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RESEARCH

SURVEY FOR THE OCCURRENCE OF GROUNDNUT ROOT ROT DISEASE CAUSED BY *MACROPHOMINA PHASEOLINA* (TASSI.) GOID IN SOUTHERN DISTRICTS OF TAMIL NADU

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Abstract: A survey was conducted in major groundnut growing areas of Tirunelveli, Thoothukudi and Tenkasi districts to assess dry root rot disease incidence and to collect infected plant samples from the surveyed areas. Maximum percent disease incidence of 54.33 was recorded in Killikulam village of Thoothukudi district. Four isolates of *Macrophomina phaseolina* namely GM1, GM2, GM3 and GM4 were obtained from the samples collected from Serndhamaram, Killikulam, Pudur and Surandai respectively. The isolates were morphologically identified by observing colony colour, colony texture, growth rate and production of sclerotia. Under pathogenicity test, all the isolates were found pathogenic and the isolate collected from Killikulam (GM2) recorded highest disease incidence by causing complete wilting of plants and proved its virulence.

Keywords: Root rot, *Macrophomina*, Groundnut

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important oil seed crop which is cultivated for consumptive use as food and oil. Groundnut production is affected both by biotic and abiotic stresses during different growth stages of crop. Diseases caused by fungi, bacteria, virus and nematode is one of the biotic stresses which hamper the groundnut production (Mayee and Datar, 1986). Soil borne fungi are causing serious yield losses in groundnut (Mathur and Cunfer, 1993). Among the different soil borne fungi, *Macrophomina phaseolina* which causes dry root rot disease is the most devastating pathogen in groundnut. Dry root rot infected groundnut plants show yellowing and drooping of the leaves. Roots of the infected plants show black lesions and bark shredding, which can be easily pulled out due to the rotting of lateral and finer roots (Palaiah *et al*., 2019).

MATERIALS AND METHODS

Survey and collection of dry root rot affected groundnut plant samples

A survey was conducted in major groundnut growing areas of Tirunelveli, Thoothukudi and Tenkasi districts *viz*., Sendhamaram, Killikulam, Pudur and Surandai to assess the incidence of dry root rot disease and to collect the diseased samples. From the selected groundnut crop fields, dry root rot affected plant samples were collected and kept in labelled polythene bags. Then, the samples were brought to the laboratory, Department of Plant Pathology, Agricultural College and Research Institute, Vallanadu for further studies. Per cent Disease Incidence was calculated for each surveyed area by using the formula given by Mayee and Datar (1986).

$$Percent\ disease\ incidence = \frac{\text{No.of plants infected}}{\text{Total No.of plants observed}} \times 100$$

Isolation, purification and maintenance of the pathogen

Isolation of the pathogen was done on the PDA medium from dry root rot affected roots of the groundnut plant by using tissue segmentation method (Rangaswami, 1972). Diseased roots were washed with the running tap water. Then, inside

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the laminar airflow chamber 2-5 mm size of diseased root tissues along with some healthy tissues were sliced out with the help of a sterilized blade. To avoid the surface contaminants, tissues were surface sterilized with 0.1 per cent mercuric chloride for about 30 seconds. After surface sterilization the tissues were washed three times with sterile water to remove the traces of mercuric chloride and blot dried in sterile blotter paper. The diseased root tissues were then transferred aseptically to the sterilized PDA medium and incubated at 28±3°C for 6 to 7 days. Mycelial growth of the pathogen from the infected tissues was observed periodically. For maintenance of pure culture, fungal hyphae grown from the infected tissues were sub cultured by transferring it into fresh sterilized PDA slants by single hyphal tip method. Then the culture was stored at 5 – 6 °C. Totally four isolates of dry root rot pathogen were maintained.

Pathogenicity test of *M. phaseolina*

Inoculum preparation

All the four isolates of the pathogen were mass multiplied on sand maize medium separately by following the method given by Riker and Riker (1936).

Mass multiplication of the pathogen

The sand maize medium was prepared by adding sand and maize powder in a ratio of 19:1 (1900g of sand and 100 g of maize powder) and moistening it with 400 ml/kg of sterile distilled water which was packed in autoclave pouches and autoclaved for two consecutive days at the pressure of 1.4 kg/cm^2. Then the medium was inoculated with nine mm actively growing seven days old culture of *M. phaseolina* and incubated at 28±3°C.

