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Short Communication

Effect of media on the growth and sclerotial formation of *Macrophomina phaseolina* (Tassi) Goid causing charcoal rot of sesame (*Sesamum indicum* L.) under *in-vitro* condition

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Abstract
Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid., is one of the most widely distributed and destructive disease of Sesame [*Sesamum indicum* (L.)], causing accountable quantitative and qualitative losses. The pathogen can induce various diseases *viz*., charcoal / root / stem rots, leaf blight etc. in many crop plants. The effects of different culture media on mycelial growth and sclerotial formation of *Macrophomina phaseolina* (Tassi) Goid inciting agent of charcoal rot of sesame were studied *in vitro*. 10 different solid media were tested for their effect on mycelial growth and sclerotial formation of test fungus. Among the solid media Potato Dextrose Agar medium had the highest mycelial growth (89.66 mm) and Excellent sclerotia formation (++++), followed by sesame root extract agar (89.30 mm and ++++) and Richard's agar (88.90 mm and ++) were the best for fungus growth as well as for sclerotial formation. Corn meal agar medium was recorded the least mycelial growth and lowest sclerotia formation (56.11 mm and +).

Keywords: Media, sclerotia, charcoal rot, *Macrophomina phaseolina*, sea same

Introduction
Sesame (*Sesamum indicum* L.) adorned as "Queen of oilseeds" is one of the oldest and most indigenous ancient edible oilseeds crops, grown in India. Sesame, which originated in Africa, is probably the most ancient oilseeds crop cultivated in many parts of the world and currently, China, India and Myanmar (Burma) are the world's largest producers of sesame (FAO, 2004). 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.
Sesame is a warm weather crop and is often grown on marginal lands and in stressed conditions. 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) [11, 7].
India ranks first in World in respect area and production of sesamum. In India Uttar Pradesh, Punjab, Jammu and Kashmir, Assam, Maharashtra, Kerala and Haryana are major sesamum growing states. In India area 1950.88 ('000 ha), production 850.07 ('000 MT) and productivity 436 kg/ ha. In Maharashtra state area is 0.28 lakh ha, production 0.60 lakh tonn and productivity 194 kg/ha. (Anonymous, 2015) [1].
Among these, charcoal 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) [12]. The disease is very important as its infection occurs from seed germination and emergence to adult stage. The pathogen being mostly soil borne and sometimes seed borne, it induces pre-emergence seed rot, as well as post-emergence seedling mortality and thereby reduction in total plant population per unit area. During drought / water stress conditions and high soil temperature, the sesame crop is more prone to charcoal rot (*M. phaseolina*) and the disease is especially widespread during extremely hot and dry season. Sclerotia (resting structures), survives on crop residues, dispersed by soil and contaminated seeds and farm equipments (Thiyagu *et al*. 2007) [10].

Macrophomina phaseolina, induces the symptoms: infected seedlings show reddish brown discoloration. Hypocotyls is girdled and seedling dies. On stem and roots, water-soaked lesions appear first and later turn dull light brown. Infected plants become weak and dry, the lower stem show a typical charcoal, grey black discoloration which often gives the whole field a black appearance. When the stem is cut open, numerous minute black speaks (sclerotia) are visible on the shredded vascular bundles and inside of the stem gives charred appearance. The pathogen *M. phaseolina* (Tassi) Goid belong the Division: Eumycota, Sub division: Deuteromycotina, Class: Coelomycetes, Order: Spaerosidales, Family: Sphaeropsidaceae, Genus: *Macrophomina* and species: *phaseolina.*

Materials and Methods

Effect of culture media

The laboratory experiments were conducted at the Department of Plant Pathology, College of Agriculture, Latur VNMKV, Parbhani, MS. India.

A total of 10 culture media (as detail under treatments) were used to study their effects on growth and micro sclerotia production of *M. phaseolina*. Autoclaved and cooled media were poured (@ 20 ml/ plate) separately in sterilized glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. On solidification of the media, these media plates (three plates / medium / replication) were inoculated by placing at the centre a 5 mm mycelial disc of actively growing 7 days old pure culture of *M. phaseolina*, incubated at 28 ± 2 °C, for a week.

