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A COMPARATIVE STUDY ON FUNGAL ACTIVITIES USING DIFFERENT SOURCES OF MEDIA

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ABSTRACT

Five fungi isolated from vegetable crop plants was grown on four different culture media viz. Potato Dextrose Agar (PDA), Sabouraud Agar (SABA), corn meal Agar (CMA) and Nutrient Agar (NA) and the result were recorded after seven days of incubation at 25 ± 1^{0}C. The colony diameter, colony character (Texture, Surface and reverse coloration, form and elevation) and Sporulation of selected test fungi were greatly affected by the media. Potato Dextrose Agar was found to be most suitable for growth of test fungi followed by CMA. Whereas Nutrient Agar was found with poor growth. All five isolates showed heavy Sporulation on Corn Meal Agar medium. These results are useful for morphological and physiological studies of fungal pathogens.

KEYWORDS : Vegetable Crop Plants, Growth Colony Characters, Sporulation

The fungal vegetable diseases are common diseases and causes major production problems in all parts of the world, Including India. All vegetable crops are suitable to causes diseases, reducing the size or a number of fruits or shortened harves period. Fungi grow on diverse habitats in nature & are cosmopolitan in distribution requiring several specific elements for growth & reproduction A wide range of media are used isolation of different groups of fungi that influence the vegetable growth & colony, morphology, pigmentation & surrounding atmospheric gas mixture (Pradeep et al., 2013; Maheshwari et al., 1999. However, the requirements for fungal growth are generally less stringent than for the sporulation.

The present study was undertaken to observe the influence of four different culture media on the mycelia growth of 5 fungi isolated from vegetable crop plants.

MATERIALS AND METHODS

A. Collection

Diseased plant parts of infected vegetable crop plants were collected from different fields of Marathwada region (Nanded, Aurangabad & Jalna).

B. Isolation of Pathogens

The pathogens were isolated from four vegetable crop plants that are Bottle gourd (*Logenaria Siceraria*), Bitter Gourd (*Momordica Charanitia*), Brinajl (*Solanum Melongene*) Tomato (*Lycopersion esulantum*) were selected as test fungi.

From the above vegetable crops the pathogens were isolated *Alternaria cucumeria*, *Fusarium oxysporum*, *Helminthosporium halodes*, *Rhizoctonia spp*, *Alternaria solani*, Isolation was done by tissue segment method. The infected host tissue from the advancing margin of the spot was selected, cut into small pieces of about 2-5 mm & transfer to sterile petriplates. The inflected pieces were surface sterilized with 0.1 mercuric chloride solution for about 1/2 minute. Immediately after the tissue sections were transfer to petridish containing PDA growth medium. The petridishes were incubated at room temperature (22-27^{0}C) & were examined daily for the growth of organism & were identified.

1. Preparation of Media

i. Potao Dextrose Agar (PDA)

Peeled potato- 200gm, Dextrose - 20gm, Agar-15gm, Distilled water - 1000 ml, ptt- 5.6.

ii. Sabouraud Agar Medium (SABA)

Dextrose - 40 gm, Peptone - 10 gm, Agar- 20 gm, Distilled water - 1000ml, pH-5.6.

iii. Corn meal Agar (CMA)

Corn-200gm, Dextrose - 20gm, Agar-20gm, Distilled water-1000ml, pH-5.6

iv. Nutrient Agar (NA)

Peptone - 10gm, Beef extract-3gm/ Yeast extract-3gm, Agar-15gm, NaCl- 5gm , Distilled water, 1000ml, pH. 5.6.

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2. Sterilization of Media

Respective media were sterilized at 15 lbs for 20 minutes under moist heat at autoclave. Media were poured in 500ml conical flask before autoclaving all the glass wares were thoroughly cleaned with dichromate acid solution & washed with sterilized distilled water & dried in oven at 15^0C for 1 ½ hour.

3. Purification of Culture

All the 5 pathogens which were isolated from diseased vegetable crop plants were purified by the single / hyphal tip method. From the fungal colonies that appeared on petriplates were examined under microscope. Then hyphal tip from identified colony were transferred to PDA slants after purification & used for further studies.

