Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(6): 1812-1816
Received: 16-09-2020
Accepted: 19-10-2020

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Studies on the detection, location and transmission of seed borne *Macrophomina phaseolina* (Tassi.) Goid causing charcoal rot disease in sesame (*Sesamum indicum L*) varieties of Tamil Nadu

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Abstract
An investigation was carried out in sesame **(*Sesamum indicum L*)** to detect the seed borne *Macrophomina phaseolina*, its location on seeds and transmission to seedlings were conducted in seed health laboratory, Seed Centre, Tamil Nadu Agricultural University, Coimbatotre. A total of ten varieties of sesame seeds were collected from various sources like seed production plots, farmers field and Research stations of Tamil Nadu during 2016-2018. The seed borne *Macrophomina phaseolina* was detected by dry seed examination, standard blotter method (SBM), agar plate method. The pathogen was detected in all the seed health test methods and the infection was recorded more in agar plate method which ranged from 3.5 to 15 per cent in all the varieties. The seed component test for the location of *M. phaseolina* revealed that the pathogen was present only on the seed coat but not in cotyledon or embryonic axis. The transmission of *M. phaseolina* from seed to seedling was observed more in water agar method which ranged from 2 to 8 per cent in the sesame varieties.

Keywords: Component plating, Location, *Macrophomina phaseolina,* SBM test, water agar, agar plate method

Introduction
Sesame (*Sesamum indicum* L.) is an ancient and traditional oil seed crop in India. India contributes the highest sesame acreage of about 17.73 lakh hectares and production of 8 lakh tonnes and productivity of 445 kg/ha. Sesame crop is affected by as many as 80 diseases which are caused by pathogens like fungi, bacteria, virus and mycoplasma among which 29 have been reported in India (Vyas *et al.*, 1984) [29]. Among the fungal diseases that affect sesame, the root rot disease also known as charcoal rot or stem rot caused by *Macrophomina phaseolina* (Tassi.) Goid is the most devastating and serious disease affecting the crop in later stages of growth (Buldeo and Rane, 1978) [24].

In India the disease is widely distributed in all the sesame growing areas in Punjab, Haryana, Uttar Pradesh, Gujarat, Madhya Pradesh, Maharashtra, Bihar, Orissa, Tamil Nadu, Karnataka, Kerala and West Bengal and the disease incidence was recorded up to 50 per cent (Chattopadyay and Kalpana Sastry, 1999) [5]. Singh *et al.* (1991) [28] surveyed sesame fields in Delhi, Haryana, Uttar Pradesh, Karnataka and Tamil Nadu for root rot incidence in fields and the disease incidence varied from 6.0 to 71.5 % (av. 17.01 %) depending on the soil conditions and crop season. One per cent increase in the incidence of *Macrophomina phaseolina* reduced seed yield by 1.8 kg/ha (Murugesan *et al.*, 1978) [21].

The most common symptom of the disease is the sudden wilting of growing plants, mainly after the flowering stage, the stem and roots become black due to severe infection. The pathogen survives as sclerotia in soil and crop residues and also has been reported as seed borne, that make it difficult to control (Maiti *et al.*, 1988) [15]. The fungus is also associated with severe infection causing both pre and post emergence damping off, including seed to seedling transmission of the pathogen (Pun *et al.*, 1998) [24]. Yu and Park (1980) [30] reported that *M. phaseolina* causes severe reduction in seed germination and seedling stand. The infected seeds produce infected plants which die immediately (Gonzalez and Subero, 1984) [12].

Materials and Methods
Collection of seed samples
A total of ten varieties of sesame samples (TMV 3, TMV 4, TMV 5, TMV 6, TMV 7, VRI 1, VRI 2, VRI 3, CO 1 and SVPR1) were collected from the Research stations and seed production fields from TNAU, Coimbatore and RRS, Virudhachalam during 2016-17. The seeds were stored in cloth bags at room temperature 22±2 ºC for further investigation.

Detection of seed-borne *M. phaseolina*

a) Dry seed examination

For dry seed examination, 400 seeds of each sesame variety were subjected to examination under stereo zoom microscope for the presence of pathogenic fruiting structures (microsclerotia) on the physical appearance of the seed as per the procedure given by Mathur and Jorgensen (1998) [19]. The per cent infections were calculated.