Experimental details

Design: C.R.D (Completely Randomized Design)
Replications: Three
Treatments: Ten (Culture media)

Treatment details

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Corn meal agar	T_6	Richard's agar
T_2	Conn's agar	T_7	V_8 juice agar
T_3	Czapek's dox agar	T_8	Sesame leaf extract (20%) agar
T_4	Potato dextrose agar	T_9	Sesame stem extract (20%) agar
T_5	Malt extract agar	T_{10}	Sesame root extract (20%) agar

Observation on radial mycelial growth/ colony diameter (mm), colony colour, colony morphology were recorded after a week of inoculation and on micro sclerotia (research microscope) after 10-12 days of incubation. Sclerotia production was graded/ categorized as per following scale (Das, 1988) [2].

Sr. No.	Grade	Description	Av. No of Sclerotia/ Microscopic field
1	-	No sclerotial formation	-
2	+	Poor sclerotial formation	10-20
3	++	Fair sclerotial formation	21-30
4	+++	Good sclerotial formation	31-50
5	++++	Excellent sclerotial formation	51 and above

Results and Discussion

Effect of culture media

Cultural characteristics *viz*., colony diameter / mycelial growth, colony characters and sclerotial population of *M. phaseolina* were studied, using 10 culture media and results obtained are narrated herein under the following sub-heads.

Colony diameter / mycelial growth

The results (Table 1, Plate 1 and Fig.1) revealed that all of the ten-culture media tested encouraged better growth and varied sclerotial production of *M. phaseolina*. Mean colony diameter/ mycelial growth with the test media ranged from 56.11mm (Corn meal agar) to 89.66 mm (Potato dextrose agar). However, it was significantly highest with Potato dextrose agar (89.66 mm), which was on par with sesame root extract agar (89.30 mm) and Richard's agar (88.90 mm). These were followed by Conn's agar (87.73 mm), Sesame stem extract agar (85.33 mm), Malt extract (82.23 mm), Sesame leaf extract agar (82.06 mm) and V_8 juice (74.23 mm). Comparatively minimum mean mycelial growth of the test pathogen was found on Czapek's dox agar (67.60 mm) and Corn meal agar (56.11 mm).

The test culture media, tested exhibited a wide range of colony characters. The mycelium produced was fluffy, thin or thick, dirty white, dark white, greyish or dark black. Reverse plate side pigmentation was mostly dirty white to dark black.

Plate 1: Effect of various culture media on mycelial growth of *M. phaseolina*

Tr. No.	Treatments	Tr. No.	Treatments
T1	Corn meal agar	T6	Richard's agar
T2	Conn's agar	T7	V-8 juice agar
T3	Czapek's dox agar	T8	Sesame leaf extract (20%)
T4	Potato dextrose agar	T9	Sesame stem extract (20%)
T5	Malt extract agar	T10	Sesame root extract (20%)

Table 1: Effect of various culture media on growth of *M. phaseolina*, causing charcoal rot of sesame

Tr. No.	Treatments	Col. Dia. (mm)*	Cultural/ colony characters	Sclerotia production**
T_1	Corn meal agar	56.11	Light brown to dark black, thin mycelial mat,	+
T_2	Conn's agar	87.73	Grayish mycelium, thick mycelial mat	++
T_3	Czapek's dox agar	67.60	Dirty white mycelium	+
T_4	Potato dextrose agar	89.66	Fluffy growth, dirty white mycelium	++++
T_5	Malt extract	82.23	Dark white mycelium, thin mycelial mat	++
T_6	Richard's agar	88.90	White to black mycelium, cottony growth at center	++
T_7	V_8 juice agar	74.23	Grayish mycelium, white at center	++
T_8	Sesame leaf extracts (20%) agar	82.06	Thin mycelial mat, white to brown mycelium	+++
T_9	Sesame stem extract (20%) agar	85.33	Thin mycelial mat white to brown mycelium	+++
T_{10}	Sesame root extract (20%) agar	89.30	Thick mycelial mat, fluffy growth mycelium, white to gray	++++
	SE ±	0.23	-	-
	CD (P=0.01)	0.68	-	-

* Mean of three replications, ** + = Poor, ++ = Fair, +++ = Good, and ++++ = Excellent

