

Cultural and morphological variability among isolates of *Myrothecium roridum* causing leaf spot of bael

ABSTRACT

This experiment was executed at College of Horticulture, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.) during 2019-21. This experiment was conducted to estimate the variation present among the isolates of *Myrothecium roridum* causing leaf spot of bael against leaf spot of bael. The fungus produces branched aseptate and greenish mycelia, conidiophores & mycelium were branched, aseptate, greenish & conidia were hyaline. Conidiophores were branching twice or thrice, and each branch gave rise to almost two to five phialides in whorls. Among all the five isolates, isolate Mr2 (6.12 & 2.16m) had the smallest length and width & Mr5 (7.80 & 3.45m) had the longest, according to the mean.

Keywords: *Myrothecium*, mycelial growth, variability, botanicals, fungicides

Introduction

Bael (*Aegle marmelos*) is a fruit crop of family Rutaceae and is among the medicinally treasured tree species of the world (Chanda, 2008) out of more than 250,000 living plant species on earth. Although it is native to northern India, its being cultivated in different countries of world such as Thailand, Bangladesh, Pakistan, Sri Lanka, and Burma. Bael is one of the most recognizable plants used in ayurvedic medicine by the Indians as well as other South Asian countries in ancient history (Jagetia and Baliga, 2004). Despite being a subtropical tree, bael is remarkably adaptable and thrives in tropical, desert, and semi-arid environments. (Singh *et al.* 2018a). While fertile and well-drained soils are best for its commercial production, trees can thrive and bear fruit on lands that are unsuitable for other crops, such as those that are rich in limestone and stones, swampy conditions, and extremes of soil pH ranging from 5 to 10 (Saroj *et al.* 2006, Singh *et al.* 2016). According to estimates, the country's enhanced bael cultivars are planted on around 1000 acres of land and produce 10,000 tonnes of fruit per year (Singh *et al.* 2018a).

This fruit has great importance in Ayurvedic medicines. The root is also a key component of "dasmula," an Ayurvedic remedy regarded to be a miraculous treatment for digestive problems, which contains 10 roots. Astringent, antidiarrheal, antidycentric, antipyretic, antiulcer, anti-diabetic, antibacterial, antiviral, antifungal, anticancer, analgesic, radioprotective, antiviral, antimicrobial, and anti-helminthic are some of the

different plant properties that are used in ethnomedicine as a result of scientists and researchers' recent efforts to identify and derive substances for treating various diseases. Purified compounds from various tree components have also shown inhibitory effects on a range of diseases (Maity et al.2009, Patkar et al.2012). For ulcers and ophthalmia, a leaf poultice is applied. Dropsy, beriberi, catarrh, and asthma are some conditions that can be treated with fresh leaves (Maity et al.2009). Bael leaf powder can be used to treat bowel syndrome (Atul et al.2012). It is applied as a corrective measure while treating beriberi. (Sampath et al.2012). There are several known medicinal effects of bael leaf essential oil (FawziMahomoodallyet al.2018). The flower is utilised in epilepsy because it has astringent and antibacterial characteristics (Gautam et al.2014). According to a study, inhibiting nitric oxide boosted prostaglandin E2 (PGE2) production and mRNA expression, which improved rat wound healing (Shirwaikaret al.2003).

Both flavonoids and polyphenols are present in significant amounts in beel. Several health-promoting polyphenols have been found to be abundant in bael fruit pulp and fruit juice. Ripe fruit juice extract reduces blood sugar levels because of its bitter flavour. Chronic gastrointestinal conditions, piles therapy, and rectum discomfort have all been found to be cured (Dhankaret al.2011, Kirtikaret al.1984). In bael pulp, alkaloids, flavonoids, phenolic chemicals, and terpenoids have gathered. Alkaloids, coumarins, polysaccharides, and carotenoids are some of the most significant polyphenols and flavonoids. The amount of polyphenols in bael varies on its stage of development (Gurjar et al.2019).

Bael is a seasonal fruit that is mostly available in May and June and may not be available at other seasons, therefore its health advantages cannot be used all year round. (Sharma et al.2007). Therefore, these issues motivate the creation of various fruit-based products. Juice can be used to make a variety of drinks. Jam, slab, and bael powder are examples of dehydrated bael products. It can also be used to make tea and wine. Making a variety of items with bael is therefore very profitable economically.

