

Original Research Article

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Survey, Virulence and Pathogenicity of Root Rot Incidence of Cowpea in Selected Districts of Tamilnadu caused by *Macrophomina phaseolina* (Tassi.) Goid

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ABSTRACT

Cowpea (*Vigna unguiculata* (L.)Walp.) is a poor men's protein source. It is one of the most ancient human food sources and an important grain legume and hay crop in many tropical and subtropical regions. It is grown in Tamil Nadu and Andhra Pradesh widely as rainfed crop. Cowpea is affected by many diseases caused by viruses, bacteria and fungi. Among the fungal diseases, the charcoal rot caused by *Macrophomina phaseolina* (Tassi.) Goid causes significant loss in yield. *M. phaseolina* is a soil borne plant pathogen with a very wide host range. The studies were initiated with survey on the dry root rot incidence of cowpea in Cuddalore, Thiruvannamalai and Vellore districts of Tamil Nadu revealed endemic nature of the root rot disease incidence of cowpea. Among the different locations of Cuddalore, Thiruvannamalai and Vellore districts surveyed for cowpea root rot incidence, Sukkanampatti (MP₁₀) registered the maximum incidence of the disease (25.84%) followed by Keelakalpoondi, Keeranur and Sivapuri. The other locations viz., Kadavacheri, Perumathur, Varakoorpettai and Simanamputhur had moderate disease incidence while the minimum root rot incidence was recorded in Thanippadi and Chittur. In general, the crop grown under rainfed conditions showed more root rot incidence when compared with the crops grown under irrigated conditions. The variations in root rot incidence could be well attributed to the difference in virulence of the isolates of *M. phaseolina* prevalent in the respective areas. The survey revealed that higher levels of disease incidence in rainfed crop than that of irrigated crop. The dry condition prevalent in the rainfed conditions might have favoured the pathogen which could be attributed as the reason for the higher level of disease incidence.

Keywords

Cowpea, Root rot, Virulence, Sclerotia, Pathogenicity, Disease incidence.

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Introduction

Cowpea (*Vigna unguiculata* (L.)Walp.) is a poor men's protein source. It is one of the most ancient human food sources (Ng and Marechal, 1985) and an important grain legume and hay crop in many tropical and

subtropical regions (Fang *et al.*, 2007). Although cowpea is cultivated worldwide, over 75% of the world production is obtained from Africa (Singh *et al.*, 2002). In Africa it is considered the second most important pulse

crop and ranked amongst the top-five most important legumes in the world (Awurum and Enyiukwu, 2013). Among the various pulse crops, cowpea (southern pea) is one of the important crop and is grown in Tamil Nadu and Andhra Pradesh widely as rainfed crop.

The name "Cowpea" probably derived from when it was an important livestock feed for cows in the United States. Cowpea is called the "hungry-season crop" because it is the first crop to be harvested before the cereal crops (Gomez, 2004).

Cowpea is a good source of food, forage, fodder, vegetable and certain snacks (Nirmalet *et al.*, 2001). Cowpea pod husks obtained after threshing are also used to feed livestock. It is a crop of low and high rainfall regions, an important component of cropping system grown as catch crop, mulch crop, intercrop, mixed crop and green crop. It has the ability to fix atmospheric nitrogen in soil at the rate of 56 kg per ha in association with symbiotic bacteria under favourable conditions through its root nodules (Ahlawat and Shivkumar, 2005; Fatokumet *et al.*, 2000). It grows well in poor soils with more than 85% sand and with less than 0.2% organic matter and low phosphorus. According to FAOSTAT (2013), worldwide green pod production in 2010 was 4.5 million tons, cultivated in approximately 8 million hectares intended for human consumption.

Area under cowpea in India is 3.9 million hectares with a production of 2.21 million tonnes with the national productivity of 683 kg per ha (Anil Kumar Singh, *et al.*, 2012).

