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CULTURAL CHARACTERISTICS OF *CERATOCYSTIS FIMBRIATA* ELL. AND HALST. ON DIFFERENT SOLID MEDIA CAUSING WILT IN POMEGRANATE

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Abstract

Pomegranate wilt disease caused by *Ceratocystis fimbriata* is one of the important diseases of pomegranate adversely affecting crop cultivation in all major growing regions of India. At present, the crop is severely affected by wilt pathogen and day by day, the wilting severity is increasing at faster rate. Growth pattern of *C. fimbriata* on different solid media showed that, growth type was raised and mycelila colour, white to grayish and colony margin in petriplate, regular to irregular. Oat meal agar and carrot agar supported more pathogen growth. Endoconidia and aleurioconidia abundantly produced in all media tested. Production of perithecia was observed in carrot agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar and V8 juice agar except Czapek's agar, host leaf agar, host stem agar, Richard's agar, V8 juice and water agar.

Key words : Pomegranate, wilt, media, *Ceratocystis fimbriata* and disease.

Introduction

Pomegranate wilt disease caused by *Ceratocystis fimbriata* is one of the important diseases of pomegranate adversely affecting crop cultivation in all major growing regions of India. At present, the crop is severely affected by wilt pathogen and day by day, the wilting severity is increasing at faster rate. It was first noticed in two areas of the Bijapur district of Kanrnatka, India in 1990 which rapidly spreaded in the entire Bijapur district. The cause was not identified until 1995, however, the fungus *C. fimbriata* was isolated from discoloured stem, root and branch tissues on wilted plants in 1996. The disease is prevalent in parts of a Maharashtra, Karnataka, Andhra Pradesh, Gujarat and Tamil Nadu States in India. Pomegranate wilt results in complete wilting of plant and is characterized by the initial symptoms as yellowing and wilting of leaves on one to several branches. Initially symptoms only occurred on shoots, but later, leaves of the whole tree turned yellow and wilted, causing extensive defoliation and dieback and the xylem of the trunk turned brown to black with a star burst-like pattern. Finally, heavy infection resulting in the whole tree dying, causing severe yield losses leading to death of affected plants in a few weeks leading to loss to the farmers. The fungus derive food and energy from the substrate upon which they grow in nature, in order to culture the fungus in the laboratory, there is no universal substrate or artificial medium upon, which all the fungi can grow and reproduce. Therefore, studies were conducted in different suitable media to identify surface medium for the growth and sporulation of *Ceratocystis fimbriata*.

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Materials and Methods

Ceratocystis fimbriata, associated with wilt was isolated from the infected stems and roots of pomegranate plant, which were collected from Ganjalli field. The sliced pieces of collected stem portions with characteristic symptoms of vascular staining were surface sterilized with 1 per cent $NaHCO_3$ (sodium hypochlorite) for about 2 minutes and washed in alcohol (70%) and twice with sterile water to remove traces of $NaHCO_3$. Pathogen isolation was made using carrot bait technique (Moller and DeVay, 1968) in which, stems were placed in between the carrot disks and kept in a humid chamber

and incubated at 25 ± 2°C under 12 hour photoperiod (Moller and DeVay, 1968). After perithecium formation, a portion of the fungi was transferred to freshly prepared PDA and oat meal agar media to allow the full development of fungi. In order to confirm the identity of the fungus, the ascospores, aleroconidia, endoconidia and perithecia were observed under the high power (40x) microscope from Raichur isolates the pure culture. The identification of studies of pathogen has done as explained by Sharma *et al*. (2010).

The cultural characters of *C. fimbriat* were studied on the following twelve different solid media *viz*., Carrot agar, Czapek's agar, host leaf agar, host stem agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar, Rhichard's agar, Sabourd's agar, V8 juice agar and water agar. Twenty ml of each medium listed above was poured in to the Petri dishes for solidification. Five mm discs of *C. fimbriata* were placed at the centre of the plate. Each set of experiment was replicated thrice and plates were incubated at 26 ± 2°C. Observations were taken on parameters such as growth type, mycelial colour, type of margin, radial growth (mm) and presence or absence of endoconidia, aleurioconidia and perithecia. When the fungus covered complete petriplate in the media. The results were analyzed statistically.