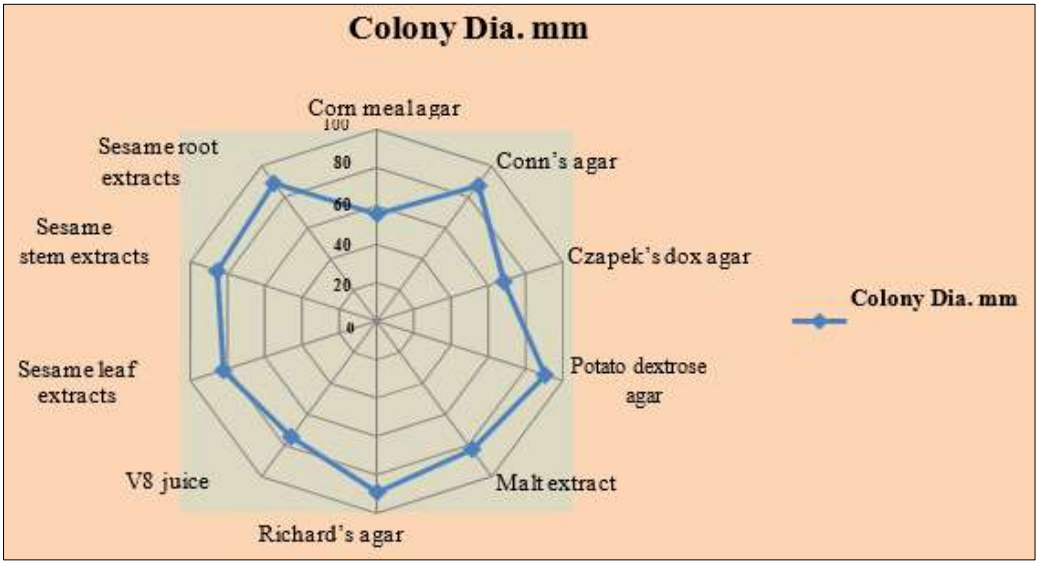

Fig 1: Effect of various culture media on mycelial growth of *M. phaseolina*

Sclerotia production

All of the 10-culture media tested, exhibited a wide range of sclerotial production of the test pathogen. However, it was excellent (++++) on Potato dextrose agar and Sesame root extract agar, good (+++) on Sesame stem extract agar and Sesame leaf extract agar, fair (++) on Conn's agar, Malt extract agar, Richard's agar and V_8 juice agar; whereas, it was poor (+) on Corn meal agar.

Similarly, Khan *et al*. (2012) [4] reported Potato dextrose agar, Chickpea root extract agar and Carrot root extract agar as better for mycelial growth of *R. bataticola*; Tandel *et al*. (2012) [9] reported Richard's agar, Oat meal agar and Czapak's dox agar as better for mycelial growth and sclerotia production of *M. phaseolina*; Deepthi *et al*. (2014) [3] reported that the culture media Malt extract agar, Potato dextrose agar and Richard's agar encouraged better mycelial growth and pycnidial production of *M. phaseolina*, causing dry root of sesame. These and many other culture media were reported to encourage better mycelial growth and sclerotia/pycnidia production of *M. phaseolina* /*R. bataticola*, earlier by many workers (Suryawanshi *et al*., 2014; Nagamma *et al*., 2015; Srinivas *et al*., 2016; Parmar *et al*., 2018) [13, 5, 8, 6].

Conclusion

All the 10-culture media tested exhibited better mycelial growth and poor to excellent sclerotial production of *M. phaseolina* (Table 1). However, Potato dextrose agar encouraged maximum radial mycelial growth (89.66 mm), with fluffy growth, dirty white mycelium. The second and third best media found were Sesame root extract (89.30 mm), with thick mycelial mat and white to gray mycelium and Richard's agar (88.90 mm), with white to black mycelium and cottony growth. Rest of the test culture media also resulted with better mycelial growth in the range of 56.11 – 87.73 mm. The sclerotial production on test culture media was poor (+) to excellent (++++). However, Potato dextrose agar and Sesame root extract resulted in excellent (++++) sclerotial production, followed by Sesame leaf extract agar and Sesame stem extract agar with good (+++) sclerotial production; Conn's agar, Malt extract, Richard's agar and V_8 juice agar with fair (++) sclerotial production; whereas, it was poor (+) on Corn meal agar and Czapek's dox agar.

Future scope

Various media has been used in this study and the result indicated that Potato Dextrose Agar Medium, followed by Sesame leaf extract agar, was shown to be the best for maximal growth and sclerotia production. These results may be useful for further studies of this pathogen at molecular level i.e. to assess the molecular diversity among different isolates of *Sclerotinia sclerotiorum*, or molecular characterization of pathogen.

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Conflict of Interest
None

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