Cowpea is attacked by many diseases caused by viruses, bacteria and fungi (Emechebe and Lagoke, 2002). In India, cowpea and other pulse crops are mostly cultivated under rainfed conditions accounting above 78% of area and being a tropical environment, favours the disease incidence. Among the

fungal diseases, the charcoal rot caused by *Macrophomina phaseolina* (Tassi.) Goid causes significant loss in yield. Concurrent heat and moisture stress favor development of charcoal or dry root rot caused by *M. phaseolina* often makes cultivation of cowpea uneconomical (Singh *et al.*, 2012). Incidence of this disease ranging from 5 to 39 per cent, cowpea crop has been reported (Ushamalini *et al.*, 2001).

M. phaseolina is a soil borne plant pathogen with a very wide host range. *M. phaseolina* attacks a variety of oil seed, legumes and vegetable crops, besides a wide range of unrelated plants. Its microsclerotia, formed in senescing shoot tissues, survive well in soil (Mayek-Perez *et al.*, 2002). The fungus is soil-borne and poses great problem in managing the disease. During the recent years this disease causes significant losses in cowpea growing areas of Cuddalore, Thiruvannamalai and Vellore Districts.

Hence, the present study was conducted with an objective to assess the prevalence and incidence of dry root rot of cowpea in Cuddalore, Thiruvannamalai and Vellore Districts, India during 2013-14 and assess the cultural characters and pathogenic variability among the isolates of *M. phaseolina*.

Materials and Methods

Survey for assessment of loss due to dry root rot disease incidence

A field survey was conducted during 2013 – 14 to assess the extent of root rot occurrence of cowpea in Cuddalore, Thiruvannamalai, and Vellore districts of Tamil Nadu state. Ten locations representing both rainfed and irrigated situations were selected for the study. The per cent disease incidence was worked out using the following formula

Per cent Disease Incidence (PDI) =

$$\frac{\text{No of diseased plants}}{\text{No of plants observed}} \times 100$$

Also, the infected plants showing the typical symptoms of root rot due to infection with *M. phaseolina* were collected along with rhizosphere soil for isolation of the pathogen. The other informations regarding the soil type in which the crop is grown and the variety of cowpea cultivated were also recorded in the respective survey fields.

***M. phaseolina* isolate**

The pathogen *M. phaseolina* (Tassi) Goid was isolated from the diseased roots of cowpea plants showing the typical root rot symptoms by tissue segment method on potato dextrose agar (PDA) medium. The axenic cultures of the different isolates of the pathogen were obtained by single hyphal tip method (Rangaswami, 1972) and these were maintained on PDA slants for subsequent experiments.

Mass multiplication of *M. phaseolina* inoculum for soil application

The isolates of the pathogen were multiplied in sand maize medium (Riker and Riker, 1936). Sand and ground maize seeds were mixed in the ratio of 19:1, moistened to 50 per cent moisture content, filled in 500ml conical flask and autoclaved at 20 psi for two h. Four actively growing mycelial discs (9 mm) of the pathogen isolates were inoculated into each flask under aseptic condition and the flasks were incubated at room temp. (28 ± 2°C) for 15 days and the inoculum thus obtained was used for the experiments.

Pathogenicity test

Pots (30cm dia) of uniform size containing sterilized soil were used for proving

pathogenicity. The inoculum of *M. phaseolina* isolates multiplied in sand maize medium was mixed with soil @ 5 % level ratio at the time of sowing (Sankar, 1994). About 2 cowpea seeds were sown in each pot and maintained in green house with need based irrigation. The PDI was assessed at 30, 60 and 90 DAS and recorded. Also the plants showing the typical root rot symptom were pulled out and the pathogen was re-isolated on PDA slants. The culture thus obtained was compared with that of the original culture and the pathogenicity (Koch postulates) was proved.

Cultural characteristics of the isolates

Morphological characters on PDA

Fifteen ml of the medium was poured into each of the 90 mm Petri dishes. One ml of streptomycin sulphate of 100 ppm strength was added to the medium just before pouring into the plates. Inoculation was made by transferring 9mm growth disc of *M. phaseolina* taken from the periphery of seven day old culture. The plates were incubated at 28±2°C. Differences in topography, type of margin, rate of growth and days to form sclerotia were recorded.

