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THE EFFECT OF DIFFERENT MEDIA ON RADIAL GROWTH OF *SCLEROTIUM ROLFSII* CAUSING CHILLI ROT.

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ABSTRACT
Chilli is grown in many states in India as a valuable trade crop. Chilli accounts for 20-30% of total Indian spices exports valuing approx Rs. 400-500 cores. Fifteen culture media Asthana and Hawker's, Carrot juice agar, Cooked vegetable agar (natural media), Conn's agar media, Czapek-dox agar, Garlic agar, Glucose-dox agar, Glucose peptone agar, Leonian agar, Oat meal agar, Malt extract peptone-dextrose agar, Potato dextrose agar, Potato sucrose agar, Richard's agar and Yeast dextrose agar were analyze for in vitro mycelial growth of *Sclerotium rolfsii.* These effects were studied using poisoning food technique. Out of the all fifteen culture media Malt extract peptone-dextrose agar was found to be the best culture medium to obtain the maximum radial growth 59 mm. It was succeeded by Potato sucrose agar was also found suitable medium with 57 mm radial growth , however (zero) 0 mm radial growth recorded on Czapek dox agar medium and Glucose dox medium after 72 hours of incubation period.

KEYWORDS: Culture media, fungal pathogen, Media effects, *Sclerotium rolfsii.*

INTRODUCTION

The Capsicum annum belong family Solanaceae is originate from Brazil. The vegetable and spice chilli contain vitamin-C (Ascorbic Acid), vitamin-6 (Pyridoxine), vitamin-A and minerals like iron, copper, potassium, but remarkable feature no-cholesterol. In India it is an important commercial crop. The disease rot of chilli cause by Sclerotium rolfsii gave major economic losses in major chilli producing state including Maharashtra. Along chilies the great financial losses found in other vegetables by fungal pathogen *Sclerotium rolfsii* diseases. Some brief reviews are as below. It has been reported that *Sclerotium rolfsii* caused about 25% seedling mortality in the groundnut cultivars JL-24 (Ingale and Mayee, 1986). In tomato this pathogen was responsible for a crop loss of 30% (Thiribuhuvanamal et, al., 1999). The fungus *Sclerotium rolfsii* is a soil borne pathogen causing rot, stem rot, diseases and more than 500 plant species of agriculture and horticultural crops throughout the world (Aycock, 1966). The pathogen caused a great economics loss on various crops. These pathogens exhibit variation in their morphological biological and immunological characteristics and pathogenesis or resistance against harm full environment.

The fungal culture media is of fundamental importance for most of the mycology, plant pathology and research paper experiments and tests to obtained pure culture for scientific studies, to grow, to weight, to cultivate selected and maintained pure culture for test fungus i.e. *Sclerotium rolfsii.* According to clinical / research laboratory stranded an optimum medium should provide good and adequate or poor and inadequate growth of the pathogenic fungus *S. rolfsii.* Without high-quality of media the possibility of achieving accurate, reproducible and repeatable microbiological test/ experiment research paper result is reduced (Sandle and Tidswell, 2010). Even with the increased use of rapid methods the majority of techniques found in the Pharmaceutical quality control laboratory require culture growth media (Cundell 2001). Morphogenic and pathogenic variation are known in many fungal pathogens and such detailed investigation was carried out on the variation with regards their nutritional factors on the mycelial growth and biomass production of *Sclerotium rolfsii.* This article is set out to show that consequence of fifteen media on radial growth of *Sclerotium rolfsii.*

MATERIALS AND METHODS

Isolation of *Sclerotium rolfsii:*
Isolation of *Sclerotium rolfsii* was carried out from diseases chilli plant collected from field of Aurangabad District.
The effect of different media on the growth of *Sclerotium rolfsii* was studied using poisoning food technique, (Dhingra and Sinclair, 1985).