4. Inoculation on Different 4 Media

Seven days old pure cultures of *Allternaria cuccumeria, Fusariam oxysporum, Helminthosporium halodes, Rhizoctonia spp*, *Allternaria solani*, were transferred on 4 media for further studies . Test media are Potato Dextrose Agar (PDA), Sabouraud Agar medium. (SABA), Nutrient Agar medium (NA) & Corn Meal Agar (CMA), The pH of test media was maintained at 5.6 being optimal for the growth & sporulation in a majority of fungi. The petridishes were incubated for 7 days at 25 ± 1^0C in BOD incubator and colony characters of each fungus were recorded (figure 1 to 5). Sporulation was assessed on glass slides by mounting a small portion of mycelia in lactophenol-cotton blue stain and observed under microscope.

RESULTS

All four culture media supported the growth of test fungi to various degrees. *Altecrnaria cucumeria* Showed maximum mycelia growth on Potato Dextrose Agar medium (8. 0 cm) and Cornmeal Agar medium (7. 4 cm) and very poor growth on Sabouraud Agar medium (6.0cm). Fusarium oxysporum showed maximum and rapid growth on PDA medium (9.0 cm) and CMA (9-0 cm) and colony

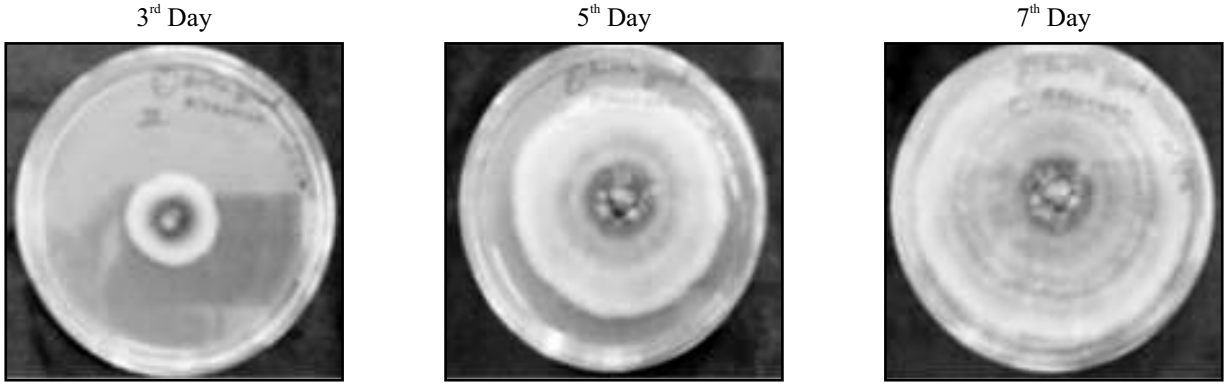

Figure 1 : *A. Cucumeria*

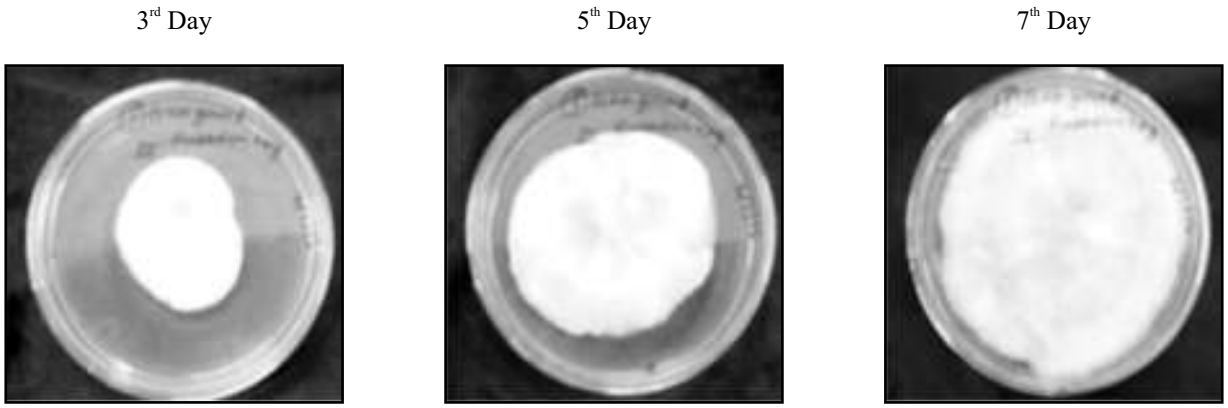

Figure 2 : *F. oxysporium*

Table 1 : Mycelial Growth , Colony Characters & Sporulation Pattern of Fungal Isolates on Four Culture Media After 7 days