Results and Discussion

The experiment on cultural characters of *C. fimbriata* on different solid media was taken up and characters such as colony diameter, colony color, presence or absence of endoconidia, aleuroconidia and perithecia were studied in this objective and results are summarized in table 1 and plate 1. The growth type of the fungus varied from flat to raised. The carrot agar, Czapek's agar, host leaf agar, host stem agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar, Rhichard's agar, V8 juice agar and water agar medium recorded flat type of growth, while *C. fimbriata* growth was raised on Sabourauds agar medium. The mycelial colour of *C. fimbriata* varied differently. The media *viz*., carrot agar, host leaf agar, oat meal agar, potato carrot agar, potato dextrose agar and V8 juice agar recorded grayish coloured growth. White colour growth was observed in Czapek's agar, host stem agar, Rhichard's agar and Sabourauds agar, while only malt agar medium showed light brown colour.

The fungus produced different type of growth margins on different media tested. Type of margin varied from regular (carrot agar, Czapek's agar, host leaf agar, host stem agar, malt agar, oat meal agar,

Table 1 : Effect of different solid media on growth of *Ceratocystis fimbriata.*

S. no.	Media	Growth type	Mycelial colour	Type of margin	Radial growth (mm)	Endoconidia	Aleurioconidia	Perethecium production
1.	Carrot agar	Flat	Grayish	Smooth, circular, regular	84.67(67.03) *	+	+	+
2.	Czapek's agar	Flat	White	Smooth, circular, regular	15.00(22.79)	+	+	-
3.	Host leaf agar	Flat	Grayish	Smooth, circular, regular	17.50(24.73)	+	+	-
4.	Host stem agar	Flat	White	Smooth, circular, regular	70.00(56.79)	+	+	-
5.	Malt agar	Flat	Light brown color	Smooth, circular,Irregular	80.33(63.68)	+	+	+
6.	Oat meal agar	Flat	Grayish	Smooth, circular, regular	90.00(71.57)	+	+	+
7.	Potato carrot agar	Flat	Grayish	Smooth, circular, regular	82.00(64.90)	+	+	+
8.	Potato dextrose Agar	Flat	Grayish	Irregular	22.27(28.16)	+	+	+
9.	Richard's agar	Flat	White	Smooth, circular, regular	12.50(20.70)	+	+	-
10.	Sabouraud's agar	Raised	White	Irregular	11.00(19.37)	+	+	-
11.	V8 juice agar	Flat	Grayish	Regular	30.00(33.21)	+	+	+
12.	Water agar	Flat	White	Smooth, circular, regular	10.00(18.43)	+	+	-
				S. Em. ±		0.49		
				C.D at 1%		1.93		

- Absent, +Present, * Figures in parenthesis arc sine transformed value.

