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Survey of *Alternaria alternate* causing Leaf Spot of Bael and its Isolation, Purification and Identification of Fungi Associated with Disease

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ABSTRACT: The bael tree, also known as the "*Aegle marmelos*", is a well-known and esteemed tree in India. The nation's cultural, religious, and medical traditions place a great importance on it. *Aegle marmelos* Correa, the fruit known as bael in India, belongs to the Rutaceae family and has chromosome number 2n=36 (n=18). Alternaria leaf spot of bael is now becoming an important menace in Uttar Pradesh and other parts of the country. Present investigations was undertaken to check area were Alterneria leaf spot of bael caused by *Alternaria alternata* is most prevailing in U.P. district is been check under survey study of alterneria leaf spot of bael, fungal isolation and purification was done to indentify the given pathogen as *Alternaria alternate* of bael. The pathogenic nature of purified *Alternaria alternata* was proved by artificial inoculation of bael leaves through spore-cum-mycelial suspension and Koch postulates was been proved. Survey was done for 16 district of Uttar Pradesh.Among the listed districts, Prayagraj consistently shows highest disease incidence and severity, with values increasing from 93.86% to 96.10% for disease incidence and from 50.10% to 51.22% for disease severity between 2022 and 2023. Least was seen in Ayodhya district for Pant Sujata germplasm lowest disease incidence and severity, with values increasing from 9.34% to 8.94% for disease incidence and from 7.84% to 7.64% for disease severity between 2022 and 2023 ND-AH-12 and ND-AH-27 also showed lower disease incidence and severity. Overall, the data shows that disease incidence and severity have generally increased across the districts and germplasms from 2022 to 2023. Some districts consistently show higher disease incidence and severity, while others maintain relatively lower levels. Further analysis is needed to identify the specific diseases and factors contributing to these patterns and to develop appropriate strategies for disease management and prevention. Survey conducted during 2022-23 showed that Alterneria blight of bael disease severity varied from 7.0 to 52.0%. The realm of arid crop research, particularly regarding the bael plant, has remained relatively unexplored. However, this survey data serves as a significant step towards filling this knowledge gap. By focusing on foliar diseases like Alterneria blight in bael, we have acquired valuable insights into the disease's prevalence and severity across various regions in Uttar Pradesh.

Keywords: *Alternaria alternata*, *Aegle marmelos* Correa, survey, isolation, purification, pathogenicity test.

INTRODUCTION

The bael tree, also known as the "*Aegle marmelos*," is a well-known and esteemed tree in India. The nation's cultural, religious, and medical traditions place a great importance on it. *Aegle marmelos* Correa, the fruit known as bael in India, belongs to the Rutaceae family and has chromosome number 2n=36 (n=18). According to John and Stevenson (1979), it is also referred to as Bengal queen, Golden apple, Japanese sour orange, Stone apple, and Wooden apple. It should be noted, nevertheless, that this deciduous tree is native to the Indian subcontinent and can be found in numerous Indian states. According to Singh and Choudhary (2012), bael is also grown and well-propagated in the wild and semi-wild in Uttar Pradesh, Rajasthan, Gujarat, Bihar, West Bengal, and Madhya Pradesh. Typically, in the districts of Mirzapur, Varanasi, Gorakhpur, Basti, Gonda, Ayodhya, and Etawah in Uttar Pradesh, as well as Sewan in Bihar (Teaotia *et al.*, 1963). Although bael plants were resilient and tolerant of biotic and abiotic challenges in antiquity, they are now seriously threatened by microbial diseases. The important diseases of bael plants are as follows. Alternaria leaf spot caused by *Alternaria alternata* (Madaan and Gupta 1985), Myrothecium leaf spot caused by *Myrothecium roridum* (Harsh *et al.*, 1989), Bacterial shot hole and fruit canker caused by

Xanthomonas bilvae (Patel *et al*., 1953), Stalk end rot caused by *Fusarium solani* (Bhargava *et al*., 1977), *Aspergillus* fruit rot caused by *Aspergillus awamori* (Arya *et al*., 1986), Fusarium fruit rot caused by *Fusarium moniliformae* (Arya *et al*., 1986), Powdery mildew caused by *Oidium* sp. and *Sphaerotheca fuligenia* (Sinha and Singh 1995), Scytalidium leaf spot (brown leaf spot) caused by *Scytalidium aeglicola* (Gautam *et al*., 2015). Among these, Alternaria leaf spot of bael is now becoming an important menace in Uttar pradesh and other parts of the country. Madaan and Gupta (1985) first time reported leaf spot disease of bael, caused by *Alternaria alternata* (Fr.) Keissler from Hisar, Haryana, and in Uttar Pradesh first time reported by Kumar *et al.* (2015) from A.N.D.U.A.T. University Kumarganj, Ayodhya, Uttar Pradesh.

