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OCCURRENCE OF ROOT ROT INCIDENCE OF SESAME AND ASSESSMENT OF CULTURAL AND PATHOGENIC VARIABILITY AMONG ISOLATES OF *MACROPHOMINA PHASEOLINA*

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Abstract

Sesame is affected by many diseases caused by viruses, bacteria and fungi. Among the fungal diseases, the charcoal rot caused by *Macrophomina phaseolina* (Tassi.) Goid is the most serious one. In the recent years, this disease causes significant losses in sesame growing areas of Krishnagiri and Cuddalore Districts and also poses great problems at such magnitude that the farmers are inclined to think about the cultivation of other crops in place of sesame. Hence, the present study was taken up to assess the damage caused by root rot disease in Krishnagiri and Cuddalore district of Tamil Nadu, India and also studied the variability in the cultural characteristics and pathogenicity among the isolates of *M. phaseolina*. The studies were initiated with survey on the dry root rot incidenceof different locations of Krishnagiri and Cuddalore district revealed endemic nature of the root rot disease incidence. Among the different locations of Krishnagiri and Cuddalore district surveyed for sesame root rot incidence, Eggoor (MP_6) village registered the maximum incidence of the disease (36.25%), followed by Maganoorpatti (MP_2) village with 32.57%, Pudhuchathiram (31.45%) while the minimum root rot incidence of 17.20 percent was recorded in Harur village. 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 soil had more root rot incidence than clay and clay loam soil. The isolates collected from different locations showed varied levels of pathogenicity. All the fifteen isolates of the root rot pathogen *M. phaseolina* showed variations with regard to mycelia growth, sclerotial size and production and also well attributed to the difference in virulence of the isolates of *M. phaseolina* prevalent in the respective areas.

Key words: Sesame, *Macrophomina phaseolina*, Eggoor (MP_6)

Introduction

Sesame (*Sesamum indicum* L.) adorned as "Queen of oilseeds" is one of the most oldest and indigenous ancient edible oilseeds crop, grown in India. Sesamum has been commonly known as Til (Hindi), Ha ma (Chinese), Sesame (French), Goma (Japanese), Tal (Gujrati), Til (Panjab, Marathi), Nuvvula (Telgu), Ellu (Tamil) and Tila (Sanskrit) etc. in different part of countries. The genus *Sesamum* belongs to the order Tubiflorea, family *Pedaliaceae* which consists of sixteen genera and sixty species, but only *Sesamum indicum* (2n=26) has been recognized as cultivated species. Sesame is a rich source of proteins (20%) and edible oil (50%) and contain about 47% oleic acid and 39% linolenic acid, seeds are rich in A, B, E vitamins, minerals like Calcium, Phosphorus, Iron, Copper, magnesium, Zinc and Potassium (Weiss, 1971; Shyu and Hwang, 2002). Sesamum crop seeds used for baking, candy making and other industries. It's oil is used for cooking, salad, margarine, in manufacture of soaps, paints, perfumes and insecticides. Sesamum seed contains two phenolic antioxidants, sesamol and sesaminol have been shown to possess cholesterol lowering effect in human and to prevent high blood pressure and increase vitamin E supplies in animals. Sesame is being grown under rainfed and as well as irrigated conditions. India ranks first in World in respect area and production of sesamum (www.iopepc.com). In India area 1950.88 ('000 ha), production 850.07 ('000 MT) and productivity 436 kg/ ha (Anonymous, 2015). Among various factors responsible for low production and productivity of sesame, the

