Variability of Growth and Sporulation of *Fusarium* Soil Isolates on Different Culture Media

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Abstract

Laboratory works were carried out to study the effects of three different culture media on mycelial growth and sporulation of Fusarium spp. isolated from an agricultural field cultivated for various crops near the Murshidabad district of West Bengal, India. The Fusarium soil isolates were identified based on their recovery on selective PCNB medium and morphological study of conidial and other reproductive structures. The growth of the isolates was found best on potato carrot agar medium after 7 days of incubation among the culture media tested. Only Fusarium SF-3 showed consistent growth and sporulation on all the culture media. Surprisingly, potato dextrose agar medium did not favor mycelial growth to the same extent as that of other two culture media. Most of the Fusarium soil isolates produced white, compact mycelia on all the culture media, expect SF-3, which developed pink pigmentation on both Czapeks Dox agar and potato dextrose agar media. All the isolates were found to be capable of producing spores at varied extent on all the culture media except SF-6 in which sporulation was found minimum or absent on the culture media tested. Again, potato carrot agar and Czapeks Dox agar media were found most conducive for ample production of both microconidia and macroconidia in Fusarium SF2-3.

Keywords:

Fusarium; PCNB medium; micrconidia; macroconidia; growth; sporulation; culture media

INTRODUCTION

The genus *Fusarium* has been considered as one of the very interesting and important group of fungus because of its diversity and cosmopolitan distribution. They are facultative parasites and live as parasites or saprophytes depending on their host. They cause vascular wilts, crown rots, head blights, scabs, root rots and cankers in many economically important plants such as banana, cotton, legumes, maize, rice, wheat, and others (Summerell et al., 2003). At least 80% of all cultivated plants are associated with at least one disease caused by a *Fusarium* species (Leslie and Summerell, 2006). Thus, they are responsible for huge economic losses due to reductions in harvest yields and/or the quality of staple foods. Furthermore most *Fusarium* species could continue living in soils or being parasites to grasses if no available host around. They produce dormant structures, mostly in the form of chlamydospores to keep on living in soils for many years before these structures are stimulated to grow. *Fusarium* species produce three types of spores viz. microconidia, macroconidia and chlamydospore. Because of their diversity and cosmopolitan distribution they have attained considerable interest by the plant pathologists worldwide. Significantly, there are some non-pathogenic or saprophytic *Fusarium* soil species which are being explored for their ability to improve plant growth by means of several mechanisms viz., auxin production, siderophore production, phosphate solubilization etc. Of late, *Fusarium* species having plant growth

promoting activities are exploited for growing agricultural needs. A high degree of variability in physiology and morphological characteristics enable *Fusarium* species to occupy diverse ecological niches in many geographic regions. Because of its wide range of distribution in soils, they are also known as soil-borne fungi.

Present work depicts the nutritional status of *Fusarium* spp. isolated from an agricultural field and effects of different culture media on growth and sporulation of the isolates to understand ecological survival of the isolates which will be helpful in laboratory evaluation.

MATERIALS AND METHODS

Isolation and morphological study of *Fusarium* spp.: The *Fusarium* spp. were isolated directly from the soil by dilution plate technique. Soil sample at a depth of 6 cm was collected from an arable land cultivated for various crops in Murshidabad district of West Bengal, India. 1 gm of soil was mixed in 10 ml sterile water to prepare the crude soil suspension. From the crude suspension, $1/10^{th}$ and $1/100^{th}$ dilutions were prepared and were subsequently inoculated on potato sucrose agar (PSA) medium [composition (g/l): potato extract 200, sucrose 20, agar 20, pH 6] supplemented with PCNB (0.1%) and chloramphenicol (0.01%) for selective growth of *Fusarium* spp. The plates were incubated at 28^0C for 5-7 days until visible sign of colony growth occurred. Reproductive structures of the isolates were studied through microscopic observation.

Effects of culture media: Following three culture media were used to find out the most suitable one for the mycelial growth and sporulation.

1. Czapek's Dox agar (CDA) medium [composition (g/l): Sodium nitrate 2, Di potassium hydrogen phosphate 1, Magnesium sulphate 0.5, Potassium chloride 0.5, Ferrous sulphate 0.01, Sucrose 30]
2. Potato Dextrose agar (PDA) medium [composition (g/l): Peeled and sliced potato 200, Dextrose 20].
3. Potato carrot agar (PCA) medium [composition (g/l): Grated potato 20, Grated carrot 20]

All the media were solidified with 2% agar. Mycelial disc (5 mm) was cut with a cork borer, placed at the center of the petri-dishes containing the culture medium and incubated at 28^0C. Mean colony diameter was measured after 7^{th} day of incubation to study the growth rate of the isolates. Consequently, growth characteristics and sporulation were also examined.

