Cultural and Morphological Variability among Different Isolates of *Macrophomina phaseolina* causing Stem and Root Rot of Sesame

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**Abstract**

The present studies were planned that the among the all ten isolates collected from different locations of Rajasthan was classified on their cultural and morphological characteristics in which isolate MPjd1 showed maximum colony diameter (90.00 mm) followed by isolate MPng2 (89.20 mm). Isolate MPjd1 showed fast growing blackish colony colour with right angle branching pattern Minimum colony growth was observed in isolate MPtn2 (64.00 mm). The colony of ten isolates varies from 90.00 to 64.00 mm with whitish, grayish and blackish colour colony appearance on PDA. Morphological observations of each isolate revealed that sclerotial size varies from 70.27-106.50 µm, in which isolate MPjd1 had maximum sclerotial size (106.50 µm) followed by isolate MPjp1 (99.00 µm). Sclerotial shape was observed oblong, ovul, irregular and round shape.

**Keywords**

Colony, Morphological, Sclerotia, Stem and root rot

**Introduction**

Sesame (*Sesamum indicum* L.) is valuable and edible seed crop belongs to family *Padaliaceae*. Seed is highly rich in quality proteins and essential amino acids, especially methionine which is considered as rejuvenative and anti-aging for human body. Sesame oil is useful for soap making, skin care industries, health food industries and cosmatic purpose. Sesame oil is cholestrol free and stable doesn't form rancid. It seed used as sweets making and medicinal forms. It is generally called as "Til" and popularly known as "Queen of oilseeds". Sesame ranked first among oil seed crops in oil content (50-52%) with significant dietary energy (6335 kcal per kg) (Kumar and Goel, 1994). Sesame mainly grown under tropical and subtropical regions of India with an annual acreage of around 19.17 lakh ha and production of about 8.66 lakh tonnes with 413 kg/ha productivity (Anonymous, 2017). Sesame area and productivity decline due to poor crop management and severe biotic and abiotic stresses. During disease development *Macrophomina phaseolina* is infect the sesame plant in any stage of growth when temperature varies from 28°C to 32°C and germination of microsclerotia showed maximal growth at 30-33°C (Viana and De Souza, 2002). The symptoms were appears...
both surface and ground level. The stem rupturing upward and becomes blackish in colour. The roots will become brittle and black colour dots appear on stem. In several form of diseased plants showed that the black capsules has immature and shriveled seed. The assortment of host species and their wide availability have revealed that *M. phaseolina* is non host specific and hetero-geneous.

Germinating microsclerotia produced germ tube and penetrate through natural openings in vascular tissues and colonized their (Bressano *et al.*, 2010, Wyllie and scott, 1988). After penetration of pathogen during initial stage no visible symptoms may occurs in aerial part of the plant and remain latent (Pratt, 2006). During growing seasons plants shows wilted and necrotic symptoms due to blockage of vascular bundles with microsclerotia and secretion of toxic substances (Gupta *et al.*, 2012). The fungus can infect 100 families with 500 plant species in overall world (Mihail and Taylor 1995 and Srivastava *et al.*, 2001). The pathogen survives in adverse conditions by formation of sclerotia and dormant mycelium on crop residues and soil. Isolates collected from different areas of Rajasthan, characterized on their colony parameters *viz.*, colony colour, colony appearances, sclerotial size and shape and their spore formation. *Macrophomina phaseolina* is seed and soil borne pathogen, so its management cultural and morphological characterstices was observed and classified into different categories.

**Materials and Methods**

**Variability of the pathogen**

Single hyphal tip cultures were raised from isolates of ten collections of *Macrophomina phaseolina* on potato dextrose agar (PDA) slants. A total of ten isolates of *Macrophomina phaseolina* were established from the surveyed districts of Rajasthan and used in present study. Isolates were transferred separately on PDA in Petri dishes to study in detail for their discernible characters on the basis of cultural and morphological characters such as the colony diameter, colour and growth patterns. For pathogenic variability, the susceptible variety (VRI-1) was inoculated with different isolates separately.

