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Standardization of suitable medium for native endophytic entomopathogenic fungus, *Fusarium oxysporum* Schltdl.

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Abstract

The effect of mycological media on mycelial growth, sporulation, viability of spores and fungal biomass production of Fusarium oxysporum, a native endophytic entomopathogenic fungus was studied. The nutritional requirement varies with the entomopathogenic fungal species and even the fungal strain. In this study, five different media were used and significant variability was observed on different media. Isolate produced maximum mean mycelial growth on SMA medium followed by SMAY and PDA. PDA medium supported maximum sporulation (1.72 x 10^5spores ml^{-1}) and viable spore count (5x10^5 cfu ml^{-1}). Maximum mean dry fungal biomass was observed in SDYB (5.811 g). Sporulation is favoured by nutritional conditions that restricted the fungal biomass production in PDB, which recorded significantly lower fungal biomass (1.345 g). Potato dextrose medium was found to be significantly superior and best suited among all media in the present investigation.

Keywords- Fusarium oxysporum; media; colony growth; cfu; sporulation; fungal biomass

I. INTRODUCTION

Fusarium is a large genus of hyaline filamentous fungi, which are ubiquitous with cosmopolitan distribution. They belong to the family Nectriaceae of the order Hypocreales within the fungal phylum Ascomycota. They can be found in air, water, plants, insects, soils and organic substrates. *Fusarium* spp. such as *Fusarium oxysporum* caused 100% of the mortalities of the insect larvae. Insect biocontrol potential of *Fusarium* is favored by their excellent soil survivability as saprophytes, and sometimes, insect-pathogenic strains do not exhibit phytopathogenicity.

Navarro-Velasco *et al.* [1] conducted a detailed study on entomopathogenicity of *F. oxysporum* and it was demonstrated that larval mortalities by *F. oxysporum* occurred through an active infection mechanism instead of a merely physical effect caused by the fungal conidia. Munoz-Gomez *et al.* [2] identified the proteins and peptides involved in an elicited immune response in the hemolymph of *G. mellonella* larva infected with *F. oxysporum* microconidia. Moreover, Brown *et al.* [3] reported the presence of antifungal peptides after larval immunization by fusaria. Gupta *et al.* [4] isolated insecticidal metabolite, beauvericin from *Fusarium* spp. Later, Logrieco *et al.* [5] and, Stępien and Waskiewicz [6] also studied the production of beauvericin by *Fusarium* species. These studies are the proof-of-concept investigations, which demonstrate the entomopathogenicity of *Fusarium* spp. like *F. oxysporum*.

In addition to the direct effect, extracts of *F. oxysporum* can be used along with existing insecticides for the management of insects [7].

The success of microbial control of insect pests depends not only on the isolation, characterisation and pathogenicity, but also on the successful mass production of the microbial agents in the laboratory. The nutritional requirement of entomopathogenic fungi varies with the fungal species and even the fungal strain under consideration. In the present study, the effect of different media on mycelial growth, sporulation, viability of spores and fungal biomass production of *F. oxysporum* was evaluated.

II. MATERIALS AND METHODS

A. Fungal isolate

This study was carried at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara during 2016-2019. Survey was conducted in the major cowpea growing areas of Kerala for isolation of endophytic entomopathogenic fungi. The endophytic isolate of *F. oxysporum* was obtained from cowpea plants collected from Kottayam district, Kerala state ((Lat. $9^{o}46'02.6"N$; Long. $76^{o}42'09.4"E$). Entomopathogenecity was confirmed using the larvae of greater wax moth, *Galleria mellonella* F. and cowpea spotted pod borer, *Maruca vitrata* F. The present laboratory study was carried out to study the influence of different media on growth, sporulation, viability and biomass production of this native endophytic entomopathogenic fungal isolate. The pure culture of fungus was subcultured and preserved at $4^{o}C$ under refrigeration. Long term preservation was done by maintaining the culture in dehydrated sterile glycerol.

