



Research Article

Biodiversity of Foliar Fungi Associated with Angiosperms of Bajaur District with Taxonomic Notes on Some New Records from Pakistan

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Abstract | Fungi are considered to be the most common and dangerous plant pathogens. Fungal diseases cause significant harm to leaves, the site of food synthesis, and thus they pose a major risk to biodiversity and global food security. The foliar fungal infections of Tehsil Khar and Tehsil Utman Khel in the Pakistani district of Bajaur, KP, were the subject of this investigation. A thorough investigation was conducted on seven distinct plant species because they exhibited foliar symptoms indicative of fungal disease. *Alternaria solani*, *Erysiphe platani*, *Helicoceras celtidis*, *Leveilulla taurica*, *Phyllactinia moricola*, *Plasmopara viticola*, *Podosphaera xanthii*, and *Pseudocercospora platanigena* were the eight fungal foliar pathogens that were isolated. *Ampelomyces quisqualis* was isolated from *E. platani*, a *Platanus* powdery mildew. All of the pathogens that have been found are new records from Pakistan, with the exception of *Alternaria solani* and *P. platanigena*. For the first time, *P. xanthii* has been found in Pakistan associated with annual weed plant "*Xanthium strumarium*". The district now has new records for each of the aforementioned taxa. Re-description and illustration of a few taxa are done.

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Introduction

It is a fact that people have been brewing and baking with fungi for thousands of years, even if systematic research on the subject is very recent less than 250 years old (Alexopoulos *et al.*, 1996). Of the estimated 2–11 million fungal species on Earth, only approximately 150 thousand have formal descriptions (Phukhamsakda *et al.*, 2022). This suggests that fungi are among the least explored of our biodiversity resources. Pathogenicity, symbiosis, soil formation, fertility for primary production,

symbiosis, and biotechnology are only a few of the many environmental, scientific, and industrial services that fungi offer (Takamatsu *et al.*, 2008; Lange, 2014; Dighton, 2018).

Powdery mildew is the most prevalent fungal infection. Obligate biotrophic pathogens are the reason; they inflict illness on over 10,000 host plant species, including major crops, and result in significant losses to horticulture, forestry, and agriculture (Braun, 2012a). Fungicides are typically used to control these diseases, although doing so may have adverse effects

on plant physiology and biodiversity in addition to causing some powdery mildew fungus to develop fungicide resistance (Németh *et al.*, 2021). The safe and all-natural substitute for dangerous fungicides is the application of biocontrol agents. Powdery mildews have been effectively controlled using the mycoparasite species *Ampelomyces* as a biocontrol agent (Németh *et al.*, 2021). The majority of research in Pakistan has focused on the Genus *Trichoderma*'s capacity for biocontrol (Ali *et al.*, 2021).

In Pakistan, fungi are among the less studied life forms (Raza *et al.*, 2022). The last comprehensive list of fungi reported from Pakistan was published Ahmad *et al.* (1997), dealing with 1219 species. Since then, several fungal species have been reported as new species and new records (Raza *et al.*, 2022). Foliar pathogenic fungi have been mainly reported from cultivated plants in Pakistan (Iftikhar *et al.*, 2010; Bux *et al.*, 2012; Sami *et al.*, 2017). A number of white blister rusts and downy mildews with taxonomic details have been provided by Abdul Haq *et al.* (2015) from Khyber Pakhtunkhwa Province.

The current study set out to look into the taxonomy and diversification of foliar pathogenic fungi connected to angiosperm intact leaves. Prior research mostly addressed issues like as pathogenicity and mitigation strategies. The researcher noted their diversity and provided descriptions of a few Pakistani fungi that had not been previously documented. This study is important since it contributes to the richness of the country's mycoflora and offers taxonomic information that will aid the future workers in the identification of these fungi.

Materials and Methods

Study area and specimen collection

During the spring and summer of 2022, infected and/or symptomatic leaves of cultivated and wild plants were gathered from various locations within the two tehsils of the District Bajaur, Khar and Utmankhel. They were placed in sterile polythene bags and sent to the Department of Botany at Government Post Graduate College Khar, Bajaur, as soon as possible, or at most within 24 hours, so that the related fungi could be isolated and identified.

