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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(8): 995-997

www.thepharmajournal.com
Received: 16-06-2021
Accepted: 22-07-2021

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In vitro efficacy of fungicides against *Myrothecium roridum* causing leaf spot of Bael

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Abstract
Leaf blight caused by *Myrothecium roridum* is a devastating disease of Bael in nursery stage. The experiments were conducted to test the efficacy of fungicides against *Myrothecium roridum* causing leaf spot of bael in *in vitro* condition. The results showed that the complete (100%) mycelial growth inhibition was recorded with Tebuconazole (50%) + Trifloxystrobin (25%) 75 WP @ 0.05%, Carbendazim (12%) + Mancozeb (63%) @ 0.2%, Propiconazole (25%) EC @ 0.1%, Tebuconazole (25%) EC @ 0.2% and Carbendazim 50% WP @ 0.1%. The minimum mycelial growth (mm) and maximum growth inhibition at 24 day after inoculation was Hexaconazole (5%) EC @ 0.1% (39.66mm and 55.93%) followed by Mancozeb (75%) WP @ 0.2% (45.66mm and 49.26%) and Azoxystrobin (18.2%) + Difenconazole 11.4% SC @ 0.2% (50.33mm and 44.07%). Maximum mycelial growth was found in control (90.00).

Keywords: Bael, *Myrothecium roridum, in vitro*, efficacy, fungicides

1. Introduction
Bael (*Aegle marmelos* Correa) is an indigenous fruit of India belongs to family Rutaceae, chromosome number is 2n = 18(36) and it is commonly known as Bengal queen, Golden apple, Japanese bitter orange, Stone apple, Wood apple (John and Stevenson, 1979) [1]. Nevertheless, the Bael tree grows in almost all the states of India; however, it is widely distributed in U.P., Bihar, West Bengal, Orissa and Madhya Pradesh (Roy *et al.*, 1992) [6].
Bael plants are widely and abundantly grown in eastern Uttar Pradesh particularly in Mirzapur, Varanasi, Gorakhpur, Basti, Gonda, Ayodhya, Etawah districts and also Sewan district of Bihar (Teaotia *et al.*, 1963) [8]. The average height of tree, 8.5 meters. Flowers, greenish white, sweetly scented, 8 mm long; diameter of a fully open flower, Fruits, yellowish green, with small dots on the outer surface, The flowering was observed to the second fortnight of June to the first fortnight of July. The fruits take almost one year to mature. The peak fruiting season is during May and June. The average yield of a wild Bael tree was 62.5 kg. (Parmer and Kaushal, 1982) [4].
In the ancient medical Ayurvedic treatise `Charaka Samhita` every part of this tree, stem, bark, root, leaves and fruit at all stages of maturity have medicinal merits and have been used as remedy for a long time. It cleanses and strengthens the intestines. Its ripe fruits flesh used for syrups or can be eaten both fresh and dried. Bael tree is considered a sacred tree, commonly grown in temple gardens in the country. It is important indigenous fruit of India. The fruit is known to possess significant therapeutic and medicinal value and is widely used in Homeopathy and Ayurveda. Bael is the most effective herbal remedy for diarrhea and dysentery. Anti-diarrheatic activity of Bael root (Pitre and Srivastava, 1984).
Bael plant is affected by fungal diseases is very serious in nursery condition like Alternaria leaf spot disease caused by *Myrothecium roridum*. The symptoms were found on leaves as small, circular or irregular in shape, brown in colour and chlorosis around the lesions were observed but later on these spots enlarges and covered the more area. Disease symptoms recorded first in rainy season (July-August) as circular brown spots on the upper surface of the leaf. The older leaf spots (15-20 days after formation of new spots) frequently exhibited concentric rings along with sporodochia arranged in a somewhat concentric pattern. At advanced stage of disease development a characteristics shot hole in the leaves were seen due to shedding of necrotic tissues.

Similar symptoms were also observed by Leath and Kendall (1983) [2] as chlorosis, purpling of leaflet margins and death of leaves and Shivaji *et al*., 2017 [7] also found the similar symptoms of Myrothecium leaf spot which is appeared on leaves as small, circular in shape which is brown in colour but later these spot enlarge and covered the more area. Chlorosis around the lesion and concentric rings may be seen. In severe condition, number and size of lesions increased, sporodochia may be seen on older lesion in circle, were black in colour. Therefore, present investigation was done to evaluate the different fungicide at different concentration in *In vitro* condition.