Mycelial growth

Fifteenml of the sterilized PDA medium was poured into sterile Petri dishes and allowed to solidify. A nine mm culture disc of *M. phaseolina* obtained from actively growing region was aseptically placed at the center of the dish and incubated at room temperature (28±2°C). The radial growth of the isolates (in mm) was measured four days after inoculation.

Sclerotial number

Four culture discs (9 mm) were cut and placed into 50 ml beakers containing 10 ml of sterile water. These beakers were kept on a

mechanical shaker at 1000 rpm for 30 min. to separate the sclerotia from the medium; then squeezed through cheese cloth; washed several times with distilled water and the sclerotia were transferred to a glass vial containing 2.5 ml of 2.5 per cent ammonium sulphate.

After 10 min. the floating sclerotia were filtered through a Whatman No. 42 filter paper rinsed with dist. water and the number of sclerotia was counted using stereo zoom microscope (Dhingra and Sinclair, 1978). The time taken by the isolates to form sclerotia was also recorded. The number of sclerotia per microscopic field and per nine mm disc were assessed and recorded.

Sclerotial size

For each isolate 100 sclerotia were collected at random. These were dried under shade for two h. and their size was measured using an ocular micrometer in a calibrated microscope.

Optimum inoculum level of *M. phaseolina*

The sand maize inoculum of the most virulent isolate (MP₁₀) of *M. phaseolina* at different levels was separately mixed thoroughly with the sterile and unsterile pot culture soil in earthen pots one week prior to sowing. Four surface sterilized seeds were sown in each pot. Three replications were maintained for each treatment and the pots were maintained under glass house conditions. The disease incidence at 30 days interval up to 90 DAS and expressed as per cent disease incidence. The optimum inoculum level identified from these experiments was used in all the subsequent experiments of this study.

Statistical analysis

The data collected were subjected to statistical analysis using computer aided AGRISTAT (V.6.2003) software.

Results and Discussion

Survey on the dry root rot incidence of cowpea in Cuddalore, Thiruvannamalai and

Vellore districts of Tamil Nadu

The data presented in table 1 on the survey in different locations of Cuddalore, Thiruvannamalai and Vellore districts revealed endemic nature of the root rot disease incidence of cowpea. Among the different locations of Cuddalore, Thiruvannamalai and Vellore districts surveyed for cowpea root rot incidence, Sukkanampatti (MP₁₀) registered the maximum incidence of the disease (25.84%) followed by Keelakalpoondi (MP₁) with 25.43 per cent, Keeranur (MP₈) with 24.41 per cent and Sivapuri (MP₄) with 23.75 per cent. The other locations viz., Kadavacheri (23.42), Perumathur (22.82), Varakoorpettai (21.48) and Simanamputhur (20.56) had moderate disease incidence while the minimum root rot incidence of 19.38 and 17.72 per cent was recorded in Thanippadi and Chittur. In general, the crop grown under rainfed conditions showed more root rot incidence when compared with the crops grown under irrigated conditions. In respect of soil type, sandy loam had more root rot incidence (17.72 to 25.84%) than red sandy (24.41%), clay loam (21.48 to 25.43%) and clay (22.82 to 23.75%).

The variations in root rot incidence could be well attributed to the difference in virulence of the isolates of *M. phaseolina* prevalent in the respective areas. The severity of the disease is also directly related to the population of viable sclerotia in the soil (Salik Nawaz Khan, 2007). The survey revealed higher levels of disease incidence in rainfed crop than that of irrigated crop. The dry condition prevalent in the rainfed conditions might have favoured the pathogen which

could be attributed as the reason for the higher level of disease incidence. Soil texture also had a significant impact on root infections. In the present survey more root rot disease incidence was observed in sandy loam as compared to clay or clay loam (Table 1). Similar to the present results, the crop grown in sandy loam soil registered higher per cent of root rot incidence than that of clay soil (Retinasababady and Ramdoss, 1999). Cruz Jimenez (2011) observed highest *M. phaseolina* root populations in sandy soils, followed by loamy sand and loam soil textures. These earlier reports lend support to the present findings.