Pathogens	Media Type	Colony diameters on 7th day in (cm)	Surface colony colour	Reverse colony colour	Texture	Spourlation	From	Elevation
1. *Lognaria siceraia Alternaria Cucumeria*	PDA	8.0	grey green	Greyish with creamish sporulation	Velvety	Heavy	Circular	Flat
	SABA	6.1	grey	dark green	Velvety Thick	Moderate	Irregular	Flat
	CMA	7.4	Creamish grey	grayish in centre with creamish surrounding	Fluccose	Heavy	Circular	Raised
	NA	6.0	Gloden brown with grey spores	Dark golden yellow	fine	poor	Circular	Flat
2.*Momordica charanita Fusarium Oxysporum*	PDA	9.0	Magenta Pink	White with orange rings	Fluccose	Heavy	Filamentous	Flat
	SABA	8.0	Cottony white	orange	Fluccose	Moderate	Filamentous	Flat
	CMA	9.0	Hyaline	colorless	light Fluccose	Heavy	Filamentous	Flat
	NA	6.7	Pinkish white	creamish orange	light velvety	Poor	Rhizoid	Flat
3. *Solamum melongene Heleminthosporiam Halodes*	PDA	9.0	Green	Dark Green	light velvety	Heavy	irregular	Flat
	SABA	6.8	Green	Dark Green	light velvety	Poor	irregular	Flat
	CMA	7.0	Green	Dark Green	velvety thick	Heavy	irregular	Flat
	NA	5.0	Green	Dark Green	light velvety	Poor	irregular	Flat
4. *Solanum melogene Rhizoctonia* spp	PDA	9.0	Cottony white	Colourless	light cottony	Heavy	Rhizoid	Flat
	SABA	7.0	Cottony white with grey sporulation	Colourless	light cottony	Heavy	Circular	Flat
	CMA	8.8	white Cottony grayish in center	Creamish	light cottony	Heavy	Circular	Flat
	NA	6.2	white Cottony greyish in center	Creamish	light cottony	Moderate	Circular	Flat
5.Lycopersicon esculatum *Alternaria solani*	PDA	7.4	Creamish grey	Brownish	Velvety thick	Heavy	Circular	Flat
	SABA	6.1	Greyish green	Dark green	Powdery	Heavy	Circular	Flat
	CMA	7.2	Greyish green	Grey with creamish sporulation	Velvety thick	Heavy	circular	Raised
	NA	5.7	Brick red with grey zonation	Brick red	Fine	Moderate	Circular	Flat

