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"In *vitro* Effect of different media on radial growth and Morphological characters of *Alternaria solani, Fusarium oxysporum* and *Rhizoctonia solani* on seven different agar media on 7th days of inoculation period"

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Abstract:

A series of laboratory experiments were conducted to determine the best culture media, as well as the appropriate environmental conditions to stimulate radial growth of *Alternaria solani*, *Fusarium oxysporum* and *Rhizoctonia solani* on 7th day of inoculation period fungus to produce conidia in the shortest period of time under laboratory conditions. Seven different culture media were used i.e. Malt extract agar Oat meal agar Czapek's agar Richard's Agar PDA (Potato Dextrose Agar) Mix vegetable leaf extract agar: The results were showed that PDA (Potato Dextrose Agar media is considered one of the best culture media in terms of the radial growth rate for *Alternaria solani, Fusarium oxysporum* and *Rhizoctonia solani* on colony, as the highest rate of colony diameter were (85),(88) (87), Whereas least radial growth colony diameter on Richard's agar (41), (46), (62) respectively. Morphological characters of *Alternaria solani, Fusarium oxysporum* and *Rhizoctonia solani* on different agar media on 7th days of inoculation period were observed, highly significant differences in colony type colony color, size, sporulation etc.

Keywords. *Alternaria solani* Tomato, Early blight, wilt disease *Fusarium oxysporum* and root rot *Rhizoctonia solani seedling* diseases of vegetables Growth behavior on Culture Media.

Keyword: *- In vitro seven media morphological character, Alternaria solani, Fusarium oxysporum* and *Rhizoctonia solani inoculation period*

Introduction:

In the world-wide different vegetables in the tomato, brinjal, radish, radish, chili, cauliflower etc. is one of the most important vegetable crops, as it is the major vegetable crops after the potato in terms of economic importance in the global.

Alternaria solani Tomato, Early blight, wilt disease *Fusarium oxysporum* and root rot *Rhizoctonia solani seedling* diseases of different vegetables, is one of the most important diseases that cause great losses in this crop a threat to the profitable cultivation of vegetable the disease causes reduction in quantity and quality of vegetable seed at germination drastically. Symptoms of the disease are characterized by brown to dark brown colored necrotic spots, wilt, and burn of seedling root rot of seedling [1]. Under humid condition in soil, these spots 1 progressed upwards and coalesced to produce the concentric zone on the plumule appearing like bull's eye [2]. Lesions on the radical observed at the stem-end which is dark,

leathery, and sunken with target board like appearance, dark brown color root rot of radical. Severe infections of the early blight root rot wilt fungi lead to defoliation, drying off of plumule and premature of seedling plant 45 to 85% losses in pant [3] Laboratory studies are among the important pillars in identifying the nature of plant diseases and choosing the most appropriate methods to control them. These fungi require several specific compounds for their growth, although the fungus is cosmopolitan in nature. In in vitro study, fungi is isolated as pure culture in specific media for studies on growth, nutrition, physiology and management of the fungus. A wide range of media can favor the isolation of the selected fungi which supports the radial growth, dry weight growth and sporulation of the fungi however the nutrient requirements for good radial growth of the fungi do not confirm the nutrient requirements for good sporulation. Various media compositions also influence the different colony morphology, physiology of fungi. The Morphological characterization is the classical approaches to several fungal species that is one of the main requisite of fungi taxonomy. In plants, carbohydrates are available in simple as well as in complex form and fungi convert the complex forms into simple water-soluble sugars of low molecular weight before utilization. It has been shown that different fungi respond differently with a particular compound and the fungi exhibit marked variation in the utilization of different carbohydrate sources. A critical and comprehensive knowledge of nutritional patterns and factors influencing the growth of fungi is a prerequisite for any study leading to the understanding of host-pathogen relationship. Not much attention has been given on the culture and growth media parameters of the pathogen. Hence, thorough knowledge on the influence of various culture media on growth of the fungi as well as sporulation and colony characteristics of the fungi isolated from early blight infected seedling tomato, wilt disease, seedling root of brinjal is needed to be developed for suitable management strategies of the disease and may help in taxonomical and physiological study of the fungus.

Material and Methods:

Effect of different media on radial growth of *Alternaria solani, Fusarium oxysporum* and *Rhizoctonia solani* on 7$^{th\ day}$ of inoculation.

To find out the effect of different media on the radial growth of selected pathogenic fungi such as, *Alternaria solani*, *Fusarium oxysporum* and *Rhizoctonia solani* seven different types of culture media were selected the composition of non-synthetic solid agar media, the composition of each media were as follows. (Table No. 17).