2. Material and methods

2.1. Isolation of pathogen

Infected leaves of Bael collected from Research farm of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya UP.

The symptom showing tip side of bael leaf brown to black spot and centric rings, The infected leaves were cut into small pieces along with healthy margins and then after surface sterilization of these cutting leaves were carried out using 1% $NaOCl_2$ for approximately 2 min., after washed three times with sterile distilled water, dried up between sterilized filter paper tissue, and then placed aseptically on the surface of potato dextrose agar (PDA) plates, supplemented with Streptomycin to avoid bacterial contamination. These plates were incubated at 25 ±1°C for one week (Zeng *et al*., 2015) [10].

2.2 Efficacy of fungicides at different concentration against *Myrothecium roridum*

Each concentration of treatments was bio-assayed against *Alternaria alternata* under laboratory condition to get out their relative efficacy for inhibiting the mycelial growth of *Myrothecium roridum*. Required quantity of each treatment was incorporated in 100 ml PDA at luke warm stage and mixed thoroughly by sacking, prior to pouring into Petri-plates. After pouring of PDA in Petri-plates, the medium was allowed to solidify and these plates were centrally inoculated with the 6 mm diameter disc of *Myrothecium roridum* at the centre of the plate which is cut by sterilized cork borer, taken from the margin of actively growing 10 days old culture. Control was used as such without treatment in the medium. Four replications of each treatment incubated at 26±2^{0}C for growth of the pathogen. The efficacy of various chemicals was observed by measuring radial growth of the fungal colony in millimeters (mm). The inhibition was evaluated in terms of per cent inhibition of fungal growth was compared to the check. The efficacy of various treatments was assessed by measuring the radial growth of the fungus after 5 and 10 days of incubation. The per cent inhibition of mycelial growth was calculated by using the following formula (Mckinney, 1923) [3]:

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

I = Percent Inhibition.

C = Colony diameter in control.

T = Colony diameter in treatment.

3. Result and Discussion

The data presented in Table no 1 showed the 100% mycelial growth inhibition was recorded with Tebuconazole (50%) + Trifloxystrobin (25%) 75 WP @ 0.05%, Carbendazim (12%) + Mancozeb (63%) @ 0.2%, Propiconazole (25%) EC @ 0.1%, Tebuconazole (25%) EC @ 0.2% and Carbendazim 50% WP @ 0.1%.

The minimum mycelial growth (mm) and maximum growth inhibition at 24 day after inoculation was Hexaconazole (5%) EC @ 0.1% (39.66mm and 55.93%) followed by Mancozeb (75%) WP @ 0.2% (45.66mm and 49.26%) and Azoxystrobin (18.2%) + Difenconazole 11.4% SC @ 0.2% (50.33 mm and 44.07%). Maximum mycelial growth was found in control (90.00). All the treatments found significantly superior over the control. Similar result was found by Zade *et al*., 2018 also reported that Mancozeb (75%) WP (62.33%) followed by Hexaconazole (5%) EC (66.66%) and Tebuconazole (25%) EC (83.33%). Shivaji *et al*., 2017 [7] also found similar result in Propiconazole @ 0.1% and followed Difenconazole and Mancozeb effective against this disease.

Table 1: Effect of fungicides at different concentrations on radial mycelia growth and inhibition of *Myrothecium roridum in vitro*

Treatments	Concentration (Per cent)	Average mycelia growth (mm)				Per cent growth inhibition			
		6 DAI	12 DAI	18 DAI	24 DAI	6 DAI	12 DAI	18 DAI	24 DAI
Tebuconazole (50%) + Trifloxystrobin (25%) 75 WP	0.05	0.00	0.00	0.00	0.00	100	100	100	100
Azoxystrobin (18.2%) + Difenconazole 11.4% SC	0.2	19.66	35.33	45.66	50.33	19.19	23.743	37.15	44.07
Carbendazim (12%) + Mancozeb (63%)	0.2	0.00	0.00	0.00	0.00	100	100	100	100
Propiconazole (25%) EC	0.1	0.00	0.00	0.00	0.00	100	100	100	100
Hexaconazole (5%) EC	0.1	12.00	20.00	29.00	39.66	50.67	56.83	60.08	55.93
Tebuconazole (25%) EC	0.2	0.00	0.00	0.00	0.00	100	100	100	100
Carbendazim 50% WP	0.1	0.00	0.00	0.00	0.00	100	100	100	100
Mancozeb (75%) WP	0.2	11.33	23.00	34.00	45.66	53.43	50.35	53.20	49.26
Check		24.33	46.33	72.66	90.00				
SE m±		5.21	9.85	14.57	17.93				
CD at 1%		0.904	1.27	1.498	1.012				
CV		5.27	3.92	3.169	1.717				