According to Sharma et al.(2007), bael is apparently incredibly powerful at fending off a variety of pathogenic organisms, including antibacterial, antiviral, anti-inflammatory, and anti-fungal agents. Bael root extract in chloroform has demonstrated promising anti-microbial efficacy against a number of pathogens that cause diarrhoea, including *Vibrio cholera*. *Shigella sp.* and *Escherichia coli* are two more microorganisms. Methods like agar dilution and disc dilution have been used to assess the activity and could be compared to the medication ciprofloxacin(Mazumder et al.2006). In another study conducted by Rani

and Khullar in 2004, it was shown that the methanolic extract of bael had an effect on *Salmonella typhi*. Its anti-inflammatory, anti-diarrheal, anti-hyperlipidemic, and anti-diabetic properties are attributed to *Ciceris*, *Alternaria alternate*, *Colletotrichum capsici*, *Curvularia lunata*, *A. brassicae*, *Fusarium oxysporum*, and Bael's mechanism (SOD, glutathione). The essential oils extracted from bael suppress the spore germination of eight diseases, including *Ustilago cynodontis* and *F. udum* (Rani & Khullar, 2004).

But it has been hampered with a lot of various fungal diseases like root rot, leaf spot, dieback caused by *Fusarium solani*, *Myrothecium roridum* and *Alternaria alternate* respectively (Anonymous, 2016), out of which leaf spot caused by *Myrothecium roridum* have proved to be a prominent hindrance in its cultivation in nursery plants (Anonymous, 2020). The main problem with this fungus is that the symptom observed on bael leaves are morphologically much more similar with *A. solani* because same symptoms with necrosis around the leaves and the concentric ring formation creates a more confusion. (Tuloch, 1972),

There are eight known species of the genus *Myrothecium*, many of which are saprophytes found in soil. *M. roridum*, which is recognised as a serious plant pathogen, has an adverse effect on more than 200 species of plants from different botanical families. Additionally, it has been recognised as a significant cucurbit pathogen that causes leaf spot and blight. *M. roridum* has been isolated from bottle gourd, Indian gourd, red gourd, sponge gourd, pumpkin, and melon seeds. *M. roridum* has purportedly been blamed for anthurium necrosis in Brazil (Quezado Duval et al. 2010). When a host cell dies, this pathogen can still produce poisons and colonise the dead cell (Murakami et al., 1999 & Murakami & Shirata, 2005). *M. roridum* has also been found in degraded plant tissues and soils, where it has been documented to infect a variety of plants including snapdragons, tomatoes, pansies, violets, cowpeas, and soybeans (Fergus, 1957). In addition to being connected to leaf spots on watermelons and soybeans in Korea, *M. roridum* has also been proposed as a potential biocontrol agent for weedy plants (Yum et al. 1990; Kim et al. 2003). However, no previous reports of the efficacy of anthurium in Korea have been made (Lee et al. 2008). In Korea, reports of the risk assessment and *M. roridum* discovery in imported anthurium plant pots are being made for the first time. When the colony of PDA-cultured medium was seen, the middle region was yellowish, while the outer edges were white. Black conidiomata appeared after more than 30 days of the fungus's incubation on the PDA. Sporodochia lacked setae and were sessile. Phase-contrast and SEM were used to see images of the conidia. Conidia resembled those of *M. roridum* in that they were

cylindrical in shape, 5-6 1–1.2 μ m in size of conidia, and had rounded ends. Despite being stained with lactophenol blue solution, the conidial mass had a green hue.

The isolated fungus was cultivated for 7 days at 25°C on a cellophane-layered PDA plate in order to identify it at the molecular level. By using a sterile knife to scrape the mycelium mat, fungal mycelia were prepared. Genomic DNA was isolated from the produced mycelium mat according to the previous description for PCR amplification (Kim et al. 1999).

A soil fungus called *Myrothecium roridum* thrives in this setting by living as a saprophyte in decomposing plant tissues (Domschet et al., 2007). *Myrothecium* is a saprophyte, yet despite this, it can still spread disease, especially in some plant species' aerial portions (Ahrazemet et al., 2000; Domschet et al., 2007). Vegetables, fruits, and ornamental plants are just a few of the many plant hosts of this facultative parasite (Murakami and Shirata, 2005). Our current study was conducted to identify the pathogen and examine its cultural and morphological diversity in light of the significance of the bael crop and the severity of this fungal disease.