RESULTS AND DISCUSSION

Isolation and morphological study of *Fusarium* spp.: Five species of *Fusarium* were isolated from the soil sample studied. They were named SF2-6. Isolates were identified based on their recovery on *Fusarium* selective PCNB medium and on the basis of macroscopic *viz.,* colonial morphology, colour, texture, shape, diameter and appearance of colony and microscopic characteristics *viz.,* septation in mycelium, presence of specific reproductive structures e.g. chlamydospore, shape and structure of conidia. All the isolates were more or less fast growing on potato sucrose agar (PSA) medium (data not shown). Colonies were characterized by the appearance of white, compact and aerial mycelium. Microscopic observation of the isolates showed 1-2 celled microconidia in abundance (all the *Fusasrium* soil isolates) and distinct chlamydospore (only *Fusarium* SF-3) on PSA medium. The chlamydospores were intercalary in position and found singly (fig. not shown).

Effects of culture media:

The effects of three culture media on growth and sporulation of the isolates were studied (Table 1, 2, 3 and fig. 5). All the culture

media supported growth of the tested *Fusarium* isolates to various degrees. The *Fusarium* isolates could exploit all the culture media tested confirming the fact that the *Fusarium* isolates were of diverse nutritional requirement. The isolates showed maximum mycelia growth on potato carrot agar and Czapek's Dox agar media but surprisingly, potato dextrose agar medium which a general fungal medium, did not favor mycelia growth to the same extent. But PDA medium supported sporulation to a considerable extent in *Fusarium* SF2-5. Sporulation was also quite satisfactory on CDA and PCA media for all the *Fusarium* soil isolates except SF-6.

Only one isolate (SF-3) was found fast growing on Czapeks Dox agar medium. The colony diameter ranges from 42-83 mm after 7 days of incubation on CDA medium (table-1). Colonies of the *Fusarium* soil isolates were characterized by smooth, circular, compact, white mycelium (fig.1). Out of five soil isolates, only *Fusarium* SF-3 and SF-5 produced faint pink and faint green pigmentations respectively. Most of the isolates produced 1-2 celled microconidia amply with considerable degrees of curvature. The shapes and the sizes of the conidia varied to a great extent (fig.2). Only one *Fusarium* isolate (SF-3) produced septate macroconidia and intercalary chlamydospore in chains with prominent wall thickness (fig. 2. b and c). *Fusarium* SF-3 also produced greater number of spores with spore number of 180/m.f. SF-2 and SF-5 produced 170 and 160 spores/m.f respectively. The sizes of microconidia, macroconidia and chlamydospore range from 5-10 µ, 35-50 µand 10-15 µ respectively. Jamaria (1972) reported maximum growth and sporulation of *F. oxysporum f. sp. vanillae* on potato dextrose agar, Richard's agar and Czapek's Dox agar.

Table 1: Growth characteristics and sporulation of the *Fusarium* soil isolates on CDA medium

Isolate no.	Colony diameter in mm	Colony morphology	Sporulation	No. of spore/microscopic field.	Pigmentation
SF-2	46	Smooth, circular and compact mycelia.	1-2 celled abundantly produced microconidia with minimum degree of curvature	170	White
SF-3	83	Circular, Compact, aerial mycelia	Microconidia, septate macroconidia and thick walled chlamydospore	180	Faint pink
SF-4	42	Circular and more compact mycelia	Sparsely produced, slightly elongated, straight to	50	Deep white

			curved microconidia.		
SF-5	60	Circular,glittering aerial mycelia	Abundant sporulation, 1-2 celled ovoid microconidia	160	Faint green
SF-6	57	Circular,aerial mycelia	1-2 celled slightly elongated microconidia with prominent curvature	60	White

SF-2 SF-3 SF-4 SF-5 SF-6

Fig. 1: Colony characteristics of the *Fusarium* soil isolates on CDA medium after 7 days of incubation