B. Media for fungal production

Five different media, Potato Dextrose Agar (PDA), Sabouraud Maltose Agar (SMA), Sabouraud Maltose Agar with Yeast Extract (SMAY), Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Agar with Yeast Extract (SDAY) were used in this study for assessing the influence of media on growth of the fungus. For studying the sporulation, viability, and biomass production of the fungus, broths of above said media were used. Five replications were maintained for each treatment.

C. Growth of fungus on different media

Twenty ml of molten sterilized medium was poured into each sterile Petri plate (9 cm diameter). A 9 mm actively grown culture of fungus was placed at the centre of each Petri plate. The inoculated plates were incubated for seven days at $28^{o}C$ in a BOD incubator. The diameter of the colonies was estimated by calculating the mean of two perpendicular measurements [8].

D. Sporulation of fungus

Actively grown culture discs of 9 mm size were inoculated into sterile test broths and incubated for 14 days. The spore count was enumerated from 14 day old fungal culture using an improved Neubauer haemocytometer and calculated using the formula,

$$\text{Spores / ml} = \frac{\text{Dilution factor x Number of spores counted}}{\text{Number of small squares observed x K}}$$

where 'K' is a constant (2.5×10^{-7}).

E. Viability of fungal spores

Viability of the cultures was assessed by taking cfu counts using dilution plate method on different media. Dilution was made upto 10^{-5} and 0.1 ml of spore suspension was poured on each Petri dish containing different media. Five replications were maintained for each treatment and each plate served as a replicate. Petri dishes were incubated at room temperature for 7 days. Number of colony forming units (Cfus) was estimated, as follows

$$\text{Number of Cfu} = \frac{\text{Number of colonies x dilution factor}}{\text{Volume of sample plated (ml)}}$$

F. **Production of fungal biomass**

The actively growing fungal discs were inoculated into 250 ml sterile test broths and incubated at 28°C for 14 days. After incubation, fungal culture in different broths was filtered through a previously dried and weighed filter paper (Whatmann No.1). The mycelial biomass collected on the filter paper was dried in a hot air oven at 70°C until a constant weight was obtained. The difference in weight gave the weight of fungal biomass produced [9].

G. Statistical analysis

All data were subjected to analysis of variance (ANOVA) in completely randomized design and the means were separated by using Duncan's multiple range test [10]. Analysis of variance (95 % confidence level) for sporulation and viability was calculated after transformation of the data to $\log_{10}$.

III. RESULTS AND DISCUSSION

3.1 Effect of media on radial growth of *F. oxysporum*

The culture medium influenced the radial growth of fungi (Table 1 & Figure 1). Results on the effect of media on mycelial growth of fungus showed that significantly higher mean growth was observed in SMA (75.33 cm), followed by SMAY (72.67 mm) and PDA (72.13 mm). Fovo *et al.* [11] reported that PDA was the next best medium to Malt Extract Agar for radial growth and sporulation. According to Jat *et al.* [12], PDA was found to be best medium for mycelial growth of *F. oxysporum*. The mean colony diameter was significantly smaller on SDAY medium (58.60 mm), followed by SDA (67.67 mm). This result indicated the influence of maltose on faster growth of fungus.

Table 1. Effect of different culture media on the mycelial growth of F. oxysporum under in vivo condition

	Diameter of fungal growth (mm)			
Media	3 DAI	5 DAI	7 DAI	Overall mean growth

T1 – PDA	50.40[b]	76.00[b]	90.00[a]	72.13[b]
T2 – SDA	40.00[c]	73.00[b]	90.00[a]	67.67[c]
T3 – SDAY	33.00[d]	58.40[c]	84.40[b]	58.60[d]
T4 – SMA	54.00[a]	82.00[a]	90.00[a]	75.33[a]
T5- SMAY	52.00[ab]	76.00[b]	90.00[a]	72.67[b]
CD (0.05)	3.221	3.430	1.072	1.530

*DAI – days after inoculation; In a column mean followed by similar letter are not significantly different at 5 % level by DMRT.