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diseases caused by biotic agents are major one. About 72 fungi, 7 bacteria 2 and one each phytoplasmal and viral pathogens are reported to attack sesame, from India (Vyas *et al.,* 1981). Sesame is known to be affected by as many as 80 diseases among which 29 have been reported in India (Vyas *et al.,* 1984). Among these, root rot / charcoal rot / stem rot caused by *Macrophomina phaseolina* (Tassi.) Goid., is one of the most devastating fungal disease, affecting the crop at all stages of crop growth and causing 5-100% yield losses (Vyas, 1981). The disease has been reported to cause more than 50% yield losses in Tamilnadu (Chattopadyaya and Shastri, 1999) and 42-45% yield losses in India (Usha Rani *et al.,* 2009). Sesame is mostly grown as a rain fed crop and under this situation, the crop is exposed to sufficient soil moisture during its initial growth stages (up to 30-35 days), the dry condition prevalent during the later stage of the crop is a predisposing factor for infection of the root rot pathogen (Balabaskar, 2006). Plants are most susceptible to infection beginning at flowering and continuing till maturity (Arora *et al*., 2012). The disease is both seed and soil borne and usually infects the crop under dry and warm conditions. During the recent years, this disease causes significant losses in sesame growing areas of Krishnagiri and Cuddalore Districts and also poses great problems at such magnitude that the farmers are inclined to think about the cultivation of other crops in place of sesame. Hence, the present study was taken up to assess the extent of damage caused by root rot disease in Krishnagiri and Cuddalore district of Tamil Nadu, India. Also, the variability in the cultural characteristics and pathogeneicty among the isolates of *M. phaseolina* was studied.

Materials and Methods

Survey on the Root Rot Incidence of Sesame in Krishnagiri and Cuddalore District. A field survey was conducted to assess the extent of root rot occurrence of sesame in Cuddalore district of Tamil Nadu State. Thirteen traditionally sesame growing locations representing both rainfed and irrigated situations were selected for the study. The per cent disease index was worked out using the 0 to 9 scale according to "Phytopathometry" by Mayee and Datar (1986) as mentioned below.

Scale 0 - No symptoms on any plant

1 - 1% or less plants killed

3 - 1-10% plants killed

5 - 11-20% plants killed

7 - 21-50% plants killed

9 - 51% or more plants killed

Per cent Disease Index (PDI) =

$$\frac{Sum\ of\ disease\ incidence}{Total\ No.of\ \ plants\ observed} \times 100$$

The other information viz., soil type and the crop variety were also recorded in the respective survey fields.

Isolation of the Pathogen

The pathogen*M. phaseolina* was isolated from the diseased roots of sesame plants showing the typical root rot symptoms by tissue segment method (Rangaswami, 1972) 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 percent 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. ($28+2^0$C) for 15 days and the inoculum thus obtained was used for the experiments.

Assessing the Virulence of *M. phaseolina* Isolates

The potting mixture was prepared by thoroughly mixing clay loam soil, sand and farm yard manure at 1:1:1 ratio. The inoculum of each isolate of *M. phaseolina* collected from different locations were separately mixed at five per cent level (w/w) with the sterilized soil filled in 30 cm earthen pots ten days before sowing (Sankar, 1994) . Surface sterilized (using 0.1% $HgCl_2$ solution for 30 sec. followed by two washings in sterile water) sesame seeds were sown @ 10 seeds pot^{-1}. Three replications were maintained in a completely randomized design and the sesame cultivar TMV3 was used throughout the study. The pots were maintained in glass house with regular, judicious and uniform watering. The root rot incidence was recorded at 45, 60, 75 DAS and at harvest and the per cent disease incidence was calculated.

Cultural Characteristics of the Isolates

Mycelial Growth

Fifteen ml 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 centre of the dish and incubated. The radial growth of the isolates (in mm) was measured three days after inoculation.

Sclerotial number

From seven day old culture of the isolates, 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 dist. 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. 1 filter paper; rinsed with dist. water and the number of sclerotia was counted using stereo zoom microscope (Dhingra and Sinclair, 1978).

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.

Results

Survey on the Root Rot Incidence of Sesame in Krishnagiri and Cuddalore District

The data presented in table 1 on the survey in different locations of Krishnagiri and Cuddalore district revealed endemic nature of the root rot disease incidence. Among the different locations of Krishnagiri and Cuddalore district surveyed for sesame root rot incidence, Eggoor (MP_6) village registered the maximum incidence of the disease (36.25%), followed by Maganoorpatti (MP_2) village with 32.57%, Pudhuchathiram (31.45%), Mathur (29.90%) and Singarapettai (28.82%) had moderate disease incidence while the minimum root rot incidence of 17.20 per cent was recorded in Harur village. The variation in the extent of the disease incidence might be due to the prevalence of the isolates of the pathogen differing in their virulence as observed in the present study. Similar such endemic nature of root rot disease of sesame in Tamil Nadu was earlier reported by Balabaskar (2015) and in Telegana by Karibasappa, (2018). In general, the crop grown under rainfed conditions showed more root rot incidence when compared with the crops grown under irrigated conditions. Muchero *et al.,* (2011) also reported that dry rainfed conditions favored higher root rot disease in sesame. The dry condition prevalent in the rainfed conditions might have favoured the pathogen which could be attributed for the higher level of disease incidence.