Pathogenicity of *M. phaseolina* isolates

The data depicted in table 2 revealed varied levels of pathogenicity with difference in isolates. Among the ten isolates of *M. phaseolina* collected from different conventional cowpea growing areas of Cuddalore, Thiruvannamalai and Vellore district, the isolate (MP₁₀) collected from Sukkanampatti was found to be more virulent and recorded the maximum incidence of 48.60 per cent (at 90 DAS) followed by MP₁ (44.75%) collected from Keelakalpoondi. The isolate MP₂ collected from Chittur was the least virulent which recorded the minimum (24.33%) root rot disease incidence.

The results of the pot culture experiment conducted by artificial inoculation of the pathogen revealed varied levels of pathogenicity with different isolates. The variation in the isolates of *M. phaseolina* from different cowpea growing areas of Udaipur was reported by Ratnoo *et al.*, (1997).

Generally, variability in the pathogenicity among the isolates of *M. phaseolina* was reported by earlier workers (Su *et al.*, 2001; Chowdary, 2009; Rayatpanah and Dalili, 2012). The above reports are in agreement

with the present investigation. Besides an increase in the root rot incidence was observed with an increase in the age of the crop. Similar to the present observation Retinasababady and Ramdoss (2000), observed significant increase in root rot incidence at 40th and 60th DAS in rice fallow blackgram.

Cultural characteristics of *Macrophomina phaseolina* isolates

Mycelial growth

All the ten isolates of the root rot pathogen *M. phaseolina* produced white, whitish grey, grey, black scanty to profusely aerial mycelial growth on Potato Dextrose Agar (PDA) medium. The isolate of MP₁₀ significantly recorded the maximum (90 mm) mycelial growth, while it was the minimum (72.60 mm) in the case of MP₂. The other isolates showed moderate mycelial growth (80.60 to 88.25 mm) (Table 3).

Similar such variation in the cultural characteristics of *M. phaseolina* on PDA was reported by Tandel *et al.*, (2012). Also, several earlier workers have reported about the variations in the mycelial growth among the isolates of *M. phaseolina* (Edraki and Banihashemi, 2010; Ijaz *et al.*, 2012). *M. phaseolina* isolates from pearl millet, sesame, horsegram and mothbean differed in their mycelial growth and showed marked variation in cultural characters (Sharma and Dureja, 2004). Shekhar *et al.*, (2006) on the basis of colony colour divided seven isolates into four groups namely grayish white, blackish gray, dark black and cottony white colonies while working with charcoal rot of maize. Further, it was observed that the isolates of *M. phaseolina* with faster mycelial growth were more pathogenic and produced higher root rot incidence.

The virulence of the isolates of *M. phaseolina* was positively correlated with their growth rate (Ghosh and Sen, 1973) and Sharmishha *et al.*, (2004) reported that the isolates of *M. phaseolina* with faster mycelial growth were found more pathogenic to cluster beans. These earlier reports corroborate with the present findings.

Sclerotial number

All the isolates of *M. phaseolina* varied in their ability to produce sclerotia on PDA medium. The maximum sclerotial number of 183.50 per nine mm culture disc was obtained from MP₁₀ which was also the most virulent isolate. This was followed by the isolates MP₁, MP₈, MP₄, MP₅, MP₆, MP₃, MP₉, and MP₇ which produced 178.25, 169.00, 168.75, 164.75, 162.50, 160.25, 159.75 and 158.25 numbers of sclerotia, respectively. The minimum number of sclerotia of 147.25 was recorded by MP₂ the least virulent isolate (Table 3). It is evident from the observations that sclerotia are the primary means of survival (Mirza, 1984) and sufficient build up of the growth is absolutely necessary for the aggressiveness of the pathogen. Generally isolates producing more sclerotia are more pathogenic and caused higher seedling mortality (Sharmishha *et al.*, 2004). Hooda and Grover (1982) observed a positive correlation between the disease intensity and the inoculum density. Similarly, the correlation between inoculum density and disease development was reported in sesame (Sankar, 1994) and in other crops (Umamaheswari, 1991; Rettinasababady, 1996) in respect of *M. phaseolina*. Generally isolates producing more sclerotia are more pathogenic and caused higher seedling mortality as reported by Sharmishha *et al.*, (2004). Also, the severity of the disease is directly related to the population of viable sclerotia in the soil (Sundravadana *et al.*, 2012). In line with these earlier reports, in the present observation also the isolate which

produced the maximum sclerotia happens to be the most virulent isolate.

