I J T A
© Serials Publications
National Academy of Agricultural Science (NAAS)
Rating : 3. 03

Survey and Virulence Studies on Dry Root Rot of Greengram [Vigna Radiate (L.) Wilczek] Incited by *Macrophomina Phaseolina* (Tassi.) Goid

*Mallaiah B[1]. and Krishna Rao V.[1]

Abstract: *Greengram (Vigna radiate L.) Wilczek is one of the important pulse crops grown in India and the crop is incited by a number of diseases. Dry root rot caused by Macrophomina phaseolina is an important disease causing yield losses up to 25 per cent. Survey conducted on the incidence of dry root rot in eight major greengram growing mandals of Chittoor district, A.P. indicated occurrence of dry root rot incidence in all the farmers fields surveyed and the incidence ranged from 5.7 to 12 per cent with low and high incidence in Pileru and Chinnagottigallu mandals, respectively. Among the three soil types surveyed, high (11.1%) while low (4.2%) incidences of dry root rot was recorded in sandy loam and clay soils respectively. Greengram cv. ML-267 recorded high dry root rot incidence (11%) while incidence was low (4.8%) in local cultivars. Virulence studies indicated an incolum level of 7 per cent (w/w) was found to be optimum infection threshold level of test pathogen, M. phaseolina with greengram cv. ML-267 in steam sterilized sandy loam soil.*

Keywords: *Greengram, Macrophomina phaseolina, Pathogencity, Survey.*

INTRODUCTION

Mungbean/green gram [Vigna radiate (L.) Wilczek] is one of the most important pulse crop and widely grown short duration legumes world wide. Green gram is an excellent source of high quality protein. It is consumed in different ways as dal, halwa, snack and so many other preparations. It is also used as green manure crop and grows in summer and kharif seasons in northern India and also in south India [1]. In India it is the third important crop after chickpea and pigeonpea. The crop is subjected to diseases caused by fungi, bacteria, and viruses. The major fungal diseases which affect the greengram are root rot (*Macrophomina phaseolina* (Tassi.) Goid, powdery mildew (*Erysiphe polygoni* DC), Cercospora leaf spot (*Cercospora canescens* Ellis and Martin) and anthracnose (*Colletotrichum dematium* and *C. lindemuthianum*) [2]. Of these diseases, root rot caused by *M. phaseolina* causes considerable yield losses in greengram [3]. *Macrophomina phaseolina* (Tassi.) Goid is one of the most virulent and destructive pathogen which incite diseases in wide range of hosts and produces seedling rot, collar rot, leaf blight in mothbean [4]. Root rot incited by this pathogen has been rated as most devasting diseases of mungbean.

The pathogen attacks on all parts of plant i.e root, stem, branches, petiole, leaves, pods and seeds. The infected seeds act as an important source of primary inoculums for new areas [4]. Therefore an attempt has been made to conduct a detailed survey to study the incidence of the pathogen and pot culture experiments to study the pathogencity.

MATERIALS AND METHODS

All the laboratory experiments were conducted at the Department of Plant Pathology, S.V Agricultural College, Tirupati, ANGRAU. The college is situated

[1] Department of Plant Pathology, PJTSAU, Agricultural Research Station, Karimnagar - Telangana 505001.
* Corresponding author. *E-mail: mallyagrico@gmail.com,* Mobil. 09440504167

at 13° North latitude and 79°E longitude and at an altitude of 182.9 m in tropical belt of South India.

Survey

Survey was conducted in eight major greengram growing mandals of Chittoor district o f Andhra Pradesh to study the incidence of dry root rot.