The isolates were coded as:

| S.No. | District | Tehsil | Village | Isolate No. |
|-------|----------|--------|---------|-------------|
| 1.    | Pali     | Sumerpur | Angor    | Mpp1        |
| 2.    | Pali     | Desuri | Sadari  | Mpp2        |
| 3.    | Jodhpur | Bilada | Bilada | Mpjd1       |
| 4.    | Jodhpur | Mandor | Mandor | Mpjd2       |
| 5.    | Nagaur  | Molasar | Molasar | Mpng1       |
| 6.    | Nagaur  | Degana | Degana | Mpng2       |
| 7.    | Tonk    | Malpura | Bagri   | Mptn1       |
| 8.    | Tonk    | Deoli | Beejwar | Mptn2       |
| 9.    | Jaipur  | Phulera | Basingpura | Mjjp9     |
| 10.   | Jaipur  | Kisangarh Renwal | Kisangarh Renwal | Mppj10   |

**Cultural and morphological variability**

All the ten isolates were grown on Potato Dextrose Agar (PDA) medium and cultural characters like colony diameter, colour and growth pattern were studied. The observation on colony colour and texture were recorded at 7th day of incubation. Required quantity of the above mentioned solid medium was prepared...
and sterilized at 1.05 kg/cm² pressure for 20 minutes. Sterilization of Petri dishes was done at 180°C for 2 h in a hot air oven. In each Petri dish, 25 ml of medium was poured. Each treatment was replicated four times. Each Petri dish was inoculated with a mycelial bit of 5 mm diameter maintained on plain agar. The inoculated Petri dishes were incubated at 25±1°C temperature and observations on mycelial growth were recorded accordingly.

**Results and Discussion**

**Variability of different isolates of *M. phaseolina* through Morphological and cultural characteristics**

The cultural and morphological characteristics such as colour and appearance of colony, mycelial growth, branching pattern, size and shape of their sclerotia of different isolates of *Macrophomina phaseolina* were recorded by growing them on PDA medium. The results were presented Table 1 revealed that there were notified variation in colony growth rates of ten isolates of fungus (measured at 7 days after inoculation) were observed and the colony growth rate of these ten isolates followed the sequence of descending order as MPjd1 (90mm) > MPng1 (89.20mm) > MPjp2 (87.50mm) > MPpa2 (84.78 mm) > MPjd2 (82.00 mm) >MPpa1 (79.15 mm) > MPng2 (78.50mm) > MPjp1 (70.00mm) > MPtn2 (67.50mm) > MPtn1 (64.00mm). Based on the colony appearance the MPpa1, MPng1, MPng2, MPjd1 and MPjp1 characterised as fast growing isolates whereas MPpa2, MPjd2, and MPjp2 as moderate growing isolates and MPtn1 and MPtn2 as slow growing isolates. The isolates also showed different type of colony colour and appearance. MPjd1 appeared as blackish in colour with excellent sclerotial formation whereas MPtn1 and MPtn2 were emerged as whitish and whitish creamy in colour, respectively without any sclerotial formation. MPng1 and MPjp1 showed round shaped sclerotia whereas MPtn1, MPtn2, MPjd2 and MPpa1 were observed oblong shaped sclerotia. The MPpa2 and MPjp2 isolates developed oval shaped whereas MPng2 and MPjd 2 were showed irregular shaped sclerotia under fluorescence microscopy. All the ten isolates showed right angle type of branching pattern. However, they had significant variation in size of sclerotia ranged from 70.27- 106.50um. The maximum size of sclerotia was reported with MPjd1 (106.50um) followed by Mppp1 (99.00um), MPtn2 (98.32um), MPng1 (93.00um), MPjd2 (90.25um) and MPtn1 (87.98um). The lowest size of sclerotia on culture media was observed in isolate MPpa1 (70.27um) and MPjp2 (77.50um). The cultural and morphological apperances of the pathogen on culture media viz, colony colour, appearance, mycelial growth, branching pattern, sclerotia shape and size were recorded for different isolates of *M. phaseolina* with the help of florescence microscopy. The results showed that isolates of *M. phaseolina* were different in their colony characters, colony colour and colony diameter.

