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IN VITRO EVALUATION OF MYROTHECIUM RORIDUM AGAINST FUNGI TOXICANTS AND BIOACTIVE BOTANICAL COMPOUNDS AGAINST LEAF SPOT OF BAEL

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ABSTRACT

Bael (*Aegle marmelos* Correa.), is one of the medicinally treasured tree species out of the 250,000 living terrestrial plant species on earth. The experiment was executed at Department of Horticulture & Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Ayodhya (U.P.) during 2019-21. The bio-efficacy of botanicals and fungitoxicants against leaf spot of bael. Among the botanicals, eleven herbal extracts: Marigold, Onion, Karanj, Garlic, Eucalyptus, Jasmine, Neem, Turmeric, Ashok, Argimone and Bhatkatiya were evaluated at 10%, 20% and 30% for their efficacy on mycelial growth inhibition *in vitro* condition. Among these, Garlic clove extract recorded 100% mycelial growth inhibition @ 20 and 30% followed by Neem @ 30% (61.47%), Jasmine @ 30% (48.60%), Argimone @ 30% (47.16%), Bhatkatiya @ 30% (46.08%) and Turmeric @ 30% (44.47%). Among the fungicides, nine fungicides: Propiconazole (Tilt); Pyraclostrobin (Headline Wg); Carbendazim + Mancozeb (Saaf); Tebuconazole (Folicur); Hexaconazole + Captan (Taqat); Tebuconazole + Trifloxystrobin (Nativo); Azoxystrobin (Amistar); Fluxapyroxad (Imbrex) and Zineb (IndofilZ-78) were evaluated at 100, 250, 500, 1000 and 1500 ppm for their efficacy for mycelial growth inhibition for controlling the *Myrothecium roridum in vitro* conditions. Among these, Propiconazole (Tilt), Pyraclostrobin (Headline Wg), Tebuconazole + Trifloxystrobin (Nativo) completely inhibited the growth of fungus @ 100ppm concentration and Tebuconazole (Folicur) was effective @ 500ppm concentration followed by Fluxapyroxad @ 250ppm (84%), Carbendazim + Mancozeb @ 500ppm (70%), all the other fungicides were not at all effective.

Key words: Myrothecium, mycelial growth, variability, botanicals, fungicides

Introduction

Bael (*Aegle marmelos*) isa 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. 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).

Both flavonoids and polyphenols are present in significant amounts in beel. 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). 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),

When a host cell dies, this pathogen can still produce poisons and colonise the dead cell (Murakami *et al.,* 1999, Murakami and 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.

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 experiment was executed at Department of Horticulture & Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Ayodhya (U.P.) during 2019-21. 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.

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 ($HgCl_2$) 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.

Management strategies using plant extracts and new fungicides

Effect of plant extracts on mycelial growth of the pathogen (*in vitro*)

The food poisoning technique was used in a laboratory experiment to determine the fungitoxicity of the eleven plant extracts listed in Table 3.2, with four replications under *in vitro* conditions to examine the inhibitory effect of these botanicals against the mycelial growth of Myrothecium roridum. Each plant's 100-gram leaves, cloves, or rhizomes were harvested, thoroughly cleaned three times with distilled water, and then let to dry for six hours at room temperature (25 1°C). Each plant's 100 g of leaves, cloves, and rhizomes were separately crushed with 100 ml of distilled water before to extraction. The extract was first sterilised by passing through a Millipore filter using a swimming filter adaptor after being filtered through muslin cloth. The extracts of each botanicals was diluted in order to achieve three concentrations *viz.*, 10, 20 and 30 per cent.

Petri-plates containing PDA supplemented with different plant extracts, each with three concentrations and replicated four times were inoculated with seven days old culture (5 mm diameter disc). A suitable check (without plant extract) was also maintained. Fungal colony was measured after 20 days of inoculation at 25±1°C. The linear growth of test fungus was recorded and per cent mycelial growth inhibition was calculated by using formula (Vincent, 1947) given below:

$$I = \frac{C-T}{C} \times 100$$

Were,

I = Percent inhibition of fungal growth C = Radial growth of colony in control T = Radial growth in treated Petri-plate

Table 2: List of botanicals used in vitro during study against Myrothecium leaf spot of bael.

