Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **3 (4):** 23-27 (2015)

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Research Article

OPEN ACCESS

Cultural Characteristics of *Ceratocystis fimbriata* ELL. and Halst. Causing Wilt in Pomegranate

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ABSTRACT

Pomegranate is one among the major fruit crops of arid zone. Cultural studies of Ceratocystis fimbriata, causal agent of wilt of pomegranate on different solid media. The fungus was grown on eight solid media. The results indicated that the best mycelial growth was made on oat meal agar (9.0 cm) followed by Richard's agar (8.4 cm) and Potato Dextrose Agar (8.3 cm). Least growth was recorded in Raulin´s media (1.4 cm) with Host leaf extract agar (2.1 cm) showing similar results. Perithecium production on PDA was faster than other media and also perithecium was produced in Malt agar, Oat meal agar and Richard's agar. On PDA abundant mycelial growth, uniformly white, uniformly dense, cottony appearance of mycelium at the periphery, very prominent pin head structure, undulated colony margin.

Key words: *Oat meal agar, Potato dextrose agar, perithecium, solid media*

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient fruit, belonging to the smallest botanical family punicaceae and pomegranate is a native of Iran. It is commercially an important fruit crop of both tropical and subtropical regions. In India, it is regarded as a "vital cash crop", grown in an area of 1, 16,000 ha with a production of 89,000 MT with an average productivity of 7.3 MT. Karnataka state has the distribution of cultivating pomegranate under tropical condition in an area of 12,042 ha with a production of 1, 29, 547 tonnes. Where this crop has spread across different districts *viz*., Bijapur, Bellary, Bagalkot, Koppal, Chitradurga, Belgaum, Davangere, Tumkur, Bangalore and Gulbarga. Pomegranate suffers from ten economically important diseases, among them bacterial blight or spot, fruit rot, anthracnose and wilt complex are severe and cause significant losses in recent years. Wilt caused by *Ceratocystis fimbriata* is the most severe disease in Karnataka which causes yellowing, drooping and death of pomegranate plant 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.*

MATERIAL AND METHODS

Pomegranate roots, stem and soil were collected from infected orchard and used for isolation of the fungus *in vitro*. The isolation of the fungus was made by following standard tissue isolation technique. Identification of the fungus was carried out based on morphological characters of the isolated fungus. Selection of the basal medium for growth, sporulation and perithecium of the fungus was done by using four non synthetic or semi synthetic medium, Potato dextrose agar, Host leaf extract agar, Oat meal agar and Malt extract agar and also four synthetic medium, Czapeck's (dox) agar, Raulin`smedia,Sabouraud`s dextrose media and Richard`s agar. The composition and preparation of the above mentioned synthetic and semi- synthetic media were obtained from Ainsworth and Bisby's 'Dictionary of the Fungi' by Hawksworth*et al*.,[1]. Twenty ml of each media was poured aseptically into Petriplates of 90 mm diameter. Five mm discs from an actively growing zone of ten days old culture was placed upside down at the centre of the solidified medium and were incubated at 27 ± 1^0C. Each treatment was replicated five times. The radial measurements of the colony of the fungus were taken when maximum growth was attained in any one of the media tested. The various cultural characters like, rate of growth, colour, sporulation on different media and perithecium production were recorded at 5, 10 and 16 days interval. The measurements of colony diameter on different media were measured.

RESULTS

The growth characters of the fungus were studied on eight different media (Table 1, Fig. 1 and Plate 1).The results revealed that both colony growth, perithecium production of *C. fimbriata* varied significantly in different media. Among the eight media, Oat meal was superior to support the colony growth (9.00 cm) followed by Richard's (8.40 cm), PDA (8.30 cm) and lowest growth in Raulin´s media (1.40 cm) followed by Host leaf extract (2.10 cm). Perithecium production on PDA was faster than other media and also perithecium was produced in Malt agar, Oat meal agar and Richard's agar. On PDA abundant mycelial growth, uniformly white, uniformly dense, cottony appearance of mycelium at the periphery, very prominent pin head structure, undulated colony margin.

Table 1: Mean colony diameter of *Ceratocystis fimbriata* on different solid media at different days of incubation

S. No.	Medium	Mean colony diameter (cm)		
		5 days	10 days	16 days
Non synthetic or semi synthetic media				
1	Host leaf agar	0.8	1.3	2.1
2	Malt agar	2.1	4.0	7.3
3	Oat meal agar	2.9	5.2	9.0
4	Potato dextrose agar	2.5	4.6	8.3
5	Sabouraud's agar	1.9	3.2	6.7
	SE m±	0.24	0.43	0.63
	CD @ 1%	1.05	1.86	2.72
Synthetic media				
6	Czapek (dox) agar	1.8	3.3	6.3
7	Raulin's media	No growth	0.9	1.4
8	Richard's agar	2.6	4.7	8.4
	SE m±	0.61	1.10	2.07
	CD @ 1%	2.66	4.79	8.95

Table 2: Cultural characteristics of *Ceratocystis fimbriata* on different solid media after 16 days of incubation