Fungal isolation and identification

The diseased samples were first cleaned of surface

impurities using running tap water and then a small portion (2-4 mm²) of the symptomatic tissues and the nearby unaffected tissues were cut with a sterile blade. Following a 30-second surface sterilization with 70% ethanol, these tiny fragments were washed 3-4 times with sterile tap water and then blotted with sterile blotting paper. They were aseptically inoculated onto Petri dishes, containing potato dextrose agar (PDA), which were supplemented with penicillin and streptomycin. For three to seven days, the plates were incubated at room temperature. The plates were routinely checked for fungal mycelial development. Additionally, foliar fungi, particularly the biotrophs, were investigated and characterized using the scotch tape method (Langvad, 1980). Using a light microscope and mycellium stained with lactophenol cotton blue, morphological characteristics were recorded. Using a mobile camera, the taxa's key traits were captured on camera. The related fungus was identified at the species level using identification keys (Thom and Raper, 1945; Ellis, 1976; Sutton, 1981; Braun, 2012; Guarro *et al.*, 2012; Watanabe, 2018). For the taxa's current name and synonymy, the online fungal database Index Fungorum (<https://www.indexfungorum.org>) was visited. The infected plant specimens were mounted on paper sheets and stored in the Department of Botany's herbarium at Government Post Graduate College Khar, KP, Pakistan (34.75535°N 71.544048°E).

Results and Discussion

A total of seven angiosperms with foliar signs of fungal infection were processed for isolation and identification of the pathogenic fungus species (Table 1). Eight fungal foliar pathogens and one fungal hyperparasite were recorded. Except for *Alternaria solani* and *Pseudocercospora platanigena*, the rest are new records for Pakistan. For the first time in the country, *Podosphaera xanthii* has been discovered in *Xanthium strumarium*. All of the aforementioned taxa are being reported for the first time from District Bajaur. The new records for Pakistan have been described.

Taxonomy

Ampelomyces quisqualis: Ces., in Klotzsch, Bot. Ztg. 10: 301 (1852) Figure 1A-E.

Synonymy: *Cicinobolus cesatii* de Bary (as '*Cicinnobolus*'), Abh. senckenb. naturforsch. Ges. 7: 431 (1870) *Cicinobolus cesatii* f. *euonymi* Tassi (as '*Cicinnobolus*'), Bulletin Labor. Orto Bot. de R. Univ. Siena 2: 150 (1899).

Table 1: List of isolated taxa and their respective host species.

S.	Pathogen	Family	Host	Tehsil	New for
1	<i>Alternaria solani</i> Sorauer	Pleosporaceae	<i>Solanum lycopersicum</i>	Arang	Bajaur
2	<i>Erysiphe platani</i> (Howe) Braun and Takam	Erysiphaceae	<i>Platanus orientalis</i>	Arang	Pakistan
3	<i>Helicoceras celtidis</i> (Biv.) Linder	Incertae sedis	<i>Celtis australis</i>	Arang	Pakistan
4	<i>Leveillula taurica</i> (Lév.) Arnaud	Erysiphaceae	<i>Capsicum annuum</i>	Khar	Pakistan
5	<i>Phyllactinia moricola</i> (Henn.) Homma	Erysiphaceae	<i>Morus. sp</i>	Khar	Pakistan
6	<i>Plasmopara viticola</i> (Berk. and Curtis) Berl. and De Toni	Peronosporaceae	<i>Vitis vinifera</i>	Arang	Pakistan
7	<i>Podosphaera fusca</i> (Fr.) U. Braun and Shishkoff	Erysiphaceae	<i>Xanthium strumarium</i>	Arang	Pakistan
8	<i>Pseudocercospora platanigena</i> Videira and Crous	Mycosphaerellaceae	<i>Platanus orientalis</i>	Arang	Bajaur
9	<i>Ampelomyces quisqualis</i> Ces.	Phaeosphaeriaceae	<i>Erysiphe platani</i>	Khar	Pakistan

Cicinobolus cesatii f. *phlomidis-herbae-venti* Unamuno, Boln Real Soc. Españ. Hist. Nat., Biologica 28: 201 (1928).

