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"Fusarium semitechtum'- A POSTHARVEST PATHOGEN OF BANANA- PRELIMINARY INVESTIGATIONS

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ABSTRACT: *Fusarium semitectum* was isolated most frequently from crown rot infected banana fruits in this study. The fungus produced creamy white to light brown in potato dextrose agar, Richards's agar. microconidia, macroconidia and chlamydo spores were observed in culture. Out of ten solid media tested, significantly maximum radial growth of the fungus was observed on potato dextrose agar (PDA)(89 mm) which was at par with Richards's agar (88. 38 mm) whereas, Richards's broth(434 mg) was preferred by the fungus among the ten liquid media tested. Excellent sporulation was noticed on PDA, Richards's agar and malt extract agar. Growth phase of *F. semitectum* recorded peak (76.00 mg) at 10th day after inoculation. Temperature had shown significant effect on growth and sporulation of *F. semitectum*, produced maximum radial growth at 25°C (89.81mm) at par with 30°C (88.05 mm). Maximum dry mycelial weight (425 mg) was recorded at 6.0 pH which was at par with at 7.0 pH (419.67 mg).
Key words: *Fusarium semitechtum*, Postharvest, Banana

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INTRODUCTION

Quality of tropical fruits is commonly affected by post harvest disease such as fruit rot mostly caused by improper handling and storage, during transportation and marketing. One of the potential pathogenic fungi that are associated with fruit rot of tropical fruits is *Fusarium*. Fusarium rot on tropical fruits could posses a potential health risk as many *Fusarium* species are known to produce mycotoxins under suitable conditions. Among the different *Fusarium* species, *F. semitectum* was found to be responsible for causing diseases like wilts, blights, root rots, and cankers in coffee, pine trees, wheat, corn, rice, cereals, carnations and grasses [1,2] reported the crown-rot disease of bananas caused by *F. semitectum.* Mikunthan and Manjunatha [3] reported the use of *F. semitectum* as a potential mycopathogen against thrips and mites in chilli. The presence of *Fusarium* species on tropical fruits received little attention and not well documented though several species have been associated with crown rot of banana. Therefore, the present study was conducted for characterization of *Fusarium* species on banana fruit rot.

MATERIALS AND METHODS

The present investigations were carried out during 2015-16. Laboratory studies on isolation, cultural and physiological characterization of the fungus were carried out in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka.

Isolation of fungi: The fruit portion showing typical symptom of diseases were used for the isolation of pathogens. Fully rotten fruits were avoided to discourage secondary fungal growth. The standard tissue isolation procedure was followed to isolate the pathogen. Fruit samples showing signs and symptoms of fruit rot were obtained from several markets and cold storages in Dharwad and Hubballi. For direct isolation, a scrape of the mycelia were extracted from the lesion by using sterile inoculation loop. Pure cultures thus obtained by single spore isolation were maintained on potato dextrose agar slants at ±25°C for further studies.

Cultural studies

Growth phase: Growth phase study was carried out in potato dextrose broth Thirty ml of broth was added in each of the 100 ml conical flasks and sterilised at 1.1 kg/cm^2 pressure for 15 min. These flasks were allowed to cool and five mm disc from actively growing cultures of respective fungi were inoculated to each of the conical flasks aseptically. Inoculated flasks were incubated at 27±1°C. Each treatment was replicated three times. Culture was filtered through Whatman No. 42 filter paper disc of 12.15 cm diameter, which was dried to a constant weight at 60°C in an electrical oven, prior to filtration. The mycelial mat on the filter paper was washed thoroughly with distilled water to remove any salts likely to be associated with it. One set of flasks was harvested on second day. Subsequent harvesting was done at an interval of two days upto twenty-eighth day. The filter papers along with mycelial mat were dried to a constant weight in an electrical oven at 60°C, cooled in a desicator, weighed immediately on an analytical electric balance and dry mycelial weight was recorded. Number of days required for maximum growth was used in further studies. Results were analysed statistically.

Effect of media on growth

Growth characters *F. semitectum* were studied on ten solid media (Table.1) *viz.,* potato dextrose agar, potato carrot agar, cornmeal agar, host extract agar, oat meal agar, malt extract agar, V-8 juice agar, Richards's agar, Czapek's agar and Sabouraud's agar. All the media were sterilized at 1.1 kg/cm^2 pressure for 15 min. To carry out the study, 20 ml of each of the medium was poured in 90 mm Petriplates, such Petriplates were inoculated with five mm disc cut from periphery of actively growing culture and incubated at 27±1^{o}C. Each treatment was replicated thrice. Colony diameter was recorded when the fungus covered complete Petriplate in any one of the media. The data on radial growth was analysed statistically. Colony colour, texture, surface elevation and sporulation were also recorded. Similar experiment was carried out with ten liquid media in 100 ml conical flasks containing 30 ml broth, dry mycilial weight was records at 10 day age for determining the best broth for growth.