Collection of Diseased Plant Samples

The farmers' fields were selected at random in eight major greengram growing mandals of Chittoor district of Andhra Pradesh *viz.*, Srikalahasti, Chandragiri, Pileru, Pakala, Pulicherla. Madanapalli, Chinnagottigallu and Nagari. In each mandal, three villages were selected at random and from each village one farmer field was surveyed. From each field, 4-6 places of 1 sq meter area (depending upon the area of the field) were selected at random on diagonal line of the field for taking diseased plant samples representing whole field. About 5-10 diseased plants showing symptoms of dry root rot incidence were collected from each field [5]. The plant samples were collected in a polythene bag and tied with a rubber band, labelled and brought to the laboratory for further studies. From the diseased plant samples the test pathogen was isolated and identified using appropriate keys. [6, 7]

Dry Root Rot Incidence

The diseased plants were recorded from 1 sq meter area in 4 to 6 places in each field and number of plants with dry root rot incidence was recorded and expressed in percentage following formula given by Singh [5].

$$\text{Dry root rot incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total number of plants}} \times 100$$

Isolation of test pathogen

The pathogen *Macrophomina phaseolina* (Tassi) Goid was isolated on potato dextrose agar (PDA) medium from greengram plant showing dry root rot symptoms. Bits of diseased portion along with healthy tissue from the infected root comprising small, black sclerotia were cut with a sterile blade and washed thoroughly under running tap water to get rid of foreign matter. These were surface sterilized with 1 percent mercuric chloride ($HgC1_2$) for 3 minutes followed by three washings with sterile distilled water. These bits were then transferred to PDA containing petriplates and were incubated at room temperature (28° ± 2) for 48 hours. A portion of the mycelium was taken from fungal growth arising from incubated bits and was transferred on to a fresh PDA plate. This fungus was purified by single hyphal tip method [8] and maintained on agar slants for further use.

Mass Multiplication of the Pathogen

The inoculum of the test pathogen, *M. phaseolina* maintained on agar slants was further multiplied on sorghum grains. One hundred grams of sorghum seeds were washed thoroughly in tap water and soaked overnight in 250 ml conical flasks with addition of 20 ml of 4 per cent dextrose. The flasks were then autoclaved for 20 min at 15 lbs. After cooling the flasks at room temperature they were shaken well to separate the sterilized grains and were inoculated with disc of 4 day old culture of *M.phaseolina* and incubated at 28 ± 2°C for seven days in BOD incubator.

Pathogenicity Studies

The test pathogen isolated from diseased plants collected during survey was used to study the effect of different inoculum levels, so as to determine optimum infection threshold level. A pot culture experiment was conducted in the green house to study the pathogenicity of the test pathogen at different inoculum levels by soil infestation following the procedure described [9] . Earthen pots of 12 inch diameter (capacity 2 kg) were filled with 2 kg steam sterilized soil.The following are the characteristics of the soil used for the pathogenicity studies.

Soil structure	= Sub angular blocky
Soil texture	= Sandy loam
pH	= 8.0
E.C	= 0.4 m mho/cm
Organic Carbon	= 0.22 (medium)

Table 1
Survey on incidence of dry root rot caused by *M.phaseolina* in major greengram growing mandals of Chittoor district, A.P.

Sl. No.	*Name of the Mandal and Village*	*Sample number/ isolate*	*Stage of the Crop (rabi)*	*Soil type*	*Variety*	*Dry root rot incidence (%)*	*Mandal average*
1.	*Srikalasti*						
	Ammapalem	1	Vegetative	Sandy Clay loam	Local	7	6.7
	Thondamandu	2	Vegetative	Sandy loam	PusaBaisakhi	8	
	Muchivolu	3	Vegetative	Sandy loam	Local	5	
2.	*Chandragiri*						
	Thondavada	4	Flowering	Clay loam	Local	3	5.8
	Sanambatla	5	Vegetative	Clay loam	Local	2.5	
	Ithepalli	6	Vegetative	Sandy Clay loam	ML-267	12	
3.	*Chinnagottigallu*						
	T.Sattevaripalem	7	Vegetative	Sandy loam	ML-267	24	12.0
	Yadanvaripalli	8	Vegetative	Clay loam	Local	3	
	Rangangariadda	9	Flowering	Sandy Clay loam	Pusa Baisakhi	9	
4.	*Pileru*						
	Reddivaripalli	10	Vegetative	Clay loam	ML-267	7	5.7
	Kotapalle	11	Vegetative	Clay loam	ML-267	6	
	Yellamanda	12	Vegetative	Clay loam	Local	4	
5.	*Pakala*						
	Mugarala	13	Flowering	Clay loam	Local	5	9.0
	Nendragunta	14	Vegetative	Sandy loam	Local	9	
	Dhanujuvaripalli	15	Vegetative	Sandy loam	ML-267	13	
6.	*Pulicherla*						
	Ramireddygaripalle	16	Vegetative	Sandy loam	Pusa Baisakhi	13	8.1
	Venkanaddivaripalle	17	Vegetative	Clay loam	Local	2.2	
	Kalluru	18	Flowering	Sandy Clay loam	ML-267	9	
7.	*Madanapalli*						
	Edigapalli	19	Flowering	Sandy clay loam	Local	5	6.0
	Pothabolu	20	Vegetative	Sandy clay loam	Local	7	
	Reddivaripalli	21	Vegetative	Clay loam	ML-267	6	
8.	*Nagari*						
	Sathrawada	22	Vegetative	Clay loam	Local	5	6.3
	Kakivedu	23	Flowering	Sandy Clay loam	Pusa Baiskhi	8	
	E. Kuppam	24	Vegetative	Sandy loam	Local	6	
	Total	*24*			*Average*	*7.4*	