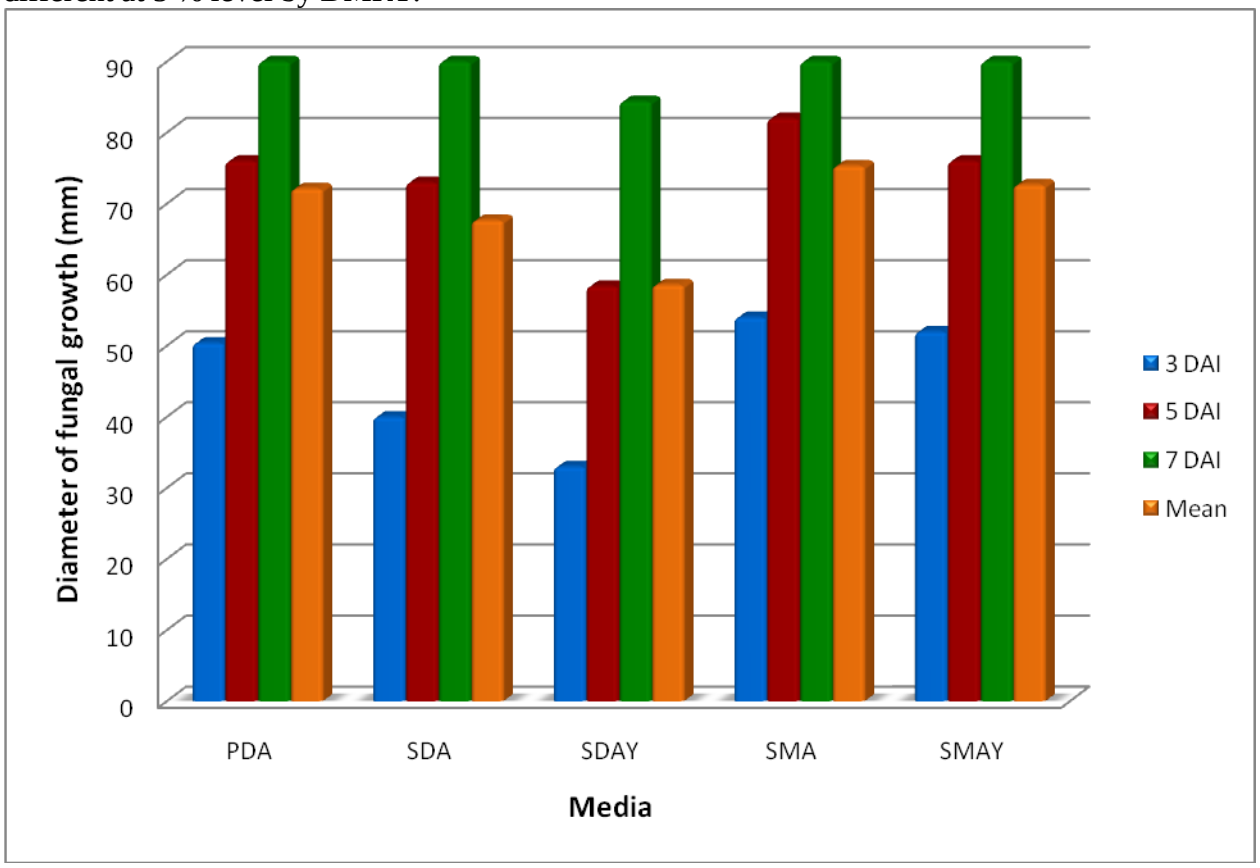

Figure 1. Effect of different media on mycelial growth of F. oxysporum

3.2 Effect of media on sporulation and spore viability

Though SMA medium was able to support maximum radial growth, maximum sporulation was observed in PDB with spore yield of 1.72 x 10^7 spores ml^{-1} followed by SMB and SMYB with 4.7 x10^6 and 3.1 x10^6 spores ml^{-1} respectively (Table 2 & Figure 2). This finding was in conformity with the earlier workers that PDA was found to be the suitable medium for growing fungus. According to Hareendranath *et al*. [13], PDA medium was found to be suitable