Sporulation: For recording sporulation three uniform bits were cut from 10 day old culture plate with the help of corkborer representing periphery, center and middle portions of culture plate. These culture bits were transferred to a sterilised test tube containing 2 ml of sterile distilled water and shaken thoroughly to dislodge the spores in water. Thereafter, 20 µl of the spore suspention was transferred to a clean glass slide and mounted for microscopic observation. Number of spores per each microscopic field (400X) were recorded likewise 10 microscopic fields were examined to obtain mean sporulation per microscopic field. The intensity of sporulation was grouped into five classes *viz.,* excellent (++++), good (+++), average (++), poor (+) and no sporulation (-) [4]. Similar procedure was followed for all the media under study and compared.

Physiological studies

Effect of temperature on growth: Potato dextrose broth was used in this experiment to compare growth of fungi in terms of dry mycelial weight. Conical flasks of 100 ml capacity and each containing 30 ml of liquid medium were inoculated with 5 mm culture disc and incubated at different temperature levels *viz.,* 15, 20, 25, 30, 35, 40 and 45°C. The dry mycelial weight at each temperature level was recorded after incubating for ten days. Linear growth of the fungi as well as sporulation were studied on potato dextrose agar and compared at different levels of temperature and in each case, three replications were maintained. Results were analysed statistically.

Effect of pH on growth: pH of the potato dextrose broth was adjusted by using 0.1N alkali (NaOH) or 0.1N acid (HCl). The pH of the medium was adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. After sterilization there was slight change in pH, which was negligible. The cultures were inoculated to each of 100 ml flask containing 30 ml of basal medium and incubated at 27±1°C for ten days. Three replications were maintained in each treatment. Dry mycelial weight was obtained as described earlier and results were analysed statistically.

RESULTS AND DISCUSSION

Fusarium semitectum was isolated most frequently from crown rot infected banana fruits in this study. *F. semitectum* showed the growth of dense aerial mycelia on PDA and Richards's agar media, initially with white to cream and later light brown in colour. Dark brown coloured pigmentation was also observed on reverse of the plates.

Microscopic examination revealed the presence of septate and hyaline hyphae. Macroconidia were abundant, mostly straight or slightly curved, generally 3 - 5 septate and measured about 28.72- 37.23 x 4.24- 6.38 µm in size. Presence of microconidia and chlamydospores also observed, microconidia these were single or two celled, measuring 7.56- 11.32 x 3.23- 4.85 µm. Chlamydospores were single celled, occurred either singly or in chains, terminal or intercalary. These findings are in conformity with the reports of Biradar [5]; Ingle and Rai [6] and Abd-Elsalam and Schnieder [7].

Cultural studies

Growth phase: Determination of optimum growth period is essential to study the physiology of fungi. In present investigations, efforts were made to study growth pattern of postharvest pathogens of banana. Maximum dry mycelial weight of *F. semitectum* (420.33 mg) was recorded on 10th day after inoculation (Fig.1). The growth declined significantly from then onwards. Meanwhile, *Fusarium semitectum* had put forth maximum growth on 12th day after inoculation as demonstrated by Biradar [5].

Growth characters on solid media: Fungi secure food and energy from the substrate upon which they live in nature. Not all the media are equally good for all fungi, nor there is a universal substrate or artificial medium upon which all fungi can grow. So, different media were tried to study the variation in growth and cultural characteristics *F.semitectum*. Among ten solid media tested for *F. semitectum*, significantly maximum radial growth was observed on potato dextrose agar (89 mm) which was at par with Richards's agar (88. 38 mm), host extract agar (87.35 mm) and oat meal agar (86.62 mm)(Table.1). The faster growth of fungal colonies could be an indicator that the respective isolate has the capability to colonize host tissues faster; such a positive correlation between growth rate of the fungus and virulence has been identified with *Fusarium* spp in a study carried out by Brennan *et al.* [8]. Growth media had also influence on colony colour and type which varied from off white to light pink in colour, areal to merge and raised to smooth colonies however, cottony white and milk white with smooth margin was frequent in most of the media tested. Colour of colony turned to light brown in potato dextrose agar, Richards's agar after ten days of inoculation. Sporulation also greatly varied in different media ranging from excellent to poor sporulation. Excellent sporulation of *F. semitectum* was noticed on potato dextrose agar, Richards's agar and malt extract agar; good sporulation in oat meal agar. Present findings are in conformity with studies of Biradar [5] who concluded that oat meal agar and Richards's agar were best media for the growth of *F. semitectum.*