Materials and Methods

The casual organism *Myrothecium roridum* was isolated from infected plants showing typical symptoms of *Myrothecium* leaf spot and was characterized culturally and morphologically following standard protocols. A total of five isolates were isolated from the samples collected from different bael growing districts of Uttar Pradesh.

Isolation, purification & identification of test fungus

To isolate the pathogen, small bits of the fungus were kept on the previously poured and solidified potato dextrose agar medium in Petri plates after the infected leaves of Bael were cut into small pieces, surface sterilised with 0.1% mercuric chloride (HgCl₂) solution, washed three times with sterile distilled water, and placed in a moist chamber. After 1–2 days had passed, fungal mycelium growth was visible. The plates were kept warm in an incubator at 25 °C. After the infected mycelium parts' mycelial growth, the plates were examined. Following sub-culture, hyphal tip purification, and maintenance of culture on PDA slant and Petri plates housed in an incubator at 25 °C, mycelial were sub-cultured (Dewangan et al. (2019)).

Isolates

The samples were taken from several Bael-growing regions in Uttar Pradesh, and a total of five isolates were isolated and designated as given in Table 1.

Table 1: Designation of *M. roridum* isolates and their place of collection

| Isolates | Place | Geographical Location | Plant part used |
|-----------------|------------------------------------------------|------------------------------------------------------|-----------------|
| Mr ₁ | National Botanical Garden, Lucknow | 26 ⁰ 51'23" N 80 ⁰ 56'55" E | Leaves |
| Mr ₂ | Block-Salon, District-Raebareli | 26 ⁰ 01'42" N 81 ⁰ 27'43" E | Leaves |
| Mr ₃ | Village-BarkheriyaJat, District-LakhimpurKhiri | 27 ⁰ 46'56" N 80 ⁰ 10'47" E | Leaves |
| Mr ₄ | Village-Dalel Ganj, Khalilabad | 26 ⁰ 47'29" N 83 ⁰ 00'23" E | Leaves |
| Mr ₅ | Village-Khushalipur, Saharanpur | 30.1615° N, 77.8424° E | Leaves |

Cultural and morphological study of *Myrotheciumroridum*

For the purpose of the cultural and morphological study, PDA medium was used to culture the five isolates of *Myrotheciumroridum*. At 25 °C and in the dark on PDA, the colony growth begins after 3–4 days. Five, ten, fifteen, and twenty days after the inoculation, the morphological and cultural characteristics (radial growth and colony character) were noted. Slides from an 18-day-old culture were made, and the quantity, variety, and dimensions of the conidia were noted. (Dewangan *et al.* (2019)).

Results and discussion -

Cultural and morphological characters of *Myrotheciumroridum*

Five distinct isolates of *Myrotheciumroridum* from pure culture had their mycelial growth measured (Table 2). Colonies on PDA media were observed as creamy white at initial stage and late on turned to yellowish white. The fungus produces branched aseptate and greenish mycelia. Conidiophores were branched repeatedly. The mycelium was branched, aseptate, greenish, conidia were hyaline, some cells were large. Conidiophores carried two to five phialides in whorls on each of its two to three branches. Similar results were also reported by Ben *et al.* (2014) where he identified *Myrotheciumroridum* to be the causal organism of Leaf Spot of *Anthuriumandraeanum* in China. Known *et al.* (2013) evaluated

Myrotheciumroridum based on its morphological characteristics, including colony shape on PDA, the microstructures of spores observed by light and scanning electron microscopy, as well as the outcomes of phylogenetic analysis. The conclusions were identical to those authors' (2013) research.

Sporulation color& Concentric zone

The sporulation colour recorded were White in Mr₁, Black brown in Mr₂, Dark green in Mr₃ and Mr₅ and Black in Mr₄. Appearance of colony was recorded as rough in Mr₁, Mr₃ and Mr₅. Whereas, smooth in Mr₂ and Mr₄. The concentric zone was recorded at Mr₂, Mr₃ and Mr₄. Whereas no concentric zone was recorded at Mr₁ and Mr₅ (Table 2). Twenty isolates of the bacterium *Myrotheciumroridum* were also subjected to cultural and morphological variability analyses by Dewanganet al. (2019). *Myrotheciumroridum* isolates were recorded as having sporulation colours of black, brownish black, green, and white on PDA.