for the growth of *F. pallidoroseum*. Similarly, Manisegarane and Letchoumanane [14] reported that PDA was the best next to Richard's medium for culturing *F. pallidoroseum.* According to Pandit and Som [15] PDA was best for growing *B. bassiana*. Similarly, Sharma *et al*. [16] also reported that PDA was the best medium for culturing *Metarhizium* isolates. In this study, lower sporulation was observed in media, SDB and SDYB ($7x10^5$ spores ml^{-1}). Higher number of viable spores was observed in PDB ($5x10^5$ spores ml^{-1}) and SDB recorded lower number of viable spores ($3x10^5$ spores ml^{-1}) (Table 2 & Figure 3).

Table 2. Effect of media on sporualtion and spore viability of F. oxysporum

Medium	Sporulation		Viability of spores	
	Spore count/ml	Log_{10} value	Cfu /ml	Log_{10} value
T_1- PDB	1.72×10^7	7.23^a	$5x10^5$	5.68^a
T_2- SDB	7.00×10^5	5.82^c	$3x10^5$	5.48^b
T_3-SDYB	7.00×10^5	5.82^c	$4x10^5$	5.60^a
T_4-SMB	4.70×10^6	6.60^b	$4x10^5$	5.60^a
T_5-SMYB	3.10×10^6	6.48^b	$4x10^5$	5.60^a
CD(0.05)		0.260		0.118

