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Characterization of a *Fusarium* isolate with respect to its pathogenecity and different growth parameters

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Studies were carried out to determine the effects of different culture media, carbon and nitrogen sources, pH and temperature on mycelial growth and sporulation of a *Fusarium* sp. isolated from an agricultural field cultivated for various crops near the Nadia district of West Bengal, India. The effect of culture filtrate was also observed on the percentage of seed germination to explore the pathogenic nature of the fungal isolate. Maximum inhibition was observed for the germination of tomato, brinjal and bean seeds. The growth of the isolate was best on Richard's agar medium among the nine culture media that were tested though the maximum sporulation was seen on Czapek's Dox agar medium with 200 conidia/microscopic field. The fungus could utilize dextrose and glycine as carbon and nitrogen sources, respectively. The *Fusarium* isolate could tolerate a wide range of pH levels (from pH 4.0 to pH 10.0). The isolate could grow well both at 40^0C and 27^0C but showed no growth at or below 5^0C.

Key words: *Fusarium*, growth, sporulation, culture media, temperature, pH, inhibition of seed germination

INTRODUCTION

Most species of the genus *Fusarium* are soil-borne fungi. 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. Because of their diversity and cosmopolitan distribution they have attained considerable interest by the plant pathologists worldwide.

Present work depicts the pathogenic property of a *Fusarium* sp. isolated from an agricultural field and the effect of different culture media, carbon and nitrogen sources, pH and temperature on growth and sporulation on *Fusarium* sp.has been determined to understand ecological survival of the pathogen which will be helpful in management strategy and laboratory evaluation.

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MATERIALS AND METHODS

Isolation and morphological study of the fungus

The fungus was isolated directly from the soil by dilution plate technique. Soil sample at a depth of 6 cm was collected from land cultivated for various crops in Nadia district of West Bengal, India. One g. of soil was mixed in 10 ml sterile water to prepare the crude soil suspension. From the crude suspension, 1/10th and 1/100th 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°C for 5-7 days until visible sign of colony growth occurred. Reproductive structures of the isolate were studied through microscopic observation.

Pathogenecity test

Pathogenicity of the isolate was assayed by observing the inhibition of seed germination taking seeds of seven different crop plants such as tomato, cucumber, brinjal, carrot, basella, bean and gram. The fungal isolate was grown in Czapek-Dox broth for 15 days of incubation. The culture filtrate was taken and the surface sterilized seeds were dipped therein overnight and next day these were placed on pre-soaked blotting papers. After five days percentage of seed germination was calculated.

Effect of culture media

Following nine 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 g 3.Nutrient agar (NA) medium [composition (g/l): peptone 5, sodium chloride 5, beef extract 1.5, Yeast extract 1.5] 4. Richards's agar (RA) medium [composition (g/l): potassium nitrate 10, potassium monobasic phosphate 5, magnesium sulphate 2.5, ferric chloride 0.02, sucrose 50] 5.Potato Carrot agar (PCA) medium [composition (g/l): grated potato 20, grated carrot 20] 6.Sabouraud's agar (SA) medium [composition (g/l): dextrose 20, peptone 10] 7.Pikovoskya's agar (PA) medium [composition (g/l): tricalcium phosphate 2.5, glucose 13, ammonium sulphate 0.5, sodium chloride 0.2; magnesium sulphate 0.1, potassium chloride 0.2, yeast extract 0.5, manganese sulphate trace, Ferrous sulphate trace] 8.Malt extract agar (MA) medium [composition (g/l): malt extract 25] 9.Asthana & Hawker's medium [composition (g/l): glucose 5, potassium nitrate 3.5, potassium dihydrogen phosphate 1.75, magnesium sulphate 0.75] 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°C. Mean colony diameter was measured after 4th and 8th day of incubation to study the growth rate of the isolate. Consequently, growth characteristics and sporulation were also examined.

Effect of carbon and nitrogen sources

To study the carbon source utilization 50 ml potato decoction broth was used supplemented with 2% of the respective carbon sources viz., maltose, dextrose, 7lactose, sucrose, mannitol, sorbitol and starch. Likewise, nitrogen source utilization study was done using nitrogen free Czapek's Dox broth supplemented with 0.2% of the various nitrogen sources viz., sodium nitrate, potassium nitrate, sodium nitrite, glycine, ammonium nitrate, peptone and glutamine. Mycelial disc of 5 mm was inoculated in each of the flask and incubated at 28°C for 7 days. The data of mycelial dry weight was taken to determine the growth of the isolate.

