Effect of different media on the growth, sporulation and chlamydospore formation of four spp. of Fusarium

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Nine culture media viz., Peptone Dextrose-Rose Bengal Agar Medium, Potato Dextrose Agar (PDA) Medium, Oatmeal Agar Medium, PCNB Agar Modified, Czapek dox Agar Medium, Asthana and Hawker's Medium, modified Asthana and Hawker's Medium 'A', Richard's Agar Medium and Glucose Asparagine medium were used to find out the most suitable one for the mycelial growth of the *Fusarium*. Out of them Rechard's medium supported best growth of the present species of *Fusarium*. It was followed by Czapek's medium in case of *F. racuminatum* and *F. solani* whereas PCNB Agar modified was second best in case of *F. racuminatum* and *F. solani* whereas PCNB Agar modified was second best in case of *F. racuminatum* and *F. solani* whereas PCNB Agar modified was second best in case of *F. racuminatum* sp. Sporulation was excellent on modified Asthana and Hawker's medium 'A', followed by Potato Dextrose-Agar and Richard's medium. Chlamydospore were also observed in a few cases and were produced in fair degree on Oat meal medium in each case. On Peptone Dextrose Rose Bengal medium only microspores were observed. The final pH of different media increased. However, a decrease in pH was recorded in case of Czepak's Dox-Agar medium and Oat meal media in every case.

Key words: Media, chlamydospore, sporulation, Fusarium sp., pH

INTRODUCTION

Fungi are dependent on the medium or the substrate for all the elements. Nutritional requirements of various fungi differ and there is no medium or substrate which can be universally suited to all the fungi. The choice of the media and environmental condition depend on the purpose of the investigation. The use of such substrate for the artificial culture of fungi continued till Pasture (1860). Roulin (1869), who has first devised a synthetic medium for the nutritional studies of some common fungi. Lilly and Barnett (1951) have found that all media are not equally suited for a particular fungus. Bilgrami (1956) working with Phyllosticta cycadina, P. artocarpina and Pestalotia mangiferae, has observed that the best growth of Phyllosticta cycadina on Richard's medium followed by Oatmeal medium, whereas P. artocarpina and Pestalotia mangiferae have showed best results on Richard's medium followed by Potato Dextrose Agar medium. The population of all of them is best on Asthana and Hawker's medium 'A' while

it is good on Potato Dextrose Agar and Oatmeal Agar media and is poor on Richard's medium.

The aim of this study has to identify an effective and cheaper medium for rapid growth of *Fusarium*.

MATERIALS AND METHODS

In the present investigation, cultural studies on F. oxysporum, F. acuminatum, F. solani and F. equiseti were studied under in vitro conditions. Following nine culture media were used to find out the most suitable one for the mycelial growth of the fungus. Each culture medium was prepared as per standard procedure in 1 liter of water and autoclaved at 120°C for 20 min. Media were inoculated with five mm disc. cut from the periphery of actively growing mycelial culture and incubated at 27±1°C. Each treatment was replicated thrice. Observations were taken when the fungus covered complete Petriplate in any one of the media. The mycelium was harvested by centrifugation for 20 min at 6000 rpm and dry mycelial weight was obtained by filtering the sample cultures through a pre-weighed filter paper (Whatman no.42) and drying in an oven for 24 hrs. at 50°C (Wang and

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Rakshit 1999). The average dry weight, degree of sporulation, chlamydospore formation and change in the pH were recorded.

solani where PCNB Agar modified was second best in case of *F. oxysporum* and *F. equiseti*. Asthana and Hawker's medium 'A' supported poor growth of all *Fusarium* spp.

RESULTS AND DISCUSSION

Results are presented in Tables 1, 2, 3 and 4. Rechard's medium supported best growth of the present species of *Fusarium*. It was followed by Czapek's medium in case of *F. acuminatum* and *F.*

Sporulation of the organisms was excellent on modified Asthana and Hawker's medium 'A', Potato Dextrose-Agar and Richard's medium. It was poor

Table. 1: Showing the average dry weight, sporulation change in pH and chlamydospore formation of F. acuminatum on different media

Media	Dry wt. in gm	Sporulation	Chlamydospor Formation	e pł Initial	Final	٠.
Asthana & Hawker's medium'A'	62.5	Excellent	Poor	5.2	6.5	
Modified Asthana & Hawker's medium'A'	74.8	Excellent	Poor	5.2	7.5	
Czapek Dox Agar medium	143.1	Good	-	7.0	6.4	
Glucose Asparagine medium	97.0	Poor	Poor	5.1	5.5	
Oatmeal Agar medium	128.9	Poor	Fair	6.2	5.5	
Peptone Dextrose Rose Bengal Agar medium	65.3	Poor	Poor	5.5	6.1	
PCNB Agar modified medium	99.7	Poor		5.4	7.4	
Potato Dextrose Agar medium	112.1	Good		6.1	6.1	
Rechard's medium	253.2	Excellent		6.4	6.2	