Culture media:
In-vitro growth of *Sclerotium rolfsii* was tested with different fifteen culture media given as Asthana and Hawker's, Carrot juice agar, Cooked vegetable agar (natural media), Conn's agar media, Czapek-dox agar, Garlic agar, Glucose-dox agar, Glucose peptone agar, Leonian agar, Oat meal agar, Malt extract peptone-dextrose agar, Potato dextrose agar, Potato sucrose agar, Richard's agar and Yeast dextrose agar.
The fifteen different media of composition (g/L) shown below.
Asthana and Hawker's:
Glucose = 5g; Potassium nitrate (KNO3) = 10g; Potassium dihydrogen phosphate (KH2PO4) = 1.75g; Magnesium Sulphate (MgSo4.7H2o) = 0.75g; Distilled Water = 1L.
Carrot juice agar:
Carrot = 200g; Agar = 20g; Distilled water = 1L.
Cooked vegetable agar (natural media):
Broth or decoction of the desired vegetables of plant parts can be prepared by 10 to 20% of the tissue in water, steamed for 30 minutes and the contents mashed and squeezed through muslin cloth. To this broth, the required quantity of agar-agar (2%) may be added. It may be required to adjust pH of the medium to desired level before autoclaving.
Conn's agar media:
Potassium Nitrate (KNO3) = 10g; Magnesium Sulphate (MgSO4.7H2O) = 1.2g; Potassium dihydrogen phosphate (KH2PO4) = 2.7g; Maltose = 2.7g; Potato starch = 10g; Agar-agar = 15g; Distilled water = 1L.
Czapek-dox agar:
Sodium nitrate NaNO3 = 2g; Potassium dihydrogen phosphate = (KH2PO4) 1g; Magnesium Sulphate (MgSO4.7H2O) = 0.5g; Ferrous Sulphate (FeSO4) = 0.01g; Sucrose = 30g; Agar-agar = 20g; Distilled water = 1L.
Garlic agar:
Garlic agar = 300g; Agar-agar = 20g; Distilled water = 1L.
Glucose-dox agar:
Sodium nitrate (NaNO3) = 2g; Potassium dihydrogen phosphate (KH2PO4) = 1g; Magnesium Sulphate (MgSO4.7H2O) = 0.5g; Ferrous Sulphate (FeSO4.7H 2O) = 0.01g; Potassium chloride (KCl) = 0.5g; Glucose = 15g; Distilled water =1L.
Glucose peptone agar:
Peptone = 2g; Glucose = 10g; Magnesium Sulphate (MgSO4.7H2O) = 50g; Potassium dihydrogen phosphate (KH2PO4) = 50g; Agar-agar = 65g; Distilled water = 1L.
Leonian agar:
Peptone (or Neopeptone) = 6g; Potassium dihydrogen phosphate (KH2PO4) = 1.25g; Maltose or Glucose = 6.25g; Magnesium Sulphate (MgSO4.7H2O) = 0.625g; Malt extract = 6.25g; Agar-agar = 20g; Distilled water = 1L.
Oat meal agar:
Blend, in a commercial blender, 60g of rolled oat in 600ml of water and then heat to 45 to 550C, add 15 to 20g agar dissolved in 400ml of water. Autoclaved it for 30 mints.
Malt extracts peptone-dextrose agar:
Malt extract = 20g; Dextrose = 20g; Peptone = 1g; Agar- agar = 20g; Distilled water = 1L.
Potato dextrose agar:
Peeled and sliced potatoes = 200g; Dextrose = 20 g; Agar-agar = 20g; Distilled water = 1L.
Potato sucrose agar:
Peeled and sliced potatoes = 200g; Sucrose = 20 g; Agar-agar = 20g; Distilled water = 1L.
Richard's agar:
Potassium nitrate (KNO3) = 10g; Potassium dihydrogen phosphate (KH2PO4) = 5g; Magnesium Sulphate (MgSO4) = 2.5g; Sucrose = 30g; Potassium chloride (KCl) = 0.5g; Agar-agar = 20g; Distilled water = 1L.
Yeast dextrose agar:
Yeast extract = 7.5g; Dextrose = 20g; Agar-agar = 15g; Distilled water = 1L.

