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ORIGINAL RESEARCH ARTICLE

Assessment of mycotic diseases attack on indigenous old mango plants grown in Vatala park, Tehsil Bernala District Bhimber, Azad Kashmir

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ABSTRACT

The current research work was explored fungal attacks on different parts of mango (*Mangifera indica* L.) trees naturally grown in Tehsil Bernala, District Bhimber Azad Kashmir. Different symptoms were observed, like stem end rot, anthracnose dieback, and blossom blight, during field survey. The identification of mycotic diseases was confirmed by further direct microscopic examination of affected parts of mango trees. Then fungal diseases of mango trees were isolated by microculture and purification methods on different media. Some other parameters, like disease incidence and disease severity rates, were measured against each identified disease through a pathogenicity test under in vitro conditions. It was indicated that maximum disease incidence (DI) was 33.73% measured against dieback disease. The lowest incidence (30.98%) was measured against blossom blight disease. It was also found that disease severity (DS) was rated from 0 to 4 on a visual rating scale.

Keywords: *Mangifera indica*; blossom blight; anthracnose; dieback; disease severity; incidence; fungal attack

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1. Introduction

The fruit known as the mango (*Mangifera indica* L.) is grown extensively around the world. It is one of the most important fruits in tropical and subtropical regions of the world. It is a member of the Anacardiaceae family. After bananas, mangoes are the second most important tropical fruit plant in Asia[1,2]. It is known as the "king of fruits" because of its outstanding, distinctive flavor, lovely aroma, and plenty of nutritionally advantageous ingredients[3,4]. Because of their great nutritional value, mangoes are recognized as a rich source of calories, fibers, vitamins, and minerals in the human diet. Foods made from mangoes include puree, slices in syrup, nectar, pickles, and chutney. Along with the leftovers from the mango food industry (peel and seed), other plant components including leaves and tree bark are also believed to be a very important source of bioactive compounds[5].

Due to a variety of biotic and abiotic factors, the mango production in Pakistan has been declined. The fungus *Ceratocystis fimbriata* is the first plant pathogen connected with mango quick decline in Brazil, Oman, and Pakistan. Mango quick decline is the most recent serious danger to the Pakistani mango industry[6–8]. One of the most harmful biotic diseases, fungal infection reduces the yield and vitality of many crops, especially mango orchards. *Lasiodiplodia theobromae* attack commonly

appeared on mango trees in Pakistan that shows decline rates varying from 8% to 61%. However, more study is required to determine the exact cause of the illness in Pakistan as well as its prevalence in areas where mangoes are produced and among the most popular mango kinds. Mango dieback syndrome is a serious disease that affects mango trees in all areas of the world that produce them[9].

The loss of mangoes yields after harvesting reaches up to 36%. While the optimum range of loss by the attack of pathogens is between 17% to 36%[10]. Many pathogens, particularly fruit rot causing pathogen are attracted toward the mango tree and cause infection on mango fruits[11]. Many fungal pathogens attack on fruit trees which caused a variety of physiological disorders on mango trees. The most common pathogens that attack on mangoes are including *Colletotrichum gloeosporioides*, *Alternaria alternata*, *Cladosporium* sp., *Dothiorella dominicana*, *Fusarium* spp. *Penicillium* sp., *Pestalotiopsis* sp., and *Phomopsis* spp. These fungi produced a variety of physiological disorders mango trees. The most common diseases of fungi named as Scab, Black spot, Leaf spots, Fruit rot, Fusarium wilt, Bud necrosis and Vascular discoloration, Twig blight and Vascular discoloration were appeared on mango plants[12].

More than 83 distinct diseases including 52 fungi, 3 bacteria, and 3 plant-parasitic nematodes of the mango trees and mango fruits have been identified that indicated huge losses globally. Some diseases were identified and isolated from mango trees in Pakistan like Anthracnose, Powdery mildew, malformation, *Fusarium wilt*, bacterial leaf spot, Crown gall, Root rot, Rhizoctonia blight and dieback. Some mango diseases were also observed in mango gardens of Sindh province, Pakistan dieback, root rot, tip dieback and plant death. These problems are widespread in orchards of various ages in Sindh province[13].