Available nitrogen = 197 kg/ha (medium)
Available P_2O_5 = 43 kg/ha (medium)
Available K_2O = 164 kg/ha (medium)

Effect of Different Inoculum Levels on Dry Root Rot Incidence

The inoculum of test pathogen multiplied on sorghum grains was added to the steam sterilized soil @ 1,3,5,7 and 9 percent (w/w) in the pots. The soil in pots without inoculum of the pathogen served as control.

Each pot was sown with 10 surface sterilized greengram seeds cv. ML-267. Six treatments with five replications for each inoculum level of test pathogen were maintained. The data on dry root rot incidence was recorded at 45 days after sowing and expressed in percentage.

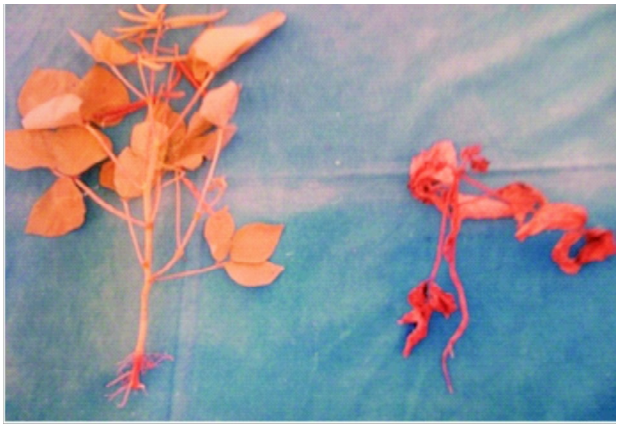

Figure 1: Greengram plant with dry root rot Symptoms

RESULTS AND DISCUSSION

Survey

A field survey was conducted to know the incidence of dry root rot caused by *Macrophomina phaseolina* in major greengram growing mandals of Chittoor district of Andhra Pradesh and the data are presented in Table 1. A total of 24 samples were collected from 24 farmer's fields representing 8 major greengram growing mandals of Chittoor district, A.P. The symptoms associated with dry root rot affected plants were leaves and stem turned straw colored, became flaccid and drooped, exhibiting wilting symptoms. Discoloration of the basal portion of the stem, rotting of underground plant parts were also observed. The taproot became dark, showing rotting symptoms and was devoid of most of its lateral and finer roots [Figure 1]. Decay of the secondary roots and shredding, brittleness of the cortex of the taproot were recorded. Minute sclerotial bodies with mycelia bits of the pathogen were seen all over the affected and shredded roots. In advanced stages drying and death of the plants was observed.

The results on the survey revealed that there was a variation in dry root rot incidence with high incidence (12%) in Chinnagottigallu mandal and low in Pileru mandal (5.6%). Similar variation in dry root rot incidence (0.5-38%) in greengram at Rajasthan also observed [10]. Reports also available with 10.8 and 24.1 per cent dry root rot incidence in Haryana and Rajasthan, respectively [11, 12]. The variation in dry root rot incidence could be due to influence of various ecological, physical and chemical characteristics of soil.