Only a small amount of information is provided in India, particularly in the state of Uttar Pradesh, according to an analysis of works done on bael diseases. Therefore, the following goals can be pursued while keeping in mind the relevance of the bael crop and the impact of leaf spot of bael diseases caused due to *Alternaria alternata*. Hence, present investigations was undertaken to check area were Alterneria leaf spot of bael caused by *Alternaria alternata* is most prevailing in U.P. district is been check under survey study of alterneria leaf spot of bael and fungal isolation and purification was done to indentify the given pathogen as *Alternaria alternate* of bael.

MATERIALS AND METHODS

Isolation of pathogen. Diseasedsamples were taken from affected beal plants at the Main Experiment Station, Horticulture and Department of Plant Pathology, Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya, Uttar Pradesh (26.5412ºN, 81.8320ºE), to isolate the pathogen, diseased samples with recognizable clinical symptoms were chosen on purpose. The selected plant components underwent a thorough washing procedure with recently sterilised water to get rid of any surface impurities or dust particles. Cutting the cleaned, infected plant parts into little pieces with care and accuracy allowed for the inclusion of some healthy components. Following surface sterilisation using a 1.0% sodium hypochlorite solution under aseptic conditions, these chopped pieces were placed in a laminar flow hood. To remove any lingering traces of sodium hypochlorite, they underwent a series of intensive washing cycles in sterilised water. The sterilised pieces were put on sterilised blotting papers to get rid of extra moisture. Sterilised needles were used to transfer the fragments to Petri dishes once they had been sterilised.A 2% potato dextrose agar medium was placed inside the Petri plates, which had previously been sterilised at 180°C for 20 minutes in an electric hot air oven. The medium had been autoclaved for 30 minutes at 15 pounds per square inch of pressure. Three to four pieces of the infected plant sections were arranged in each Petri plate at equal intervals. The Petri plates were properly labelled with the isolate number, isolation date, and other pertinent information using a gloss marking pencil. After that, the Petri plates were moved to an incubator that had been preheated to 25°C.

Pure-culture of the pathogen. The single spore isolation method was employed to purify the fungus. In Petri plates, a diluted spore suspension was equally distributed, forming a thin layer that encouraged the spores to adhere to the agar surface. Individual spores were carefully separated from one another once they had settled, examined under a microscope, and marked with a dummy cutter inside the Petri plates. The tagged spores and agar blocks were then put onto Petri dishes with 2% potato dextrose agar medium that had been sterilized. Regular sub-culturing was done to keep an eye out for any potential contamination until a pure culture was established after the fungi obtained from the single spore culture demonstrated appropriate growth. For additional investigation, these cultures were kept on potato dextrose agar slants and sub-cultured every 15 days while being refrigerated at temperatures between 6 and 8°C.

Maintenance and preservation of culture. To maintain various cultures of *Alternaria alternata*, sterilized Potato Dextrose Agar (PDA) was utilized as the growth medium. Petri dishes were inoculated with a small portion taken from 10-day-old cultures that had been grown on PDA at a temperature of 25±1ºC. Following an incubation period of 20 days, one set of each pathogen was regularly sub-cultured on PDA. Additionally, a separate set of pure cultures was stored in the refrigerator to preserve their virulence, which could be compromised by frequent sub-culturing. To encourage fungal sporulation, the cultures were placed in an incubator set at a temperature of 25±1ºC.

Identification of the fungus. The identification process of the pathogen, *A. alternata*, involved the analysis of its morphological and cultural characteristics. These characteristics were observed and recorded on Potato Dextrose Agar (PDA) after a period of seven days following inoculation. To facilitate examination, temporary slides were prepared using lactophenol and stained with cotton blue. The slides were then examined under a compound microscope with binoculars, utilizing both low and high magnification. The morphological features of the pathogen, including its length, width, color, shape, size, as well as the number of septa in the conidiophores and conidia of *A. alternata*, were carefully observed and documented.

