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ISOLATION OF FUNGUS PHYTOPATHOGEN *MACROPHOMINA PHASEOLINA* FROM INFECTED STEM PARTS OF *SORGHUM BICOLOR (L.)*

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Interaction of plants with environmental factors and microbial world is a dynamic process. As part of this ever-changing process new disease emerges or minor disease may become major with time. This paper majorly reports a very brief account of well-studied old diseases of sorghum and emphasizes on emerging diseases with particular reference to India. Description of sorghum diseases caused by fungi, bacteria, and their transmission through seed implies quarantine significance. The fungus pathogen *Macrophomina phaseolina* was successfully isolated from the basal portion of the charcoal rot of infected Sorghum stem collected from the fields of National Research Centre for Sorghum (NRCS) Rajendranagar, Hyderabad. In this present study small bits of sclerotia bearing strands were surface sterilized by immersing in 0.1 percent mercury chloride for two minutes. The surface sterilized strands washed in three changes of sterile distilled water. They were planted on PDA under aseptic conditions and incubated at 25^0C in SEW, BOD incubator. After 4days of incubation the fungus was transferred on a fresh PDA plate and was purified through single sclerotia isolation purified culture of *M. phaseolina* was maintained on PDA slants at room temperature (26-28^0C).

Key words: Isolation, Fungal Pathogen, *M. phaseolina* Sclerotia, aseptic and Charcoal rot and *Sorghum bicolor (L.)*

Introduction

The word “sorghum” typically refers to cultivated sorghum (*Sorghum bicolor* [L.] Moench subsp. bicolor), a member of the grass family Poaceae, tribe Andropogoneae, and subtribe Sorghinae (Clayton and Renovoize, 1986) that is grown for its grain (grain sorghum), its sugary sap (sweet sorghum) or as a forage (forage sorghum). A variety of common names are used in different regions to refer to cultivated sorghum, including great millet, guinea corn, broomcorn, kaffir corn, durra, mtama, milo, jowar or kaoliang (FAO, 1995).

Sorghum is a cereal of high importance, being in fifth place in the world after the production of grains (57.6 million tonnes) after corn, wheat, rice and barley (FAO 2017). It has a great development due to its use in human nutrition, especially in the semi-arid areas of the world, where pedo climatic conditions offer limited conditions for agriculture. Such situations are mainly in Africa, Asia and Latin America, areas that are frequently affected by drought (Taleon, *et.a.,* 2012), , here sorghum is one of the most important crops representing the main source of energy and nutrition for people (Xiong *et.al.,* 2019). In western countries, such as the United States, Mexico and Australia, sorghum is mainly grown for animal feed, but there is a growing interest in its use for biofuel production, as well as for use in human nutrition, having given its chemical composition, which is beneficial for the development of healthy and functional foods. (Rooney *et.al.* 2013).

*Sorghum bicolor (L.) i*s an important crop for human consumption and animal fodder. It is grown principally for grain in the tropical and subtropical areas of the Indian sub-continent. Sorghum attains the fourth place in India among the staple food crops. Sorghum crop suffers from many diseases and are caused by various organisms like bacteria, viruses, mycoplasma and fungal pathogens. Among fungal diseases during post rainy season charcoal rot is one of the important disease caused by *M. phaseolina (Tassi). Goid*. It is commonly referred as charcoal rot, due to presence of soot black sclerotia of *M. phaseolina* in lodged plants which cause severe yield loss.

Material and methods

The pathogen *M. phaseolina* was successfully isolated from the basal portion of the charcoal rot of infected Sorghum stem collected from infected Jowar (*Sorghum bicolor* (L.) stem segments randomly from different areas of Agraharam Rajanna Siricilla District Telangana Sate. The Infected samples were the first surface sterilized by washing under running tap water to remove dirt such as sand. A flamed blade was used to cut partly diseased and partly healthy portions of the sample, the cut portions were then surface sterilized using 70% alcohol after which they were rinsed in successive changes of sterile distilled water. They were then inoculated on Potato Dextrose Agar (PDA). This was done for all the samples from different areas of Agraharam Rajanna Siricilla District Telangana Sate, the plates were incubated at 28^0C±2. Fungal growth was observed daily. After six days of incubation, a small portion of mycelium from each fungal colony was transferred aseptically into fresh plates containing the medium used. The fungi were purified by repeated sub-culturing in the Research Laboratory Department of Botany Osmania University, Hyderabad.

In this present study small bits of microsclerotia are visible on host tissue isolation on culture media is easily accomplished using a dissecting scope. If microsclerotia are not evident, isolation should be attempted from the areas of the plant likely infected.

