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Geographic Distribution and Genetic Diversity of *Podosphaera xanthii* in Pakistan

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ABSTRACT

Background and Objective: *Podosphaera xanthii* is one of the most commonly occurring powdery mildew pathogens, with a broad host range and wide distribution. Despite its prevalence, an updated record of its host plants in Pakistan was lacking. This study aimed to update the host range of *P. xanthii* based on recent surveys, confirm its occurrence through morphological and molecular analyses, and highlight its diversity and regional spread in Pakistan. **Materials and Methods:** Field surveys were conducted across five regions of Pakistan Battagram, Fairy Meadows, Khanspur, Mansehra, and Swat between 2022 and 2024. Infected plant samples were collected and analyzed morphologically, focusing on both anamorphic and teleomorphic stages. Molecular confirmation was done through sequencing of the internal transcribed spacer (ITS) region. Descriptive statistics were used to report host range diversity and geographic occurrence. **Results:** A total of ten plant species were confirmed as hosts of *P. xanthii*, based on morphological characteristics and ITS-based molecular validation. Among these, members of the Asteraceae family were dominant. The fungus was found in all surveyed regions, indicating a wide ecological range and adaptability. The simultaneous observation of both reproductive stages supports its active and persistent lifecycle across diverse climatic zones. **Conclusion:** This study provides an updated account of *P. xanthii* host plants in Pakistan, confirming its widespread occurrence and significant diversity. The findings underscore the need for targeted management strategies to mitigate its impact, especially on susceptible plant families like Asteraceae.

KEYWORDS

Asteraceae, Battagram, Fairy Meadows, fungal pathogen, host specificity, ITS region, powdery mildews

INTRODUCTION

Powdery mildew fungi belonging to the family Erysiphaceae are obligate biotrophic parasites that grow on both sides of leaf surfaces, petioles, and stems. They are characterized by a whitish powdery mass that obtains nutrients by means of haustoria[1]. They may develop in open fields and greenhouses and reduce the crop yields up to 40%[2]. The family Erysiphaceae is composed of five major tribes, including *Erysipheae* Tul. & C. Tul., *Golovinomyceteae* U. Braun, *Cystotheceae* Katumoto, *Phyllactinieae* Palla, and *Blumerieae* Cook. The tribe *Cystotheceae* is further composed of *Cystotheca* Berk. and Curtis, *Podosphaera* Kunze, and *Sawadaea* Miyabe. *Podosphaera* is a pathogenic fungus of herbaceous plants and trees, while *Cystotheca, Sawadaea* are pathogens of trees[3].

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The genus *Podosphaera* is capable of both sexual and asexual reproduction. In the teleomorphic stage, fruiting bodies (chasmothecia) develop and contain a single ascus. Formation of a single ascus is the distinguishing feature of the genus *Podosphaera,* which distinguishes it from other powdery mildew fungi. Another distinguishing teleomorphic character of *Podosphaera* is the formation of myceloid, uncinate, circinate, or dichotomously branched hyphal appendages. Anamorphic characters of *Podosphaera* include the formation of multiple conidiospores per condiophore and are almost similar to other genera of Erysiphaceae[4-7].

Among various *Podosphaera* species, *P. xanthii* mostly infects trees and herbaceous plants and is reported on numerous hosts of the family Asteraceae, Balsaminaceae, Fabaceae, Solanaceae, Verbenaceae in North, South America, Asia, Australia, Africa, Europe, and New Zealand[3]. This fungus is causing enormous economic losses in members of the family Cucurbitaceae. The nomenclature and host range of *P. xanthii* have changed repeatedly and were reported for the first time under the name *Sphaerotheca fuliginea* Schltdl. by Sawada in 1919, causing infection on 15 different plant species. Braun in 1987 reduced this species to *Sphaerotheca fusca* (Fr.) S. Blumer and in 2001 re-introduced this species as *P. xanthii* after examining its infection on hosts of various plant families[3-8]. Despite the widespread occurrence of *Podosphaera xanthii* and its economic impact, data on its host range and distribution in Pakistan remain sparse. In this study, new host records of *P. xanthii* from Pakistan based on recent field collections have been documented. These records are supported by morphological characterization of both anamorphic and teleomorphic stages, as well as molecular identification using DNA sequence analysis. This work contributes to the understanding of the biodiversity, host specificity, and biogeography of *P. xanthii*, and serves as a foundation for future research on its management and taxonomy.

MATERIALS AND METHODS

Collection and preservation: In the years 2022-2024, the symptoms of powdery mildew disease were observed on the leaves of *Bidens alata* Melchert., *Chrysanthemum coronarium* L., *Cirsium arvense* (L.) All, *Cucurbita maxima* L., *C. pepo* L., *Impatiens bicolor* Royle., *Momordica charantia* L., *Salvia officinale* (L.) All. *Senecio chrysanthimoides* DC., and *Xanthium strumarium* L. during phytopathological surveys conducted in Battagram, Fairy Meadows, Khanspur, Mansehra, Swat, Pakistan. Infected plant samples were collected and shade-dried on blotting papers. Then, these were preserved in paper envelopes and deposited in the herbarium of the Institute of Botany, University of the Punjab, Lahore (LAH38549, LAH38326, LAH38560, LAH38202, LAH38037, LAH39550) and Department of Botany, Hazara University, Mansehra (HUP16751, HUP16733, HUP16768, HUP16753). Sampling sites are featured in Fig. 1.