S. No	Medium	Growth characters	Pertheciumproduction
Non synthetic or semi synthetic media			
1	Host leaf agar	Very poor mycelial growth with grayish centers and erect margins.	-
2	Malt agar	Abundant mycelial growth,unifomely dense growth with light brown colour centre, grayish white growth pheriphery, prominent pin head structure, undulated colony margin	+
3	Oat meal agar	Abundant mycelial growth, grayish white colour,not uniformly dense, moderate pin head growth at centre, moderate cottony appearance.	+
4	Potato dextrose agar	Abundant mycelial growth, uniformly white, uniformly dense, cottony appearance of mycelium at the pheriphery, very prominent pin head structure, undulated colony margin	+
5	Sabouraud's agar	Abundant mycelial growth, uniformly white, uniformly dense, cottony appearance of mycelium with smooth edge	-
Synthetic media			
6	Czapek's (dox) agar	Uniformly dense growth, ash colour mycelium prominent pin head structure at center with undulated colony margin.	-
7	Raulin's media	Very poor mycelial growth with grayish white colour	-
8	Richard's agar	Abundant mycelial growth, white colored mycelium, dense growth at the centre and moderate growth at pheriphery, prominent pin head at center, cottony appearance of mycelium at pheriphery.	+

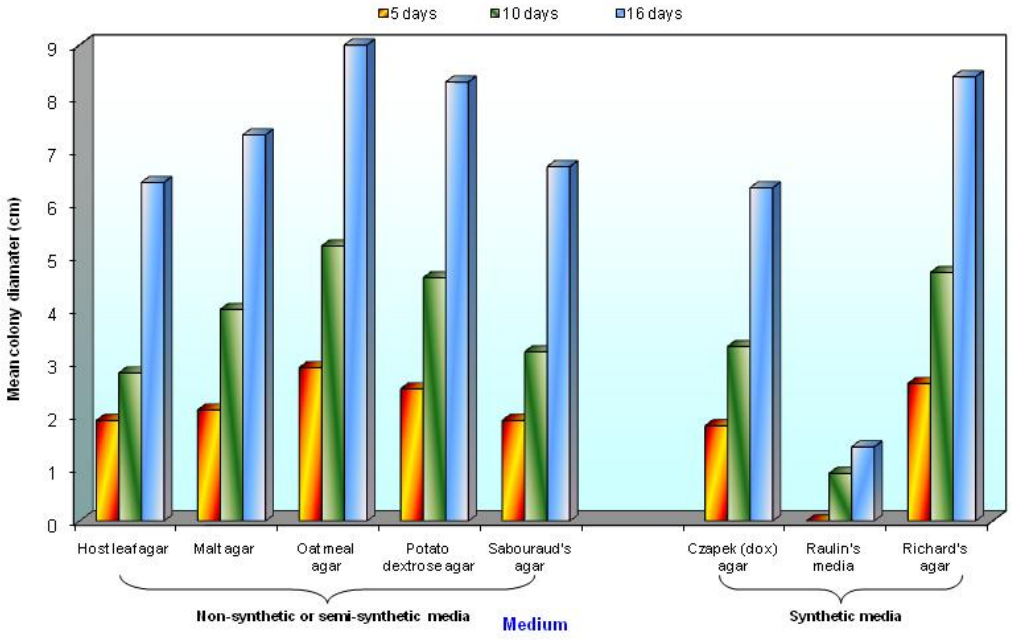
\- Absent
\+ Present

Plate 1: Growth of *Ceratocystis fimbriata* on different solid media at 5, 10 and 16 days of incubation

Synthetic Media

Non-Synthetic/Semi Synthetic Media

Fig. 1: Growth of *Ceratocystis fimbriata* on different solid media at 5, 10 and 16 days of incubation



DISCUSSION

Every living being requires food for its growth and reproduction and fungi are not exception. Fungi derive the food from the substrate upon which they grown. In order to culture fungi artificially, it is necessary to supplement in the medium, those essential nutrients needed for their growth and development. So, to find out the best source of nutrients for the fungus, different synthetic and non-synthetic media were tested. The radial growth of the fungus was used to determine growth on solid media.

The fungus was grown on eight solid media. The results indicated that the best mycelial growth was made on oat meal agar (9.0 cm) followed by Richard's agar (8.4 cm) and Potato Dextrose Agar (8.3 cm). Least growth was recorded in Raulin´s media (1.4 cm) with Host leaf extract agar (2.1 cm) showing similar results. Similar observations were made by many workers on *C. paradoxa*[2,3].

There was no much difference in the measurements of colony diameter taken five days after inoculation on different media. However, the difference was substantial at 10 and 16 days after inoculation. A comparison of oat meal agar, Richard's agar and Potato Dextrose Agar at sixteen days after inoculation revealed that the presence of fungal bit in the medium almost doubled the diameter of fungal colony. This observation is in direct relation to the preference shown by *C. paradoxa*[4].

Perithecium production was observed in PDA. Oat meal agar, Malt agar and Richard's agar. In *C. paradoxa*, sporulation was observed by Yadahalli[4].

Every living organism has a definite pattern, in which it attains a maximum growth and declines thereafter. In the present study, the fungus *C. fimbriara* attained maximum growth after sixteen days of incubation in Potato Dextrose Broth and thereafter a decline in dry mycelial weight was observed[4].

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