Radial Growth Measurements-
(On solid Media)
Assessment of radial growth of *Sclerotium rolfsii* on solid was studied. All selected fifteen media for studies poured into each sterilized petriplate, at about 15-18 ml. Three replication of each medium were maintained. In three petriplate for each tested medium, inoculated by placing a 4 mm diameter potato dextrose agar disc taken from margin of the

freshly grown colony of Sclerotium rolfsii, with the help of sterilized cork borer. All the inoculated petriplates were incubated at 280C temperature, radial growth and morphogenic effect of the pathogen was measured after every 12 hours till 72 hours.

RESULTS

The morphgenic studies of Sclerotium rolfsii growth in diameter on selected fifteen media's were presented in Table 1 can look at in Fig.1 and studies detailed in Graph1.

Table 1: Morphogenic effect of different media on growth in diameter of *Sclerotium rolfsii.*

Sr.No.	Media	Diameter	of	Colony	In	mm	
		12 Hours	24 hours	38 Hours	48 hours	60 hours	72 hours
1	Asthane & Hawker's Agar	1.5	3	4.8	9.6	15	30
2	Carrot Agar	3.6	7.3	12	24	34	42
3	Conn's Agar	2.1	4.3	11	21	22	43
4	Cook Vegetable Agar	1.1	2.3	12	24	33	43
5	Czapek dox Agar	0	0	0	0	0	0
6	Garlic Agar	2	4	9.6	19	24	49
7	Glucose dox Agar	0	0	0	0	0	0
8	Glucose peptone Agar	0	0	1.1	2.3	2.8	5.6
9	Leonian Agar	1.8	3.6	8.8	17	20	40
10	Malt extract peptone dextrose Agar	4.5	9	15	29	44	59
11	Oat meal Agar	1.1	2.3	5.5	11	16	31
12	Potato sucrose Agar	2.6	5.3	14	29	42	57
13	Potato dextrose Agar	2.3	4.6	14	29	36	43
14	Richard's Agar	1.6	3.3	4.8	9.6	11	23
15	Yeast dextrose Agar	2.1	4.3	7.3	14	20	40

*Values are Average of three replicates.
*After deducting the inoculums disc of 4 mm in diameter.

From the results recorded in Table.1, it showed that zero radial of S.rolfsii has been recorded on Czapek dox agar medium and Glucose dox medium after 72 hours of incubation. Whereas Glucose peptone agar medium also gave zero radial growth, but it is restricted to 24 hours only. Glucose peptone agar medium and Richard's agar medium has been found to be very poor supported of radial growth. Media's like Richard's agar, Asthane and Hawker's agar, Oat meal agar media, Leonian agar and Yeast dextrose agar showed better radial growth of S. rolfsii, in these Leonian agar and Yeast dextrose medium as were observed same radial growth of diameter after 72 hours of incubation.

Investigate those medium were proved to be best radial growth in diameter 72 hours of incubation were Carrot agar, Conn's agar, Cooked vegetables agar, Potato dextrose agar and Garlic agar medium.
The best with significantly superior radial growth has been found in Potato sucrose agar media and Malt extract peptone dextrose agar medium i.e. with 2mm dereferences only.