Growth in liquid media: For isolation and characterization of fungi it is necessary to know their nutrient requirements. In the radial measurements, it is not possible to consider the amount of submerged mycelium. The fungus was grown in different liquid media and dry mycelial weight was recorded at 10th day after inoculation in present study. The maximum dry mycelial weight of *F. semitectum* was obtained in Richards's broth (434 mg) which was significantly superior over all other media under study (Table.2). This was followed by potato carrot broth (413.67 mg) and potato dextrose broth (397 mg) which were at par with each other. Host extract broth could result in dry mycelial weight of only 119.67 mg though marked linear growth was recorded. Colour of fungal growth was off-white to cream colour in all liquid media. Results of present study are in harmony with findings of Biradar [5] in case of *F. semitectum* causing fruit rot of custard apple.

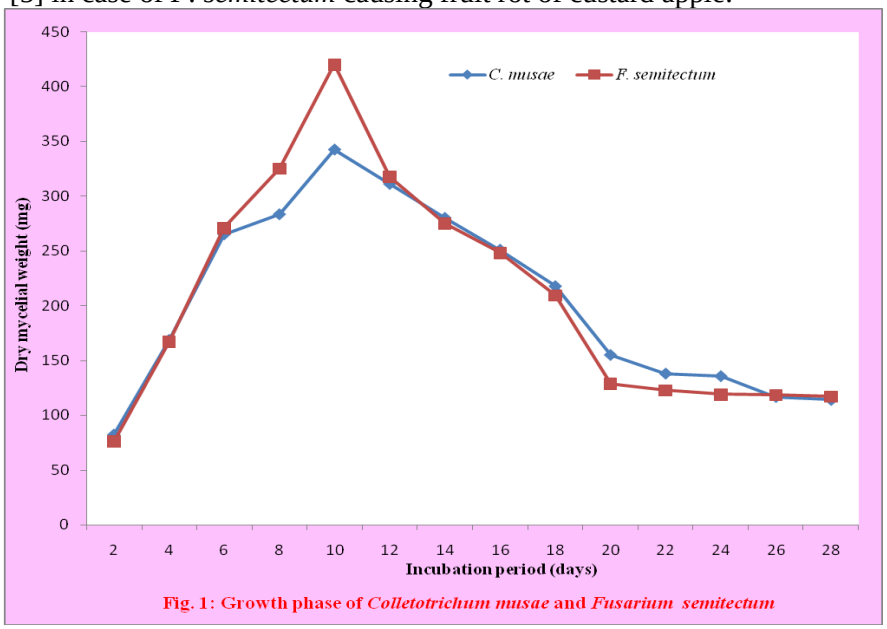

Fig. 1: Growth phase of *Colletotrichum musae* and *Fusarium semitectum*

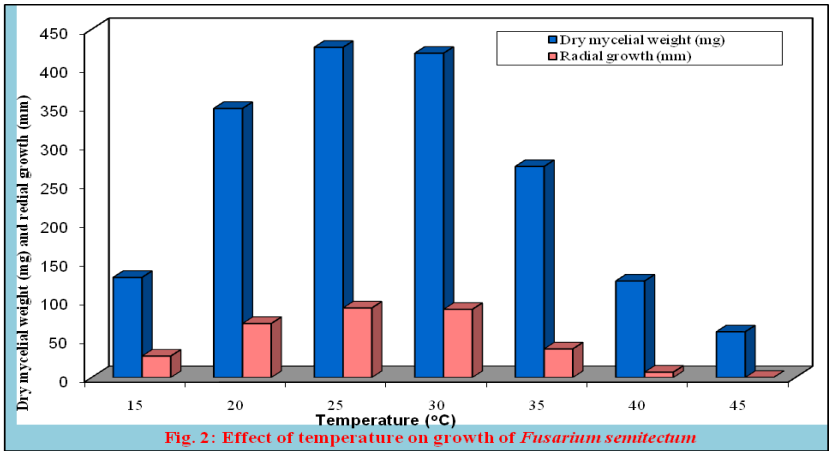

Fig. 2: Effect of temperature on growth of *Fusarium semitectum*

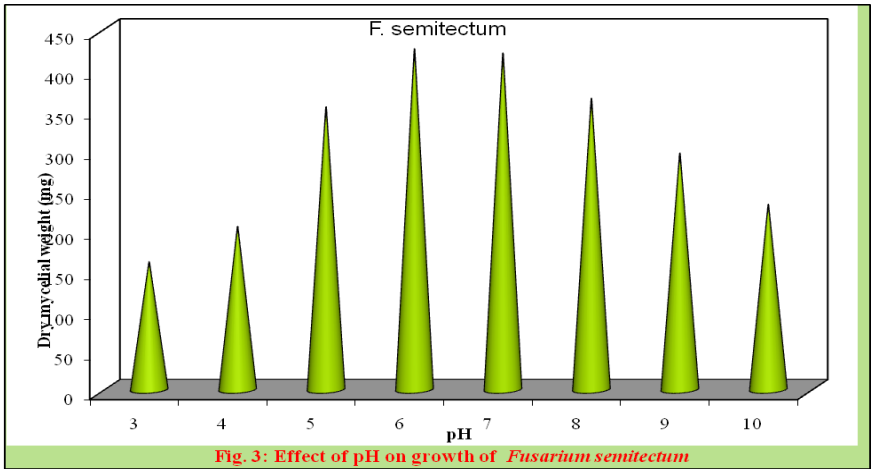

Fig. 3: Effect of pH on growth of *Fusarium semitectum*

Table 1: Cultural characters of *Fusarium semitectum* in different solid media

Media	Colony characters				Sporulation
	Radial growth (mm)	Type of growth	Colony margin	Colony colour	
Corn meal agar	41.72	Merged	Irregular, rough	Transparent white	+
Czapek's agar	52.85	Aerial	Circular, wavy	Cottony white	+
Host extract agar	87.35	Aerial	Circular, rough	Dull white	+
Malt extract agar	62.17	Submerged	Circular, smooth	Light pink	++++
Oat meal agar	86.62	Aerial	Circular, smooth	Creamy white	+++
Potato carrot agar	80.80	Raised	Circular, smooth	Milky white	++
Potato dextrose agar	89.00	Raised	Circular, rough	Dull white	++++
Richards's agar	88.38	Raised	Circular, smooth	Cottony white	++++
Sabouraud's agar	68.08	Aerial	Circular, wavy	Off white	++
V-8 juice agar	76.85	Submerged	Circular, smooth	Milky white	+
S.E m ±	**1.20**				
CD at 1%	**4.82**				
CV%	**2.83**				

Sporulation	Macro conidia/ microscopic field (400X)
++++	>40
+++	26-40
++	11-25
+	≤10

Table 2: Growth of *Fusarium semitectum* in different liquid media

Media	Dry mycelial weight (mg) of *F. semitectum*