Pathogenicity test

Pathogenicity test for *M. phaseolina* isolates *viz*., GM1, GM2, GM3, GM4 was done by employing sick pot method (Choudhary *et al*., 2011). For this, autoclaved and cooled potting mixture of soil: sand: FYM (2:1:1) was filled into the nursery pots. The *M. phaseolina* isolates multiplied in the sand maize medium was inoculated at 50g Kg^{-1}of potting mixture and mixed thoroughly. Then the pots were maintained in the glass house for two weeks for the multiplication of the pathogen. In each pot four groundnut seeds (VRI 8) were sown and three replications were maintained along with control (without inoculum). Four weeks after sowing, dry root rot disease incidence was recorded and re-isolation of all the isolates was made. Among the four isolates, GM2 recorded highest Percent Disease Incidence which was selected for further studies.

RESULTS AND DISCUSSION

Survey and collection of dry root rot affected groundnut plant samples

A survey was conducted during 2019-2020 to estimate the disease incidence of groundnut dry root rot and to collect diseased plant samples from major groundnut growing areas of Tirunelveli, Thoothukudi and Tenkasi districts *viz*., Serndhamaram, Killikulam, Pudur and Surandai. The collected samples were used to isolate *M. phaseolina*. Upon survey, the disease incidence in surveyed areas ranged between 36.00 per cent to 54.33 per cent. Maximum disease incidence was found in Killikulam (54.33 %) followed by Serndhamaram (47.00%), Pudur (42.00%). Among all the surveyed area, least disease incidence was recorded from Surandai (36.00%) (Table 1; Picture 1). The results were in accordance with the results given by Muthukumar *et al*. (2014). They conducted a survey in Cuddalore district to assess the dry root rot incidence of groundnut. They reported that maximum incidence of 30.33 per cent in Sivapuri village. Mohanapriya *et al*. (2017) performed a survey to assess the dry root rot incidence of cowpea in Cuddalore district and reported that the incidence was ranged between 17.72 per cent and 25.84 per cent.

Symptomatology

Dry root rot affected groundnut plants showed dark lesions around the stem and collar portion during initial stage. When the affected plants were uprooted, the root portions were severely disintegrated and prominent black colored pycinida were found. In later stage, infected plants showed wilting and premature dying (Picture 2). Kumar and Thirumalaisamy (2016) reported that the dry root rot infected groundnut plants showed necrotic lesions on stem and shredding, rotting on the tap root. They also observed the presence of numerous sclerotia on the infected kernels.

Seethapathy *et al*. (2017) also observed similar type of symptoms in charcoal rot affected pulses plants and they reported the presence of dark minute pinhead sclerotia on root portions.

Isolation of groundnut dry root pathogen *M. phaseolina*

Groundnut dry root rot pathogen was isolated from diseased samples by tissue segment method aseptically. Four isolates of *M. phaseolina* were isolated from the infected samples. The isolates were named as GM1, GM2, GM3 and GM4. Pure culture of the pathogenic isolates was maintained (Picture 3). The pathogen was confirmed based on the morphological characters and cultural characters *viz*., colony colour, texture, mycelial branching pattern and shape of the sclerotia (Picture 4a; Picture 4b). Similar results were described by Claudino and Soares (2014). They isolated this pathogen from different plant species like castor, sesame, sunflower, cotton, peanut which exhibited typical dry root rot symptoms. Manjunatha and Saifulla (2018) isolated twenty

isolates of *M. phaseolina* from the chickpea plants collected from ten different states of India.

Identification of virulent isolate

Morphological and cultural characters of dry root rot pathogen were studied in PDA medium. Among the four isolates, the isolate GM1 produced olive green colored mycelium and covered the plate within nine days. GM2 isolate produced intense black coloured mycelium and full growth of mycelium was observed in the plate on seventh day of inoculation. GM3 isolate produced grey coloured mycelium and covered the plate within ten days. GM4 isolate produced greyish white mycelium and covered the plate within ten days. Among the four isolates, GM2 produced a greater number of sclerotia and GM4 produced a smaller number of sclerotia (Picture 5). Identical results were reported by Shekhar *et al*., (2010). They studied the phenotypic characters of ten isolates of *M. phaseolina* from maize plants and concluded that this pathogen produced, coloured mycelium and profuse aerial mycelia with abundant sclerotial production.

