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Proving Pathogenicity 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. Pathogenicity test was carried out on six-month old pomegranate plants C. fimbriata pathogen was inoculated on pomegranate. The time of appearance of first symptoms like yellowing of leaves in some twigs or branches, followed by drooping and drying of leaves as the external signs as observed during present investigation. The leaves turned pale yellow starting from lower branches and progressed upwards. Later, partial wilting of the plant with drying and death of some branches took place. The isolate produced wilting symptoms 40 days after inoculation.

Key words: *Pomegranate, Wilt, Pathogenicity, Day and Symptom*

INTRODUCTION

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. Pomegranate is a crop of high impact and is a vital cash crop of India gaining greater economic importance. In India, it is grown in an area of 113 ha with a production of 745 milloin tons[1]. Among the different states growing pomegranate, Maharashtra is the largest producer occupying 2/3rd of total area in the country followed by Karnataka, Andhra Pradesh, Gujarat and Rajasthan. In India, it is regarded as a "vital cash crop". The fruit is symbolic for its cool, refreshing juice and valued for its medicinal properties. Pomegranate wilt disease caused by *Ceratocystis fimbriata* Ell. and Halst. is one of the important diseases of pomegranate adversely affecting crop cultivation in all major growing regions of India.

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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.

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[5] 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[5]. 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*[6].

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.

Pathogenicity

Pathogenicity tests were conducted on six-month-old seedlings of pomegranate *cv*. Kesar, raised in plastic pots (30 x 45 cm). Potting mixture was sand: red soil: FYM (1:2:1) and it was sterilized in autoclave at 1.1 kg/cm^2 (121^0 C) pressure for 30 min. successively for two days. Roots were superficially wounded by peeling 1 mm deep and 0.5 cm long to the epidermis with sterilized razor blade. The wounded area in each plant was inserted with *C. fimbriata* culture using a sterilized needle and wrapped with cotton cloth (moistened with

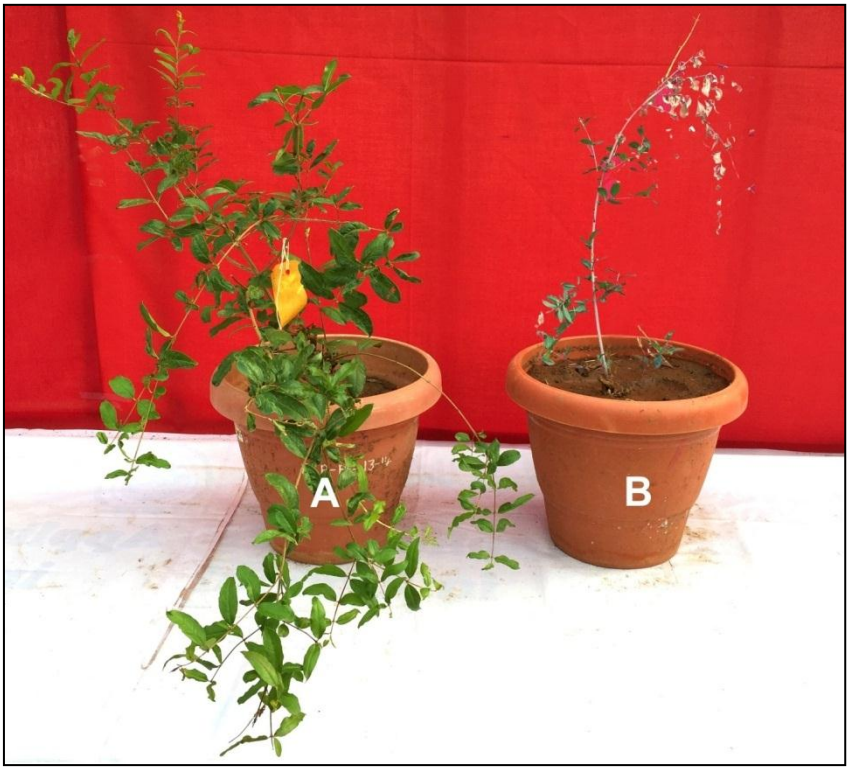
sterile distilled water) and plastic film. The method was replicated thrice with inoculation on other two plants under glasshouse condition. Plants which were inoculated with distilled water served as control. The inoculated plants were kept in glass house (average temperature of 27°C) for further observation. Once the artificially inoculated plants develop typical symptoms, the disease samples were collected and the organism was re-isolated on potato dextrose agar medium thus confirming the Koch's postulates to establish the pathogen and proving pathogenicity.

RESULTS AND DISCUSSION

Pathogenecity for the *C. fimbriata* was carried out. The time of appearance of first symptoms like yellowing of leaves in some twigs or branches, followed by drooping and drying of leaves as the external signs as observed during present investigation. The leaves turned pale yellow starting from lower branches and progressed upwards. Later, partial wilting of the plant with drying and death of some branches took place. The isolate produced wilting symptoms 40 days after inoculation (Plate 1). Again, the fungus was re-isolated from such wilted plants from pots and was found to resemble the original culture of *C. fimbriata* thus proving the pathogenicity.

To prove the Koch's postulates, *Ceratocystis fimbriata* was isolated from affected tissue of root and inoculated to plants under glass house condition. After 40 days, pathogen produced typical symptoms of the diseases such as yellowing of foliage of one or two branches of plant followed by yellowing and drooping of foliage of the entire plant within 72 days. When split open the affected root, dark grayish-brown streaks like brown black discoloration in vascular and adjoin cortex tissues were observed. The pathogen was re-isolated from such symptoms and compared with original culture for its conformity. Pathogen produced mycelial growth which was whitish grey in colour and endoconidia, aleurioconidia and perithecium were also produced by re-isolated pathogen. Similarly pathogenicity was proved by earlier workers with description that was observed during the present study[2,3,4,7,8].

Plate 1. Prooving pathogenicity



A) Healthy plant **B) Wilted plant**

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