Sclerotial size

The isolates of *M. phaseolina* produced varying sizes of sclerotia on PDA. The most virulent isolate MP₁₀ produced the biggest sclerotia with a size of 104.20 μ (Table 3) and the smallest sclerotial size of 78.20 μ was recorded with MP₂, which was the least virulent isolate. This was followed by other isolates *viz.*, MP₁, MP₈, MP₄, MP₅, MP₆, MP₃, MP₉, and MP₇ which produced sclerotia with the size of 96.50, 89.60, 86.20, 83.60, 82.40, 80.80, 80.28 and 80.06 μ , respectively. Similar variation in the sclerotial size of *M. phaseolina* was observed by several workers (Suriachandraselvan and Seetharaman, 2003; Tandel *et al.*, 2012). Significant differences in mycelial development, size of sclerotia and pathogenicity of different isolates of *M. phaseolina* from cotton was observed by Vilela *et al.*, (1987). All these above reports corroborate with the present findings.

The isolates producing bigger sclerotia caused more root rot incidence in cotton even at low inoculum level (Monga and Sheo Raj, 1994). In the present study also the isolate (MP₁₀), which produced the biggest sclerotia caused the maximum root rot incidence. The possibility of containing more food materials and subsequent production of more germ tubes by bigger sclerotia might have resulted in more aggressiveness of the isolate.

Effect of inoculums level of against *M. phaseolina* on the root rot incidence of cowpea

The data depicted in table 4 revealed that the inoculum level of *M. phaseolina* on the root rot incidence of cowpea showed variation in the level of root rot incidence with difference in the inoculum level.

Table.1 Survey on the incidence of Dry root rot of cowpea in Cuddalore, Thiruvannamalai and Vellore Districts

	Isolate number	Villages	Districts	Soil type	Variety	Situation	Dry root rot incidence (%)
1	MP ₁	Keelakalpoondi	Cuddalore	Clay loam	Local variety	Rainfed	25.43
2	MP ₂	Chittur	Cuddalore	Sandy loam	Local variety	Irrigated	17.72
3	MP ₃	Varakoorpettai	Cuddalore	Clay loam	Co 6	Rainfed	21.48
4	MP ₄	Sivapuri	Cuddalore	Clay	Local variety	Rainfed	23.75
5	MP ₅	Kadavacheri	Cuddalore	Clay	Local variety	Rainfed	23.42
6	MP ₆	Perumathur	Cuddalore	Clay	Local variety	Rainfed	22.82
7	MP ₇	Thanippadi	Thiruvannamalai	Sandy loam	Co 6	Irrigated	19.38
8	MP ₈	Keeranur	Thiruvannamalai	Red sandy	Paiyur 1	Rainfed	24.41
9	MP ₉	Simanamputhur	Vellore	Sandy loam	Local variety	Rainfed	20.56
10	MP ₁₀	Sukkanampatti	Vellore	Sandy loam	Local variety	Rainfed	25.84