Table 2: Cultural variability of different isolates of *Myrotheciumroridum* on potato dextrose agar

| Isolates | Mycelial colour | Colony character | | | |
|----------|-----------------|--------------------|--------------------|--------------|-------------------------|
| | | Sporulation Colour | Circular/Irregular | Smooth/Rough | Concentric Zone |
| Mr1 | Creamy white | White | Circular | Rough | Without concentric zone |
| Mr2 | White | Brown black | Circular | Smooth | Concentric Zone |
| Mr3 | Yellowish white | Dark green | Irregular | Rough | Concentric Zone |
| Mr4 | Greyish black | Black | Circular | Smooth | Concentric Zone |
| Mr5 | White | Dark green | Irregular | Rough | Without concentric zone |

Morphological characters

Conidia (Shape & Size)

Isolate Mr5 had the largest length and width (7.80 & 3.45 μm) followed by Mr4 (7.20 & 3.72 μm), Mr3 (6.96 & 2.87 μm), Mr1 (6.72 & 2.75 X) and Mr2 (6.12 & 2.16 μm) according to the mean. Conidia were described as being one-celled, ovoid to elongate, hyaline to slightly dark, and having rounded ends. Conidia length and breadth ranged from 5.04 to 8.40 X 2.40 to 4.08 μm , respectively. The results were consistent with Cabraet al. (2009) evaluation, which determined that the five fungal isolates were acquired and identified as *Myrotheciumroridum* with conidia size 6.4 to 7 X 1.7 to 2.3 μm and phialide length ranging from 10.3 to 11.2 μm . The conidia shape revealed that isolates Mr₁, Mr₂, Mr₃ gave creamy white and white mycelial colour respectively. The shape of mycelia was circular in Mr₁, Mr₂ and Mr₄ and irregular in Mr₃ and Mr₅ (Table 3).

Table 3: The morphological diversity of different *Myrotheciumroridum* isolates

| Isolates | Conidia size (μm) | | | | Conidia shape |
|---------------|--------------------------------|------|-------------|------|----------------------------|
| | Length | | Width | | |
| | Range | Mean | Range | Mean | |
| Mr1 | 6.00 - 7.44 | 6.72 | 2.40 - 3.12 | 2.75 | Cylindrical with round end |
| Mr2 | 5.04 - 7.20 | 6.12 | 1.92 - 2.40 | 2.16 | Rod shaped with round end |
| Mr3 | 6.24 - 7.68 | 6.96 | 2.80 - 2.95 | 2.87 | Rod shaped with round end |
| Mr4 | 6.72 - 7.68 | 7.20 | 3.36 - 4.08 | 3.72 | Cylindrical with round end |
| Mr5 | 7.20 - 8.40 | 7.80 | 3.30 - 3.60 | 3.45 | Cylindrical with round end |
| CD (0.01%) | | 0.67 | | 0.31 | |
| CV (%) | | 7.20 | | 7.70 | |
| SEm \pm | | 0.22 | | 0.10 | |

Conclusion: -

On the basis of experimental findings described in preceding following conclusions were made: There was visible cultural variability in aerial and radial mycelium growth, colour & length among all five isolates of the test pathogen i.e, *Myrothecium roridum*. Among all the five isolates, isolate Mr2 (6.12 & 2.16m) had the smallest length and width of conidia & Mr5 (7.80 & 3.45m) had the longest, according to the mean.

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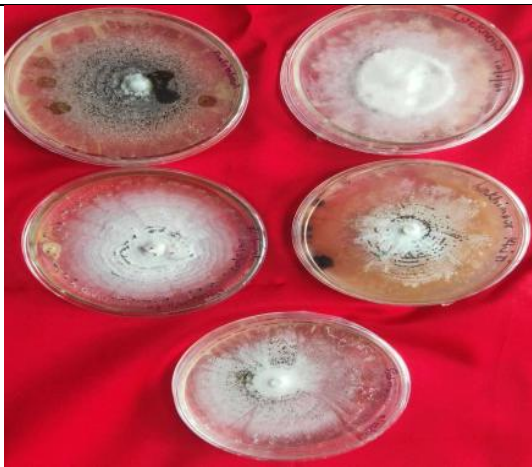


Fig. 1: - Five isolates of different districts



Fig. 2: - Microscopic image of conidia (40x)



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Fig. 3: Symptoms of *Myrothecium roridum*

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