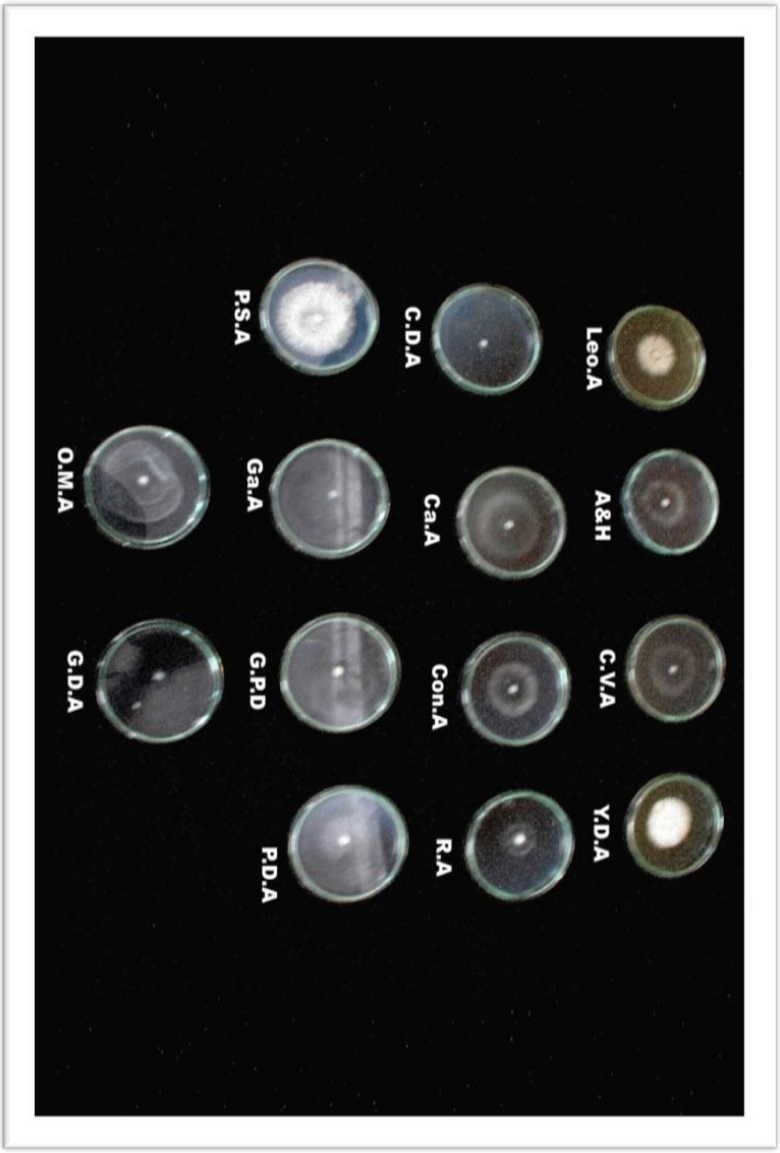


Fig 1.1- Screening radial growth of *Sclerotium rolfsii* in fifteen different media at after uninterruptedly 12 hours.

*In Fig 1-Leo.A.M.-Leonian agar medium, A & H.A.M.-Asthane & Hawker's agar medium, C.V.A.M.- Cook vegetable agar medium, Y.D.A.M.-Yeast dextrose agar medium, C.D.A.M.-Czapek dox agar medium, Ca.A.M.-Carrot agar medium, Conn's.A.M.-Conn's agar medium, R'S.A.M.- Richard,s agar medium, P.S.A.M.-Potato sucrose agar medium Ga.A.M.-Garlic agar medium, Gl.P.D.A.M.- Glucose peptone dextrose agar medium, P.D.A.M.-Potato dextrose agar. medium, Om.A.M.-Oat meal agar medium, G.D.A.M.-Glucose dox agar medium,

And in Fig 2 - Me.P.D.A.M.-Malt extract peptone dextrose agar medium.

*In Fig 1& 2- word M i.e. medium is hiding for short space, the medium name end with agar.