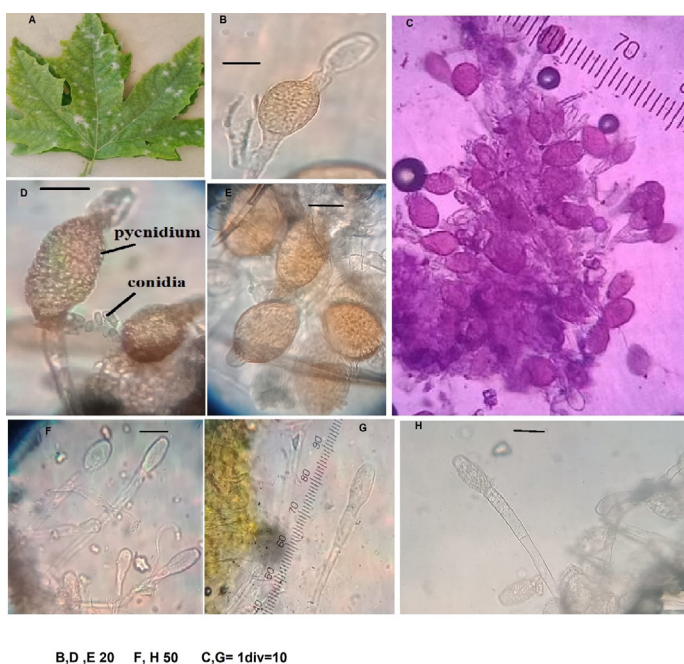


Figure 1: A: Leaf infected by *E. platani*; B-E: pycnidia of *A. quisqualis*; F-H Conidia and conidiophores of *E. platani* (bar B, D and E=20 um, F-H=50 um, C and G 1div=10 um).

Specimens examined: GPK109 Department of Botany, GPGC Khar, Bajaur, KPK, Pakistan, *Platanus orientalis* leaf infected by *Erisiphe platani* in GPGC Khar Boys Hostel.

Sexual state was not observed: The conidiomata were pycnidial, which were stipitate, scattered, mostly solitary, superficial, uniloculate, globose or elongated to pyriform, pale brown, ostiolate, sometimes papillate. The pycnidial wall was thick, composed of pale brown cells with angular arrangement, measuring 42.5-60.0 (\bar{x} =50.1) x 28.0-42.5 (\bar{x} =32.4) um. The pycnidial stipe was measuring 50-100. Conidia were amerosporous,

cylindrical to fusiform, straight to curved, pale brown, thin and smooth-walled, measuring 3-5 x 1-3 um. Hyphae were hyaline and septate; are present within the hyphae, conidiophores, and conidia of infected *E. platani*.

Ampelomyces has long been reported in various species of the Erysiphaceae. It was among the first mycoparasites to be studied in detail and also among the first fungi used as potential biocontrol agents of economically important plant pathogens (Manjunatha et al., 2020). *Ampelomyces quisqualis* has been found on more than 64 species of powdery mildew on 256 species of plants (Kiss et al., 2004). *Ampelomyces* species could be distinguished into slow and fast-growing types. Based on the rDNA ITS region phylogenetic analysis, Kiss et al. (2004) concluded that strains from the two types of differentiated growth rate were not congeneric. The slow-growing isolates, obtained from intracellular pycnidia in powdery mildew mycelia was *Ampelomyces* sensu stricto. While fast growing isolates from sessile pycnidia on mildew-infected leaves, were closely related to *Phoma* species. The genus *Ampelomyces* have stipitate pycnidia, while pycnidia of *Phoma* sp. are sessile (Sutton, 1981).

***Erysiphe platani*:** (Howe) U. Braun and S. Takam., Schlechtendalia 4: 12 (2000) Figure 1F-H.

Synonymy: *Microsphaera platani* Howe, in Bessey, Bull. Torrey bot. Club 5: 4 (1874)

Specimens examined: HA103 Department of Botany, GPGC Khar, Bajaur, KPK, Pakistan, *Platanus orientalis* leaves showing powdery mildew symptoms Boys Hostel, GPGC, Khar in the Tehsil Khar

The mycelium displayed an amphigenous growth

pattern, forming colonies of varying density. The hyphae were branched, transparent, and smooth, and featured lobed mycelial appressoria. The conidiophores were upright, measuring 125–275 µm in length, and could have up to 3 septa. They had straight to flexuous foot cells and produced mature conidia singly. The primary conidia had rounded apices and subtruncate bases, were transparent, and measured 29–40 x 14–22.5 µm. The secondary conidia were ellipsoid to doliform, with slightly convex ends, transparent, and measured 30–38 x 15–22.5 µm. Chasmothecia were not observed.

White powdery mildew colonies were apparent primarily on the upper leaf surface of leaves. Stunting and distortion of mature and terminal leaves of growing shoots were observed. Defoliation in severely affected plants was noted. It was observed in some plants that this pathogen was hyper-parasitized by *A. quisqualis*.