Soil texture also had a significant impact on root rot infections. In the present survey more root rot disease incidence was observed in sandy loam (36.25 to 20.50%) as compared to clayloam (28.82 to 17.90%) and clay (27.25 to 17.20 %). (Table 1). Similar to the present results, Karibasappa, (2018) also reported that disease was more prevalent in sandy loam soil areas followed by clay loam and minimum in clay soil areas. Cruz Jimenez, (2011) observed highest *M. phaseolina* root populations in sandy soils, followed by seedlings planted in loamy sand and loam soil textures. Higher incidence of the disease in sandy soils might be attributed to the less competitive saprophytic ability (CSA) of the pathogen at high moisture holding capacity (MHC) associated with heavy soils like clay (Umamaheswari, 1991) and reduction in the germination of sclerotia of *M. phaseolina* at high MHC (Ali and Ghaffar, 1991). The local cultivars of sesame recorded comparatively lesser (17.20 to 29.90%) root rot incidence than the improved cultivars *viz.,* TMV_3 and TMV_4 (36.25 to 31.45% and 22.998 to 17.90% of disease incidence respectively). Similarly, Balabaskar *et al.,* 2015 reported that the root rot disease caused by *M. phaseolina* in sesame was recorded maximum root rot incidence in improved cultivars *viz*., CO1, TMV3 and TMV4than the local cultivars.

Virulence of different isolates of *M. phaseolina*

Generally the isolates collected from different locations showed varied levels of pathogenicity (Table 2). Among the fifteen isolates of *M. Phaseolina* collected from different conventional sesame growing areas of Krishnagiri and Cuddalore districts, the isolate (MP_6) collected from Eggoor village was found to be more virulent recording the maximum incidence of 79.93 per cent (at harvest) followed by MP_2 (75.93%) collected from Maganoorpatti village. The other isolates *viz.,* $MP_{13,}$ MP_3, $MP_{7,}$ MP_9, MP_{12}, MP_4, MP_{11}, MP_{15}, MP_8, $MP_{14,}$ MP_1 and MP_{10} recorded 73.93, 67.75, 67.24, 64.82, 63.05, 62.93, 68.35, 61.83, 61.05, 60.04, 53.07, 52.45 and 49.82 per cent root rot incidence respectively. The isolate MP_5 collected from Harur village was the least virulent which recorded the minimum (48.30%) disease incidence at the time of harvest. The MP_9 and MP_7 isolates recorded 23.00 and 26.21 percent root rot incidence at 45 DAS itself. The variability in the pathogenecity among the isolates of *M. phaseolina* was reported by several workers (Karunanithi, 1996; Rayatpanah and Dalili, 2012). Sathiyasivananthanmoorthy (2017) recorded 35.76 to 67.23 percent root rot incidence of blackgram with different isolates of *M. phaseolina*. Sobti and Sharma, (1992) recorded 13 to 63 percent root rot incidence of groundnut with different isolates of *R. bataticola*. The

Table 1: Survey on incidence of dry root rot of sesame in Krishnagiri and Cuddalore district.

