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Morphological Characteristics of *Ceratocystis fimbriata* Ell. and Halst. Causing Wilt in Pomegranate

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ABSTRACT

The Pomegranate (Punica granatum L.), an ancient and commercially important fruit of both tropical and subtropical countries, belongs to the smallest botanical family punicaceae. It is known as the 'fruit of paradise' native of Iran, but spread to the Mediterranean countries at an early date. On oat meal agar C. fimbriata margin colour was grayish and colony diameter was 90 mm after 16 days of incubation at room temperature, with age owing to production of aleurioconidia, endoconidia, ascospores and perithecium. Black colored perithecia with globose base was observed with size of 181.1 x 131.2 µm, exuding small, hyaline and hat shaped ascospores from the apex of the perithecium which measure 5.13 x 4.27 µm. Endoconidia were hyaline, cylindrical and average size was 23.6 x 4.90 µm. Aleurioconidia were thick walled ellipsoidal or pyriform with size of 18.5 x 10.10 µm.

Key words: *Pomegranate, Wilt, Media, Cultural and Morphological*

INTRODUCTION

Pomegranate (*Punica granatum*) is an important fruit crop, belonging to the family Punicaceae. Pomegranate is a good source of carbohydrates and minerals such as calcium, iron and sulphur. It is rich in vitamin C and citric acid is the most predominant organic acid. Glucose (5.46%) and fructose (6.14%) are the main sugars with no sucrose in fruits. The fruits of pomegranate are known to possess pharmaceutical and therapeutic properties. Sweet varieties are mildly laxative, sour types are good source for curing inflammation of stomach and heartache. The flower buds are very useful in Ayurveda for managing bronchitis. The bark of the stem, root and rind of the fruit is used for slimming, control of dysentery, diarrhea and killing tape worms. Successful cultivation of pomegranate in recent years has met with different traumas such as pest and diseases. At present, 15 to 20% crop is severely affected by wilt pathogen and day by day the wilting severity is increasing at faster rate.

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Pomegranate wilt is an important disease which results in complete wilting of plant. The disease is prevalent in parts of a Maharashtra, Karnataka, Andhra Pradesh, Gujarat and Tamil Nadu, states in India in Jammu and Kashmir reported recently. 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 results 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. Wilt is a destructive disease of many economically important crops caused by the soil borne fungus *Ceratocystis fimbriata* Ell. and Halst. and is a common soil pathogen and saprophyte that feeds on dead and decaying organic matter.

MATERIAL AND METHODS

Isolation of the pathogen

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[4] 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[4]. 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*[5].

Hyphal tip isolation

This method was followed for maintaining of pure culture. Hyphal tip isolation was done on water plates. Dilute spore suspension of the pathogen was prepared in sterilized distilled water containing eight to ten spores per ml from 15 days old culture. One ml of such suspension was spread uniformly on two per cent solidified water agar plates and observed for spores under the microscope. Single spore was marked with a marker on backside of the Petri plate and it was allowed to germinate. Such plates were periodically observed for spore germination under microscope. The hyphae growing from each cell of the single spore was traced and marked with marker. The tip of the hyphae was cut carefully and transferred to PDA plates and incubated at 25 ± 2^o C for 15 days. Later, mycelial bits of the fungus were transferred in the centre of petri plates containing PDA and incubated at 25 ± 2^o C for 15 days. Saltation or sectoring was observed in the culture to confirm the pure culture of the fungus.

Maintenance of the culture

The hyphal tip cultures of the fungus were sub-cultured on potato dextrose agar slants and kept in laboratory at 25 ± 2^o C for 15 days. Such mother culture slants were preserved at 5^o C in refrigerator. Further, these cultures were sub-cultured once in a month and used for future studies.

Morphological characters

C. fimbriata was morphological characterized for production of aleurioconidia, endoconidia, ascospore and perithecia. For this, the growth of *C. fimbriata* was selected from 21 days old pure culture and kept on a clean sterile glass

slide using sterilized needle. With the help of fluorescent microscope, the length and breadth of aleurioconidia, endoconidia, ascospore and perithecia in µm were measured. Three observations were recorded from the pure culture of fungus to maintain. Ten aleurioconidia, endoconidia, ascospores and perithecia were picked up randomly to determine the diameter and *C. fimbriata* was characterized for colony color and growth pattern on oat meal agar. The mycelial disc of 5 mm diameter was cut from periphery of actively growing culture of *C. fimbriata* and transferred aseptically to a 90 mm Petri dish containing 20 ml of oat meal agar and incubated for a period till the fungal growth covered the complete petri plate in the media at 26 ± 2^{o} C. The colony was characterized for phenotype and growth pattern. Different morphotypes colony colour, type of colony, type of margin, margin colour and colony growth were observed *in vitro*.