Helicoceras celtidis: (Biv.-Bernh. ex Sprengel) Linder, 1931, Ann. Mo. Bot. Gdn, 18:3. [Figure 2E, F](#).

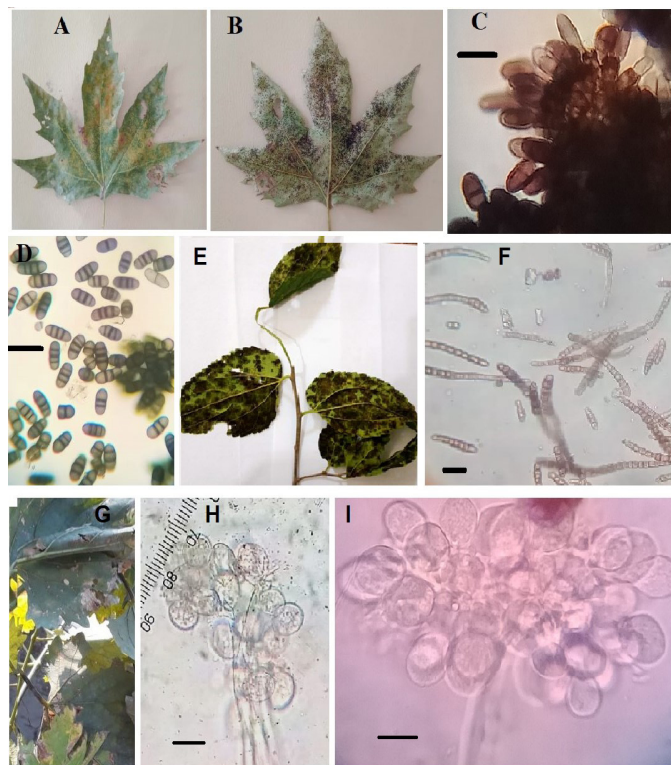


Figure 2: A-D *Pseudocercospora platanigena*. A, B: infected leaves; C: Sporodochium; D: conidia; E, F: *Helicoceras celtidis*; E: infected leaf; F: conidia; G-I: *Plasmopara viticola*; G: infected leaf; H-I: Sporangia and sporangiophore (bar=10 um).

Synonymy: *Gyrocera celtidis* (Biv.) Mont. and Ces. (as ‘celtis’), in Montagne, Syll. gen. sp. crypt. (Paris): 308 (1856).

Monilia celtidis Biv. (as ‘celtis’), Stirp. Rar. Sic. 3: 18, pl. 3, Figure 3 (1813)

Sirosporium celtidis (Biv.) M.B. Ellis, Mycol. Pap. 87: 4 (1963)

Torula celtidis (Biv.) Sacc., Atti Soc. Veneto-Trent. Sci. Nat. 2(1): 178 (1873)

Specimens examined: RA108 Department of Botany, GPGC Khar, Bajaur, KPK, Pakistan, *Celtis australis* leaves showing reddish to dark brown spots in the Tehsil Arang.

The colonies exhibited a hypophyllous growth pattern, appearing as reddish brown to dark blackish brown and possessing a velvety texture. The conidiophores were erect or ascending, measuring 4-8 µm thick. They were smooth and pale brown near the base, often becoming verrucose and darker at the apex. The conidia were cylindrical or sometimes obclavate, with a tendency to be curved or coiled. They had a smooth, rugulose, or verrucose texture and can range from sub-hyaline to golden or reddish brown in colour. The conidia typically had 4-38 transverse septa, occasionally featuring 1 or 2 longitudinal or oblique septa. They were slightly constricted at the septa, measuring 17-150 µm in length, 7.5-10 µm in thickness at the broadest part, and 2.5-5 µm in width at the base.

Leaves showed reddish to dark brown velvety irregular spots, later becoming greyish brown on the upper surface. This fungus has been reported in Algeria, India, Israel, Italy, Japan, Morocco and Portugal (Ellis, 1971). It has been recently reported by the same host from Indian-held Kashmir (Dar et al., 2015).

Phyllactinia moricola: (Henn.) Homma, Trans. Sapporo nat. Hist. Soc. 11(3): 174 (1930) (1929) [Figure 3A-H](#).