Malt extract agar:

Sucrose	20 gm
Malt extracts	25 gm
Agar	20 gm
Distilled water	1000 ml

Oat meal agar:

Sucrose	20 gm
Oat	100 gm
Agar	20 gm
Distilled water	1000 ml

Czapek's agar:

Sucrose	30 gm
Sodium nitrate	2 gm
Dipotassium phosphate	1 gm

Magnesium sulfate	0.5 gm
Potassium chloride	0.5 gm
Ferrous sulfate	0.01 gm
Agar-agar	20 gm
Distilled water	1000 ml
Richard's Agar:	
Sucrose	30 gm
Potassium nitrate	10 gm
Dipotassium phosphate	5 gm
Magnesium sulfate	2.5 gm
Ferric chloride	0.02 gm
Agar-agar	2 0 gm
Distilled water	1000 ml
PDA (Potato Dextrose Agar)	
Potato	200 gm
Agar	20 gm
Dextrose	20 gm
Distilled water	1000 ml
Nutrient agar:	
Peptone	5gm
Beef extract	0.5 gm
Yeast extract	1.5 gm
Sodium chloride	5 gm
Agar-agar	20 gm
Distilled water	1000 ml
Mix vegetable leaf extract agar:	
Mix vegetable leaf extract	100gm
Glucose	1 gm
Di potassium phosphate	0.5 gm
Distilled water	1000 ml

Different type of media were prepared by taking respective ingredients as noted above, media of each type plugged with non-absorbent cotton and sterilized in autoclave at 15 lb. pressure (120^0C) for 20 minutes, sterile media of each type taken out from autoclave and allowed to cool for some extent and added in sterile Petri plates in sterile condition three Petri plates were poured with each type of medium and inoculated with 4 mm disc of vigorously growing *Alternaria solani* disc was removed with the help of sterile borer in sterile condition then disc was inoculated at center of media amended Petri plate, three Petri plates were inoculated for each type of fungus and medium. Similar procedure was used for *Fusarium oxysporum* and *Rhizoctonia solani* effect of different media by Somnath koley and Shyama Sundar Mahapatra (2015) Evolution of cultural media for growth characteristics of *Alternaria Solani* causing early blight of tomato. Balkishan Choudhry and (Sanjeev *et al.*, 2018) Effect of different media on growth and sporulation of *Fusarium u.* causing wilt of pigeon pea.

Experimental Result

Effect of different media on radial growth of selected plant pathogenic fungi on 7th day of inoculation period.

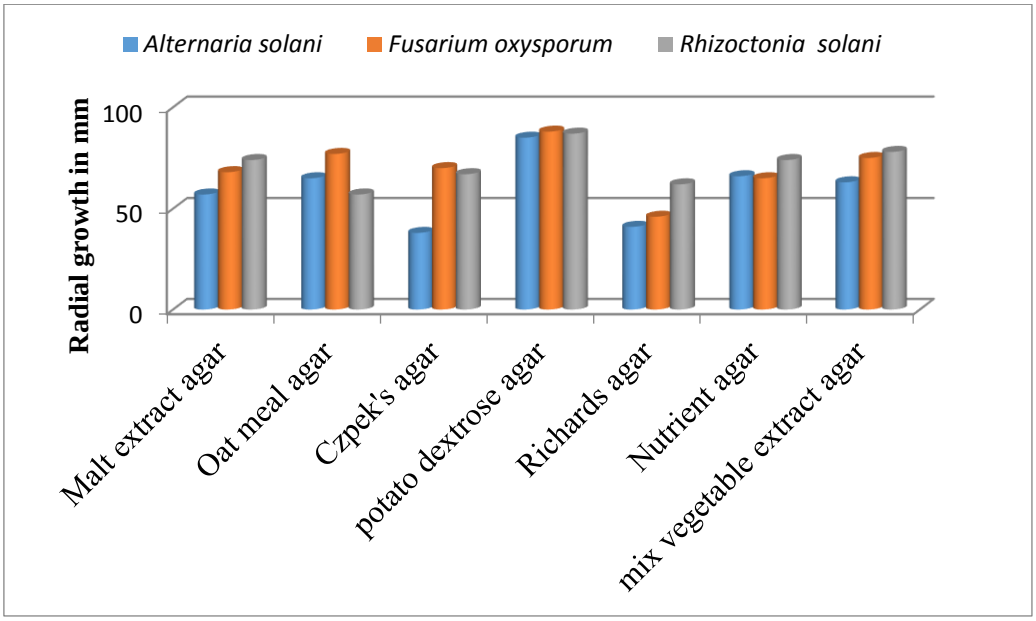
Sr. No.	Media	Radial growth in 7th day mm		
		Alternaria solani	***Fusarium oxysporum***	***Rhizoctonia solani***
1	Malt extract agar	57	68	74
2	Oat meal agar	65	77	57
3	Czapek dox agar	38	70	67
4	Potato Dextrose agar	85	88	87
5	Richard's agar	41	46	62
6	Nutrient agar	66	65	74
7	Mix vegetable extract agar	63	75	78
	S. D.	16.05	12.93	10.13
	S. Em ±	6.07	4.89	3.83

Values: Average of three replicates.
*The result is significant at $p < 0.01$.
* The result is significant at $p < 0.05$.

