Original Research Article

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

ABSTRACT

The experiment was executed at College of Horticulture, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.) during 2019-21, to estimate the variation present among the isolates of *Myrothecium roridum* causing leaf spot of bael and to see the bio-efficacy of botanicals and fungitoxicants 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

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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 impotance 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, redioprotective, antiviral, antimicrobial, and anti-helmintic are some of the different plant parts 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 ophthalimia, 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 (Fawzi Mahomoodally et 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 (Shirwaikar et 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 (Dhankar et al. 2011, Kirtikar et 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 symtpoms 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 m in size, 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 (Domsch et al., 2007). Myrothecium is a saprophyte, yet despite this, it can still spread disease, especially in some plant species' aerial portions (Ahrazem et al., 2000; Domsch 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.

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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-Lakhimpur Khiri	$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 *Myrothecium roridum*

For the purpose of the cultural and morphological study, PDA medium was used to culture the five isolates of Myrothecium roridum. 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.

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Results and discussion -

Cultural and morphological characters of *Myrothecium roridum*

Five distinct isolates of *Myrothecium roridum* 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 *Myrothecium roridum* to be the causal organism of Leaf Spot of *Anthurium andraeanum* in China. Known et al. (2013) evaluated

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Myrothecium roridum 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 Mr_1, Black brown in Mr_2, Dark green in Mr_3 and Mr_5 and Black in Mr_4. Appearance of colony was recorded as rough in Mr_1, Mr_3 and Mr_5. Whereas, smooth in Mr_2 and Mr_4. The concentric zone was recorded at Mr_2, Mr_3 and Mr_4. Whereas no concentric zone was recorded at Mr_1 and Mr_5 (Table 2). Twenty isolates of the bacterium *Myrothecium roridum* were also subjected to cultural and morphological variability analyses by Dewangan et al. (2019). *Myrothecium roridum* isolates were recorded as having sporulation colours of black, brownish black, green, and white on PDA.

Table 2: Cultural variability of different isolates of *Myrothecium roridum* 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 & 3.72µm), Mr3 (6.96 & 2.87µm), Mr1 (6.72 & 2.75 X) and Mr2 (6.12 & 2.16µm) 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 m, respectively. The results were consistent with Cabra et al. (2009) evaluation, which determined that the five fungal isolates were acquired and identified as *Myrothecium roridum* with conidia size 6.4 to 7 X 1.7 to 2.3 m and phialide length ranging from 10.3 to 11.2 m. The conidia shape revealed that isolates Mr_1, Mr_2, Mr_3 gave creamy white and white mycelial colour respectively. The shape of mycelia was circular in Mr_1, Mr_2 and Mr_4 and irregular in Mr_3 and Mr_5 (Table 3).

Table 3: The morphological diversity of different *Myrothecium roridum* 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±		0.22		0.10	

Conclusion: -

On the basis of experimental findings described in preceding followingconclusionswere 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 & Mr5 (7.80 & 3.45m) had the longest, according to the mean.

Declarations

Ethical approval: Not applicable.

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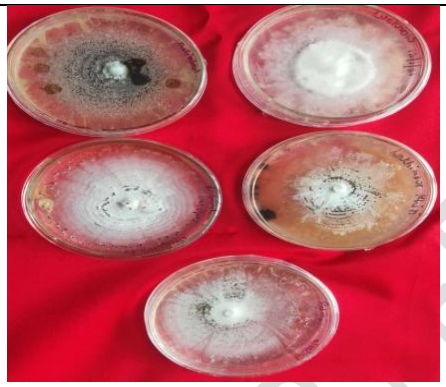

Fig. 1: - Five isolates of different districts

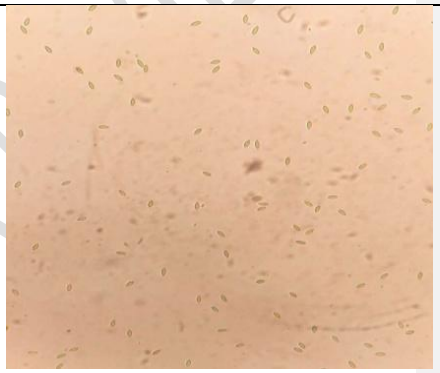

Fig. 2: - Microscopic image of conidia (40x)

Fig. 3: Symptoms of *Myrothecium roridum*