b) Standard Blotter Method

Seed samples were analysed for the detection of seed-borne *M. phaseolina* by blotter method following ISTA, 1993 [13]. In this method three layers of blotter paper were soaked in sterilized water and placed in the bottom Petri plates. Four hundred seeds were sterilized with 2% sodium hypochlorite solution for 1 to23 minutes. Twenty-five seeds were placed at equal distance aseptically on the moist blotter paper with 16 seeds on the outer circle, 8 seeds in the inner circle and 1 seed in the middle. The plates were then incubated for 7 days at 22± 2 º C under 12 h of alternating cycles of light and darkness. Four replications with 100 seeds per replication were maintained. After incubation, the *M. phaseolina* infection on each seed was examined under stereo microscope for the seed infection. Also, temporary slides were prepared from fungal colony and observed under compound microscope for proper identification of the fungus. The fungus was identified as per the keys suggested by Malone and Musketee 1964; Booth 1971, Ellis 1971; Chidambaram and Mathur 1975 and Neergaard and Saad 1962 [16, 3, 11, 6, 23]. The per cent disease incidence was worked out by using the formula

$$\text{PDI} = \frac{\text{No. of seeds infected}}{\text{Total No. of seeds}} \text{ x } 100$$

c) Agar plate method

The sesame seeds were surface sterilized with 2% sodium hypochlorite solution and then washed thrice with sterile distilled water, Then the sterilized seeds were plated on potato dextrose agar medium and the plated seeds were incubated for 5-7 days at 22-25°C under 12h alternating cycles of light and darkness. At the end of the incubation period, fungi growing out from seeds on the medium are examined and identified. Identification is based on colony characters and morphology of sporulating structures under a compound microscope.

Location of the *M. phaseolina* in sesame

Component plating method

The location of the pathogen on the sesame seeds were investigated by the component plating method (Du-Hyunglee *et al.*, 1984) [10]. The sesame seeds were surface sterilized with 2% sodium hypochlorite (NaOCl) for two minutes and soaked in sterile distilled water for five hours. Then the individual seed components *viz*., the seed coat, cotyledons and embryonic axis (plumule and radicle) were dissected aseptically using forceps and needles on blotter. Each component was dipped separately in 2% sodium hypochlorite solution (NaOCl) for one minute and was then plated on blotter paper using SBM method and on the agar medium (Agar plate method) and incubated as described the above. One hundred seeds were dissected in each sample and four replications of 25 seeds each were maintained. After eight days the plates were observed under stereo binocular microscope for the presence of pathogen on the components of seed. Fungal infection in different seed components was determined based on the appearance of the fungus on the SBM and agar plate and the percentage of infection was calculated.

Seed to seedling transmission of *Macrophomina phaseolina*

a) Rolled paper towel method

The sesame seeds infected with *M. phaseolina* (seeds showing microsclerotia on seed coat) were selected for disease transmission. One hundred seeds of each sample were used for determining the seed to seedlings transmission in the rolled paper towel method. The seeds were placed in between the moist blotter paper rolls in four replicates of 25 seeds each. The rolls were kept at 23º $\pm$ 2ºC in seed germinator and incubated for 7 days. The observations were recorded on the incidence of infected seedlings and confirmed through the isolation of the *M. phaseolina* from infected young plant.

b) Sand method

The infected **s**eeds were planted in a uniform layer of sterilized moist sand and then covered to a depth of 1 to 2 cm with sand. The seeds were placed at equal spacing on the sand and moistened with 50% of its water holding capacity. Then the germination trays were placed under the controlled temperature conditions for a period of 7 days (ISTA testing). The observations were recorded on the incidence of infected seedlings and confirmed through the isolation of the *M. phaseolina* from infected young plant.

c) Water agar method

The infected sesame seeds were placed individually in 1% water agar slant and covered with aluminium foil /cotton plug, The tubes were then incubated at 20°C± 2°C for 14 days under 12h alternating cycles of artificial day light and darkness. The aluminum foil/cotton plugs must be removed the day seedlings start touching them. The seeds were tested for the seed to seedling transmission of *M. phaseolina* infection. Four replications with 25 seeds each were maintained for each variety. The seedlings were observed for the infection and per cent infection was recorded.

Results and Discussion

Detection of seed borne *Macrophomina phaseolina*

The sesame varieties subjected to various seed health testing methods *viz*., dry seed examination, standard blotter paper method and agar plate method revealed that the per cent seed infection was recorded more in agar plate method which ranged from 3.5 per cent to 15 per cent compared to other two methods of detection (Fig. 1). Among the sesame varieties which were screened for the detection of seed borne *M. phaseolina*, the sesame variety SVPR 1 recorded maximum infection of 6.5%, 3.5% and 15% in standard blotter paper method, dry seed examination and agar plate method respectively. The sesame variety VRI 3 recorded nil incidence of *M. phaseolina* seed infection in all the three methods of detection. The pathogen appeared as greyish mycelial growth on sesame seeds in both agar plate (Fig. 2) and standard blotter paper method whereas in dry seed examination, the infected seeds show microsclerotia on the seed coat (Fig.3). The above results are in association with Manthachitra, 1971; Mohalay, 1988; Shakir and Mirza, 1992; Shakir *et al.* 1995 [17, 14, 26, 27] who reported the *M. phaseoloina* from the seeds of cucumber, bottle gourd, bitter gourd, pumpkin, Indian gourd, red gourd and sponge gourd.