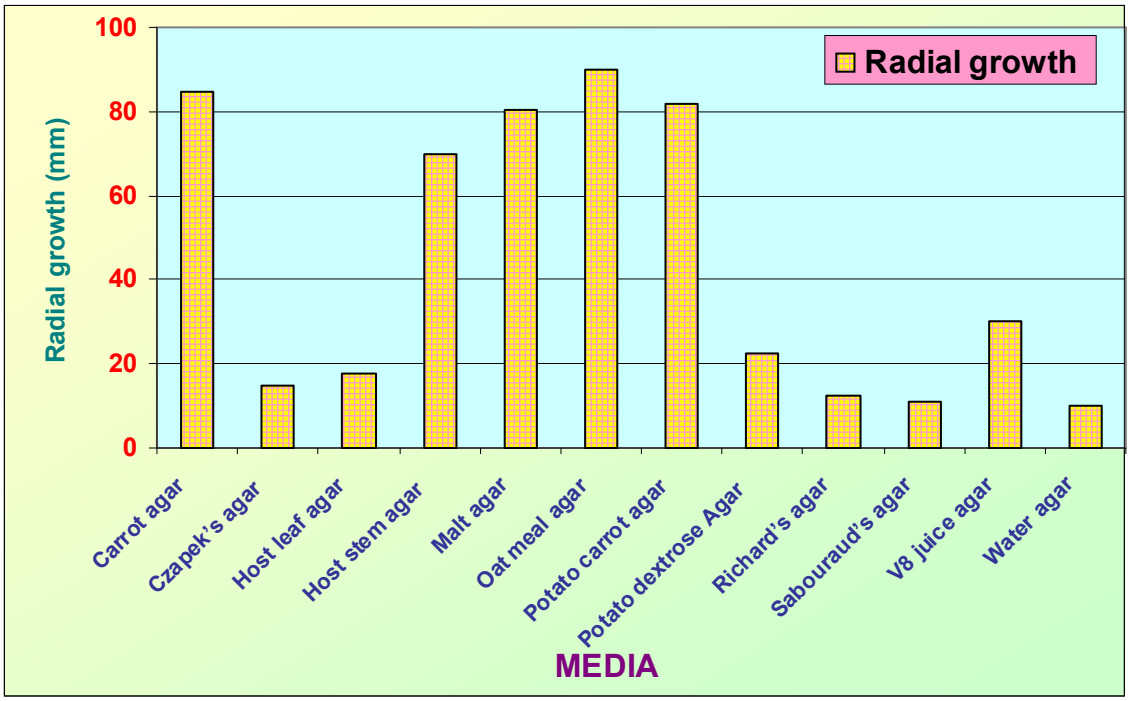

Fig. 1 : Mycial growth of *Ceratocystis fimbriata* on different solid media.