Table.2 Pathogenicity of *M. phaseolina* isolates

S.No.	Isolates	Root rot incidence (%)			Mean
		30 DAS	60 DAS	90 DAS	
1	MP ₁	21.00 (27.28)	36.75 (37.33)	44.75 (42.00)	34.16
2	MP ₂	12.22 (20.47)	18.45 (25.65)	24.33 (29.55)	18.33
3	MP ₃	15.75 (23.39)	24.60 (29.73)	35.88 (36.79)	25.41
4	MP ₄	18.40 (25.50)	28.10 (32.03)	38.24 (38.19)	28.24
5	MP ₅	18.00 (25.10)	26.88 (31.24)	38.00 (38.06)	27.63
6	MP ₆	16.80 (24.20)	26.20 (30.79)	36.25 (37.03)	26.42
7	MP ₇	12.25 (20.50)	22.00 (27.97)	32.00 (34.45)	22.08
8	MP ₈	20.80 (27.13)	34.00 (35.67)	38.60 (38.41)	31.13
9	MP ₉	14.90 (22.17)	22.75 (28.50)	34.10 (35.73)	23.92
10	MP ₁₀	25.90 (30.59)	38.65 (38.45)	48.60 (44.20)	37.72
	S.Ed.	0.08	0.09	0.10	-
	C.D. (p=0.05)	0.18	0.19	0.22	-

Data in parentheses indicate arcsine transformed values

DAS – Days after sowing

Table.3 Cultural characters of *M.phaseolina* isolates

S.No.	Isolate number	Mycelial growth(mm)	Number of sclerotia (9 mm disc)	Sclerotial size (μ)	Mycelial characters
1	MP ₁	88.25	178.25	96.50	Black grey profusely growth
2	MP ₂	72.60	147.25	78.20	Black profusely aerial growth
3	MP ₃	83.50	160.25	80.80	Black grey profusely aerial growth
4	MP ₄	86.75	168.75	86.20	Grey profusely aerial growth
5	MP ₅	84.25	164.75	83.60	Light grey scanty aerial growth
6	MP ₆	84.11	162.50	82.40	Black scanty aerial growth
7	MP ₇	80.60	158.25	80.06	Light grey profusely aerial growth
8	MP ₈	87.00	169.00	89.60	White grey profusely aerial growth
9	MP ₉	82.50	159.75	80.28	Black grey profusely aerial growth
10	MP ₁₀	90.00	183.50	104.20	Black grey profusely aerial growth
	S.Ed.	0.07	0.10	1.01	-
	C.D. (p=0.05)	0.14	0.22	2.12	-

Table.4 Effect of inoculum level of *M. phaseolina* on the root rot incidence of cowpea (Pot culture)

Tr.No.	Inoculum level (%)	Per cent infection			Mean
		30 DAS	60 DAS	90DAS	
1	1.0	10.04	12.00	18.70	13.58
2	3.0	21.81	30.00	34.40	28.73
3	5.0	25.65	38.00	51.04	38.23
4	7.0	26.64	39.00	52.10	39.24
5	10.0	25.54	38.12	51.24	38.30
6	Control	00.00	00.00	00.00	00.00
	S.Ed.	1.22	1.84	2.61	-
	C.D. (p=0.05)	2.72	4.12	5.84	-

DAS – Days after sowing

Among the five different levels of inoculums tested, the 7 per cent inoculum conc. (T₄) recorded the maximum per cent infection at 30, 60 and 90 DAS (26.64, 39.0 and 52.1%) which was on par with that of five per cent level of inoculum. The one and three per cent levels recorded only moderate incidence.

The subsequent studies were carried out with five per cent level of the inoculum. Similar to the present observations McCain and Scharpf (1989) and Moradia (2011) have found that increasing sclerotial population of *M. phaseolina* increased the infection and colonization in conifers sunflower and groundnut respectively. Umamaheswari *et al.*, (2002) observed that in groundnut root infection was severely increased by increasing inoculums density of *M. phaseolina*. Similarly, Suriachandraselvam and Seetharaman (2003) reported that increase in the inoculums level of *M. phaseolina* by artificial inoculation increased the root rot incidence in blackgram. In spite of being a mono-specific genus, *M. phaseolina* exhibits a high degree of morphological (Mayek-Perez *et al.*, 1997), pathogenic (Su *et al.*, 2001), physiological (Mihail and Taylor, 1995) and genetic (Babu *et al.*, 2007) variability probably due to the presence of heterokaryosis (Beas-Fernandez *et al.*, 2006).

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