Symptomatology. The symptoms of the disease were examined critically on leaves of bael plants of different age at 15 days intervals to elucidate the course of development of the disease in the field.

Pathogenicity test. A pathogenicity test was conducted on three fungi in a net house, using plants that were 6 month old. Soil sterilization was performed by collecting soil from the Main Experimental Station, Horticulture, of A.N.D. University of Agriculture and Technology Kumarganj, Ayodhya (U.P.). Sandy loam soil mixed with farmyard manure in a 2:1 ratio was filled in each pot and then sterilized in an autoclave at 15 psi for one hour. Prior to inoculation, the plants were exposed to 95% humidity for 24 hours. They were then inoculated using an atomizer, with a conidial suspension containing 20-25 conidia per microscopic field. Following inoculation, the plants were placed in a glasshouse. Control plants were also maintained, receiving only sterile distilled water by spraying. Symptoms began to appear after 4-5 days. The pathogen was subsequently re-isolated from the artificially infected leaves, and the obtained culture was compared with the original culture, thus confirming the identification of the isolated pathogen.

Survey of Alternaria blight of bael in different districts of Uttar Pradesh. To find out the prevalence of the Alternaria blight disease of bael caused by *Alternaria alternata,* an extensive roving, survey data was taken for two year 2022 and 2023. It was conducted in the nearby districts of A.N.D. University of Agriculture and Technology, located in Kumarganj, Ayodhya, Uttar Pradesh, were bael is grown, survey was conducted in months of March, as depicted in the Table 1.

Table 1: Survey for the Alternaria blight disease from different Districts of U.P.

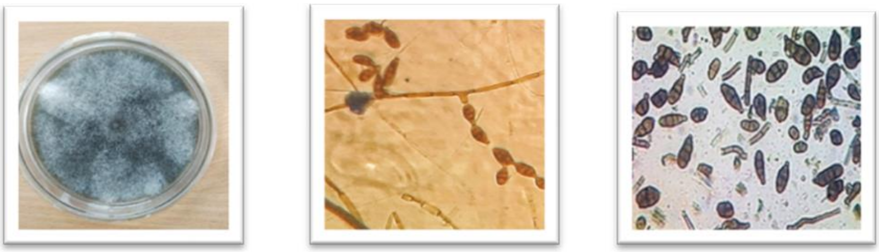
District	Longitude & latitude	Plant parts used
Shidharth nagar	27.2716° N, 82.8210° E	Infected leaves
Basti	26.8140° N, 82.7630° E	Infected leaves
Gonda	27.1340° N, 81.9619° E	Infected leaves
Raibareli	26.2145° N, 81.2528° E	Infected leaves
Gorakhpur	26.7606° N, 83.3732° E	Infected leaves
Mau	25.9463° N, 83.5610° E	Infected leaves
Banaras	25.3176° N, 82.9739° E	Infected leaves
Prayagraj	25.4358° N, 81.8463° E	Infected leaves
Khaliabaad	26.7658° N, 83.0834° E	Infected leaves
Deoria	26.2145°N,81.2528°E	Infected Leaves
Sultanpur	26.2585° N, 82.0660° E	Infected Leaves
Ayodhya	26.7922° N, 82.1998° E	Infected Leaves
Jaunpur	25.7464° N, 82.6837° E	Infected Leaves
Kanpur	26.4499° N, 80.3319° E	Infected Leaves
Amethi	26.1541° N, 81.8142° E	Infected Leaves
Lucknow	26.8467° N, 80.9462° E	Infected Leaves

RESULTS AND DISCUSSION

Collection, isolation, purification, pathogenicity and identification of the pathogen. Associated pathogen was isolated on potato dextrose agar medium and kept in BOD at 25±1ºC for proper growth and its further detailed studies of the pathogen. The single spore technique was followed to purify the associated cause of the disease. The young culture of the fungus was light grey in colour but after three days, it was dark green to black with a white margin. At 7th day of incubation, the whole surface of the fungal colony changed into black color. Under microscopic examination of fungus, brownish hyphae with light brown conidiophores at the top of each branch were observed. Acropetal chains of pale brown to light brown, obclavate or ellipsoid conidia were noticed on the conidiophores. Multicellular conidia were beakless or had short and stout conical beak at the tip. Conidia were muriform with 3-5 transverse and 0-3 longitudinal septa (Plate 2). Based on these cultural and morphological characteristics and literature of Alternaria Maurya *et al.* (2016); Kumar *et al.* (2015) from A.N.D.U.A.T university Kumarganj Ayodhya, Uttar Pradesh who first reported this disease in bael plant in Uttar Pradesh. The fungus was identified as *Alternaria alternata.*