Table 2
Influence of soil types on the dry root rot incidence assocaited with greengram in major green gram growing mandals of Chittoor district A.P.

S. No.	*Soil type*	*Total samples*	*Mean per cent dry root rot*
1.	Sandy loam	7	11.1
2.	Sandy clay loam	7	8.1
3.	Clay loam	10	4.3
	Total	*24*	

Soil Type

The soil type also influence the dry root rot incidence (Table 2), a high incidence of dry root rot was observed in sandy loam (11.1%) followed by sandy clay loam (8.1%) and least in clay soils (4.3%). In light sandy soils, the incidence of dry root rot due to *M. phaseolina* was reported to be high. Similar results with the same pathogen were also earlier observed [12] and also in chick pea and black gram, respectively [13]. The fungal activity is influenced by soil aeration and soil texture. The amount of free oxygen obviously decides the activity of soil borne fungus. A critical stage for oxygen competition between plants and microorganisms arises during seed germination. Sandy soils with more number of macropores compared to clay soils can hold adequate air though they are poor in water holding capacity [14]. This could be the probable reason for high percentage of dry root rot incidence in sandy looms when compared to clay looms.

Cultivars

The dry root rot incidence was found in all the cultivars (ML-267, Pusa Baisakhi, and local) surveyed (Table 3). Out of three cultivars surveyed greengram cv. ML-267 recorded a high dry root rot incidence (11%) followed by Pusa Baisakhi and least in local cultivars (4.8%). The incidence of dry root rot (4.8%) was also recorded in on all the cultivars surveyed [15]. There is a yield loss of 10.8 and 24.1 per cent due to *M. phaseolina* in mungbean from the states of Haryana and Rajasthan respectively [11, 12].

A detailed survey was conducted on the incidence of root rot disease of rice fallow black gram caused by *M. phaseolina* in Karaikal region and revealed the incidence of 16 to 33 per cent [13]. In

Table 3
Influence of cultivars on the dry root rot incidence associated with green gram in major green gram growing mandals of Chittoor district A.P.

S. No.	Cultivars	Total samples	Mean per cent dry root rot
1.	Pusa Baisakhi	4	9.5
2.	ML-267	7	11.0
3.	Local	13	4.8
	Total	*24*	

Table 4
Effect of different inoculum levels of *Macrophomina phaseolina* on dry root rot incidence of green gram cv. ML-267

S. No.	Inoculum density per cen (w/w)	*Dry root rotincidence (%)
1.	Control	0.0 (0.0)
2.	1	12.5 (20.40)
3.	3	25.0 (29.89)
4.	5	37.5 (37.73)
5.	7	78.2 (62.17)
6.	9	82.7 (65.65)
	S.Em +	2.344
	CD at 5%	6.960

* *Mean* of five replications. Figures in parentheses are arc sine transformed values.

higher percentage of root rot incidence than in clay soil and also observed twenty four per cent of mungbean *(Vigrza radiata)* seed samples collected from 11 districts of Rajasthan during 1996-97 showing 0.5 to 38 per cent *M.phaseolina* infection [10].

Isolation and Identification of the Pathogen

The pathogen associated with diseased plants collected from different farmers fields during survey was isolated on PDA and purified by single hyphal tip technique. The detailed characteristics of pathogen isolated from T. Sattevaripalem village of Chinnagottigal mandal (isolate number-7) was studied as the dry root rot incidence rate was highest among the fields surveyed. The same isolate was used for subsequent studies in the present investigation. Pathogenicity was established by inoculation and reisolation of the pathogen from infected plant parts.