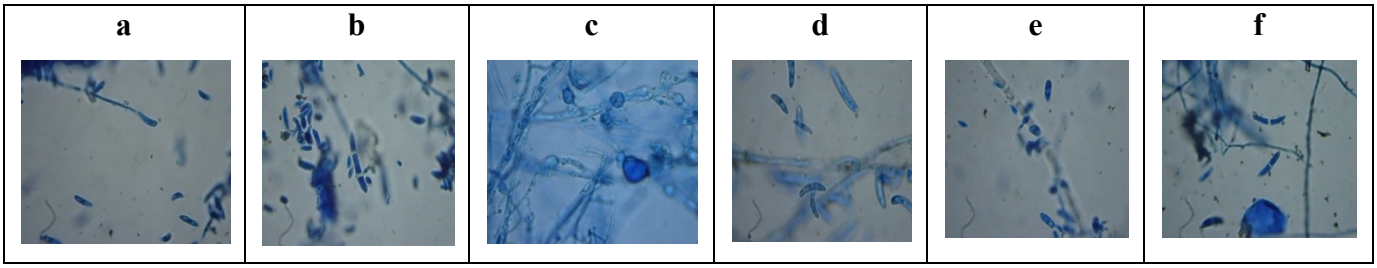

Fig. 2: Conidial morphology of the *Fusarium* spp. on Czapeks Dox agar (CDA) medium a. *Fusarium* SF-2, b and c. *Fusarium* SF-3, d. *Fusarium* SF-4, e. *Fusarium* SF-5, f. *Fusarium* SF-6

On PDA medium, one isolate (SF-3) was found moderately fast growing with colony diameter of 61 mm after 7 days of incubation. Rest of the *Fusarium* isolates were slow growing and their colony diameter ranged from 21-19 mm after the same days of incubation on potato dextrose agar medium (table-2). All the isolates produced smooth, circular, compact white mycelia except SF-3 which developed deep ping pigmentation on PDA medium (fig.3). On the contrary, the medium was quite favourable for sporulation of most of the *Fusarium* isolates. The *Fusarium* SF-2, 3 and 5 profusely produced 1-2 celled microconidia having variable degrees of curvature. *Fusarium* SF-2 did sporulate best on PDA medium having 170 spores/m.f. *Fusarium* SF-4 showed sparsely produced ovoid microconidia and SF-6 did not produce any spore on PDA medium. Markedly, these results were not in

confirmation with Ingole (1995) who reported that PDA and Richard's agar supported best mycelial growth of *F. udum*. Khare et al., (1975) also reported maximum growth of *Fusarium oxysporum f. sp. lentis* on PDA followed by lentil extract and Richard's agar. Anjaneya Reddy (2002) observed maximum growth of *F. udum* on Richard's agar and potato dextrose agar. Naik *et al*. (2004) reported PDA and Richards agar as best growth medium for *Fusarium oxysporum* f. sp. *vanillae.* Recently Imran Khan et al., (2011) studied effect of media on *F. oxysporum f.sp. ciceri* and found that PDA is best for the growth of different isolates. But here, surprisingly the isolates were found slow growing on PDA medium.

Table 2: Growth characteristics and sporulation of the *Fusarium* soil isolates on PDA medium

Isolate no.	Colony diameter in mm	Colony morphology	Sporulation	No. of spore/microscopic field.	Pigmentation
SF-2	27	Smooth, circular mycelia	Abundant sporulation, only 1-2 celled microconidia with prominent degree of curvature	170	White
SF-3	61	Circular, Compact, mycelia	Microconidia in abundance with noticeable degree of curvature	160	Deep pink
SF-4	21	Circular and smooth lawn of mycelia	Sparely produced microconidia, ovoid to slightly elongated.	50	White
SF-5	29	Circular and smooth lawn of mycelia	Amply produced 1-2 celled microconidia	120	White
SF-6	26	Circular and compact mycelia	Absence of sporulation	0	White

SF-2 SF-3 SF-4 SF-5 SF-6

Fig. 3: Colony characteristics of the *Fusarium* soil isolates on PDA medium after 7 days of incubation

The *Fusarium* soil isolates grew best on potato carrot agar medium. On PCA medium, all the isolates seemed to be fast growing. The colony diameter of the isolates ranged from 73-88 mm after 7 days of incubation (table-3). *Fusarium* SF-6 was the most fast growing fungus among the five *Fusarium* soil isolates on PCA medium with a colony diameter of 88 mm on 7^{th} day of incubation. All the fungal isolates produced smooth, circular, white mycelia, but to the exception, SF-3 and SF-5 produced profuse white aerial mycelia on PCA medium (fig.4). None of the fungal isolates produced any pigmentation on PCA medium. Among the soil isolates, *Fusarium* SF-3 was found to sporulate best on PCA medium producing both micro and macroconidia abundantly. *Fusarium* SF-5 also produced both types of conidia on PCA medium, but to a moderate extent. *Fusarium* SF-3 and SF-5 produced 150 and 90 spores/m.f respectively on PCA medium. For the other *Fusarium* isolates the medium was found to be reasonably to least effective with regards to the ability to sporulate. *Fusarium* SF-2, 4 and 6 only produced 1-2 celled microconidia sparsely with variable sizes and shapes.