Location of *M. phaseolina* in sesame seeds

The location of *M. phaseolina* in sesame seeds assessed by component plating method revealed that the pathogen was

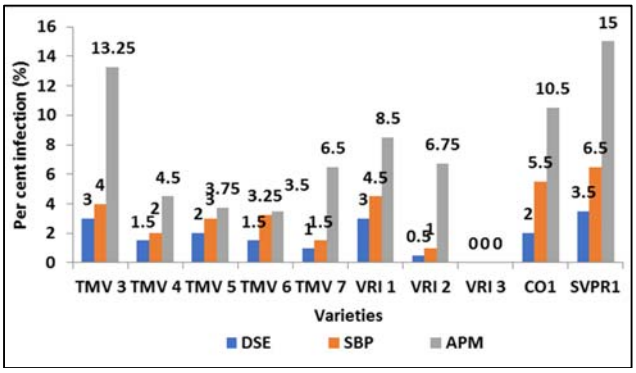
recovered only from the seed coat but not in the cotyledons and the embryo (Fig. 4). The results are in conformity with those of Chilkuri Ashwini and Giri (2014) [7] who reported that the maximum colonisation of *M. phaseolina* in seed coat of green gram. Shakir *et al.* (1995) [26] isolated *M. phaseolina* from testa, tegmen and embryo of sponge gourd seeds. The equal infection level of *M. phaseolina* in the pericarp, endosperm and embryo of sunflower seeds were reported by Bhutta *et al.* (2009) and Dawar (1994) [8]. Nasreen Sultana (2009) [22] reported the location of *M. phaseolina* in cucumber seeds and its infection level decreased with the depth and was very low in cotyledons and embryo.

Seed to seedling transmission of *Macrophomina phaseolina*

Seed to seedling transmission of *M. phaseolina* in sesame was experimentally demonstrated by seedling symptom test invitro where the pathogen moves from infected seeds to hypocotyls and seedlings and was confirmed by isolating the pathogen from the roots and hypocotyls of young seedlings. The results revealed that among the three tests, *viz*., paper towel method, sand method and water agar method, the maximum seedling infection was recorded in water agar method which recorded 2 to 8 per cent infection in sesame varieties. The maximum detection was recorded in the sesame variety SVPR 1 (8%) and VRI 3 recorded nil incidence (Fig. 5, 6). Dhingra & Sincliar (1975) [9] reported that *M. phaseolina* carried on the seed coat either did not show seed germination or produced seedlings that may die soon after emergence due to post emergence damping off. Reuveni *et al.*, (1983) [25] detected *M. phaseolina* in seed coat and cotyledons of melon and found the fungus to penetrate the frut via peduncle to infect the seeds. *Macrophomina phaseolina* has been reported to be transmitted from seed to seedling of sunflower (Dawar, 1996; Bhutta et.al., 1996) [8, 2] and soybean (Anwar *et al.*, 1995) [1]. Nasreen Sultana (2009) [22] reported that the fungus *M. phaseolina* was transmitted to seedling and caused pre and post emergence infection. *Macrophomina phaseolina* was found transmissible from seed to plant causing seedling blight in green gram (Chilkuri Ashwini and Giri, 2014) [7]. Mota et.al. (2019) [20] reported that *Macrophomina phaseolina* was transmitted from the seeds to seedlings of lima bean with a transmission rate of 76.46 per cent causing symptoms in various aerial parts.

Acknowledgements

The financial assistance received from All India Co- ordinated Research Project- National Seed Project (Crops) for the work is greatly acknowledged.



DSE-Dry Seed Examination ; SBP- Standard Blotter Method ; APM-Agar Plate Method

Fig 1: Detection of *Macrophomina phaseolina* from sesame varieties by different methods