Due to their high moisture content and nutritional reserve, mango fruits are particularly susceptible to various pathogenic fungi between harvest and eating. Mango fruits are extremely perishable; thus, they must be sold as soon as they are produced. Numerous pathogens, particularly those that cause fruit rot after harvest, are attracted to the mango tree and its fruit in particular[14]. Mango anthracnose (*Colletotrichum gloeosporioides*) is a significant pre- and post-harvest disease that adversely impacts quality and marketing as well as yield in the field and packing facilities. For context on the various disease management techniques[15]. High humidity and damp surroundings are the main contributors to the development and spread of anthracnose. *Lasiodiplodia theobromae* is a member of the globally recognized *Botryosphaeriaceae* family. They have been connected to severe losses owing to host plant mortality, root necrosis, gummosis, and leaf spots all over the world[12]. Blossom blight, which can destroy blossoms and a sizable amount of the panicle, can have a substantial impact on fruit set and productivity.

The State of Pakistan's District Bhimber includes the lovely Barnala as a subdivision. It is situated in the border-making buffer zone between the territorial region and Pakistan. It is situated between 32°58'60" N and 74°04'0" E latitude and longitude. The climatological characteristics indicate that the valley has 850 mm of precipitation annually and temperatures that range from 5 °C to 50 °C. Even during the rainy season, humidity is still high (66%). These environmental conditions are suitable for mycotic diseases[16].

2. Objectives

This research work has following objectives: To identify the fungal diseases of *Mangifera indica* L. which are naturally grown in Vatala part of Tehsil Bernala, District Bhimber Azad Kashmir (BAK). To assess the incidence of fungal diseases of *Mangifera indica*. To assess the severity of fungal diseases of mango from the study area.

3. Methods

3.1. Sample collection

Samples were gathered inside closed paper envelops rather than plastic bags to prevent establishing a microclimate for the specimen following procedure of Delhove and Qasim[17]. The gathered diseased leaves were divided into envelops based on the various kinds. The variations were also listed on each envelope, along with identification (series numbers 1–5). Each location yielded a minimum of four different mango kinds, with the exception of a larger area. Five leaves were randomly selected from each envelope and identified (i.e., serially numbered based on the presence of spots/lesions on the leaf) using masking tape. The collected leaves were transported for variety verification to the mycology lab for further isolation and diagnosis of the fungus pathogens.

3.2. Isolation of fungal pathogens

All of the mango kinds that were brought from the field were sorted individually in the lab. According to the variety and place found, isolation was completed. The effected parts of leaves were surface sterilized and put in in petri dishes onto PDA (potato dextrose agar). The PDA media was plated in 17 Petri dishes altogether to test mango leaf effected parts. Then the plates were placed at room temperature (25 °C–28 °C) for three days. After that the fungal colonies appears on media were identified by mycological literature and microscopic identification.

3.3. Direct microscopic examination

It is possible to separate off little pieces of decaying plant tissue, color them, and look at them under a microscope. Some fungus exhibits specific morphological traits. Spores, for instance, can occasionally be seen on the surface of diseased above-ground plant sections. This method is frequently used to identify fungal diseases such as downy mildew, powdery mildew, grey mold and leaf spots. Affected material may produce sporangia, fungal growth or spores that can be directly identified under a microscope by incubating it on trays in moist plastic bags.