The pathogenic nature of purified *Alternaria alternata* was proved by artificial inoculation of bael leaves through spore-cum-mycelial suspension. Thus, the suspension of *Alternaria alternata* was applied separately by atomizer on the pin pricked plants. Proper humidity was maintained by using humidifier to insure proper disease development. Observations on disease expression and symptom development were recorded after 10 days of inoculation till full expression of the symptom on inoculated plants were presented in the. The re-isolation of the fungus was made from the infected leaves of artificially inoculated plants and compared with original isolate of *Alternaria alternata* and Koch's postulates were proved (Plate 3). After 10 days of inoculation, inoculated leaves showed diseased symptoms which were similar to those observed in the orchard under natural conditions. Symptoms initially started from leaf margin and tip of leaves as irregular which are light brown in colour, later converted into dark brown to grey. In severe condition several spots coalesce to form large necrotic spots and covers large area (Plate 1). On young leaves, the disease produces minute brown to black spots. Symptoms can appear in as little as after infection. Lesions usually continue to expand and large areas of the leaf. Similar paintings changed into additionally achieved through Shingne *et al.* (2020) as he additionally discovered signs and symptoms of *A. alternata* after 6-8 days at the healthful leaf changes was discovered (Plate 4).

Plate 1. Typical severe symptoms of Alternaria leaf spot of bael.



(A) Pure culture (B) Conidia and Conidiophores (C) Muriform Conidia

Plate 2. Morphological characteristics of *Alternaria alternate.*

Inoculated plant Un-Inoculated plant

Plate 3. Pathogenicity test of *Alternaria alternata* on bael.

Plate 4. Different type of symptoms of *Alternaria alternata* on bael.

Survey of Alternaria blight disease of bael in different districts of Uttar Pradesh. Across all districts and germplasms, there is a general trend of increased disease incidence and severity from the year 2022 to 2023, based on the pooled data of both years From Table 2 following finding are been revealed as listed below

Disease Incidence and Severity. Among the listed districts, Prayagraj consistently shows highest disease incidence and severity, with values increasing from 93.86% to 96.10% for disease incidence and from 50.10% to 51.22% for disease severity between 2022 and 2023, followed by Deoria shows high disease incidence and severity, with values increasing from 92.14% to 94.38% for disease incidence and from 49.24% to 50.36% for disease severity between 2022 and 2023, followed by Jaunpur consistently shows high disease incidence and severity, with values increasing from 90.66% to 92.90% for disease incidence and from 48.50% to 49.62% for disease severity between 2022 and 2023. While in Ayodhya for Pant Sujata germplasm lowest disease incidence and severity, with values increasing from 9.34% to 8.94% for disease incidence and from 7.84% to 7.64% for disease severity between

2022 and 2023 ND-AH-12 and ND-AH-27 also showed lower disease incidence and severity, followed by Basti shows low disease incidence and severity, with values increasing from 66.76% to 69.00% for disease incidence and from 36.55% to 37.67% for disease severity between 2022 and 2023, followed by Amethi consistently shows low disease incidence and severity, with values increasing from 75.66% to 77.90% for disease incidence and from 41.00% to 42.12% for disease severity between 2022 and 2023. It was concluded that Prayagraj, Deoria, Jaunpur and Gorakhpur show consistently high disease incidence and severity in both 2022 and 2023. Raibareli, Banaras, Khaliabaad, Sultanpur, Lucknow and Gonda districts exhibit moderate levels of disease incidence and severity, with values fluctuating slightly from 2022 to 2023. Ayodhya, Basti, Amethi, Shidharthnagar, Kanpur and Mau districts have relatively lower disease incidence and severity compared to the other districts, and these values remain relatively stable from 2022 to 2023. In Ayodhya district, both the germplasms (Pant Sujata and ND/AH-12) show relatively low disease incidence and severity compared to other districts. However, there are still slight increases in both parameters from 2022 to 2023. The germplasm ND/AH-27 in Ayodhya district stands out with significantly high disease incidence and severity, with values increasing from 49.86% to 50.98% for disease incidence and from 28.10% to 28.66% for disease severity and this pattern persists in both 2022 and 2023.