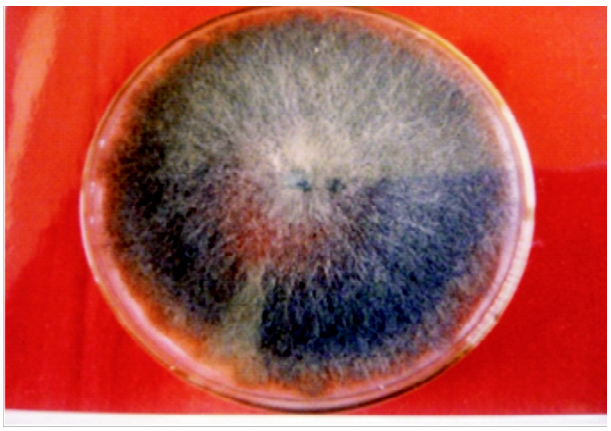

Figure 2. Growth of M. *phaseolina* on PDA medium

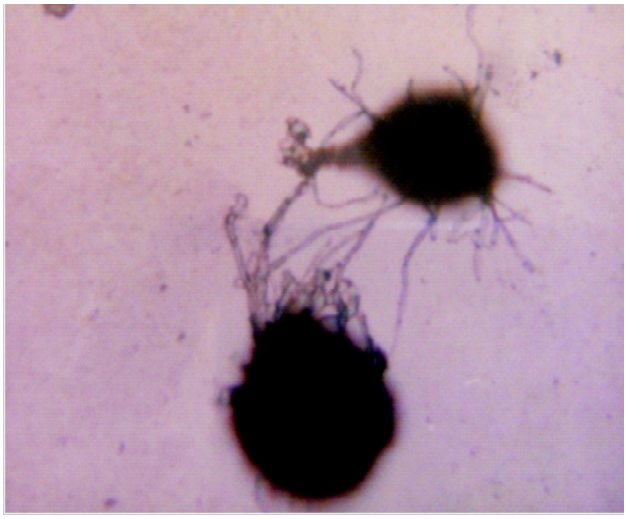

Figure 3: Sclerotia of pathogen

Characteristics of the Pathogen

The growth of mycelia was observed 24 h after inoculation on PDA medium. Within 4 days, the colonies became fluffy, carbonaceous, brown to black in colour with concentric zonation covering the entire plate. With time numerous sclerotia developed throughout the colony. The pycnidia were not observed on PDA medium.

Mycelia were hyaline to grey or brown in colour, septate, branched, dendroid and frequently ran parallel to each other with right angled hyphae. Initially the hyphae was thin and became thicker with age. Sclerotia were black, varied from spherical to irregular in shape and measured 75 to 110 m in diameter. Based on cultural and morphological characters the pathogen was identified as *Rhizoctonia bataticola/M.phaseolina* [Figure 2, 3]. The morphology of the pathogen observed was in accordance with the description given for greengram *M.phaseolina* [16].

general soils of sandy loam/sandy clay recorded

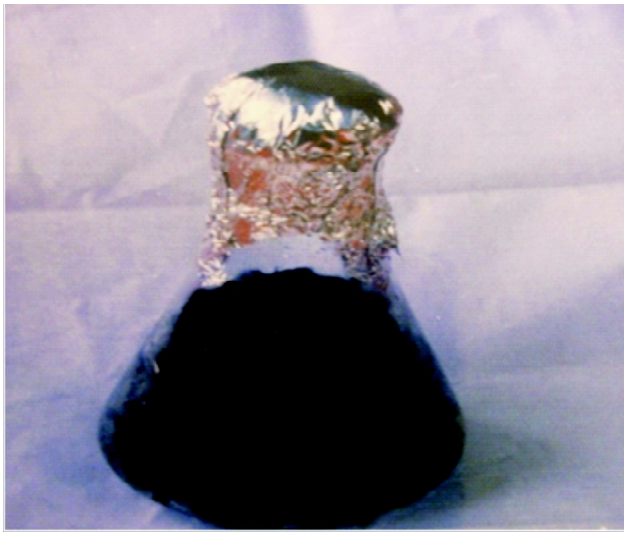

Figure 4: Mass multiplication of M. *phaseolina* on sorghum grains

Virulence Studies

The pathogen isolated was purified and mass multiplied on sorghum grains (Figure 4) and further tested at different inoculums levels so as to identify the optimum infection threshold levels of the pathogen using greengram cv. ML-267 in pot culture studies. The dry root rot incidence was recorded based on the following symptoms associated with greengram.