3.4. Pathogenicity tests

The pathogenicity test was conducted in lab for the assessment of fungal severity rate. The collected samples of mango leaves were surface sterilized with 5% sodium hypochlorite under laminar flow. Then the spot effected parts of leaves were washed with distilled water and shifter onto potato dextrose media that already poured into sterilized petri plates. The effected part of mango leaves after wishing with distilled ware were inoculated on the media under laminar flow bench. The incubated Petri plates were saved for ten to fifteen days on a laboratory bench at room temperature (25 °C–30 °C) for sub culturing selected fungi. The pure cultivated fungi were centrifuged and re-suspended the spores in three changes of sterile distilled water, spore suspensions were created. Each of the three fungi's spore suspensions (*Botryodiplodia theobromae*, *Macrophoma mangiferae*, and *Pestalotiopsis mangiferae* were separately applied to the lower epidermis of the leaves using a rocking sprayer. Also employed as an inoculum was a mixture made from 50 mL of each of the three fungi's spore suspensions. The experiment was conducted three times on various tree branches. Spraying sterile distilled water on one healthy branch served as the control. For five weeks, sterile cellophane bags were placed over every branch; when the disease symptoms indicated, the bags were taken off and exposing the leaves to the elements. After those results were measured.

3.5. Measurement of disease incidence and disease severity

To measure the frequency of disease incidence (DI) and disease severity (DS) of foliar fungal diseases of mango from the selected study area. The field survey of mango foliar diseases was conducted systematically

from different mango-growing regions of the study area. For this analysis, Twenty (20) mango trees were selected randomly in each sampling site. The sampling was followed by some modifications according to Awa and his colleagues[18], Abubakar[19] techniques with a minor adjustment. The scale 0 to 4 was used for the analysis instead of 1 to 5 scale. scale 0 represents no leaf lesions/spots, 1 represents one to three lesions, 2 represents four to six lesions, 3 represents seven to fifteen lesions and 4 indicted more than 70 per cent of leaf surface is of diseased leaves. The DS (percentage of area affected on the leaf on average) were then obtained by using the following formula: 1. No disease symptoms on leaves (rated zero). The 1%–25% age of leaf area covered with spots scored as 1 while 26%–50% of leaf area covered with spots scored as 2. Similarly, 51%–75% of leaf area covered with spots scored as 3% and 76% or more scored shown with 4.

$$\mathrm{DI} = \frac{\text{No. of infected trees}}{\text{Total number of trees assessed}} \times 100$$

$$\mathrm{DS} = \frac{\Sigma(a + b)}{NZ} \times 100$$

where,

Σ (a + b) indicated the Sum of diseased leaves of mango and their corresponding score, while,

N showed the total number of sampled mango trees,

Z indicated maximum rating on the selected scale and,

X expressed number of diseased mango trees[18].

The DI (%) of mango trees showed by disease symptoms and calculated by counting number of infected leaves out of total assessed leaves on mango. The result was documented in form of DI (%). The second parameter was Disease severity (DS). It was also measured in percentage from the lesion area on the leaves surface via evaluated from visible lesions. The scale 1 to 7 was used by scoring; 0 = No disease, 1 = 1%–2% disease, 2 = 5% disease, 3 = 10% disease, 4 = 20% disease, 5 = 40%, 6 = 60% and 7 indicated more than 80% DS. This method was followed by Hadthamard et al.[20] with some key modifications.

4. Results

The current study was conducted from January 2022 to August 2023 in the Mucology and Plant Pathology Lab. Department of Botany, Mirpur University of Science and Technology (MUST), Bhimber Campus, Azad Kashmir. The results of this article showed that different symptoms appeared including necrotic (leaf spot and leaf blight) and chlorotic during the study of mango. The aim of this study was to provide the information about the fungal diseases of indigenous mango varieties and their possible control in the area of Bernala so that the people of this area are aware of these diseases and their control. Leaf spot and leaf blight symptoms showed variable incidence and severity among the endophyte isolates. There is an influence of the endophyte fungi isolates on the leaf spot and leaf blight incidence and severity where the influence was highly significant. The combined treatment was expressed the most effective results to reduce leaf spot DI with 4.94% value. Although, all isolates' treatments were not indicated significant difference value. but they were shown significantly difference value with compared with control treatments. It was showed the best isolates to inhibit the DS of the symptoms appeared on leaf in form of leaf spot with 1.08% DS (**Figure 1**). Hence, all observed endophytic fungi treatments were not indicated significant difference. The study's findings also demonstrated after inoculation with the pathogen *L. Pseudotheobromae*, a variety of symptoms have been appeared including necrotic leaf spot, leaf blight and chlorotic spot. These were developed on both the treated and control mango plants by using formulas mention in the method.