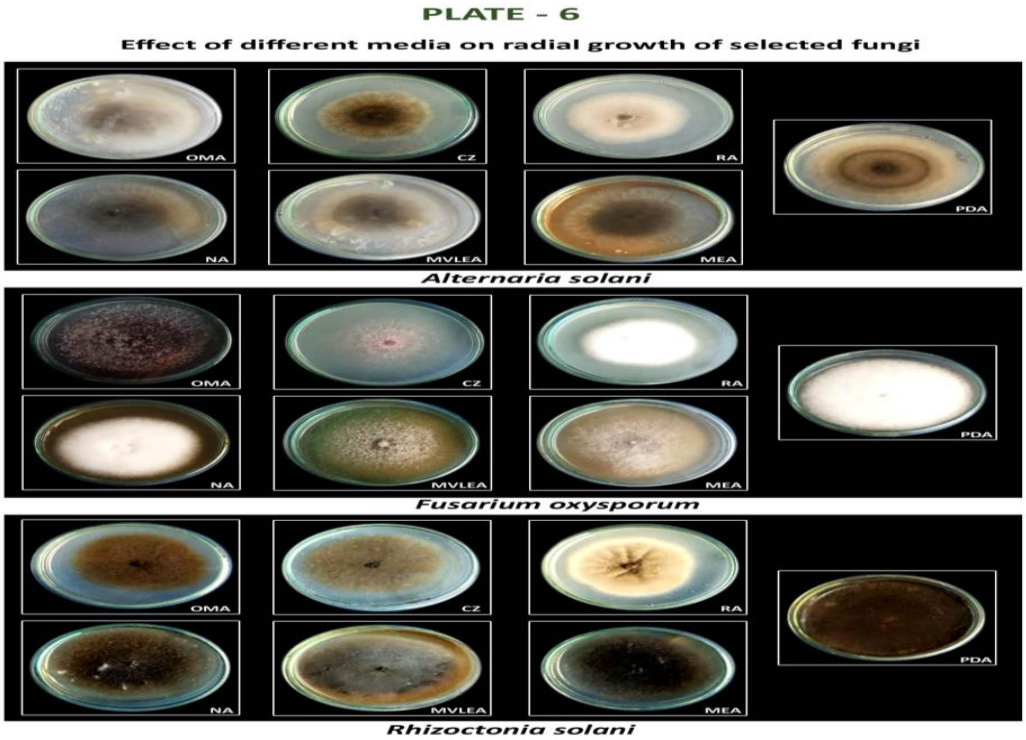
In order to studied that radial mycelial growth of *Alternaria solani*, *Fusarium oxysporum* and *Rhizoctonia solani*, on 7th day of inoculation period, on seven different types of solid cultural media (Malt extract agar, at meal agar, Czapek dox agar, Potato dextrose agar, Richard's agar, nutrient agar and mix vegetable extract agar). The data represented in Table No. 17, Photo plate 6 and **graph** 22. Result revealed that PDA (Potato dextrose agar) medium was highly significant the maximum radial mycelial growth of *Alternaria solani* that was 85 mm, *Fusarium oxysporum* 88 mm and *Rhizoctonia solani* 87 mm. Followed by oat meal agar in which *Fusarium oxysporum* (77 mm), in *Alternaria solani* (65 mm) and *Rhizoctonia solani* showed least growth (57 mm). followed by Mix vegetable extract agar and nutrient agar, which showed second largest radial growth on agar medium that is *Alternaria solani* (63 mm, 66 mm) *Fusarium oxysporum* was 75 mm, 65 mm. And Rhizoctonia *solani*, (78 mm, 74 mm) respectively, the growth of fungi on malt extract agar was *Rhizoctonia solani* (74 mm) *Fusarium oxysporum* (68 mm) and *Alternaria solani* (57 mm) respectively, Czapek dox agar medium was supported lowest mycelial growth of fungi such as, *Alternaria solani* 38 mm, *Fusarium oxysporum* 70 mm and *Rhizoctonia* solani 67 mm. and also Richard's agar medium was supported lowest mycelial radial growth of fungi viz. *Alternaria solani* 41 mm, *Fusarium oxysporum* 46 mm and *Rhizoctonia solani* 62 mm.