Synonymy: *Phyllactinia suffulta* var. *moricola* Henn., Jahrb. Syst. 28(3): 271. 1900

Phyllactinia suffulta f. *moricola* Jacz. (Jaczewski, 1927: 434)

Phyllactinia moricola (Henn.) Sawada, Rep. Dept. Agric. Gov. Res. Inst. Formosa 49: 84. 1930

Anamorph: *Ovulariopsis moricola* Delacr., Bull. Soc. mycol. Fr. 19: 345 (1903)

Specimens examined: GPK107 Department of

Botany, GPGC Khar, Bajaur, KPK, Pakistan, *Morus Sp.* leaves showing powdery mildew symptoms in Botanical Garden GPGC Khar

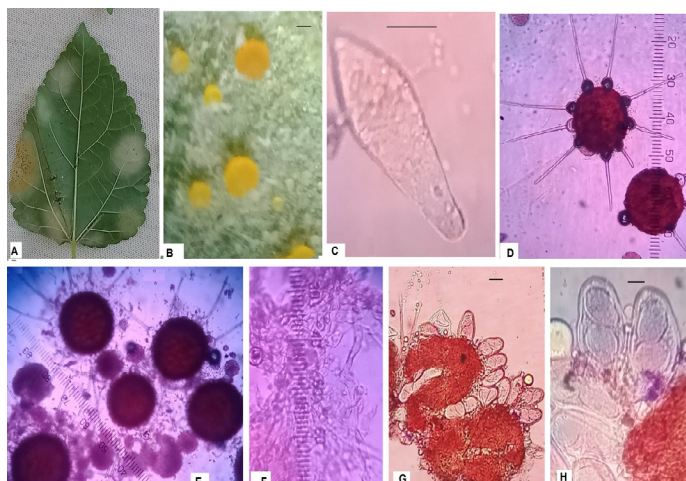


Figure 3: *Phyllactinia moricola*. **A:** infected leaf; **B, D, E:** cleistothecia; **F:** penicillate cells; **G, H:** asci and ascospores (bar B, C, E, G, H = 20 μ m, 1 div. D, F = 10 μ m).

Mycelium on the leaves was hypophyllous, thin to moderately thick, effuse, white to greyish in colour and evanescent to persistent. The hyphae were flexuous, branched, septate and 3-7 μ m wide. The conidiophores were 3-5 celled, having dimensions of 60-350 x 5-8 μ m. Conidia formed singly, clavate or fusiform, apex rounded or protruding. 40.90 x 15-32.5 μ m. Cleistothecia were hypophyllous, mostly scattered, yellow when young and turned brownish black when matured, measuring 130-250 μ m in diameter. Each had 7-20 acicular appendages, with a bulbous base of 30-40 μ m diameter. The appendages were 1-2.5 times as long as the cleistothecial diameter, penicillate unbranched and 100-187 μ m long. The asci were 6-30 per ascocarp; broadly clavate, measuring 62-137 X 30-37.5 μ m, and 1-2 spored. The spores were ellipsoid-ovoid; 25-35 x 15-25 μ m.

The powdery mildew disease of mulberry (*Morus sp.*), caused by *Ph. moricola* was observed in autumn. The fungus only affected juvenile plants. The disease first manifested as small, characteristic white-coloured powdery masses on the lower surface of leaves, accompanied by corresponding chlorotic lesions on the upper surface. As the disease progressed, the size of the patches increased and eventually coalesced to cover the entire abaxial surface of leaves, transforming the patches from brown to black and the leaves from green to yellow. Only three species of the genus: *Ph. guttata* on *Betula utilis* from Gilgit, *Ph. mali* (Duby) U. Braun (Syn. *Ph. mespeli*) on *Cotoneaster*

bacillaris and *Prunus amygdalus* from Quetta and *Ph. cornicola* on dogwood trees from Azad Jammu and Kashmir, have been reported from Pakistan (Ahmad *et al.*, 1997; Zafar *et al.*, 2023). The fungi found on *Morus* were designated as *P. guttata* by Braun (1987) and Zheng and Yu (1987), while Nomura (1997) and Braun (2012) classified them as *P. moricola*. In their phylogenetic analysis utilizing combined 28S and ITS sequences, Takamatsu *et al.* (2008) categorized *Phyllactinia* species from diverse host families into five groups. Subgroup 1B includes fungi parasitic on hosts of the Moraceae family. Accepting a broad species concept, Shin (2000) proposed that the fungi on Moraceae could be considered a single species, specifically *Ph. moricola*. The phylogenetic analysis based on ITS sequence presented by Wang *et al.* (2014) and Meeboon *et al.* (2018), the isolate of *Ph. guttata* from the Genus *Morus* clustered with that of *Ph. moricola*, however, the isolate reported from other plants showed phylogenetic distance. The isolate was identified by comparing it with the description of *Ph. guttata* (Sharma *et al.*, 2011) and *Ph. moricola* (Homma, 1937; Meeboon *et al.*, 2018; Heluta and Korytnianska, 2021; Jayawardena *et al.*, 2021).