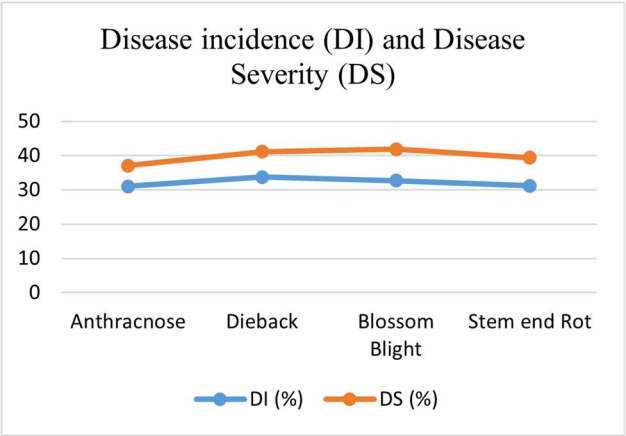

Figure 1. The graph indicated disease incidence (DI) and disease severity (DS) of four diseases of mango trees on the basis of morphometric characteristics.

The DI and DS of leaf spot and leaf blight symptoms varied across the endophytic strains. The incidence and severity of leaf spot and leaf blight are affected by the endophyte fungal isolates, and this influenced quite substantially indicated in **Figure 1**.

The most effective isolates only increase the severity of the leaf spot symptom by 7.4%. Treatments for endophyte fungal isolates did not differ considerably from one another. However, none of the isolate treatments significantly differed from the pathogen-only control, which exhibited no symptoms (**Table 1**).

Table 1. Disease incidence (DI) and disease severity (DS) according to symptoms on leaves.

Mycotic diseases	DI (%)	DS (%)
Anthracnose	30.98	6.12
Dieback	33.73	7.4
Blossom blight	32.68	9.17
Stem end rot	31.20	8.17

The incidence and severity of leaf blight are reduced by the most efficient isolates by 50.15% and 11.10%, respectively. However, all isolate treatments significantly differed from the pathogen-containing control, and a small percentage of the non-pathogen-containing control plants also exhibited symptoms of blight (**Table 2**).

This showed endophytic fungi from mango stems tissue can inhibit one of the causal agents of mango dieback, *L. pseudotheobromae*, on the mango stems. The results indicated that all the isolates of the endophytic fungi completely inhibited different symptoms produced by *L. pseudotheobromae*, i.e., leaf spot, and leaf blight. The exploration of DI and DS of four fungal diseases were also mentioned symptomatically in pie graph (**Figure 2**).

Table 2. Disease incidence and severity according to necrotic symptoms on leaves (leaf blight).

Mycotic diseases	Disease incidence (%)	Disease severity (%)
Anthracnose	54.35	16.01
Dieback	52.25	14.12
Blossom blight	50.15	11.10
Stem end rot	54.20	15.11

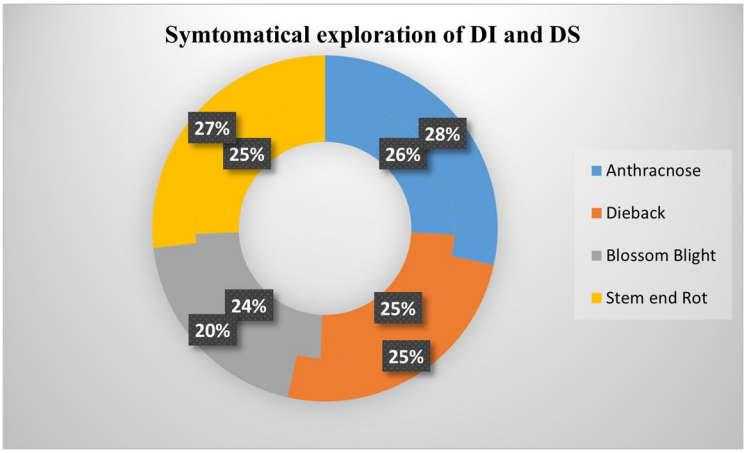

Figure 2. The pie graph indicated disease incidence and severity according to necrotic symptoms on leaves of mango trees.