S. No.	Common Name	Botanical name	Plant parts used	Family
1.	Neem	*Azadiracta indica*	Leaves	*Meliaceae*
2.	Garlic	*Alium sativum*	Clove	*Amaryllidaceae*
3.	Marigold	*Tagetes erecta*	Leaves	*Asteraceae*
4.	Turmeric	*Curcuma longa*	Leaves	*Solanacceae*
5.	Onion	*Allium cepa*	Bulb	*Amaryllidaceae*
6.	Eucalyptus	*Eucalyptus obliqua*	Leaves	*Myrtaceae*
7.	Ashok	*Saraca asoca*	Leaves	*Fabaceae*
8.	Argimone	*Argemone maxicana*	Leaves	*Papaveraceae*
9.	Bhatkatiya	*Solanum torum* and stem	Leaves	*Solanaceae*
10.	Jasmine	*Jasminum officinalum*	Leaves	*Oleaceae*

Table 3: List of fungicides used under *in vitro* study against *Myrothecium* leaf spot of bael.

Trade name	Active ingredient	Producing company	Used form
Tilt	Propiconazole 25 EC	Syngenta India Ltd.	Liquid
Headline WG	Pyraclostrobin 20% WG	BASF Corporation	Granules
Saaf	Carbendazim 12% + Mancozeb 63% WP	UPL Limited REGD.	Powder
Folicur	Tebuconazole 25 EC	Bayer Crop Science Ltd.	Liquid
Taqat	Hexaconazole 5% + Captan 70% WP	Rallis Tata Enterprise	Powder
Nativo	Tebuconazole 25% + Trifloxystrobin 50% WG	Bayer Crop Science Ltd.	Granules
Amistar	Azoxystrobin 18% SC	Syngenta India Ltd.	Liquid
Imbrex	Fluxapyroxad 250g SC	BASF Corporation	Liquid
Indofil Z-78	Zineb	Bayer Crop Science Ltd.	Powder

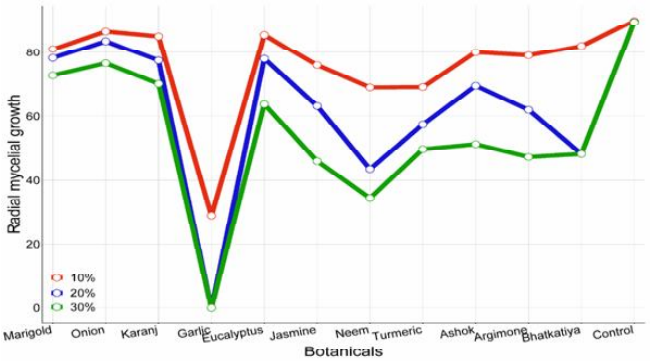

Fig 1: Line graph showing relationship between different concentrations of botanicals on growth of *M. roridum.*

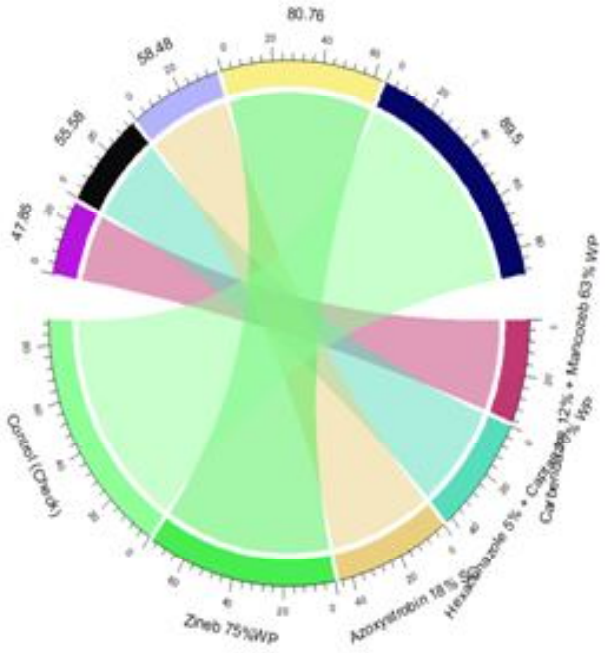

Fig-2: Chord diagram showing relationship between different concentrations of fungicides on growth of *M. roridum.*

Effect of fungicides on mycelial growth of the pathogen (*in vitro*)

In order to determine mycelia growth and the percentage of the pathogen that was inhibited by the poisoned food technique, the following fungicides were tested against Myrothecium roridum at three concentrations (500, 1000, and 1500 ppm) in a lab setting (Umamaheshwari, 2018). The appropriate amount of each fungicide was added to sterilised PDA medium, thoroughly mixed by shaking, and then poured into sterilised Petri plates, where they were left to set. These Petri dishes were filled with a 5 mm-diameter disc of the pathogen's 20-year-old culture, and they were then incubated at 251°C. Four times with an appropriate control, each treatment was duplicated. It was assessed how effective the fungicides were in

each treatment and the average of four replications. Mycelial growth inhibition percentage was obtained using the provided formula.