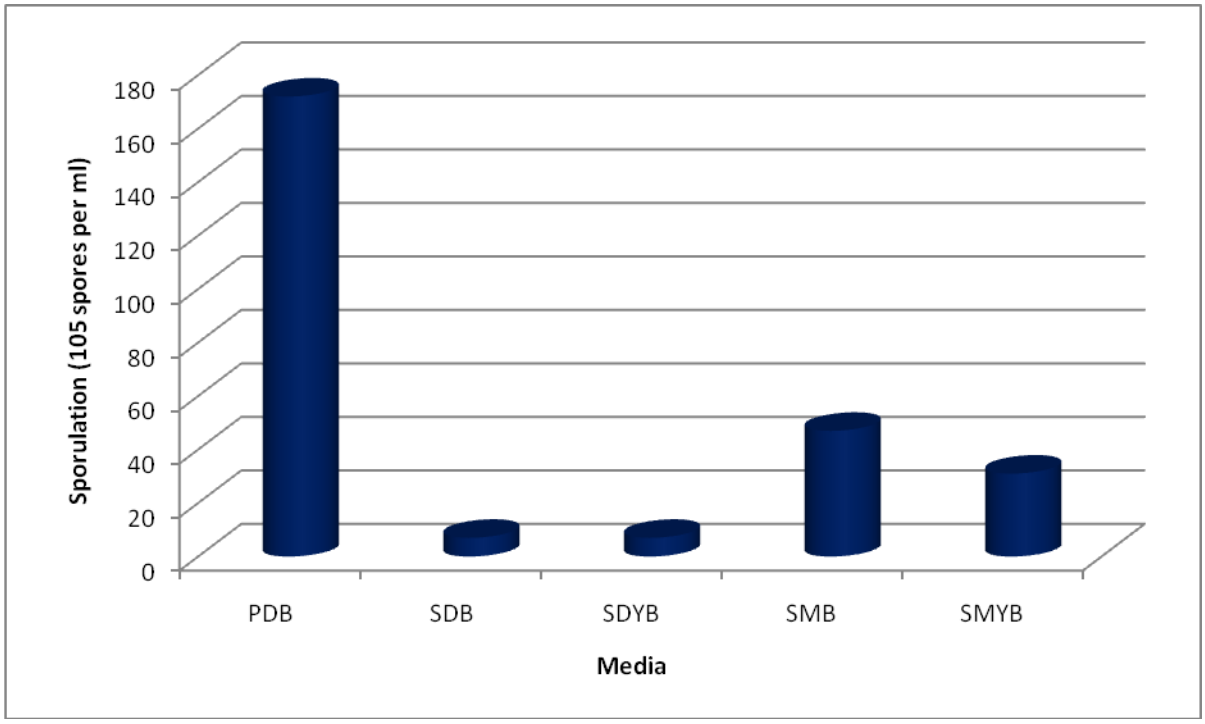

Figure 2. Effect of different media on sporulation of F. oxysporum

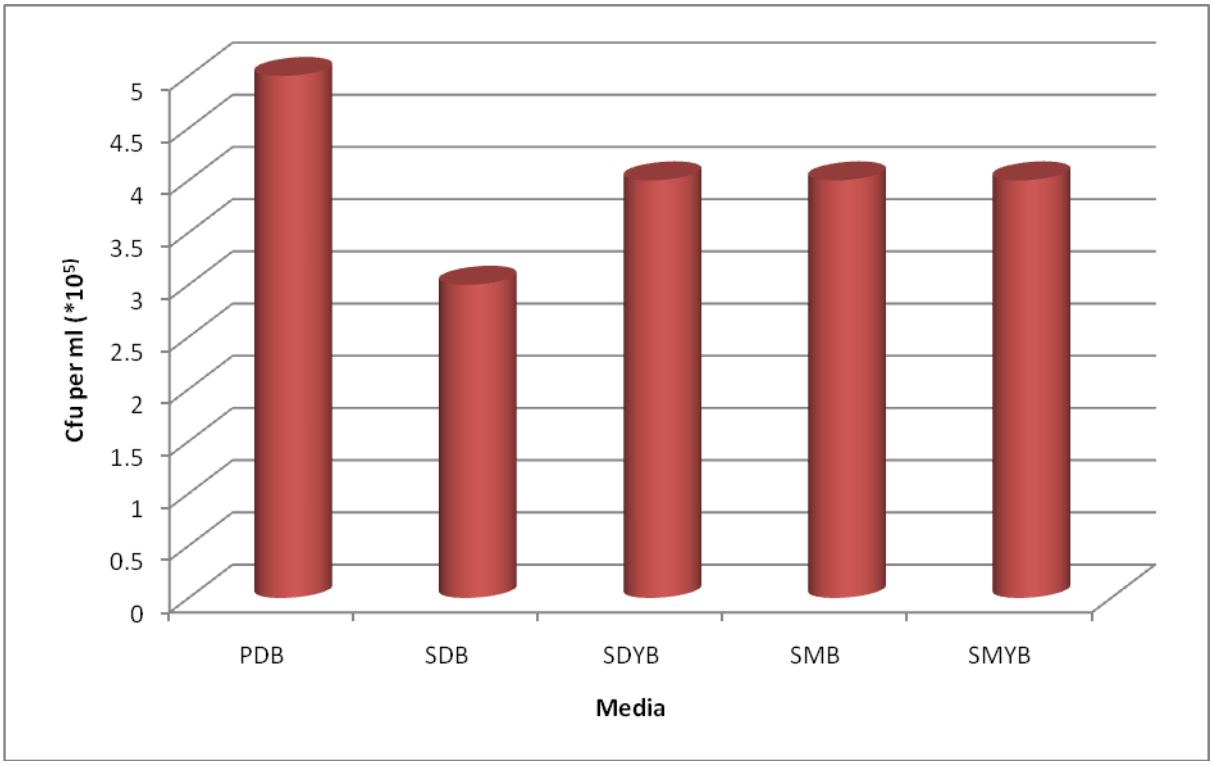

Figure 3. Effect of different media on viability of fungal spores

3.3 Effect of media on fungal biomass of *F. oxysporum*

Biomass production is significantly influenced by different culture media (Table 3 and Figure 4). The data on dry fungal biomass of isolate on broth media revealed that mean dry fungal biomass was maximum (5.811 g) on SDYB medium. Good fungal biomass production in SDYB might be due to the presence of peptone as nitrogen source [16]. Minimum mean dry fungal biomass recorded in PDB (1.345 g) on dry weight basis. This finding was in consonance with Senthamizhlselvan *et al.* [18] who reported the lowest biomass production of *F. pallidoroseum* bhendi fruit borer isolate with respect to PDB. In several studies, it is observed that sporulation is favoured by nutritional conditions that restrict growth [17] and in this study also, maximum sporulation was recorded in PDB. Jat *et al.* [12] observed variability in fungal biomass production, and sporulation of *F. oxysporum* in different culture media. Mathur and Prasad [19] also observed variability in sporulation and relative production of conidia in different media.