Fungal isolates were identified using taxonomic keys. Disease severity was rated from 0 to 4, based on a visual scale, where 0 is an asymptomatic inflorescence, 1 is equal to 1% to 25% of diseased area, 2 equals to 26% to 50% of diseased area, 3 equals to 51% to 75% of diseased area, and 4 is greater than 76% of diseased area the percent range midpoint was taken directly for each estimated interval of the visual scale, a zero-disease severity (asymptomatic) was replaced by the value of 0.001 (**Table 3**). The bar graph indicated number of inflorescences and midpoint measurement on the basis of disease severity visual scale and H-B scale during this analysis (**Figure 3**).

Table 3. Number of inflorescences was evaluated using a disease severity visual scale and converted to a midpoint based on H-B scale during two surveys.

Disease severity scale	Midpoint based on H-B scale	Number of inflorescences
0	0.03	230
1	13.0	142
2	38.0	23
3	63.0	22
4	88.0	35

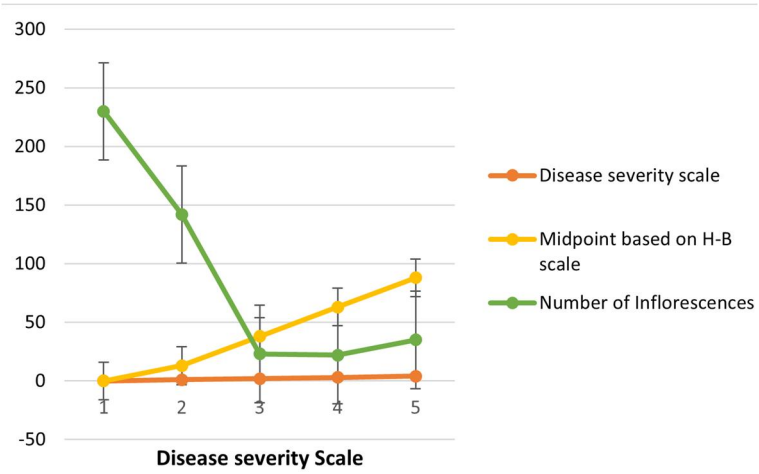

Figure 3. The graph indicated number of inflorescences evaluated using a disease severity visual scale and converted to a midpoint based on H-B scale during assessment.

Table 4 was elaborated field survey of four sites from the study area that indicated estimation of mango trees population and count their effected ratio. According to this study, total 434 mango trees were observed in the area. Out of these trees, only 80 mango trees were infected with fungal species. The trees were planted

on the different sites of Tehsil Bernala of District Bhimber, Azad Kashmir which supplied appropriate moisture for the most part of the year. A sprawling urban center provided a huge demand for the mango fruits. Furthermore, it was depicted that the site Zardari gardens of mango infected with the highest disease infection rate 28.57% while lowest infection rate 13.18% was measured from Koil. The overall fungal infection rate was 18.43% was documented from the four observed sites of Tehsil Bernala, District Bhimber, Azad Kashmir (**Table 4**).

Table 4. The survey of infected mango trees in four mango trees growing dense areas of Tehsil Bernala.

Location	Estimated mango tree population	Estimated infected mango trees	Infection rate (%)
Moil	112	27	24.11
Rehana	67	14	20.89
Sardari	35	10	28.57
Koil	220	29	13.18
Total observed trees	434	80	18.43

The disease severity (DS) and lead score (LS) from different locations was also measured and documented as mentioned in **Table 5**. It was indicated that the site Kotjamel shown maximum DS (56.75%) and LS (2.75). While the lowest DS (31.65%) and LS (1.65) measured from Moil. The ecoclimatic conditions of Moil less supported to cultivation of fungi and the environment of Kotjamel was more suitable for the expansion of fungi. The mango gardens of the Kotjamel surrounded by diverse vegetation. Hence, fungi need high humidity and low temperature for their effective growth and spread. Therefore, the area depicted more humidity and low temperature. These two climatic factors supported to grow fungo more on mango gardens.