Pathogenicity test

Pathogenicity test for all the isolates of *M. phaseolina* was done by soil inoculation method by using sand maize medium. All the isolates were found to be pathogenic. Among the isolates, GM2 resulted in complete wilting, dying of plants and recorded the highest disease incidence of 67.50 per cent. Hence, GM2 isolate of *M. phaseolina* was identified as most virulent isolate and used for further studies (Table 2; Plate 6). The present results were in line with the previous work done by Muthukumar *et al*. (2014). They reported about seven isolates of *M. phaseolina* from dry root rot infected groundnut plants and concluded that isolate from Sivapuri village of Cuddalore district recorded with maximum disease incidence of 30.33 and found to be virulent.

Table 1. Survey and incidence of groundnut dry root rot

Sl. No.	Village	District	Isolate	*Percent Disease Incidence
1	Serndhamaram	Tirunelveli	GM1	47.00 (43.27)[b]
2	Killikulam	Thoothukudi	GM2	54.33 (47.48)[a]
3	Pudur	Thoothukudi	GM3	42.00 (40.32)[c]
4	Surandai	Tenkasi	GM4	36.00 (36.66)[d]
SE(d)				**1.79**
CD(p=0.05)				**4.20**

*Means of four replications

Values in the parentheses are arc sine transformed values

Values in the parentheses followed by different alphabets are significantly different from each other

Table 2. Pathogenicity test for *M. phaseolina* isolates

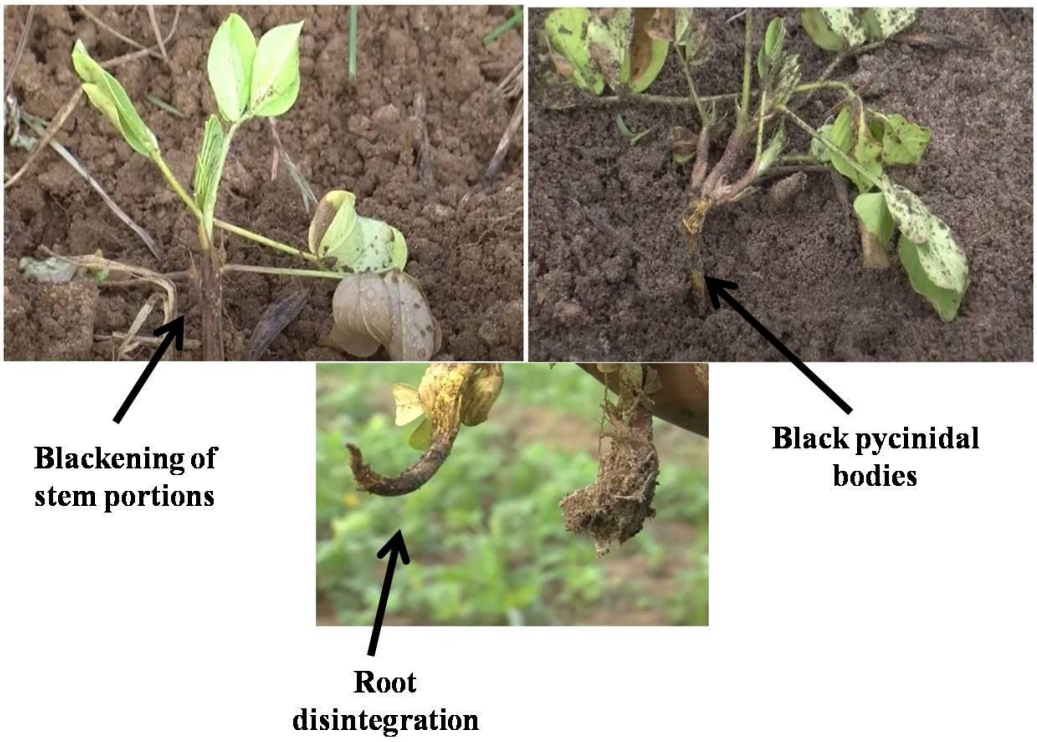
SL No.	Isolates	Percent Disease Incidence*
1.	GM1	55.00 (46.44)[b]
2.	GM2	67.50 (55.28)[a]
3.	GM3	27.50 (31.55)[c]
4.	GM4	15.00 (22.50)[d]
5.	Control	0.00[e]
SE(d)		**2.51**
CD(p=0.05)		**5.47**