CD= 9.83at 5% level of significant

Table. 2: Showing the average dry weight, sporulation change in pH and chlamydospore formation of F. oxysporum on different media

Media	Dry wt. Spor	Sporulation	Chlamydospore	pH		
,	in gm		Formation	Initial	Final	
Asthana & Hawker's medium'A	43.7	Good		5.1	6.2	
Modified Asthana & Hawker's medium'A'	56.6	Excellent	Poor	5.0	6.8	
Czapek Dox Agar medium	132.6	Good		7.5	6.8	
Glucose Asparagine medium	57.0	Poor	Poor	5.0	5.2	
Oatmeal Agar medium	124.9	Poor	Fair	6.5	5.5	
Peptone Dextrose Rose Bengal Agar medium	57.7	Poor	Poor	5.5	6.0	
PCNB Agar modified medium	156.0	Fair	() em	5.5	8.8	
Potato Dextrose Agar medium	150.0	Excellent		6.2	6.5	
Rechard's medium	248.3	Excellent		5.0	6.5	3 3

Table. 3: Showing the average dry weight, sporulation change in pH and chlamydospore formation of F. solani on different media

Media	Dry wt. in gm	Sporulation	Chlamydospore Formation	рН	
				Initial	Final
Asthana & Hawker's medium'A'	47.1	Good	Poor	5.1	7.0
Modified Asthana & Hawker's medium'A'	60.5	Excellent	Poor	5.0	7.9
Czapek Dox Agar medium	160.1	Excellent	1 7977 14	7.2	6.8
Glucose Asparagine medium	85.2	Poor	Poor	5.5	6.2
Oatmeal Agar medium	119.8	Poor	Fair	6.5	6.0
Peptone Dextrose Rose Bengal Agar medium	65.2	Poor	Poor	6.0	6.5
PCNB Agar modified medium	130.0	Fair		6.0	8.5
Potato Dextrose Agar medium	142.3	Excellent		6.5	6.5
Rechard's medium	247.2	Excellent		5.0	6.5

CD= 2.5 at 5% level of significant

Table. 4: Showing the average dry weight, sporulation change in pH and chlamydospore formation of F. equseti on different media

*	Media	Dry wt.		Chlamydospore Formation	р	Н	
					Initial	Final	
	Asthana & Hawker's medium'A'	40.1	Good		5.5	6.5	
	Modified Asthana & Hawker's medium'A'	53.6	Excellent	Poor	5.1	6.7	
	Czapek Dox Agar medium	130.0	Poor	-	7.5	6.5	
	Glucose Asparagine medium	55.2	Poor		5.2	5.5	
	Oatmeal Agar medium	120.3	Poor		6.5	5.5	
	Peptone Dextrose Rose Bengal Agar medium	55.8	Poor	Poor	5.3	5.8	
G.	PCNB Agar modified medium	153.1	Fair	-	6.0	6.3	
	Potato Dextrose Agar medium	143.0	Excellent		6.5	6.0	
	Rechard's medium	245.5	Excellent		5.4	6.9	

CD= 3.03 at 5% level of significant

on Glucose Asparagine, Oat meal and Pepton-Dextrose Rose Bengal Agar were medium. Sporulation of *Fusarium* ranged from fair to good on other media, (chlamydospore were also observed in few cases and were produced in fair degree on Oat meal medium. In Peptone Dextrose Rose Bengal medium only microspores were observed. The final pH of different media increased. However, a decrease in pH was recorded in case of Czepak's Dox-Agar medium and Oat meal media in every case. Martin, (1950) found that counting of soil fungi were higher in the media containing rose Bengal; than other media. Wu *et al.*, (2009) observed that