Results and Discussion

To study the efficacy of botanicals and fungicides against Myrothecium roridum *in vitro*

Efficacy of botanicals against Myrothecium roridum *in vitro* conditions

Among the botanicals, eleven herbal extracts were used *viz.* Marigold (*Tagetes erecta*); Onion (*Aliium cepa*); Karanj (*Millettia pinnata*); Garlic (*Allium sativum*); Eucalyptus (*Eucalyptus globulus*); Jasmine (*Jasminum* spp.); Neem (*Azadirachta indica*); Turmeric (*Curcuma longa*); Ashok (*Saraca asoca*); Argimone (*Argimone mexicana*) and Bhatkatiya (*Solanum verginianum*) were evaluated at 10%, 20% and 30% for their efficacy on mycelial growth inhibition *in vitro*. Among these, T_4 (Garlic clove extract) recorded 100% mycelial growth inhibition @ 20 and 30% followed by T_7 (Neem) @ 30% (61.47%), T_6 (Jasmine) @ 30% (48.60%), T_{10} (Argimone) @ 30% (47.16%), T_{11} (Bhatkatiya) @ 30% (46.08%) and T_8 (Turmeric) @ 30% (44.47%). Umeshwari *et al.*, (2018) also experimented that the efficacy of Twelve plant extracts viz. *Azadirachta indica, Allium cepa, Ocimum sanctum, Aegle marmelos, Allium sativum, Cymbopogan flexiosus, Lantana camara, Ipomoea batatus, Pongamia pinnata, Nyctanathes arbortristis, Eucalyptus citriodora* and *Mentha arvensis* were experimented in-vitro at 10% and 20% concentrations by Poison food technique. This investigation also showed that, compared to controls, all plant extracts significantly outperformed both concentrations in reducing the development of *Myrothecium roridum*. *Aegle marmelos* experienced the least growth reduction, or 5.8%, while *Nyctanathes arbortristis* experienced the largest reduction of growth, or 74%, at a 10% concentration. *Mentha arvensis* plant extract showed a 100% growth inhibition at a 20% concentration, while *Nyctanathes arbortristis* showed a 79.8% growth inhibition at the same concentration. *Cymbopogan flexiosus*, with a 48.6% inhibition rate, recorded the lowest level. The antifungal effectiveness of the five medicinal plant species—Neem, Karanj, Datura, Ashok, and Besharam—was also studied by Akso in 2019. At 10 DAI, *M. roridum* growth was inhibited by a percentage ranging from (48.46 to 84.04%). The extract of Karanj showed the greatest growth inhibition at 10 DAI (84.04%). Neem (68.65) and Besharam (48.46%) had the least amount of inhibition.