*Means of four replications

Values in the parentheses are arc sine transformed values

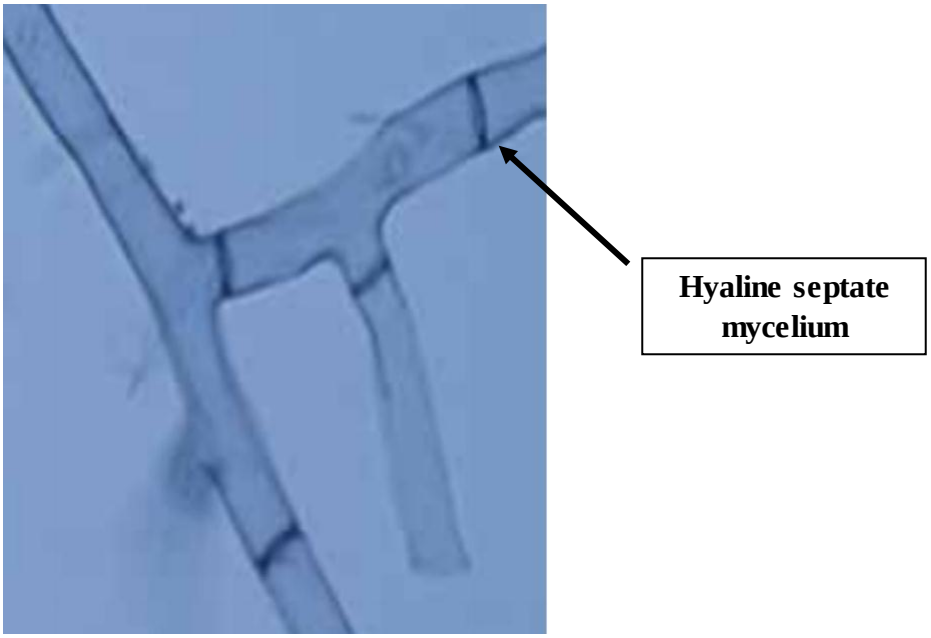
Values in the parentheses followed by different alphabets are significantly different from each other

Picture 1. Survey on the incidence of groundnut dry root rot disease



Picture 2. Symptoms on the dry root rot infected groundnut plants

Picture 3. Different isolates of *Macrophomina phaseolina*



Picture 4a. Mycelial branching pattern of *M. phaseolina*

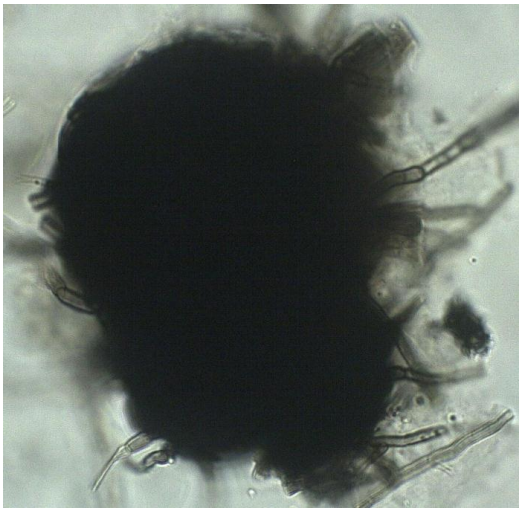


Plate 4b. Sclerotia of *M. phaseolina*

Picture 5. Pathogenicity test for *M. phaseolina isolates*

CONCLUSION

Dry root rot infected plant samples were obtained from areas *viz.*, Serndhamaram, Killikulam, Pudur and Surandai of Tirunelveli, Thoothukudi and Tenkasi districts of Tamil Nadu. In the surveyed areas, the percent disease incidence of dry root rot ranged between 36.00 and 54.33 per cent.

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AUTHORS CONTRIBUTION

NR, KE carried out the implementation and development of the work. JS corrected the work and analyzed the data. All authors read and approved the final manuscript. All the authors read and approved the final manuscript.

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