S. No.	Isolates	Village	Soil type	District	Situation	Variety	Root rot incidence (%)
1	MP_1	Andiyur	Red sandy loam	Krishnagiri	Irrigated	TMV 4	18.70 (25.62)
2	MP_2	Maganoorpatti	Sandy loam	Krishnagiri	Rainfed	TMV 3	32.57(34.79)
3	MP_3	Mathur	Sandy loam	Krishnagiri	Irrigated	Local	29.90(33.14)
4	MP_4	Pochampalli	Clay loam	Krishnagiri	Rainfed	Local	24.40(29.60)
5	MP_5	Harur	Clay	Krishnagiri	Irrigated	Local	17.20(24.50)
6	MP_6	Eggoor	Sandy loam	Krishnagiri	Rainfed	TMV 3	36.25(37.01)
7	MP_7	Singarapettai	Clay loam	Krishnagiri	Irrigated	Local	28.82(32.46)
8	MP_8	Karapattu	Sandy loam	Krishnagiri	Rainfed	TMV 4	20.50(26.92)
9	MP_9	Vadakkumangudi	Clay	Cuddalore	Rainfed	Local	27.25(31.46)
10	MP_{10}	Sivapuri	Clay loam	Cuddalore	Rainfed	TMV 4	17.90(25.02)
11	MP_{11}	Annuvampattu	Clay loam	Cuddalore	Rainfed	Local	23.65(29.09)
12	MP_{12}	Theithampalayam	Sandy loam	Cuddalore	Rainfed	Local	25.80(30.52)
13	MP_{13}	Pudhuchathiram	Sandy loam	Cuddalore	Rainfed	TMV 3	31.45(34.11)
14	MP_{14}	Koththa	Clay loam	Cuddalore	Irrigated	Local	19.30(26.06)
15	MP_{15}	Periyapattu	Sandy loam	Cuddalore	Rainfed	TMV 4	22.98(28.64)

variation in the isolates of *M. phaseolina* from different cowpea growing areas of Udaipur was reported (Ratnoo *et al.,* 1997). *M. phaseolina* isolated from different host species differ in their morphological and cultural characters and even differences occur in the isolates from various parts of same host (Sundravadana *et al.,* 2012). The above reports are in agreement with the present investigation. In the present study, incidence of root rot was the maximum at harvest stage plants when compared to 75, 60 and 45 days old plants (Table 3). Similar observations were made by Sankar, (1994) and Thirurnaryanan, (2017). A significant increase in root rot incidence at 40th and 60th DAS was observed in rice fallow blackgram by Rettinasababady and Ramadoss (2000). Davet and Serieys (1987) reported decreased level of reducing sugars at later stages of plant growth. The susceptibility of sesame plant to the disease at the later stages of growth might be due to the decline in the *in situ* defence mechanisms.

Cultural Characteristics

Mycelial Growth

All the fifteen isolates of the root rot pathogen *M. phaseolina* produced white, whitish grey, grey and black scanty to profusely aerial mycelial growth on Potato Dextrose Agar (PDA) medium. The isolate MP_6 and MP_2 recorded themaximum (90 mm) mycelial growth at 5 days after inoculation, while it was the minimum (73.95 mm) in the case of MP_5. The other isolates showed moderate mycelial growth (87.82 to 74.86 mm) (Table 2).

Sclerotial number

All the isolates of *M. phaseolina* varied in their ability to produce sclerotia on PDA medium. The maximum sclerotial number of 186.50 per nine mm culture disc was obtained from MP_6 which was also the most virulent isolate. The isolates (MP_2, MP_{13}, MP_3, MP_7, MP_9, MP_{12}, MP_4, MP_{11}, MP_{15}, MP_8, MP_{14}, MP_1 and MP_{10}) produced 184.34, 183.72, 180.25, 179.31, 178.62, 178.20, 177.36, 176.10,169.72, 168.53, 168.26 and 167.75 numbers of sclerotia, respectively. The minimum number of sclerotia of 165.28 was recorded the isolates MP_5 which was the least virulent isolate (Table 2).