Effect of pH and temperature

Growth characteristics of the isolate were studied to evaluate the suitable pH and temperature required or preferred by the pathogen for its growth and multiplication. Different sets of Czapek's Dox agar medium were prepared and pH of the medium was maintained from 6 to 10. To study the growth in lower pH the organism was inoculated in two CD broths having pH 4 and 5 respectively. After inoculation the isolate was incubated at 28°C for 9 days. Similarly, to study the effect of temperature, the organism was inoculated on CDA medium and incubated at 5°, 15°, 27° and 40°C

for 5 days. Thereafter, the growth and sporulation were studied.

RESULTS AND DISCUSSION

Isolation of the Fusarium sp.

Only one species of *Fusarium* was isolated from the soil sample studied. Microscopic observation of the isolate showed 1-2 celled microconidia and distinct chlamydospore on PSA medium. The chlamydospores were terminal, intercalary, single and occasionally in chains as well. The size of microconidia and chlamydospore ranges from 5-10 μm and 10-20 μm respectively.

Table 1 : Inhibitory effects of the culture filtrate on germination of seeds

Name of seeds	Control (% of germination)	Inoculated (% of germination)
Tomato*	50	00
Cucumber	96	88
Brinjal*	62	00
Carrot	84	72
Basella	77	61
Bean*	82	19
Gram	75	37

* Maximum inhibition of seed germination

Effect of culture filtrate on seed germination

The inhibitory effect of the culture filtrate of the isolate on the germination of seeds of seven different plants was studied (Table1). The fungal isolate seemed to be pathogenic at least to a few members of the Solanaceae (tomato and brinjal) as far as inhibition of germination was concerned. The inoculated seeds of tomato and brinjal showed no germination whereas in control the percentage of germination was more than 50.The fungus was also able to impede the germination of bean seeds to a considerable extent (Table 1). Pathogenic *Fusarium* spp. produce different toxic compounds such as fusaric acids which plays important role in disease development. The obstruction of seed germination might be due to production of toxin produced by the isolate.

Effect of culture media

The effect of nine culture media on growth and sporulation of the isolate was studied (Table 2). The fungus could exploit various culture media establishing the fact that the *Fusarium* isolate was of diverse nutritional requirement. The isolate showed maximum colony diameter in Richard's agar (78 mm) and Czapek's Dox agar (74 mm) medium after 8 days of incubation. Naik *et al.* (2004) reported PDA and Richards agar as best growth medium for *Fusarium oxysporum* f. sp. *vanillae.* The fungal isolate produced white, compact mycelia with loose aerial hyphae at times in most of the media tested but to the exception, the fungus developed deep pink pigmentation in both Sabouraud's agar and Pikovoskya's agar media. The fungus showed luxuriant growth in a number of culture media that were tested, but surprisingly, sporulation was most satisfactory in potato carrot agar (PCA) medium, as typical sickle shaped/elongated macroconidia (75-90 μm) with prominent 8-10 septa were abundantly produced along with tiny microconidia and chlamydospores. Statistically, though the sporulation was recorded maximum in Czapek's Dox agar medium with 200 microconidia/microscopic field. Sporulation was satisfactory to a great extent in Potato dextrose agar, Nutrient agar and Richard's agar media as well. Production of macroconidia was only supported by potato carrot agar medium but the rest of the media only encouraged the generation of 1-2 celled microconidia to various extents. Interestingly, chlamydospore formation was hastened by a number of media viz., Sobouraud's agar, Malt extract agar and Pikovoskya's agar that were not conducive to the production of either micro or macroconidia. The chlamydospores showed prominent wall thickness.

Effect of carbon and nitrogen sources

Effects of each of the seven different carbon and nitrogen sources on the growth of the isolate was recorded and depicted in the Table 3. The isolate had spectacular potential of utilizing various carbon and nitrogen compounds. The *Fusarium* isolate was found to be capable of utilizing all the seven carbon and seven nitrogen sources to a varied extent, but dextrose (carbon source) and glycine (nitrogen source) were most preferred by the fungus. On the other hand, lactose and sodium nitrite were least utilized as carbon and nitrogen sources respectively by the fungal isolate.