Culture medium is source of food (nutritional) for the promote growth, development and reproduction of fungi. Which is used to cultivate fungi in laboratories? Pathogenic fungi are nutrition specific in nature. Hence the response of fungus to different types of media in variable. Present study supported by **(**Somnath and Shyama Sundar 2015). Suggested PDA (Potato dextrose agar) medium supported the highest diameter growth of *Alternaria solani*. Followed by oat meal agar medium, Corn meal agar medium and Hansen's agar medium. (Balkishan and Sanjeev *et al.*, 2018). Reported that potato dextrose agar media (PDA) are

highly significant. And maximum growth and sporulation of *Fusarium u.* causing wilt of pigeon pea.



Graph No. 13: Effect of different media on radial growth of selected plant pathogenic fungi on 7th day of inoculation period.



Morphological characters of *Alternaria solani, Fusarium oxysporum* and *Rhizoctonia solani* on different agar media on 7th days of inoculation period.

Morphological characters of *Alternaria solani*:

Growth characters of *Alternaria solani* was studied on seven different agars medium on 7th days of inoculation period, on the basis of cultural agar media. were observed color of colony type, radial growth pattern of colony, beak, length, sporulation characters are studied

blackish, grey and thin colony was observed in malt extract and oat meal agar in case of and Czapek dox agar medium colony showed normal growth in middle part circular black ring like structure are formed and border line whitish colored but maximum supported growth of *Alternaria solani* on PDA compare to other medium in Richard's agar colonies are thick, whitish cottony, hairy like mycial structure was observed as on mix vegetable leaf extract agar colony was normal growth pattern yellowish green colored.

Morphological character of Fusarium *oxysporum*

Effect of different agar media on growth characters of *Fusarium oxysporum* on 7th days of inoculation period was observed, highly significant differences in colony type colony color, size, sporulation etc. In malt extract agar was observed 68mm radial growth but hyphae grown weekly color of yellow, in oat meal agar media colony was not cottony grow but fresh hyphae are appeared, the color of colony was off white, on Czapek dox agar medium colony are not well developed. It is thin, hyaline, sparced hyphae and normal pinkish pigments color was formed. The *Fusarium oxysporum* showed maximum growth on PDA medium compare to other medium. On PDA whitish colored colony and attractive growth was formed. Mature hyphae produce pink colored pigmentation appeared. In Richards agar medium colony was a whitish, cottony, irregular shaped and thick. Nutrient agar medium was colony growth of *Fusarium oxysporum* was normal, regular, white cottony. Whereas on mix vegetable leaf extract agar medium colonies were thin and yellowish green in colors.

This indicates that there is great variation in the form, and colony type due to change in the composition of medium the *Fusarium oxysporum* is nutrition specific, it requires a particular type of ingredients for well-established growth and development. the experiment was calculated in order to select a more favorable medium for the growth of *Fusarium oxysporum*, to perform further experiments during research work. The potato dextrose medium was found more favorable for the growth of *Fusarium oxysporum* as it showed 88 mm colony diameter on 7th day of inoculation period.

Morphological character of *Rhizoctonia solani*

The *Rhizoctonia solani* growth characters of seven different media on 7th days of inoculation period, was observed that on oat meal agar, nutrient agar and mix vegetable leaf extract agar colony is normal, dark brown and yellowish green formed, malt extract and PDA medium was highest biomass of fungi color blackish to brown, yellowish grey, normal regular and full growth of colony are formed, whereas Czapek dox agar medium are irregular colony, grayish brownish. Richard's agar medium was highly significance and difference the color colony white, oval, marginal are formed, and result noted there was no sporulation observed on *Rhizoctonia solani*.

Discussion

It was clear from the Table. Different media plays the important role in growth, Development and sporulation of *Alternaria solani*, *Fusarium oxysporum* and *Rhizoctonia solani*. Potato Dextrose agar (PDA) media shows ideal media for *Alternaria solani* (85 mm), *Fusarium oxysporum* (88 mm) and *Rhizoctonia solani* (87 mm). Present study supported by research worker Somnath koley and Shyama Sundar Mahapatra (2015), studied that Evolution of cultural media for growth characteristics of *Alternaria solani* on different solid media showed that PDA medium supported the highest diameter growth. (Balkishan et al., 2018) reported that potato dextrose agar media (PDA) are highly significant and maximum growth, sporulation of *Fusarium u.* causing wilt of pigeon pea.

Summary

The potato dextrose agar (PDA) medium was supported maximum radial growth of *Alternaria solani,* (85 mm), *Fusarium oxysporum,* (88 mm) and *Rhizoctonia solani* on (87 mm) fungi on 7th day of inoculation period, followed by oat meal agar on *Fusarium oxysporum* (77 mm), in *Alternaria solani* (65 mm), in *Rhizoctonia solani.* Lowest growth (57 mm), and other agar medium also supported growth of fungi. The morphological characters of *Alternaria solani, Fusarium oxysporum* and *Rhizoctonia solani* on seven different agar media various from each other.

Conclusion

It has a concluded that potato dextrose medium has found highly suitable for the growth of all three fungal genera selected for study.

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