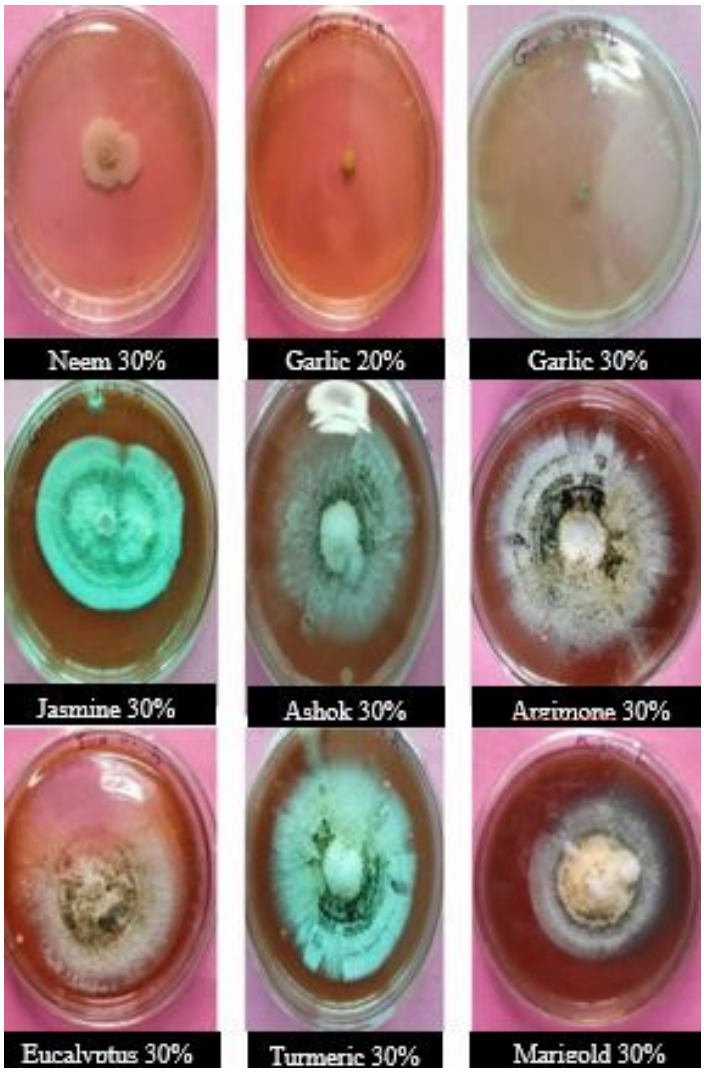

Fig 3: Efficacy of botanicals against *Myrothecium roridum in vitro*

Efficacy of fungicides against Myrothecium roridum *in vitro*

Among the fungicides, nine fungicides: Propiconazole (Tilt); Pyraclostrobin (Headline Wg); Carbendazim + Mancozeb (Saaf); Tebuconazole (Folicur); Hexaconazole + Captan (Taqat); Tebuconazole + Trifloxystrobin (Nativo); Azoxystrobin (Amistar); Fluxapyroxad (Imbrex) and Zineb (IndofilZ-78) were evaluated at 100 ppm, 250 ppm, 500 ppm, 1000 ppm, 1500 ppm for their efficacy for mycelial growth inhibition for controlling the *Myrothecium roridum in vitro* conditions. Among these, Propiconazole (Tilt), Pyraclostrobin (Headline Wg), Tebuconazole + Trifloxystrobin (Nativo) completely inhibited the growth of fungus @ 100ppm concentration and Tebuconazole (Folicur) was effective @ 500ppm concentration followed by Fluxapyroxad @ 250 ppm (84%), Carbendazim + Mancozeb @ 500ppm (70%), All the other fungicides were not at all effective. Similar

Fig 4: Efficacy of fungicides against *Myrothecium roridum in vitro*

type of result was also obtained by Dewangan and Kurre (2018) experimented that the fungicides such as Tebuconazole, Hexaconazole, Mancozeb, Pyraclostrobin, Fluxapyroxad, and Propiconazole were extremely effective in lowering the percent disease index over control when applied topically to soybeans to treat myrothecium leaf spot. Pyraclostrobin (73.58%) and Propiconazole (78.90%) were the two drugs that had the most effectiveness in lowering the percent disease index when applied foliarly. Tebuconazole (35.75%) had the lowest percent illness index over control, followed by Fluxapyroxad (38.99%). The same result was reached by Mishra et al. (2017), who tested the effectiveness of fungicides in in vitro conditions and found that Carbendazim (83.31%) showed growth inhibition for up to 24 hours. However, growth inhibition lasting up to 48 hours was seen in plates treated with carbendazim (79.76%) and mancozeb (87.05%). For 72 hours, Carbendazim and Mancozeb treatments showed growth inhibition (69.54 and 86.00%). Growth inhibition was observed in the plates treated with carbendazim, mancozeb, and copper oxychloride (65.00, 82.80, and 95.320%), whereas growth inhibition was shown in the plates treated with propiconazole and difenconazole for up to 96 hours.

Conclusion

Garlic clove extract and Neem extract were found to be most inhibitory to the test pathogen i.e, *Myrothecium roridum*, whereas Onion extract was found to be the least effective. Propiconazole (Tilt), Pyraclostrobin (Headline Wg), Tebuconazole + Trifloxystrobin (Nativo), and Tebuconazole (Folicur) were the only fungicides that completely inhibited the radial growth of the test pathogen *i.e., Myrothecium roridum*.