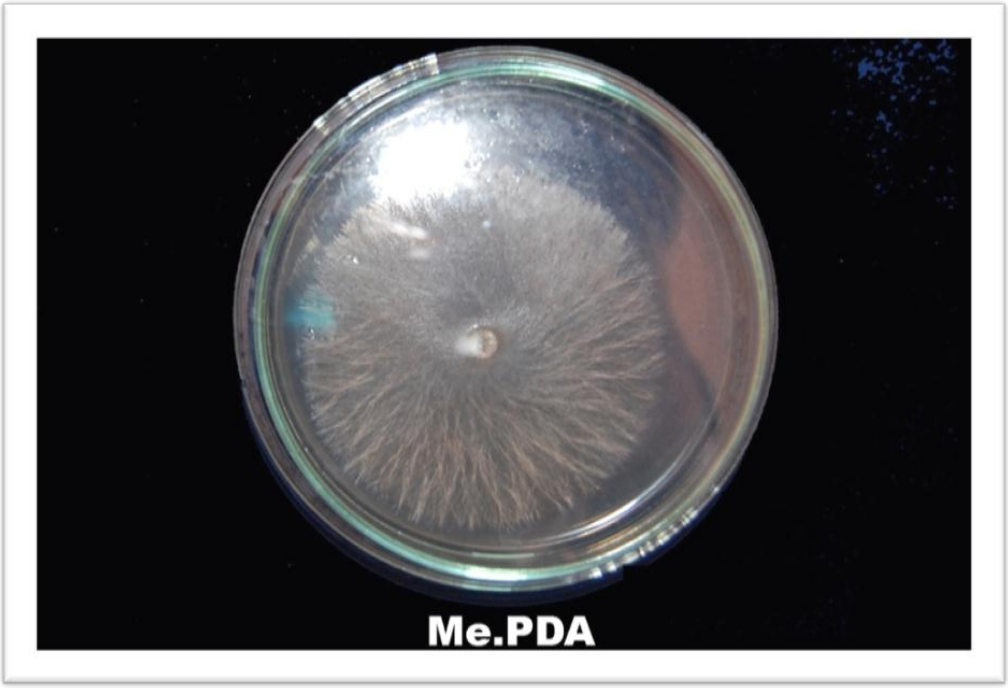
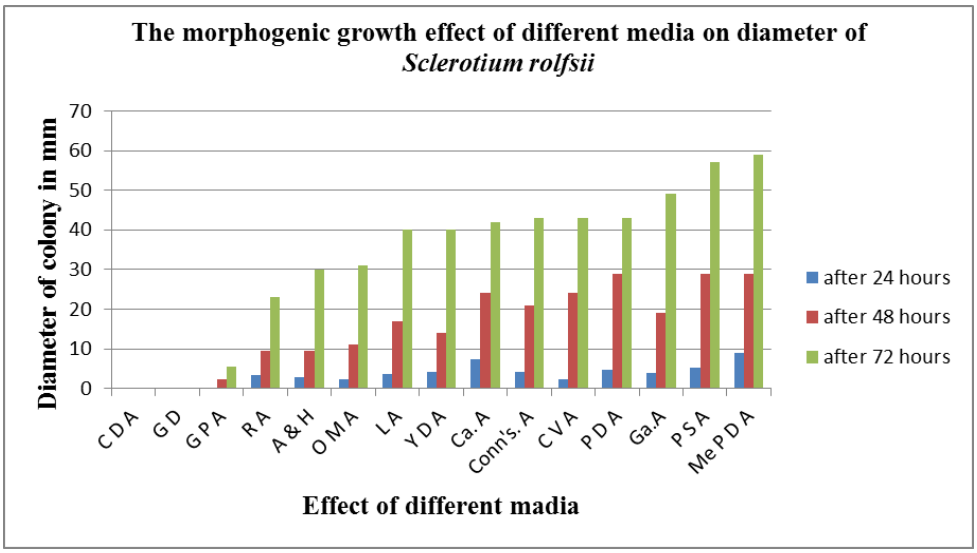


Fig 1.2 - Screening radial growth of *Sclerotium rolfsii* in fifteenth (15th) Me.P.D.A (Malt extract peptone dextrose agar) media at after uninterruptedly 12 hours.