RESULTS AND DISCUSSION

Morphological features of *C. fimbriata* were described by growing the isolates on oat meal agar as described in Table 1, Plate 1a and Plate 1b. On oat meal agar, the colour of colony of *C. fimbriata* was grayish on oat meal agar with flat type of colony growth and regular type of margin. The margin colour was grayish and colony diameter was 90 mm after 16 days of incubation at room temperature. The colour of colony changed to grayish colour with age owing to production of aleurioconidia, endoconidia, ascospores and perithecium. Black colored perithecia with globose base was observed with size of 181.1 x 131.2 µm, exuding small, hyaline and hat shaped ascospores from the apex of the perithecium which measure 5.13 x 4.27 µm. Endoconidia were hyaline, cylindrical and average size was 23.6 x 4.90 µm. Aleurioconidia were thick walled ellipsoidal or pyriform with size of 18.5 x 10.10 µm (Table 1).

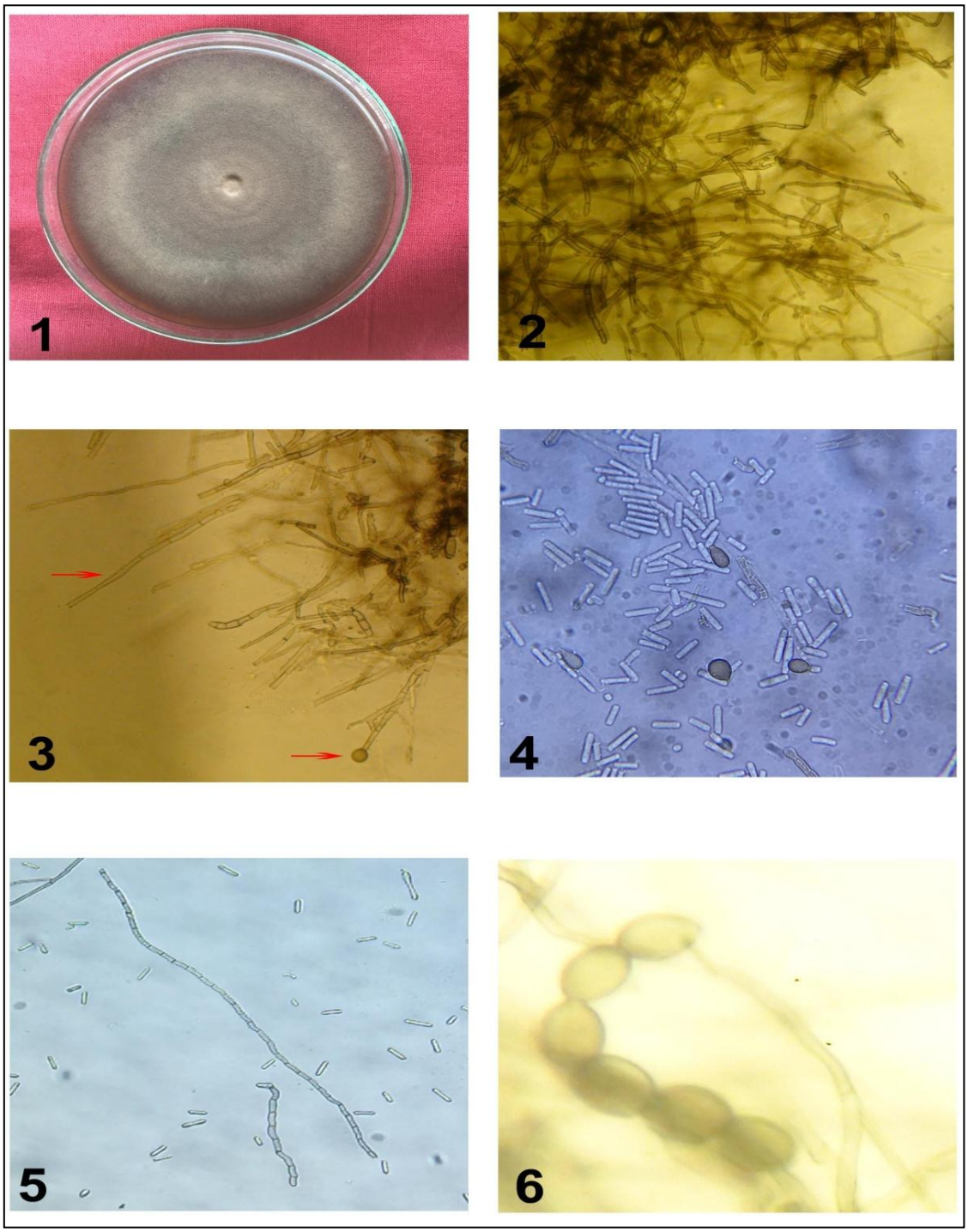
The *C. fimbriata* produced grayish coloured colony with flat type with regular type of margin on oat meal agar. In the present investigation, it was observed that the margin colour was grayish and colony diameter was 90 mm after 16 days of incubation at room temperature. The colour of colony changed to grayish colour with age owing to production of aleurioconidia, endoconidia, ascospores and perithecium. Black colored perithecia with globose base was observed with size of 181.1 x 131.2 µm, exuding small, hyaline and hat shaped ascospores from the apex of the perithecium which measure 5.13 x 4.27 µm. Endoconidia were hyaline, cylindrical and average size was 23.6 x 4.90 µm. Aleurioconidia were thick walled ellipsoidal or pyriform with size of 18.5 x 10.10 µm. Similar results with respect to morphological characters were reported by several workers[1,3,6,7]. Faisal *et al*[1]., explained similar morphological characteristics of the fungus which showed perithecia brown to black with globose base, necks almost 800-900 µm long with ostiolar hyphae. Ascospores elliptical 4-8 x 25 µm, hyaline, non septate, hat shaped appearance. Conidiophores hyaline, septate up to 150 µm long. Conidia cylindrical, sometimes in chains, truncate at the ends.