Table 5. Measurement of disease severity of mango trees in different sites of study areas.

Location	No. of spot	1–25	26–50	51–75	75–100	Leaf score	Disease severity (%)
Moil	4	6	5	3	2	1.65	31.65
Rehana	0	2	4	3	1	2.30	45.50
Sardari	4	4	6	5	1	1.75	34.15
Koil	10	4	6	2	8	1.80	37.00
Khairowal	1	6	6	10	2	2.24	44.48
Kotjamel	0	3	5	6	6	2.75	56.75
All locations	19	25	32	29	20	2.05	41.02

5. Discussion

The current study showed endophytic fungi from mango stems tissue that inhibited the causal agents of mango dieback (*L. pseudotheobromae*). Similar findings were previously explored from mango trees particularly dieback disease[9]. The findings showed that leaf spot, leaf blight, and chlorosis, three symptoms caused by *L. pseudotheobromae*, were totally prevented by the endophytic fungi[21].

The endophytic fungi used are of the genus *Aspergillus*. Similar findings were also explained previously[22]. Aspergillus species have been described as endophytes with antifungal activity and the capacity to create a variety of secondary metabolites. Through a variety of inhibitory activities, mango endophytic fungi can defend mango plants from phytopathogens. Additionally, according to Yang et al.[23] endophytic fungi from mango tree tissue can colonize mango plant tissue and can react to biotic and abiotic stresses in the plant. Endophytic fungi from the tissue of mango stems and leaves effectively reduced the incidence and severity of

disease caused by the fungus *L. pseudotheobromae*. But a broader study area would yield more information, especially in the outdoors.

In my research, it was discovered that the indigenous microorganisms that live epiphytically on mangoes have a negative impact on *C. gloeosporioides*. This supports the hypothesis that epiphytes are an effective source of biocontrol for eradicating plant diseases. Because *Colletotrichum gloeosporioides* can infect a variety of crops and result in significant economic losses, as observed by Arzanlou et al.[24]. It has been thoroughly examined.

6. Conclusion

This is the first comprehensive study to identify fungal pathogens of inflorescences, leaves, stems, and mango fruit at different developmental stages in four mango cultivars of Barnala. Necrosis, powdery mildew, blossom blight, anthracnose, stem end rot, and tip blight were the most common diseases identified in mango trees. Disease incidence and severity caused by the fungus *L. pseudotheobromae* were effectively reduced by endophytic fungi from mango tree stems and leaves. The results indicated that all the isolates of the endophytic fungi completely inhibited different symptoms produced by *L. pseudotheobromae*, i.e., leaf spot and leaf blight. Biomanagement was done by using different chemical, biological, and physical treatments for preventing, suppressing or delaying disease symptoms during storage.

7. Recommendations

Several fungal pathogens can cause various diseases in mango trees or fruits. During ripening, the fruit becomes susceptible to various fungal pathogens that switch from the endophytic stage to the necrotrophic stage and cause diseases like SER, dieback, and anthracnose. Although SER disease leads to significant fruit loss, the basic science of fruit SER is largely unknown. Nevertheless, studies have shown that different treatments can decrease the incidence of SER and anthracnose by directly inhibiting the fungal growth, indirectly inducing host resistance, or indirectly changing the stem-end micro-biome to a more diverse and less pathogenic community. With the increased availability of new tools such as deep sequencing, new studies are expected to emerge, leading to a better understanding of the host-pathogen interaction, which could lead to the development of new means to reduce fruit, stem, and inflorescence diseases in mango.

Author contributions

Conceptualization, TH and RS; methodology, TH; software, FAK; validation, TH, RS and FAK; formal analysis, FAK; investigation, TH; resources, TH; data curation, TH; writing—original draft preparation, TH; writing—review and editing, FAK; visualization, TH; supervision, TH; project administration, TH; funding acquisition, TH. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declared no conflict of interest.

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