.49	19.50_h
8.	JP_8	13.64	23.61	36.33	24.53_f

CONCLUSIONS

Among the eight different villages of Jaipur district, the maximum per cent disease incidence was recorded 25.67 Percent from Bhojpura kalan in Jobner block (JP1) and lowest 12.67 Percent from Kalwar in Jhotwara block (JP7). It was determined that *M. phaseolina* was the causative agent of cowpea root rot disease. Hence, Mycelial growth was highest in isolate JP1 (90.54 mm) and the isolate JP7 had the smallest sclerotia size, quantity, and growth (79.34 mm, 152.93, and 70.66 µm). Similar to the present observations Moradia (2011) have found that increasing sclerotial population of *M. phaseolina* increased the infection of root rot and colonization in groundnut.

FUTURE SCOPE

Review literature revealed that stem and root rot disease of cowpea is affecting its production in Jaipur district due to climate change and very limited information is available on management of this disease according to current climatic requirements. Information is needed to properly implement effective disease management strategy. Considering the increasing importance of stem and root rot of cowpea, it is proposed to investigate on survey aspects.

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How to cite this article: Nisha Nitharwal, Mahabeer Singh, Sonali Meena, Shri Kishan Bairwa, Shankar Lal Bijarniya and Harish Kumar Bijarniya (2025). Survey, Cultural Variation and Pathogenicity of *Macrophomina phaseolina* causing Stem and Root Rot of Cowpea (*Vigna unguiculata* (L.) Walp.) in Jaipur District of Rajasthan. *Biological Forum – An International Journal, 17*(1): 107-112.