Table 1. Morphological characteristics of *C. fimbriata* on oat meal agar

Sl. No.	Aleurioconidia (µm) (L x B)	Endoconidia (µm) (Lx B)	Ascospore (µm) (L x B)	Perithecia (µm) (L x B)*
1	18.5 x 10.10	23.6 x 4.90	5.13 x 4.27	181.1 x 131.2

*L-length, B-breath

Plate 1a. Morphological characters and asexul structures of *Ceratocystis fimbriata*



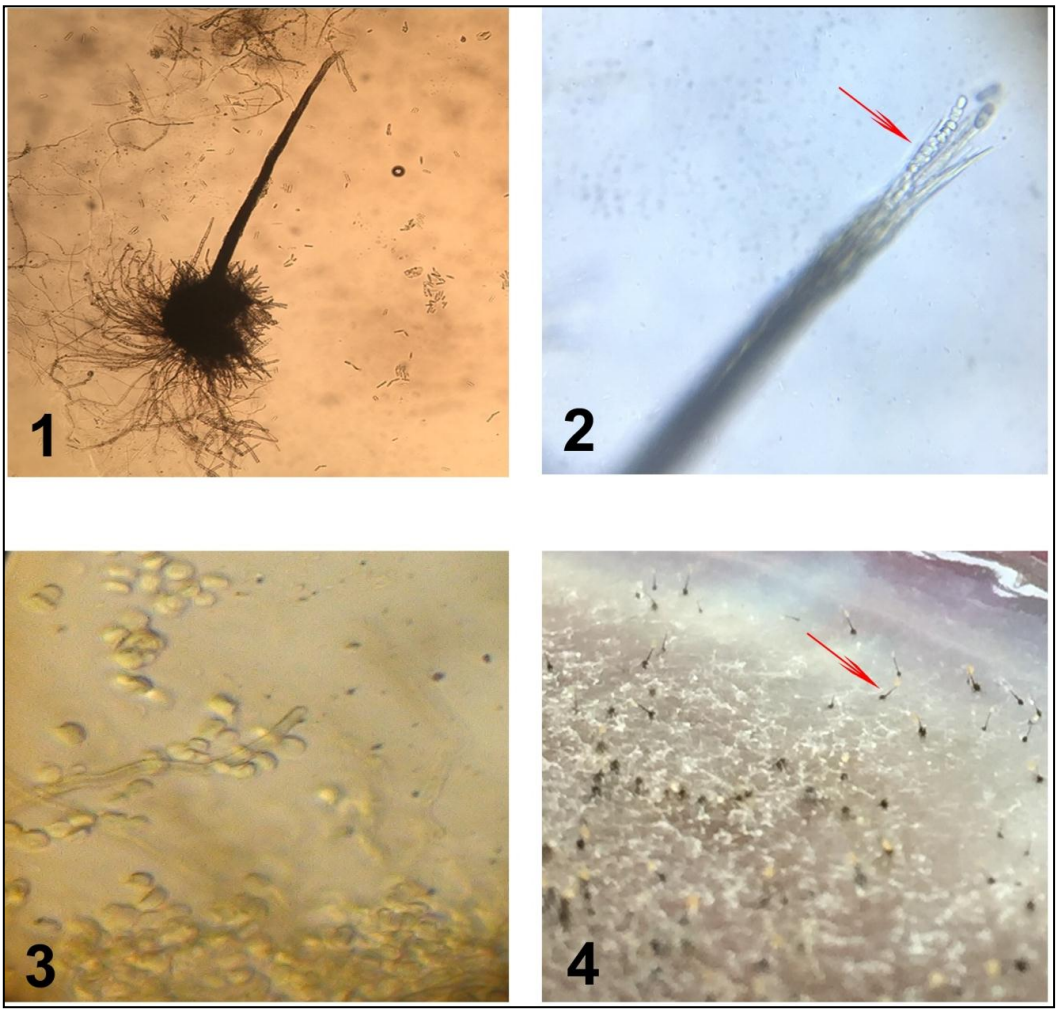
1) Pure culture of pathogen 2) Mycelia (40x)

3) Conidiophore producing both cylindrical endoconidia and aleurioconidium (40x)

4) Endoconidia and aleurioconidium (40x) 5) Chain of endoconidia (10x)

6) Chain of aleurioconidia (40x)

Plate 1b. Sexual structures of *Ceratocystis fimbriata*



(1) Perithecia (2) Ostioles and ascospores

(3) Hat-shaped ascospores (100x) (4) Sticky droplets of ascospores on perithecia

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