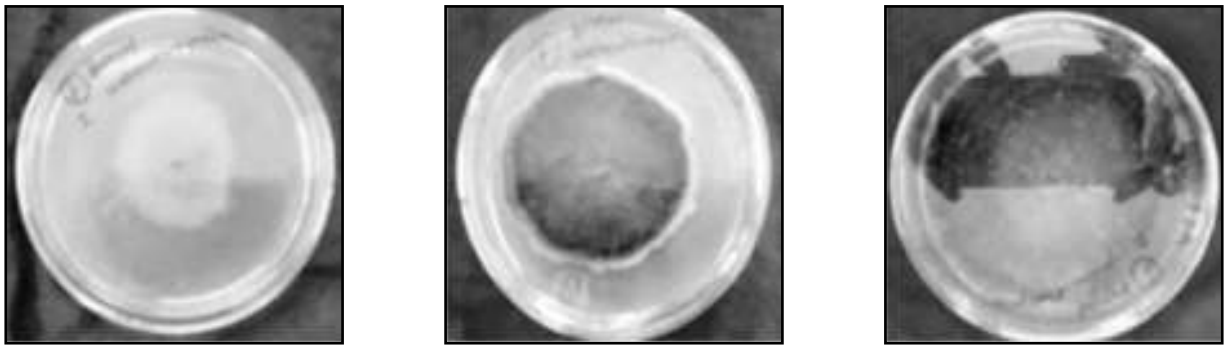

Figure 3 : *H. halodes*

3rd Day | 5th Day | 7th Day

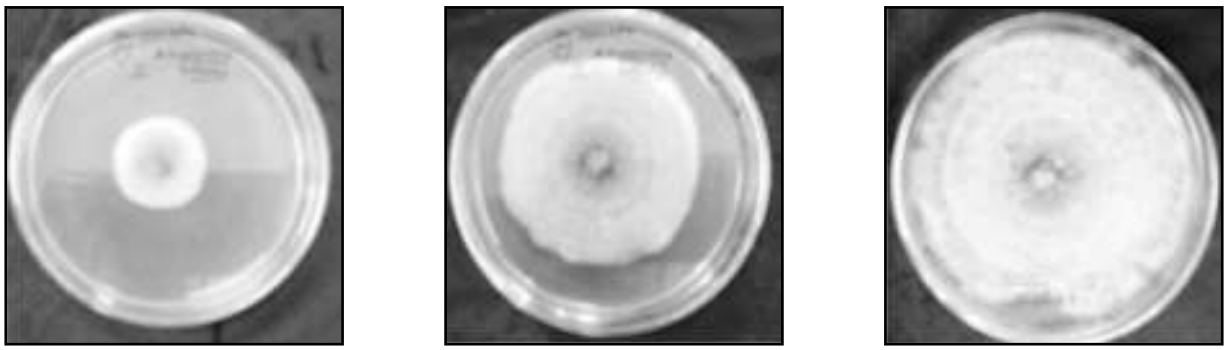

Figure 4 : *A. Solani*

Figure 5 : *Rizoctonia* spp.

was completed on 5th day and slow growth on NA (6.7 cm). Helminthosprium halodes showed maximum growth on PDA (9.0cm) and on CMA (7.0) and slow growth on NA (5.0cm). *Rhizoctonia* spp. showed maximum growth on PDA (9.0cm) and slower on NA (6.2 cm). *Alternaria solani* showed rapid growth on PDA & CMA (74. & 7.2) & slower on NA (5.7 cm) (table 1)

Media were also effected on colony colours of fungal pathogens. Fusarium oxysporum showed more colour variations (showed Magenta pink on PDA, Cottony white on SABA, Hyaline on CMA and Pinkish white on NA) (figure 6). followed by *Alternaria cucumeria* (showed grey green on PDA, creamish gray on CMA, Golden brown on NA) . Whereas less color variation shwoed by Alternaia solani and *Rhizoctonia* spp.No colour variations showed by *Helminthosporium haldoes*. (figure 9)

There for the result of all the pathogens fungi recorded on Nutrient Agar Medium had great variation in colour changes while the colony showed velvety, cottony and floccose appearance on CMA.