Table 3. Effect of different media on biomass production of F. oxysporum

Medium	Weight of fungal biomass (g)
T_1- PDB	1.345^{d}
T_2- SDB	2.127^{c}
T_3-SDYB	5.811^{a}
T_4-SMB	2.564^{b}

T_5-SMYB	2.643^b
CD(0.05)	0.127

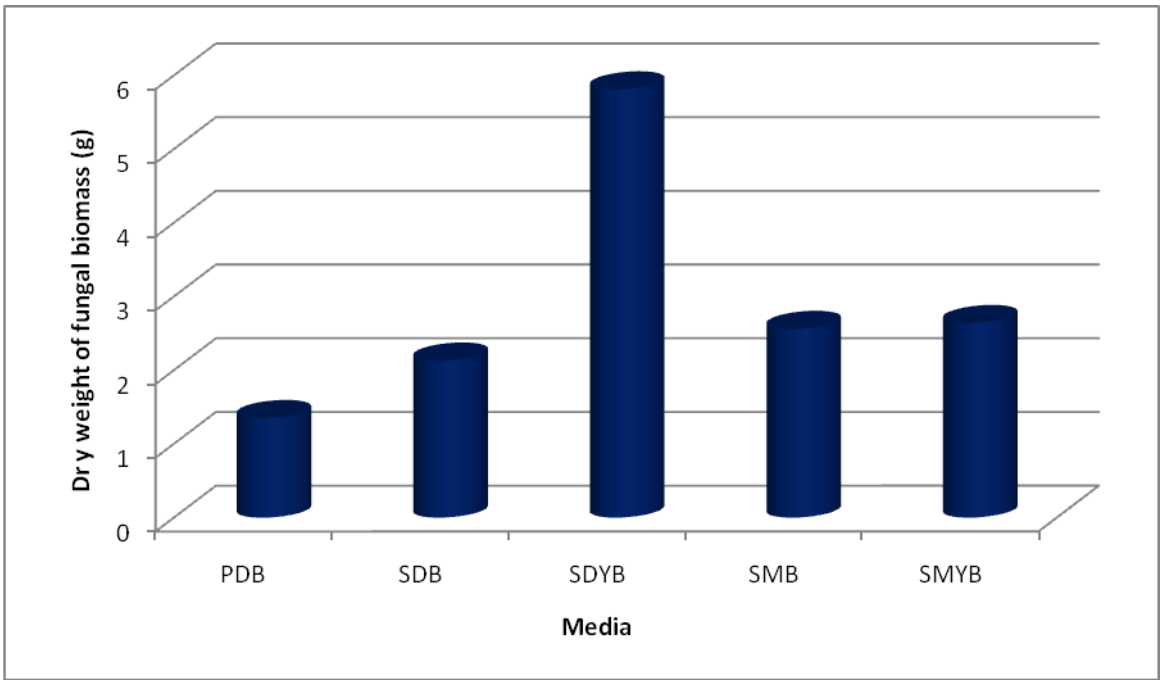

Figure 4. Effect of different culture media on fungal biomass production of F. oxysporum

IV. CONCLUSION

This study revealed that culture media differentially influence the growth, sporulation, viability of spores and fungal biomass production of endophytic entomopathogenic fungus, *F. oxysporum*. Among the test media used in the present study, PDA was found to be most suitable for mass multiplication of *F. oxysporum*, as it supported maximum sporulation and viable spore production. Tetarwal *et al.* [20] observed cultural variation of *Fusarium* isolate in different media thereby, confirming the findings of present investigation.

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