

Screening of Different *Macrophomina phaseolina* Isolates on Susceptible (RMG-62) Variety of Mungbean

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ABSTRACT

Dry root rot caused by *Macrophomina phaseolina* is of wide occurrence in sandy soil of Rajasthan where climatic conditions are dry and temperature remains high. Dry root rot is the most devastating disease in all the mungbean growing districts of Rajasthan particularly in Bikaner, Jaipur, Bhilwara, Bharatpur, Sri Ganganagar, Jodhpur, Kota and Udaipur. In the present investigation ten isolates of *Macrophomina phaseolina* were tested for their pathogenic nature, using susceptible RMG-62 variety of mungbean. The dry root rot incidence (%) in sterilized soil and unsterilized soil varied from 33.33 to 100 and 26.67 to 83.33, respectively. Isolate Bikaner was most virulent followed by Churu, Hisar, Sri Ganganagar, Junagarh-6486, IARI-5156, Sawaimadhapur, Delhi, Jalana-5156 and Narnaul. Bikaner isolate showed 83.33 per cent disease incidence in unsterilized soil which was less than sterilized soil i.e 100 per cent.

Key words: *Macrophomina phaseolina*, Dry root rot, Mungbean, Incidence (%).

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] also known as green gram belongs to family Leguminosae. Mungbean believed to be originated from India, is a source of protein (25%) with high quality of lysine (4600 mg/g N) and tryptophan (60 mg/g N). In India, it is the third important pulse crop after chickpea and pigeonpea². Mungbean is grown in almost all parts of the country in summer and kharif season in Northern and Southern India. The major mungbean growing states in India are Andhra Pradesh, Orissa, Maharashtra and Rajasthan. The important mungbean growing districts in the state are Jaipur, Bhilwara, Bharatpur, Sri Ganganagar, Jodhpur, Kota and

Udaipur³. Mungbean is being infected by several fungal, bacterial and viral diseases. But, dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid. Is considered as the most devastating disease in all the mungbean growing areas of country. The fungus *M. phaseolina* infects more than 500 plant species worldwide^{10,13} and causes charcoal rot disease in several agronomically important crops including soybean, maize, sorghum and cotton⁸. The disease is quite wide spread across the Rajasthan state due to congenial weather conditions and causes considerable yield losses^{5,7}. The pathogen may infect almost all parts of plants i.e. root, stem, branches, petioles, leaves and pods.

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Seed infection due to *M. phaseolina* ranges from 2.2 to 15.7 per cent which may cause losses in grain yield to the extent of 10.8 per cent and protein content of 12.3 per cent⁶. The infected seeds act as an important source of primary inoculum for new areas. Plant stand is affected due to pre and post-emergence infection of the crop. In pre-emergence stage, the fungus causes seed rot and mortality of germinating seedlings while in post emergence stage seedlings get blightened due to soil and seed-borne infection. In later stages of crop growth decay of secondary roots and shredding of the cortex region of the tap roots are commonly observed. The fungus produces dark brown lesions on the epicotyls and hypocotyls of seedlings and seedling death follows because of obstruction of xylem vessels and wilting. In adult plants, the pathogen causes red to brown lesions on roots and stems, and produces dark mycelia and black microsclerotia¹. The stem shows longitudinal dark lesions and the plant becomes defoliated and wilted. *M. phaseolina* is a heat tolerant pathogen since sclerotia could withstand a temperature range of 60–65°C. The seed-borne nature of the disease has also been demonstrated⁹. The pathogen being soil-borne and its propagules distributed randomly in soil is difficult to be controlled by fungicide. Moreover, the fungicides are effective only on the active metabolic stage of the propagules and not on resting structure. The disease is of wide occurrence in sandy soil of Rajasthan where climatic conditions are dry and temperature remains high. Therefore, it was very important to undertake the studies to find out the cause for disease development.

MATERIALS AND METHODS

Dry root rot infected mungbean samples were collected from different field locations, viz. Bikaner, Sawaimadhopur, Churu, Hisar, Delhi, Sri Ganganagar, IARI-5143, Jalana (Maharashtra)-5156, Junagarh-6486 and Narnaul. The root samples of diseased plants were used for isolation. The roots were thoroughly washed with tap water to remove soil. Small pieces of about 0.5 cm length were

surface sterilized with 0.1 per cent mercuric chloride solution for 2 minutes. Three washings with sterilized distilled water given, placed on Potato Dextrose Agar (Peeled potato 200 g, dextrose 20 g and agar agar 20 g in 1000 ml distilled water) slant in a laminar flow and incubated at $28 \pm 1^\circ\text{C}$ temperature for growth for seven days. To maintain the pure culture of *M. phaseolina* single hyphal tip isolation technique was adopted. One ml of suspension having 5-6 pieces of hypha per 10 x microscopic field were spread over 2 per cent plain agar in Petri dishes evenly by tilting the Petri dishes clockwise as well as anticlockwise. The excess amount of suspension was decanted and Petri dishes were incubated at $28 \pm 1^\circ\text{C}$ for 24 hours. The single piece of hypha was demarcated under low power of microscope (10 x) and cut with help of mechanical cutter. Individual piece of hypha was transformed on PDA slants with the help of an inoculating needle. The inoculated slants were kept in B.O.D. incubator for growth at $28 \pm 1^\circ\text{C}$ for 7 days. Thus, the purified cultures were maintained by periodical transfers on PDA slants and used for further studies.