Preparation of Potato Dextrose Agar

1. Suspend 39 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. 2. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before dispensing.

3. In specific work, when pH 3.5 is required, the medium should be acidified with sterile 10% tartaric acid.

4. The amount of acid required for 100 ml. of the sterile, cooling medium is approximately 1ml.

5. Do not heat the medium after addition of the acid Molds will grow as filamentous colonies of various colors.

The cultural characteristics viz, colony characters, morphology, mycelia growth and sclerotia production of *M. phaseolina* were studied on different culture media viz, potato dextrose agar, maize meal agar, oat meal agar and sorghum meal agar used. Autoclaved and cooled (40^0C) media were poured into sterilized glass petriplates and allowed to solidify, upon solidification of the media, plates were inoculated aseptically with 5mm culture disc obtained from5-6 days old culture of *M. phaseolina.* Each treatment was replicated thrice and plates were incubated at room temperature.

Observations on radial mycelia growth, sclerotial production and cultural characteristics were recorded and presented in the (Table-1)

Results and Discussion

M. phaseolina is a root–inhabiting fungus (Garrett, 1956) with little or no saprophytic growth in either soil or dead host cells of infected plants (Edmunds, 1964).In absence of host plants, it survives or over seasons predominantly as small black sclerotia root and stem debris or in soil after decay of the plant material in which they are formed. (Smith, 1969, Bhattacharya and Sammaddar, 1976). Thus the primary source of inoculum is sclerotia in the soil. (Cook *et al.*, 1973). Meyer *et al.*, 1973) reported that after 16 months in soil, (23%) of sclerotia from stalks have germinated. Populations of sclerotia in a maize field ranged from zero to 100 grams of soil (Papavizas and Klag, 1975).This variation in inoculum density in soil is one of the factor responsible for highly variable incidence of charcoal rot in the field..

The isolation of *M. phaseolina* from the surface sterilized sections illustrates that this fungus penetrated, some of these dead tissue in the presence of other competitive and antagonistic soil microorganisms. There are only few reports on the quantitative isolation of *M. phaseolina* from soil as well as from diseased plants.

In the present study, the pathogen *M. phaseolina* was isolated from basal portion of the charcoal rot affected sorghum stems. The fungus culture maintained on PDA slants at room temperature. The isolation method involved in above study was reported earlier by Cook et al., (1973) Meyer et al.,(1973)., Papavizas and Klag, (1975) and Dhingra and Sinclair,(1973).

Effect of different media on the growth of the *M. phaseolina,* Watanabe *et al.,* (1970*)* developed a differential flotation technique for assaying populations of *M. phaseolina sclerotia* in pine nursery soils. Meyer *et al.* (1973) described two selective media with rice agar, the basal medium to isolate *M. phaseolina* from soil. Papavizas and Klag, (1975) reported the selective media and a method was developed for the direct isolation of *M. phaseolina* from soil. The similar reports are observed with reference to ergot pathogen *Claviceps sorghi* by Kumar and Arya, (1978). *M. phaseolina* survives in debris and soil as very small black sclerotia. The soil borne sclerotia caused greater disease incidence than mycelium. Selective media are needed to accurately assay all of the propgules of plant pathogens since saprophytic fungi are omini present in soil quickly over grow on agar plates and prevent recovery of important pathogens. There are only few reports on the quantitative isolation of *M. phaseolina* from soil as well as from plant debris.