Overall, the data shows that disease incidence and severity have generally increased across the districts and germplasms from 2022 to 2023. Some districts consistently show higher disease incidence and severity, while others maintain relatively lower levels. Further analysis is needed to identify the specific diseases and factors contributing to these patterns and to develop appropriate strategies for disease management and prevention. Survey conducted during 2022-23 showed that Alterneria blight of bael disease severity varied from approx. 7.00% to 52.00%.

Similar result was also reported by Anonymous (2020 and 2022) survey of bael orchard was carried out for recording incidence and severity of various diseases at different places at M.E.S. Horticulture, A.N.D.U.A.T, Ayodhya, Sultanpur and Mrizapur district on different farmer field of U.P. from August to December 2021, of Alternaria leaf spot of Bael. The maximum incidence and severity of *A. alternata* in Pant sujata was found 6.0 and 0.50 per cent respectively. Rain *et al*. (2019); Balai *et al*. (2013) he has undertaken the roving method of survey of the pigeon pea crop to assess the severity of Alternaria blight disease during 2009-10 and 2010-11 in nine districts of eastern Uttar Pradesh *viz.* Azamgarh, Ballia, Bhadohi, Chandauli, Ghazipur, Jaunpur, Mau, Mirzapur, Sohanbhdra and Varanasi and five adjacent districts of Western Bihar viz., Sivan, Buxar, Arah, Bhabhua and Aurangabad. The disease intensity in areas varied from 16.93 to 38.59% during 2009-10 and 15.12 to 38.86 during 2010-11.

Table 2: Survey for the Alternaria blight disease of bael in different districts of Uttar Pradesh.

Districts	Germplasm	Year 2022		Year 2023		Pooled data of 2022-23	
		Disease incidence (%)	Diseases severity (%)	Disease incidence (%)	Diseases severity (%)	Disease incidence (%)	Diseases severity (%)
Shidharthnagar	Unknown	72.16	39.25	74.40	40.37	73.28	39.81
Basti	Unknown	66.76	36.55	69.00	37.67	67.88	37.11
Gonda	Unknown	75.34	40.84	77.58	41.96	76.46	41.40
Raibareli	Unknown	88.16	47.25	90.40	48.37	89.28	47.81
Gorakhpur	Unknown	90.14	48.24	92.38	49.36	91.26	48.80
Mau	Unknown	72.66	39.50	74.90	40.62	73.78	40.06
Banaras	Unknown	87.22	46.78	89.46	47.90	88.34	47.34
Prayagraj	Unknown	93.86	50.10	96.10	51.22	94.98	50.66
Khaliabaad	Unknown	86.08	46.21	88.32	47.33	87.20	46.77
Deoria	Unknown	92.14	49.24	94.38	50.36	93.26	49.80
Sultanpur	Unknown	75.90	41.12	78.14	42.24	77.02	41.68
Ayodhya	Pant Sujata	9.34	7.84	8.94	7.64	9.14	7.74
	ND/AH-12	13.86	10.10	13.96	10.15	13.92	10.13
	ND/AH-27	49.86	28.10	50.98	28.66	50.42	28.38
Jaunpur	Unknown	90.66	48.50	92.90	49.62	91.78	49.06
Kanpur	Unknown	72.64	39.49	74.88	40.61	73.76	40.05
Amethi	Unknown	71.66	39.00	73.90	40.12	72.78	39.56
Lucknow	Unknown	75.66	41.00	77.90	42.12	76.78	41.56

CONCLUSIONS

This study sheds light on the growing concern of Alternaria leaf spot, caused by *Alternaria alternata*, in bael trees, particularly in Uttar Pradesh, India. The bael tree holds immense cultural, religious, and medicinal significance in the region, making it imperative to address the threat posed by this disease. The research involved a comprehensive survey covering 16 districts of Uttar Pradesh, revealing varying degrees of disease incidence and severity.This newfound understanding not only aids in shedding light on the state of Alterneria blight but also lays the foundation for more targeted and comprehensive future studies. Armed with this data, researchers can make informed decisions about where to concentrate their efforts, ultimately advancing our understanding of this disease and its impact on bael crops.

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Conflict of Interest. None.

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