Earthen pots (25 cm diameter) were filled with sterilized soil collected from mungbean cultivated areas of Agronomy Farm, College of Agriculture, Bikaner. Sand maize flour medium (10 g maize flour, 90 g sand and 20 ml distilled water in each flask) was autoclaved in 250 ml Erlenmeyer's flasks. Each flask was inoculated with pure culture isolate of *M. phaseolina* separately and incubated at $28 \pm 1^\circ\text{C}$ for 15 days. Fungus infested sand maize flour medium was mixed in soil of each pot with a ratio of 1: 200. The pots were watered regularly and kept moist for two days. Similarly, the pots filled with unsterilized soil were also made sick for testing the pathogenicity. The susceptible mungbean variety RMG-62 was used for testing pathogenicity. The seeds surface disinfected with 0.1 per cent mercuric chloride solution for one minute to remove the possibility of any fungus present on the surface of seed was sown in each pot. After 40

days of sowing root rot appeared in plants. *M. phaseolina* was isolated and purified. Koch's postulates were proven and pathogenic nature of each isolate was established.

Statistical Analysis

Each treatment in all experiments was in triplicate and data analysed statistically with the help of Completely Randomized Design (CRD) or Factorial in CRD. The per cent value was transformed into angles corresponding to that as follows¹¹.

$$\text{Angles} = \text{Arcsin } \sqrt{\text{Percentage}}$$

RESULTS AND DISCUSSION

Ten isolates of *M. phaseolina* tested for their pathogenic nature, using susceptible RMG-62 variety of mungbean were found virulent in sterilized as well as unsterilized soil. Koch's postulates were proven for isolates. The dry root rot incidence (%) in sterilized soil and unsterilized soil varied from 33.33 to 100 and 26.67 to 83.33, respectively. Isolate Bikaner was most virulent followed by Churu, Hisar,

Sri Ganganagar, Junagarh-6486, IARI-5156, Sawaimadhopur, Delhi, Jalana-5156 and Narnaul. Bikaner isolate showed 83.33 per cent disease incidence in unsterilized soil which was less than sterilized soil i.e 100 per cent. In general, the mortality of plant in unsterilized soil was reduced over sterilized soil. Symptoms of dry root appeared after 30 days of germination in sterilized soil while it was delayed up to 45 days in unsterilized soil. In unsterilized soil the reaction of isolates was less and delayed as compared to sterilized one. Devi and Singh⁴ categorized 56 isolates of *M. phaseolina* from blackgram and greengram crops from different parts of India and observed higher incidence of root rot in sterilized soil than in unsterilized soil as investigated in the present studies. Similarly, Umer and Tariq¹² isolated 65 isolates of *M. phaseolina* from Punjab and Khyber Pakhtun khwa provinces of Pakistan and found variation in their pathogenic nature, thereby confirming the present findings.

Table 1: Pathogenicity of Ten isolates of *M. phaseolina* on RMG-62 variety of mungbean in sterilized and unsterilized soil

Isolate	Sterilized	Unsterilized	Average
Bikaner	100 (90)*	83.33 (66.14)	91.67 (78.07)
Churu	80 (63.43)	66.67 (54.78)	73.34 (59.11)
Delhi	56.67 (48.85)	53.33 (46.92)	55 (47.89)
Hisar	73.33 (59)	63.33 (52.78)	68.33 (55.89)
IARI-5143	60 (50.77)	56.67 (48.85)	58.34 (49.81)
Jalana-5156	50 (45)	43.33 (41.15)	46.67 (43.08)
Junagarh-6486	63.33 (52.78)	53.33 (46.92)	58.33 (49.85)
Narnaul	33.33 (35.22)	26.67 (30.79)	30 (33.01)
Sawaimadhopur	60 (50.85)	46.67 (43.08)	53.34 (46.97)
Sri Ganganagar	70 (57)	66.67 (54.78)	68.34 (55.89)
Average	64.67 (55.29)	56 (48.62)	60.33 (51.95)

* Values in parentheses are angular transform value

	S.Em.±	CD (P=0.05)	CD (P=0.01)
Isolates	1.76	5.00	6.66
Soil types	0.79	2.23	2.98
Isolates x Soil types	2.49	7.07	9.42
CV (%)	8.29		

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