During virulence studies failure of germination, seedling necrosis, stunted growth, wilting and drying of leaves and stem was observed. Tap root exhibited blackening and formation of numerous black sclerotia on the infested roots. Shredding of the cortex and absence of secondary roots were observed. The data revealed that dry root rot incidence of greengram cv. ML-267 increased from 12.5 to 82.7 per cent as the inoculum level increased from 1 to 9 per cent (Table 4). The optimum infection threshold level was found to be 7 per cent inoculum causing 78.2 per cent dry root rot incidence. There was no significant difference in disease incidence with inoculums level from 7-9 per cent. This gives an indication that infection threshold of 7 per cent is optimum for maximium dry root rot incidence.

Similar pathogenicity studies were conducted by earlier workers but a variation in dry root rot incidence with respective inoculums level was observed. In a report 10 per cent inoculum density caused 71 per cent seedling mortality in greengram and black gram [17] .

CONCLUSION

The present study indicates that dry root rot is a highly devastating disease which needs immediate attention as it occurs in all types of soils and all available cultivars of greengram emphasizing the need of developing effective management strategy. Further this information can also be utilized for development of resistant varieties.

References

Yadava DS (1992), Mungbean-pulse crops(Production technology). (1 stedn). Kalyani Pub, New Delhi, Ludhiana.

Grewal JS (1988), Diseases of pulse crops. An overview. *Indian Phytopathology* 41:1-4

Raguchander T., R. Samiyappan and G.Arjunan, (1993), Biocontrol of Macrophomina root rot of mungbean. *Indian Phytopathology* 46, 379-382.

Sandhu A, Singh RD (1998), Role of seed borne inoculums in development of charcoal rot of cowpea. *Journal Mycology and Plant Pathology*17: 154-157.

Singh R S (1984), Assessment of disease incidence and loss In: introduction to Principles of Plant Pthology. *Oxford and IBH Publishing Company* pp 315-333.

Gillam (1957), A manual of soil fungi. *IBH publishers*, Calcutta p.227

Barnett (1962), Illustrated generaof imperfect fungi. *Berges Publishing* Company, Minneapolis, USA p.225

Rangaswami G (1958), An agar block technique for isolating soil microorganisms with special reference to pythiaceous fungi. *Science and Culture* 24: 85.

Haware M.P. (1980), Methods of artificial inoculation and disease rating of root pathogen in phytopathological techniques (ed.) pp.32-35

Kratisharma and Tribhuwan Singh (2000), Seed and seedling infection of *Rhizoctonia bataticola* in *Vigna radiata. Journal of Mycology and Plant Pathology* 1: 15-18

Kataria H R and Grover R K (1977), Comparison of fungicides for the control of *Rhizoctonia solani* causing damping off of mungbean (*Phaseolus aureus*). *Annals of Applied Biology* 83: 79-85

Tyagi R N S, Mathur A K, Gaur V K, Chitley K, Bansal R K and Pathak A K (1988), Pathological status of pulse crops in Rajasthan. *Indian Phytopathology* 41: 280.

Rettinassababady C and Ramados S N (1999), Occurrence of root rot in rice fallow black gram (*Macrophomina phaseolina). Legume Research* 23(2): 139-140.

Baver L D, Walter H, Garden W and Gardner R (1962), *Soil Physics*. John Wiley Company p.199

Sahu A K and Jena N (1997), Seed Microflora of greengram (*Phaseolus aureus Roxb)* cultivars of Orissa and their impact on seed germination. *Journal of Mycological Research*. 1997: 35:(2) 93-97

Phillip C T, Kartha K K, Joshi R K and Neema K G (1969), A new Rhizoctonia disease of mung (*Phaseolus aureus* Roxb.) in Madhya Pradesh. JNKVV Research Journal 3: 40-43.

Prameela Devi T and Singh R H (1998), Studies on virulence of *Macrophomina phaseolina* isolates from blackgram and greengram. *Journal of Mycology and Plant Pathology* 22(2): 196-198.