Graph1. Graphically illustrate the morphogenic effect of fifteenth different media on diameter of *Sclerotium rolfsii* in following graph below.

*In graph C.A.M.-Carrot agar medium, C.V.A.M.- Cook vegetable agar medium, G.A.M.-Garlic agar medium, Om.A.M.-Oat meal agar medium, L.A.M.-Leonian agar medium, G.D.A.M.-Glucose dox agar medium, Conn's.A.M.-Conn's agar medium, A&H.A.M.-Asthane & Hawker's agar medium, C.D.A.M.-Czapek dox agar medium, Y.D.A.M.-Yeast dextrose agar medium, P.D.A.M.-Potato dextrose agar. medium, Gl.P.D.A.M.- Glucose peptone dextrose agar medium, R'S.A.M.- Richard,s agar medium, P.S.A.M.-Potato sucrose agar medium and Me.P.D.A.M.-Malt extract peptone dextrose agar medium.

*In graph word M i.e. medium is hiding for short space, the medium name end with agar.

DISCUSSION

On Radial Growth

In the present investigations, morphogenic effect of different fifteen media on diameter of *Sclerotium rolfsii* growth were studied from results Table1, screened in photographically in fig 1.1, 1.2 and graphically in Graph 1, it's clear that Glucose dox agar media behave not suitable for the growth of *Sclerotium rolfsii*, this complete inhibition in growth may be due to presence of nitrogen sources, complex organic sources and other Carbon sources.

It were observed that growth varies with different sources of Carbon like potato, starch, dextrose, sucrose, glucose and maltose were tested on the growth of *Slcerotium rolfsii* . Chaurasia.et.al.2013 also reported similar opinion.
These results are in agree with investigator's, Chet.et.al.(1966), Chet and Henis (1968), Bozarth and Tweedy (1971), Melhuish and Bean (1971), Okon, et.al (1972). Ercegovich et.al. (1973), Le Tourneall (1976, 1978), Igwegbe et.al. (1977), Fellmen and Tourneall (1983) who founded that the *S.rolfsii* in culture is inhibited by low concentration of some unrelated chemicals of which reduced mycelial growth. Our studied medium for the most suitable for the better growth of *Slcerotium rolfsii* was Malt extract peptone dextrose media then Potato sucrose agar media.

According to Zape.A.S.et.al. (2013) most suitable medium for better growth of *S.rolfsii* was Potato dextrose agar medium, Malt extract dextrose media and potato sucrose agar. These view of Zape et.al. (2013) are in match with Pandey (1984), Akram et.al. (2007), Rajalakshmi et. al. (2006) and Nene and Sheila (1995). Who have the same opinion that potato dextrose agar was best for *S. rolfsii* growth. The mycelial growths of *Slcerotium rolfsii* were tested as nitrogen source including both organic and inorganic. The relative differences were found to vary with peptone yeast extract as effect of complex organic sources. The inorganic sources show significant in results. Our investigation demonstrates Malt extract peptone dextrose medium gives best medium for growth of *Sclerotium rolfsii*.

CONCLUSION

The result of the effect of fifteen different culture media on the radial growth in *Scleorium rolfsii* is presented in Table 1. It is evident from the data that the radial mycelial growth of *Sclerotium rolfsii* was greatly influenced by the nutrient of different culture media. Although earlier works proved that potato dextrose agar medium found to best, but in our result analysis showed Malt extract dextrose agar, media are most suitable for better growth of *Sclerotium rolfsii* as compared to potato dextrose agar medium. This may be due to Malt extract peptone dextrose agar media contain peptone like complex organic sources, with one Carbon sources dextrose consume for nutrition. On the other hand Potato dextrose agar media carried only one Carbon sources as compared to Malt extract peptone dextrose agar medium. These finding in our studies concluded that in *Sclerotium rolfsii* maximum radial growth found in Malt extract peptone dextrose agar media, then second ranked goes to Potato sucrose agar media.

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