Table 3: Cultural characteristics of various isolates of *M. phaseolina.*

S. No.	Isolates	Mycelial character	Mycelial growth(mm)	No. of. Sclerotia	Sclerotial size (µ)
1	MP_1	Blackish grey, profuse aerial growth	76.46	168.26	91.90
2	MP_2	Deep black, fluffy growth	90.00	184.34	102.27
3	MP_3	Greyish white, fluffy growth	86.57	180.25	100.32
4	MP_4	Blackish grey, profuse aerial growth	81.45	177.36	97.52
5	MP_5	Greyish white, flat growth	73.95	165.20	90.50
6	MP_6	Blackish grey, flat aerial growth	90.00	186.50	105.75
7	MP_7	Greyish white, fluffy growth	85.83	179.31	99.65
8	MP_8	Blackish grey, slightly fluffy growth	78.43	169.72	93.41
9	MP_9	Black profuse, aerial growth	84.70	178.62	98.90
10	MP_{10}	Greyish white, flat growth	74.86	167.75	91.34
11	MP_{11}	Blackish scanty, aerial growth	81.30	176.09	95.23
12	MP_{12}	Deep black, flat growth	82.25	178.20	98.10
13	MP_{13}	Blackish grey, profuse aerial growth	87.82	183.72	101.20
14	MP_{14}	Medium black, flat growth	77.85	168.53	92.50
15	MP_{15}	Blackish grey, flat growth	80.47	176.10	94.65

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

SI. No	Isolates	Root rot incidence (%)				Mean
		45 DAS	60 DAS	75 DAS	At harvest	
1	MP_1	14.98 (22.77)	25.47 (30.30)	35.79 (36.74)	52.45 (46.40)	32.17
2	MP_2	29.79 (33.07)	46.96 (43.25)	57.69 (49.42)	75.93 (60.61)	52.59
3	MP_3	25.60 (30.39)	36.25 (37.01)	49.52 (44.72)	67.75 (55.39)	44.65
4	MP_4	21.04(27.30)	30.64 (33.60)	45.46 (42.39)	62.93 (52.49)	40.04
5	MP_5	14.35 (22.26)	25.29 (30.19)	34.92 (36.22)	48.30 (44.02)	30.71
6	MP_6	32.45 (34.72)	49.96 ()44.97	65.69 (54.14)	79.93 (63.38)	57.07
7	MP_7	27.79 (31.81)	43.28 (41.13)	55.69 (48.26)	67.24 (55.08)	50.17
8	MP_8	17. 72 (24.89)	29.07 (32.62)	40.09 (39.28)	60.04 (50.79)	36.73
9	MP_9	23.00 (28.65)	34.53 (35.98)	49.35 (44.62)	64.82 (53.62)	43.65
10	MP_{10}	15.17 (22.92)	26.52 (30.99)	39.25 (38.79)	49.82 (44.89)	33.50
11	MP_{11}	20.15 (26.67)	32.78 (34.92)	45.87 (42.63)	61.83(50.79)	40.43
12	MP_{12}	22.60 (28.38)	35.06 (36.30)	47.60 (43.62)	63.05 (52.56)	42.52
13	MP_{13}	26.21 (30.79)	37.28 (37.63)	50.82 (45.46)	73.93(59.29)	45.66
14	MP_{14}	16.36 (23.85)	27.27 (31.48)	39.72 (39.06)	53.07 (46.76)	33.95
15	MP_{15}	18.52 (25.48)	31.20 (33.95)	42.02 (40.40)	61.05 (51.38)	38.20

Sclerotial size

The isolates of *M. phaseolina* produced varying size of sclerotia on PDA. The most virulent isolate MP_6 produced the biggest sclerotia with a size of 105.75µ (Table 2) and the smallest sclerotia size of 90.50µ was recorded by $MP_{5,}$ which was the least virulent isolate.

Variations in the mycelial growth among the isolates of *M. phaseolina* (Edraki and Banihashemi, 2010; Ijaz *et al.,* 2012; Mohanapriya *et al*., 2017). The virulence of the isolates of *M. phaseolina* was positively correlated with their growth rate. Similarly, the isolates of *M. phaseolina* with faster mycelial growth were found more pathogenic and produced higher disease incidence was stated by several workers (Sharmishha Purkayastha *et al*., (2004) in cluster bean; Vinothini, (2015) in cowpea and Thirunaryanan, (2017) in sesame). In the present study also the isolate (MP6), which produced the biggest sclerotia caused the maximum root rot incidence. The possibility of containing more food materials and subsequent production of more pathogenic germ tubes by bigger sclerotia might have resulted in more aggressiveness of the isolate.

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