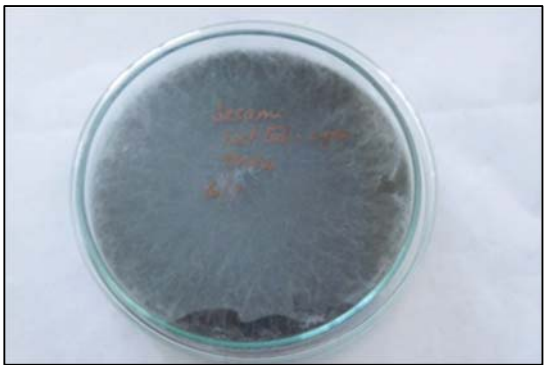


Fig 2: Detection of *M. phaseolina* by Agar plate method

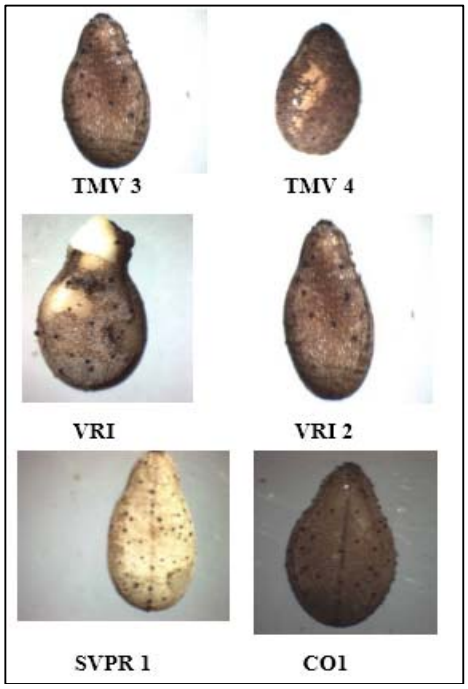


Fig 3: Sesame seeds showing microsclerotia of *M. phaesolina* on seed coat

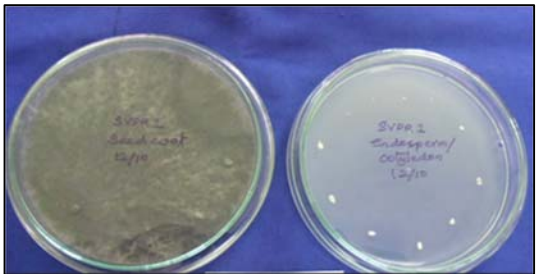


Fig 4: Location of *M. phaseolina* by component plating method

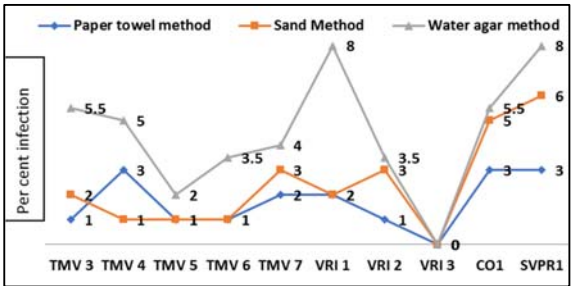


Fig 5: Seed to seedling transmission of *M. phaseolina* in sesame by various methods

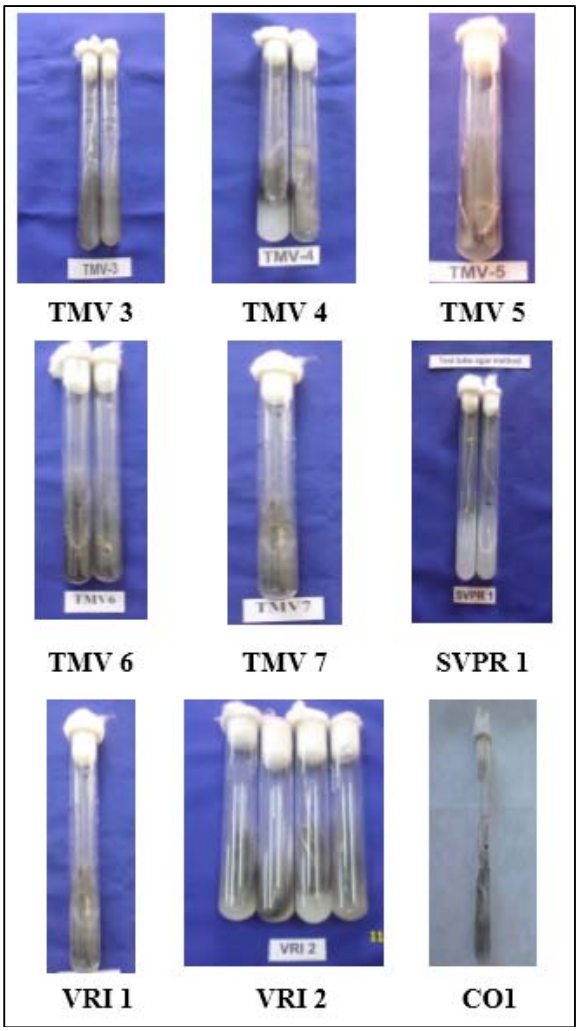


Fig 6: Seed to seedling transmission of *M. phaesolina* by water agar method

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