Corn meal broth	84.00
Czapek's broth	219.33
Host extract broth	119.67
Malt extract broth	320.33
Oat meal broth	386.67
Potato carrot broth	413.67
Potato dextrose broth	397.00
Richards's broth	434.00
Sabouraud's broth	305.00
V-8 juice broth	211.00
S.E m ±	**4.62**
CD at 1%	**18.59**
CV%	**2.77**

Physiological studies

Effect of temperature: Temperature is the most important physical environmental factor for regulating vegetative and reproductive activity of the fungi. Effect of temperature on the growth of postharvest pathogen of banana was studied in present investigation, which had shown significant role in growth of *F. semitectum* (Fig.2). Growth and sporulation of *F. semitectum* was significantly influenced by temperature. Pathogen grew well at temperature levels 25^{o}C (426.67 mg) and 30^{o}C (419 mg) producing significantly maximum dry mycelial weight. Least growth was recorded at 45^{o}C. Optimum range of temperature for linear growth of *F. semitectum* was also found to be 25^{o}C (89.81 mm) and 30 ^{o}C (88.05 mm) which were statistically on par and superior over other levels of temperature. Sporulation of *F. semitectum* was better supported at 25 ^{o}C and 30 ^{o}C. Results of present study are in conformity with findings of Biradar [5] who concluded that optimum temperature required by *F. semitectum* was 30 ^{o}C. Meanwhile, Ingle and Rai [6] reported that different isolates of *F. semitectum* grew well at 28°C on potato dextrose agar.

Effect of pH: Different fungal pathogens require a particular range of pH for their growth and development. It was felt necessary to study pH as it might be one of the factors influencing pathogenesis. Maximum dry mycelial weight of *F. semitectum* was obtained at pH 6.0 (425 mg) which was on par with pH 7.0 (419.67 mg) and significantly superior over all other levels of pH and followed by pH 8.0 (363.33 mg) (Fig.3). Mycelial growth was raised and milky white to light brown in colour in most of the treatments. Similar results were obtained by Biradar [5] and Rawal *et al.* [9].

CONCLUSION

F. semitectum was a potential pathogen on banana causing crown rot, it grew well on potato dextrose agar, Richards's agar and Richards's broth. Optimum and growth conditions were 25-30 °C temperature and 6 - 7.0 pH.

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