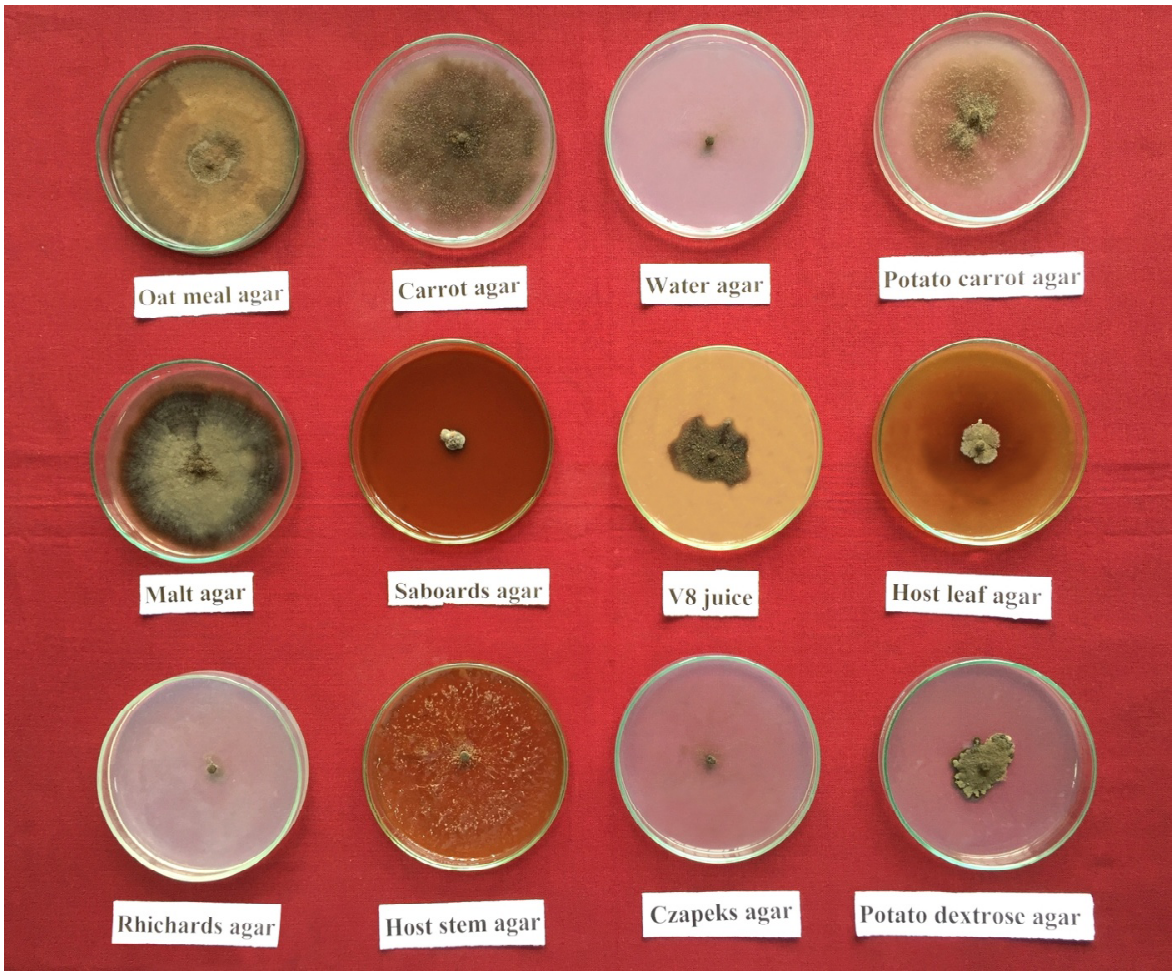

Plate 1 : Cultural characters of *Ceratocystis fimbriata* on different solid media.

potato carrot agar, Rhichard's agar, V8 juice agar and water agar) to irregular (Sabourd's agar and potato dextrose agar). Among the various solid media, significantly more growth of *C. fimbriata* was observed on oat meal agar with mean colony diameter of 90 mm followed by carrot agar (84.67 mm) and least mean colony diameter of 10.00 mm was observed in water agar. The results indicated that endoconidia and aleurioconidia abundantly produced in all media (carrot agar, Czapek's agar, host leaf agar, host stem agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar, Rhichard's agar, V8 juice agar, water agar media and Sabourauds agar medium). Production of perithecia was observed in carrot agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar and V8 juice agar and some are not produce perithecia in Czapek's agar, host leaf agar, host stem agar, Rhichard's agar, Sabourd's agar and water agar after 13-16 days.

Fungi secure food from the substrate upon which they live in. In order to culture the fungus in the laboratory, it is necessary to furnish the essential elements and compounds in the medium, for their growth and other life processes. All media are not equally good for all fungi, nor there can a universal substrates or artificial medium, upon which all fungi to grow. Therefore, twelve different solid media were tried to find out superior media for the growth of the pathogen. During the study, there was variation in the colony characters of isolate *C. fimbriata* with respect to growth type, mycelia colour, type of margin, endoconidia, aleurioconidia and perethecia production. The growth type of the fungus varied from flat to raised. The growth of fungus was flat in carrot agar, Czapek's agar, host leaf agar, host stem agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar, Rhichard's agar, V8 juice agar and water agar medium, while it was raised on Sabourauds agar medium. Further, carrot agar, host leaf agar, oat meal agar, potato carrot agar, potato dextrose agar and V8 juice agar recorded grayish coloured growth. White colour growth was observed in Czapek's agar, Host stem agar, Rhichard's agar and Sabourauds agar, while only malt agar medium showed light brown colour. The fungus produced different type of growth margins on different media tested. Type of margin varied from regular to irregular in different media. Significantly more growth was observed on oat meal agar fallowed by carrot agar (fig. 1). El-Wakil *et al*. (1985) concluded that glucose to be the best carbon source for linear growth of the fungus. Endoconidia and aleurioconidia abundantly produced in all media tested. Production of perithecia was observed in carrot agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar and V8 juice agar and Czapek's agar did not produce perithecia. Similar findings were reported by several workers (Rokibah *et al*., 1988; Bachiller, 1998 and Chaudhari *et al*., 2016). Sonyal *et al.* (2015) evaluated the eight solid media, among them the best mycelia growth was on oat meal agar (9.0 cm) followed by Richards agar (8.4 cm) and potato dextrose agar (8.3 cm).

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