DAI= Day after Inoculation

PGI= Per cent Growth Inhibition

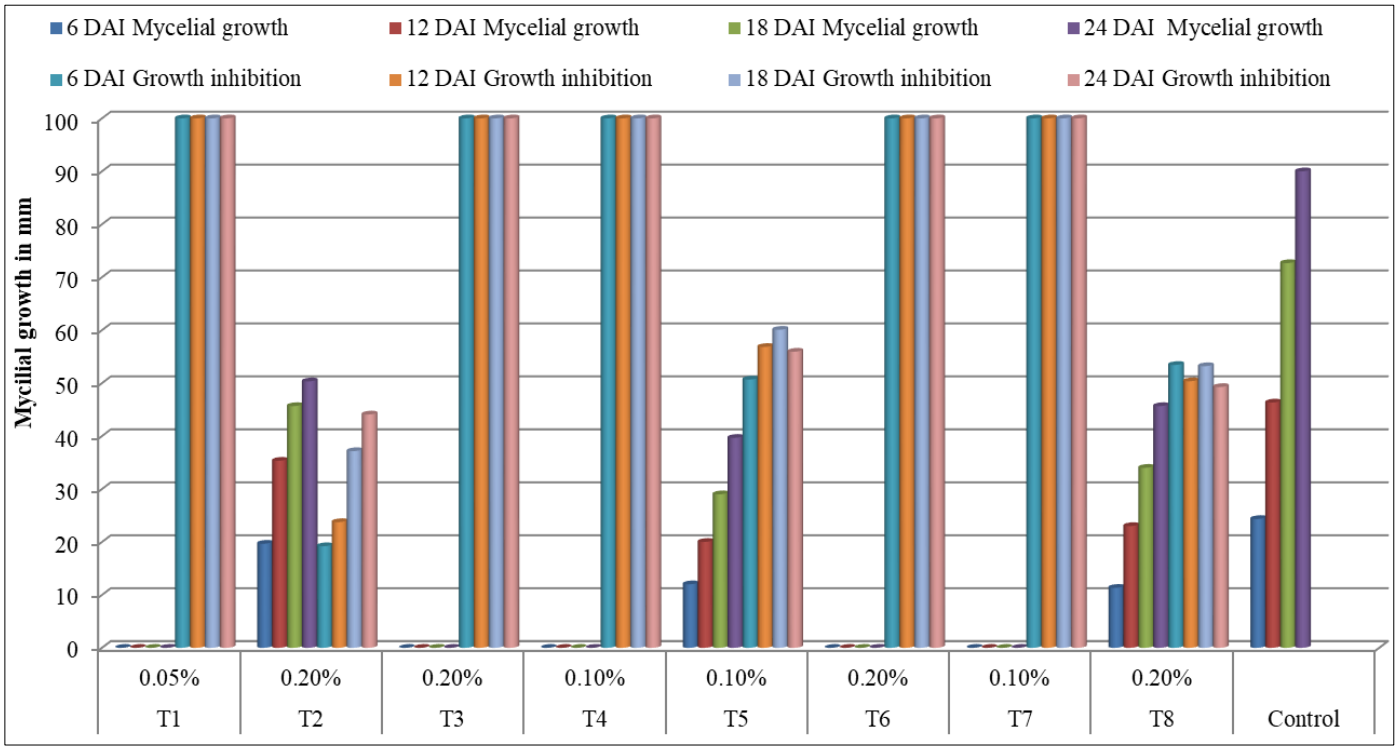

Fig 1: Treatment and concentration

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