The present investigations were supported by Riaz et al., (2007) in sunflower. Shekhar et al., (2012) observed a direct correlation between sclerotial production and virulence of isolates against charcoal rot on maize and collected seven isolates were characterized on their cultural and pathogenic characteristics. Kaur et al., (2013) worked on sixty one isolates of *M. phaseolina* in pigeon pea and differentiated on the basis of their morphological and cultural characters. Iqbal and Mukhtar (2014) reported sixty five isolates and analyzed that the radial growth of pathogen ranged from 32.00 to 87.17mm and significant variation detected among sixty five isolates on their radial growth, sclerotial size, weight as well as pathogenicity test.
Table 1 Cultural and morphological characteristics of different isolates of *Macrophomina phaseolina*

| S. N. | Isolates | Colour colony | Colony appearance | Mycelial growth (mm) | Branching pattern | Sclerotial size (um) | Sclerotial shape | Sclerotial Formation |
|-------|----------|---------------|-------------------|----------------------|-------------------|---------------------|------------------|---------------------|
| 1     | MPpa1   | Whitish grey  | Fast growing      | 79.15                | Right             | 70.27               | oblong           | ++                  |
| 2     | MPpa2   | Brownish grey | Moderate to less growing | 84.78               | Right             | 80.50               | ovul             | ++++                |
| 3     | MPjd1   | Blackish      | Fast growing      | 90.00                | Right             | 106.50              | oblong           | +++                 |
| 4     | MPjd2   | Blackish      | Moderate growing  | 82.00                | Right             | 90.25               | Irregular        | ++++                |
| 5     | MPng1   | Creamy brown  | Fast growing      | 89.20                | Right             | 93.83               | Round            | ++++                |
| 6     | MPng2   | Whitish grey  | Fast growing      | 78.50                | Right             | 81.74               | Irregular        | ++++                |
| 7     | MPjp1   | Grayish white | Fast growing      | 70.00                | Right             | 99.00               | Round            | +++                 |
| 8     | MPjp2   | Blackish brown| Moderate growing  | 87.50                | Right             | 77.50               | Ovul             | +++                 |
| 9     | MPtn1   | Whitish       | Slow growing      | 67.50                | Right             | 87.98               | Oblong           | +++                 |
| 10    | MPtn2   | Whitish creamy| Slow growing      | 64.00                | Right             | 98.32               | Oblong           | ++++                |

0 = no sclerotial formation; 1-10 (+) = Poor, 11-20 (++) = Moderate, 21-30 (+++) = Good, >30 (++++) = Excellent

Satpathi and Gohel (2018) and Wagan et al., (2018) also worked on same pathogen and collected different isolates, the isolates were categorized on their morphological and cultural features and observed the colony colour, radial growth, shape and size of sclerotia.

In conclusion for management of stem and root rot pathogen we need know out their variability and survivability in soil. Different isolates were isolated from samples collected from different sesame growing surveyed areas of Rajasthan and classified on their morphological and cultural characteristics and seen variability between different collected isolates. Variation among ten isolates is colony appearance, colony colour, mycelia growth, branching pattern sclerotial size, shape and their formulation were recorded. Isolate MPjd1, MPjp1, MPng1, MPng2, MPpa1 and MPpa2 showed 100% mycelial growth after seven days of inoculation on PDA plates, colony appeared less to fast growing mycelial growth with right angle branching pattern. Colony coloured varied from whitish grey to blackish in colour and formed sclerotia. Sclerotial size varies from 70.27-106.50 um. Shape of sclerotia was oblong, round, ovul and irregular type.

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