Figure 6 : Colour Variation on *F. oxysporium*

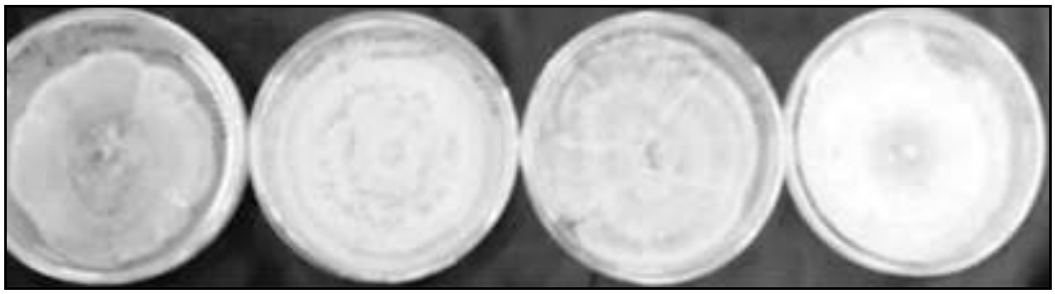

Figure 7 : *A. solani* on All 4 Media

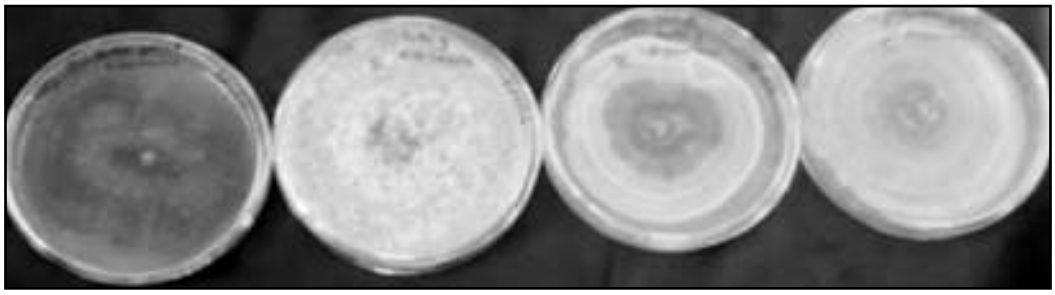

Figure 8 : *A. cucumeria* on All Media

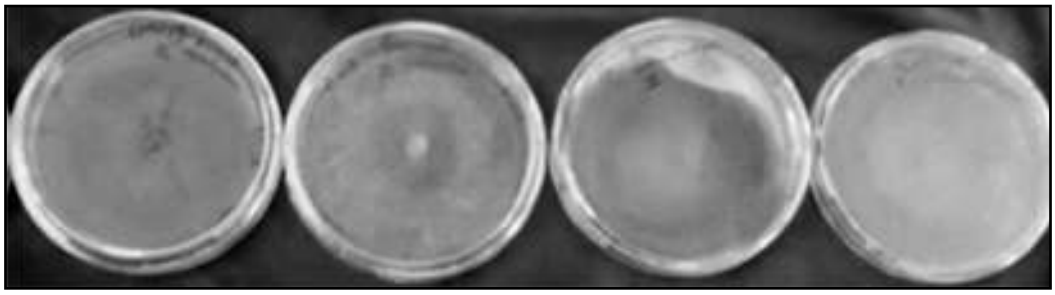

Figure 9 : No Colour Changeon *H. halodes*

All test fungi showed more colour variation on NA so colony colours of fungi ware most influenced by NA. All five test fungi were observed with more velvety, cottony and floccose texture on CMA medium. All test fungi recorded almost same texture and same color on PDA and SABA (Figure 7 and 8) Heavy sporutation was seen on PDA and CAM media where as poor sporulation was seen on NA medium. (table1)

DISCUSSION

Growth and sportulation were high in the present study. Several workers have recognized the importance of

reproductive structures for inoculums production. The present study have been conducted on the effects of various media components along with important physiological parameters that lead to maximum sporulation. This result coincide with the reports of Sharma and Pandey (2010), Gupta et al., (2012). Type of culture media and their chemical compositions significantly affected the mycelia growth rate & sporulation.

PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of Fungi. PDA was found to be the best medium for culturing. Reports of Rabhani et al., 2011 were supported to the result. The colour, growth, texture and sporulation in fungal colonies were found to be influenced by the culture media used. The results are more relevant to observations recorded by Sharma and Pandey (2010).

PDA showed excellent average colony diameter which was observed and the same result were recorded by Puyam Anita et al., 2013, Soltani et al., (2014), Islam (2015).

CONCLUSION

Our findings revealed that culture media differentially effect on growth, colony characters & sporulation of the test fungi.

1. Out of four media Potato Dextrose Agar was found to be most suitable for growth of test fungi followed by Corn Meal Agar and poor growth was observed on Nutrient Agar media.
2. Corn Meal Agar was found to be suitable for sporulation of test fungi.
3. Most colour variations was showed by NA medium where as rest of three media were showed less colour variations.
4. Most velvety texture was observed by Corn Meal Agar medium.
5. It is concluded that Potato Dextrose Agar and Corn Meal Agar media are more appropriate for routine cultural and different colony characters studies and these result are useful for morphological and physiological studies of fungal pathogens.

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