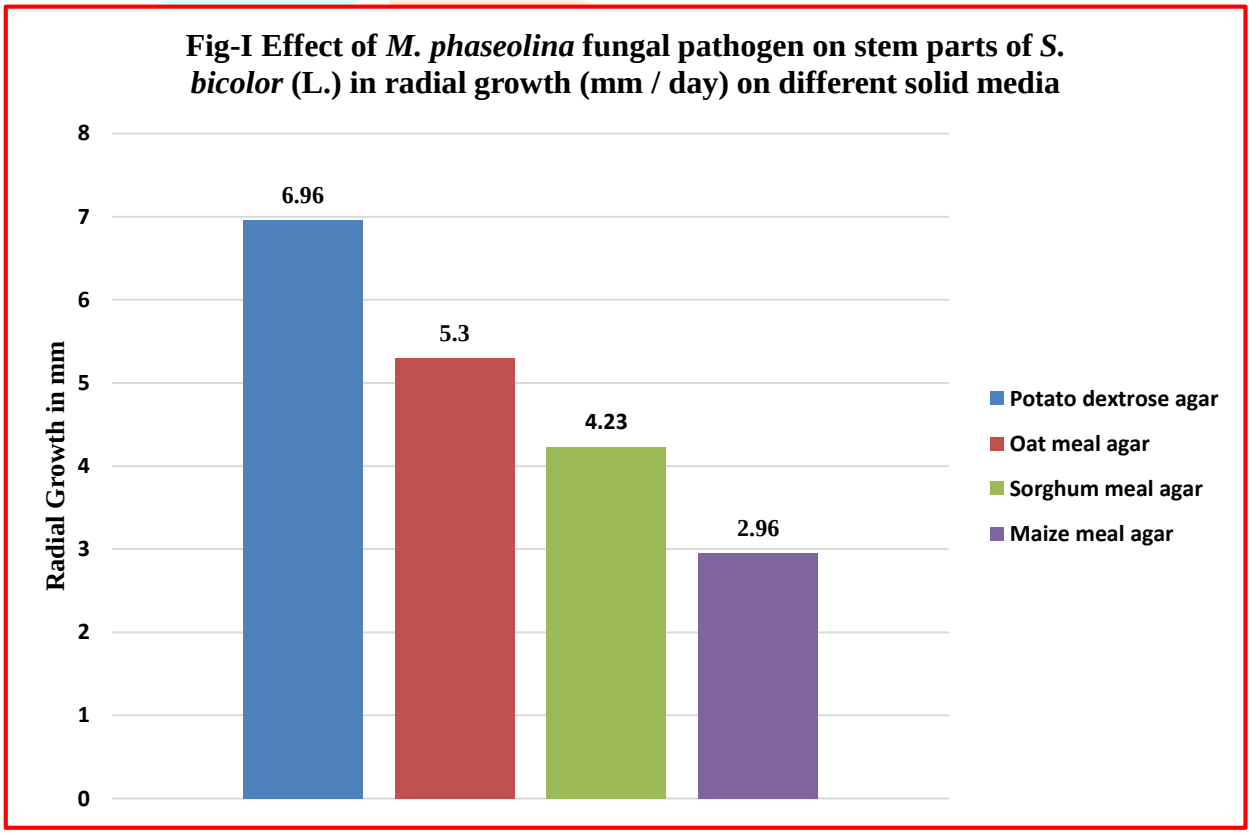
In the present study, PDA was used as common medium for the growth and maintenance of the fungus. The effect of different media i.e. potato dextrose agar, oat meal agar, sorghum meal agar and maize meal agar was studied. The results are presented in the (Table -1) (Fig-I)

Table-1 Effect of *M. phaseolina* fungal pathogen on stem parts of *S. bicolor* (L.) in radial growth (mm / day) on different solid media

S. No.	Media	Radial Growth(mm)
1	Potato dextrose agar	6.96
2	Oat meal agar	5.30
3	Sorghum meal agar	4.23
4	Maize meal agar	2.96

from the results obtained, it is clear that potato dextrose agar supported highest rate of growth of the fungus and least growth was observed in maize meal agar medium. There was no significant difference in the growth rate of fungus on oat meal agar and sorghum meal agar medium.

Fig-I Effect of *M. phaseolina* fungal pathogen on stem parts of *S. bicolor* (L.) in radial growth (mm / day) on different solid media



Conclusion:

The charcoal rot fungus was isolated in pure culture from basal portion of the diseased stems and studied for various aspects as there is little information avaliable in the literature. The growth of the *M.phaseolina* was studied in the meal obtained from sorghum and maize grains in addition to a non-host plants namely oats. PDA which is mostly used for the growth and maintenance of the fungus was used as a standard to compare the pathogen behaviour in the meals of grains of maize, sorghum and oat.

The growth rate of *M.phaseolina* per day was found to be maximum in PDA(6.96mm), next best growth was found in oat meal agar(5.30mm), least growth was observed in Sorghum meal agar(4.3mm)

and maize meal agar(2.96mm). The increased growth in PDA could be attributed to 1. The rich nutrients of potato extracts and 2. The presence of essential carbon compounds namely glucose. Oat meal agar is widely used medium next to PDA medium for the cultivation and maintenance of many fungii especially *Pythium spp* and *Colletotrichum spp*. This could to due to the availlability of adequate nutrients for the growth of fungii. But not to the extent available in PDA. Cereal meal media like maize and sorghum did not encourage the growth of the pathogen, which obviously projects the either lack of certain essential nutrients in the media or the presence of high carbohydrate contents in these meals which would have inhibited the growth of *M.phaseolina.*

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