Gallic acid when added in media, inhibited the growth of *F. oxysporum* f.sp.*niveum*. The colony diameter, the conidia germinating rate and the conidia yield were reduced by 5.7-22.9%, 35.8-55.6% and 38.9-62.2 per cent respectively. Chittem and Kulkarni (2008) reported that the maximum dry mycelial weight of *F. oxysporum* f. sp. *gerberae* on Oat meal broth (450 mg) which was significantly superior over all the media tested. This was followed by Richards's broth (340 mg) and Potato dextrose broth (310 mg). Sharma *et al.* (2005) found that *Fusarium oxysporum* f. sp. *lini* grew well on linseed and maize meal media. Nareah *et al.* (2009) reported that the Richard media

supported best growth of the pathogen Bipolaris sorokiniana. Kim and Xiao (2005) reported that Oatmeal agar (OMA) was most suitable for production of pycnidia and conidia of Sphaeropsis pyriputrescens, the causal agent of Sphaeropsis rot of pears and apples, but Cornmeal agar was not suitable for either mycelial growth or pycnidial production. Castella et al. (1997) found that Malachite Green Agar 2.5 ppm (MGA 2.5) is a potent selective medium for isolation and enumeration of Fusarium spp. Farooq et al., (2005) found that the Czapeck Dox agar and Chickpea Seed meal Extract Agar media were the best for the radial growth of F. oxysporum as this fungus gave maximum growth of 85 and 80 mm, respectively, followed by Cornmeal agar and Malt extract agar media which showed growth of 70 and 65 mm, respectively. Vladimir et al., (2002) observed that the myclobutanil-based medium showed the highest selectivity to growth of Fusarium spp. but required a slightly longer incubation time (>5 d) than peptonepentachloronitrobenzene-based agar (PPA) (< 5 d). PPA allowed a faster fusarial growth but also permitted the growth of other moulds.

Among the nine media used, modified Asthana and Hawker's medium 'A' was selected as basal medium for the present four species of *Fusarium* as it provided fair growth and excellent sporulation.

REFERENCES

Bilgrami, K.S. 1956, *Physiological and pathological studies of some fungi causing leaf spot diseases*. D.Phil. Thesis University of Allahabad pp73.

- Castella, G., Bragulat, M.R., Rubiales, M.V. and Cabanes, F. J. 1997, Malachite green agar, a new selective medium for Fusarium spp. Mycopathol. 137: 173-178.
- Chittem, Kishore and Kulkarni, Srikant 2008, Effect of Media on the Growth of Fusarium oxysporum f. sp. gerberae and Fusarium oxysporum f. sp dianthi Karnataka J. Agric. Sci., 21: 303-304.
- Farooq, Sajid, Iqbal, Sh. Muhammad and Rauf, Ch.Abdul 2005. Physiological Studies of Fusarium oxysporum F. Sp. Ciceri. Int. J. Agri. Biol. 7: 275-277.
- Kim, Y.K., and Xiao, C.L. 2005, Influence of culture media and environmental factors on mycelial growth and pycnidial production of Sphaeropsis pyriputrescens. Mycologia 97: 25-32.
- Lilly, V.G. and Barnett, H.L. 1951, Physiology of the fungi, Mc Graw Hill Book Co. Inc., New Yark, pp 464.
- Martin, James P. 1950, Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Science* 69: 215-232
- Naresh, Prem, Biswas S.K., Kumar, Upesh and Rajik. Mohd. 2009, Effect of Media, pH, Temperature, Host range and Fungicides on *Bipolaris sorokiniana*. *Annals of Pla. Prot. Sci.* 17: 95-98.
- Pasteur, L. 1860, Note relative on *Penicillium glaucum* et al. dissymmetric molecuaire des produits organiques naturals. *Comt. Rend. Acad. Sci.* 51: 298-299.
- Raulin, J. 1869, Etudes chimiques sur vegetation. Ann. Sci. Nat. Botan.11:223-229.
- Sharma R.L., Singh, B.P., Thakur, M.P. and Thapak S.K. 2005, Effect of Media, Temperature, pH and Light on the Growth and Sporulation of Fusarium oxysporum f sp. Lini (Bolley) Snyder and Hensan. Annals of Pla. Prot. Sci., 13: 71-73,
- Vladimir Vujanovic, Chantal Hamel, Suha Jabaji-Hare, and Marc St-Arnaud 2002, Development of a selective myclobutanil agar (MBA) medium for the isolation of *Fusarium* species from asparagus fields. *Can. J. Microbiol.* **48**: 841–847.
- Wang, X.D. and Rakshit, S.K. 1999, Improved extracellular transferase enzyme production by Aspergillus foetidus for synthesis of isooligosaccharides. Biproc eng 20: 429-434.
- Wu, H. S., Wang, Y., Zhang, C.Y., Bao, W., Ling, N., Niu D.Y and Shen, Q. R. 2009, Growth of in vitro Fusarium oxysporum f. sp. niveum in chemically defined media amended with gallic acid. Bio Res. 42: 297-304.