***Plasmopara viticola*:** (Berk. and M.A. Curtis) Berl. and De Toni, in Berlese, De Toni and Fischer, Syll. fung. (Abellini) 7(1): 239 (1888) Figure 2G-I.

Synonymy: *Botrytis viticola* Berk. and M.A. Curtis, J. hort. Soc., London 6: 289 (1851)

Rhysotoheca viticola (Berk. and M.A. Curtis) G.W. Wilson, Bull. Torrey bot. Club 34: 407 (1907)

Specimens examined: HA104 Department of Botany, GPGC Khar, Bajaur, KPK, Pakistan, *Vitis vinifera* leaves showing downy mildew symptoms in Arang Tehsil Utmankhel.

The sporangiophores emerging through the stomata are branched perpendicularly and up to 700 μ m long and up to 9 μ m wide at the base. The sterigmata up to 8 μ m. carry. The sporangia hyaline, spherical, ovoid or pyriform, measuring 10-15 x 12-22 (mean 12.5-16.5) μ m. Sexual structures were not found.

Plasmopora viticola is an obligate parasite, belonging to the oomycetes family Peronosporaceae (Dick, 2013). The fungus has a narrow host range, parasiting the cultivated grapevine (*Vitis vinifera*) and a few other *Vitis* species (Langcake and Lovell, 1980). The

pathogen attacks all green parts of the grapevine. In susceptible vines, this disease can develop and spread rapidly during a single season. The most damage occurs on inflorescence and leaves. In Bajaur the disease was observed in the July-September of the year 2022. The lesions appeared on leaves as yellow or reddish brown areas on the upper leaf surface corresponding to white downy fungal growth on abaxial surface. Some downy mildews have been already reported from the area (Abdul Haq *et al.*, 2015) and this is the first report of *P. viticola* from Pakistan.

***Pseudocercospora platanigena*:** Videira and Crous, in Videira, Groenewald, Nakashima, Braun, Barreto, de Wit and Crous, Stud. Mycol. 87: 410 (2017) [Figure 2A-D](#).

Synonymy: *Stigmina platani* (Fuckel) Sacc., Michelia 2(no. 6): 22 (1880)

Specimens examined: GPK102 Department of Botany, GPGC Khar, Bajaur, KPK, Pakistan, *Platanus orientalis* leaves showing powdery mildew symptoms Main Library, GPGC, Khar.

Sporodochia hypophyllous, black, at first punctiform and scattered, later confluent and forming extensive colonies. Conidiophores up to 12.5-25 (\bar{x} =18.8) x 5-8 μ m. Conidia ellipsoidal or cylindrical, rounded at the apex, truncate at the base, mid to dark brown, smooth, with 2-4 (usually 4) transverse and occasionally no longitudinal or oblique septa, 10-20x5-10 μ m.

It has been already reported as *Stigmina platani* from Pakistan on leaves of *Platanus* leaves (Ahmad *et al.*, 2014). It is also reported from the same host in Europe, India and N. America (Ellis, 1976).

Conclusions and Recommendation

This research was undertaken primarily to record the diversity and taxonomy of fungi linked to the leaves of flowering plants. This study is significant because it reports some new records of fungi from Pakistan and provides a taxonomic details of selected taxa. This is a departure from earlier works which were mainly focused on disease reports. The prevalence and variety of powdery mildew make them the most common and diverse foliar pathogenic fungi. The presence of *Ampelomyces*, in the natural fungal population is a good

sign and can be used as biocontrol agent. Nonetheless, it's important to note that this study has limitations and is not comprehensive as a large number of fungi are waiting for discovery. Incorporating DNA molecular techniques can significantly enhance the robustness of such works.

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Novelty Statement

The paper provides addition to the fungal diversity of Pakistan. It also provides taxonomic description of some phytopathogenic fungal taxa.

Author's Contribution

Rafi Ullah and Hamid Ullah: Performed experimental work, analyzed the data, wrote the manuscript.

Muhammad Abdul Haq: Supervised the study

Aminul Haq: Co-supervised the study

Conflict of interest

The authors have declared no conflict of interest.

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