Table 3: Growth characteristics and sporulation of the *Fusarium* soil isolates on PCA medium

Isolate no.	Colony diameter in mm	Colony morphology	Sporulation	No. of spore/microscopic field.	Pigmentation
SF-2	81	Smooth, circular	Moderate sporulation, microconidia with prominent degree of curvature and very few macroconidia	80	White
SF-3	85	Circular, slightly aerial mycelia	Abundant sporulation with both types of conidia having greater degree of curvature	150	White
SF-4	73	Circular and	Sparse	40	White

		aerial mycelia	sporulation with only microconidia.		
SF-5	82	Circular, smooth mycelia	Modrate sporulation with both micro and macrconidia	90	White
SF-6	88	Profuse aerial mycelia	Sparsely produced, 1-2 celled slightly curved microconidia	30	White

SF-2 SF-3 SF-4 SF-5 SF-6

Fig. 4: Colony characteristics of the *Fusarium* soil isolates on PCA medium after 7 days of incubation

Conclusion: The present study indicated that potato carrot agar was best medium for growth of *Fusarium* isolates. Our findings also revealed that culture media differentially influenced the growth, colony characters and sporulation of the tested *Fusarium* soil fungi. Out of the three culture media employed in our study, CDA and PCA media were found to be most suitable for profuse sporulation of the fungal isolates while CDA produced most visible colony morphology. It can be concluded that instead of using a particular culture medium, a combination of two or more culture media will be more appropriate for routine characterization of fungi.

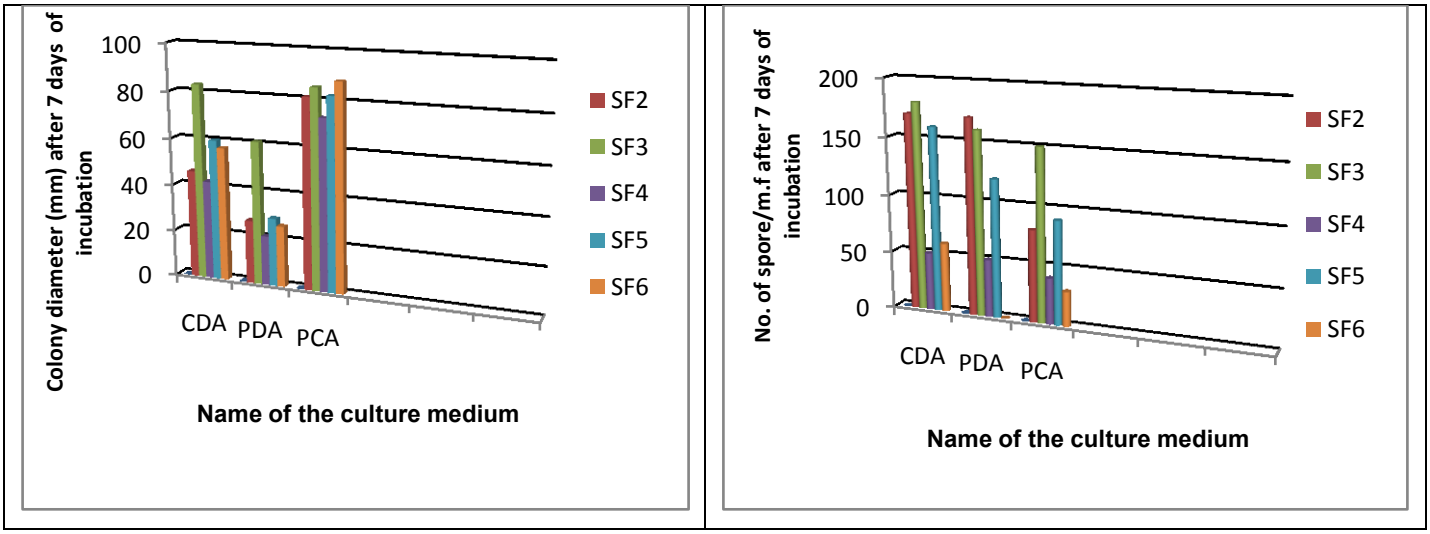

Fig.5: Effect of culture media on growth (left) and sporulation (right) of the *Fusarium* spp.

Acknowedgements. The work was partially supported by the grant received from University of Kalyani.

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B.Sc (Hons.) in Botany (1st class 1st), M.Sc in Botany (1st class 1st) from Kalyani University with specialization in "Cytogenetics and Plant Breeding". The author was felicitated with "Durgabati Memorial Gold Medal" for standing 1st class 1st in M.Sc by honorable governor of West Bengal Mr. M. K. Naraynan in 2010 at the annual convocation of Kalyani University. The author qualified Joint CSIR-UGC NET in 2011 and started research on "*Fusarium* soil isolates" in Mycology and Plant Pathology Laboratory of Deptt. of Botany, K.U under supervision of Dr. Bejoysekhar Datta. The author is presently pursuing his Ph.D.

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