Effect of pH and temperature

Effects of different pH and temperature on growth

Table 2 : Growth and sporulation of the *Fusarium* isolate on different culture media

Media	Mean colony diameter (mm)		Growth characters after 8th day	Total no. of spore/ microscopic field and spore types found after 8th day
	Day 4	Day 8		
Czapek's Dox agar	27	74	White, dense, compact aerial mycelia.	200 tiny 1-2 celled microconidia.
Potato dextrose agar	31	36	Light, brown mycelia.	100 elongated microconidia.
Nutrient agar	35	62	Loose, aerial, whitish mycelia.	150 microconidia with a few chlamydospores.
Richard's agar	32	78	Dense, white, compact mycelia	85 tiny microconidia.
Potato carrot agar	36	71	Mycelia forms a faint-white lawn	120 micro-, macroconidia & chlamydospores.
Sabouraud's agar	30	33	Compact mycelia with deep pink pigmentation.	10 microconidia with chlamydospores.
Pikovoskya's agar	33	38	Compact mycelia with deep pink pigmentation.	20 microconidia with chlamydospore.
Malt extract agar	20	56	Faint lawn of mycelia, dense at centre, gradually thins at periphery.	15 tiny microconidia with chlamydospores.
Asthana & Hawker's medium	22	37	White and compact mycelia.	5 tiny microconidia.

Table 3 : Utilization of various carbon and nitrogen sources by *Fusarium* isolate

Carbon sources	Mycelial dry weight (g)	Nitrogen sources	Mycelial dry weight (g)
Maltose	0.244	Sodium nitrate	0.285
Dextrose *	0.377	Potassium nitrate	0.267
Lactose	0.140	Sodium nitrite	0.121
Sucrose	0.288	Glycine *	0.297
Mannitol	0.275	Ammonium nitrate	0.242
Sorbitol	0.282	Peptone	0.278
Starch	0.157	Glutamine	0.236
Control	0.050	Control	0.020

* Best utilization of carbon and nitrogen sources

and sporulation of the isolate were studied (Tables 4 and 5). The fungus could tolerate both acidic (pH 4 and 5) (data not shown) and alkaline pH (up to 10) although maximum radial growth and sporulation were observed at pH 6 and pH 7 (Table 4). Extreme pH didn't favour sporulation. The temperature 27° and 40°C were most favourable for the growth of the fungus (Table 5). The fungal isolate showed no growth at the temperature 5°C. Interestingly, low and high temperatures favoured

chlamydospore formation in addition to sparsely scattered microconidia, whereas moderate temperature was permissible to the ample production of only microconidia. Singh and Kumar (2011) have shown that a temperature of 25°C, pH of 5.0 and Potato dextrose medium was most suitable for the growth of *Fusarium oxysporum* f. sp. *chrysanthemi* and the isolate was failed to grow both at 40° and 45°C. Variability in the temperature requirements of *Fusarium* has been reported by Daami-Remadi *et al.* (2006) and Fayzalla *et al.* (2008). Khilare and Ahmed (2012) examined effect of different culture, media, pH and temperature levels on mycelia growth of *Fusarium oxysporum* f.sp. *ciceri* and observed that the fungus grew best on Czapek Dox agar and PDA media and the most suitable pH level for growth of fungus was 6.0 and 6.5 and temperature was 30°C. From our study it may be concluded that the *Fusarium* isolate which inhibited seed germination of tomato and brinjal could utilize a wide range of culture media, carbon and nitrogen sources, and tolerate both acidic and alkaline pH and preferred higher temperature (40°C) for its multiplication under *in vitro* condition.

Table 4 : Effects of pH on growth and sporulation of the isolate

pH	Radial diameter (mm)	Sporulation (total no. of spore/ microscopic field)
6*	81	200
7	78	190
8	73	140
9	70	70
10	68	60

* Best pH for luxuriant proliferation of the isolate
Growth of the fungus in CD broth having pH 4 and 5 was also observed.

Table 5 : Effect of different temperatures on the growth and sporulation of the isolate

Temperature (°C)	Radial diameter (mm)	Total no. of spore/ microscopic field and spore types
5	0	0
15	32	50 microconidia with abundant chlamydospores.
27	36	200 microconidia.
40	47	50 microconidia with abundant chlamydospores.

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