Declarations

Conflict of interest: All authors declare that they have no conflict of interest.

Ethical approval: Not applicable.

References

Ahrazem, O., Gómez-Miranda, A.P., Bernabe, M., Leal, J.A., (2000). Heterogeneity of the genus Myrothecium as revealed by cell wall polysaccharides. Archives of Microbiology. 173, 296-302. DOI: 10.1007/s002030000149.

Anonymous (2016). Production of bael in 2016-2017. National Horticulture Board (NHB).

Anonymous (2020). The cause of wilting/drying in bael plants. Annual Report of AICRP on Arid Zone Fruits (ICAR). Central Institute for Arid Horticulture, Bikaner. Rajasthan.

Chanda, A. (2008). Phytochemical and pharmacological activity of *Aegle marmelos* as a potential medicinal plant: an overview. *Pharmacognosy Research*, **6(1):** 3.

Domsch, K.H., Gams, W. and Anderson, T. (2007). Compendium of soil fungi. 2nd Edition. IHW Verlag. Eching, Germany, 672.

Fergus, C.L. (1957). Myrothecium roridum on Gardenia. *Mycologia*, **49:** 124–127.

Gurjar, P.S., Bhattacherjee, A.K., Singh, A., Dikshit, A. and Singh, V.K. (2019). Characterization of nutraceuticals in bael powder prepared from fruits harvested at different developmental stages. *Indian Journal of Traditional Knowledge*, **18:** 724–730.

Kirtikar, B.D. and Basu, K.R. (1984). Indian medical plants, Goyal Offset Printers, **3:** 2143–2155.

Kim, S.H., Uzunovic, A. and Breuil, C. (1999). Rapid detection of Ophiostoma piceae and O. quercus in stained wood

by PCR. *Applied and Environmental Microbiology,* **65:** 287-290.

Lee, H.B., Kim, J.C., Hong, K.S. and Kim, C.J. (2008). Evaluation of a fungal strain, Myrothecium roridum F0252, as a bioherbicide agent. *The Plant Pathology Journal,* **24:** 453-260.

Murakami, R. and Shirata, A. (2005). Myrotoxin B detection from mulberry leaves infected with *Myrothecium roridum*, cause Myrothecium leaf spot of mulberry, and possible role in pathogenicity. *Japan Journal of Phytopathology,* **71:** 91-100.

Murakami, R., Yasui, H., Shirata, A. and Kato, A. (1999). Production of myrotoxin B by *Myrothecium roridum* isolated from Myrothecium leaf spot of mulberry in Japan. *The Journal of Sericultural Science of Japan,* **68:** 469-477.

Murakami, R. and Shirata, A. (2005). Myrotoxin B detection from mulberry leaves infected with *Myrothecium roridum*, cause *Myrothecium* leaf spot of mulberry, and possible roles in pathogenicity. *Japanese Journal Phytopathology,* 71.

Quezado, D.A.M., Henz, G.P., Paz-Lima, M.L., Medeiros, A.R., Miranda, B.E.C., Pfenning, L.H. and Reis, A. (2010). New hosts of *Myrothecium* spp. In Brazil and a preliminary in vitro assay of fungicides. Brazilian. Journal of Microbiology, 41.

Saroj, P.L., Singh, R.S. and Singh, A.K. (2006). Bael (Aegle marmelos), pp. 21–38. Advances in Arid Horticulture, Vol 2.(Eds) P.L. Saroj and O.P. Awasthi. *International Book Distributing Co.,* Lucknow.

Singh, A.K., Singh, S. and Saroj, P.L. (2018a). The Bael (Production Technology). Technical Bulletin No.CIAH/ Tech./ Pub.No. 67, ICAR-CIAH, Bikaner, pp 1–55.

Singh, A.K. (2018). Propagating arid fruit commercially. *Indian Horticulture,* **63(5):** 82–8.

Singh, A., Sharma, P.C., Meena, M.D., Kumar, A., Mishra, A.K., Kumar, P., Chaudhari, S.K. and Sharma, D.K. (2016). Effect of salinity on gas exchange parameters and ionic relations in bael (Aegle marmelos Correa). *Indian Journal of Horticulture,* **73:** 48–53.

Yum, K.J. and Park, E.W. (1990). Soybean leafspot disease caused by *Myrothecium roridum* Tode ex Fries. *Korean Journal of Plant Pathology,* **6:** 313-7.