Microscopic analysis: Host plants were identified by comparison with healthy preserved plants present in the Herbarium, Hazara University, Mansehra (HUP); Institute of Botany, University of the Punjab, Lahore (LAH). Morphological features were observed under a stereomicroscope (EMZ-5TR, Meiji Techno Co., Ltd., Japan). Conidiophores, conidia, foot cells, and appressoria were stripped from the leaf surface with a clean needle and mounted in lactic acid. The slides were observed under a light microscope (LABOMED, Labo America, Inc., USA) with attached camera (HDCE-90D). Measurements of microscopic features were taken with the help of Scope Image 9.0(X5). Twenty-five measurements each for anamorph (conidiophores, conidia, foot cells, appressoria) and teleomorph (chasmothecia, asci, ascospores) were made after observing their shapes.

Molecular analysis: Fungal mycelia were scratched from the infected plant samples while observing under the stereomicroscope. Autoclaved and ice-chilled pestles and mortars were used along with liquid nitrogen for crushing the material and were preserved in Eppendorf tubes at -18°C.

Genomic DNA was extracted using GeneJET Plant Genomic DNA Purification Mini Kit. #K0791 (Thermo Scientific, Vilnius, Lithuania) according to the manufacturer's instructions. The Internal Transcribed Spacer (ITS) region was amplified using primers i.e, Forward primer PMITS1 (5´-TCGGACTGGCC(T/C)AGGGAGA-3´)

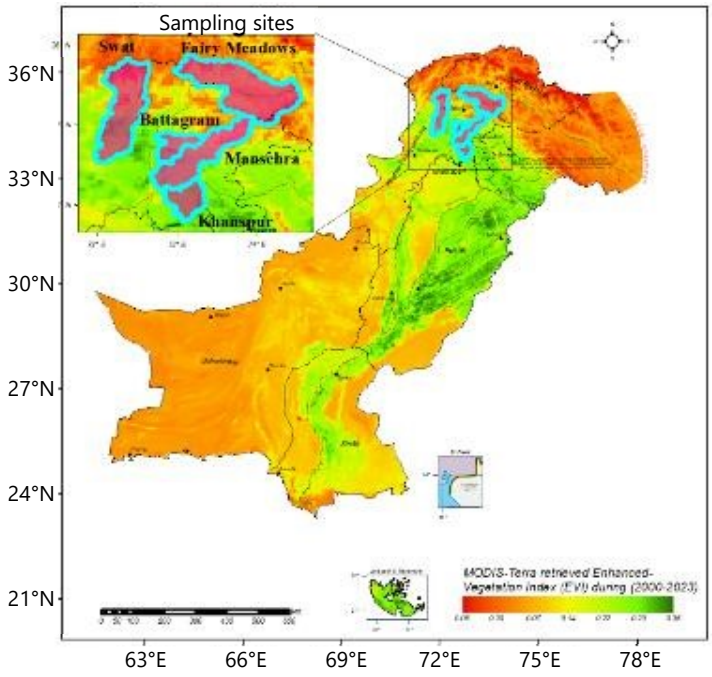

Fig. 1: Location map showing sampling sites, Battagram, Khanaspur, Mansehra, Swat, Khyber Pakhtunkhwa; Fairy Meadows, Gilgit Baltistan, Pakistan (By the software ArcGIS, version 10.3)

and Reverse primer PMITS2 (5´-TCACTCGCCGTTACTGAGGT-3´)[9]. Sanger dideoxy sequencing was done by Tsingke in China. Consensus was made using forward and reverse sequences on Bioedit[10]. Consensus sequences were then run in NCBI BLAST to search and collect similar sequences of the ITS regions from the GenBank database. Sequences of different published *Podosphaera* taxa were obtained from GenBank to determine the phylogenetic position of the sample. Multiple sequence alignment was performed using MAFFT. Trimming of sequences was done using BioEdit at their conserved sites. Software MEGA 7.0 was used for the reconstruction of a phylogenetic tree based on the Kimura 2-parameter model[11]. One thousand bootstrap replicates were run to infer the evolutionary history of the species. *Phyllactinia guttata* and *Phyllactinia populi* were used as an outgroup (Fig. 2).

RESULTS AND DISCUSSION

***Podosphaera xanthii* (Castagne) U. Braun & Shishkoff, Schlechtendalia 4: 31, 2000:** First record of *Bidens alata* melchert (Asteraceae) in Pakistan (Fig. 3a-i).

Material examined: On *Bidens alata* Melchert. (Asteraceae), with anamorphic stage, Battagram, Khyber Pakhtunkhwa, Pakistan, 1850 m.a.s.l., 13th October, 2022, AFE072, Voucher No. HUP 16751, GenBank Accession No. PQ2691820 (ITS).

Mycelium amphigenous, stems, in irregular patches or effuse, thin to dense, evanescent to±persistent; hyphae thin walled, smooth or almost so, (3-)5-8(-10) µm wide; chasmothecia scattered to gregarious, (70-)80-110(-115) µm diam (Fig. 3c-e); peridium cells conspicuous, large, (10-)15-55 µm diam., shape irregularly polygonal to daedaleoid, i.e. with sinuous walls; chasmothecial appendages few to numerous, in the lower half, mycelioid, simple or irregularly branched, often interwoven with each other and the mycelium, length variable, 4-12 µm wide, hyaline, later brown throughout or paler towards the tips, short, coarse, brown appendages often mixed with longer, paler appendages, septate, walls thin, smooth to rough (Fig. 3d); ASCUS broadly ellipsoid-ovoid to subglobose, 60-80×50-65 µm, sessile, wall up to 3 µm thick, terminal oculus (-10)15-25 µm diam., (6-)8-spored, mostly immature (Fig. 3f); ascospores

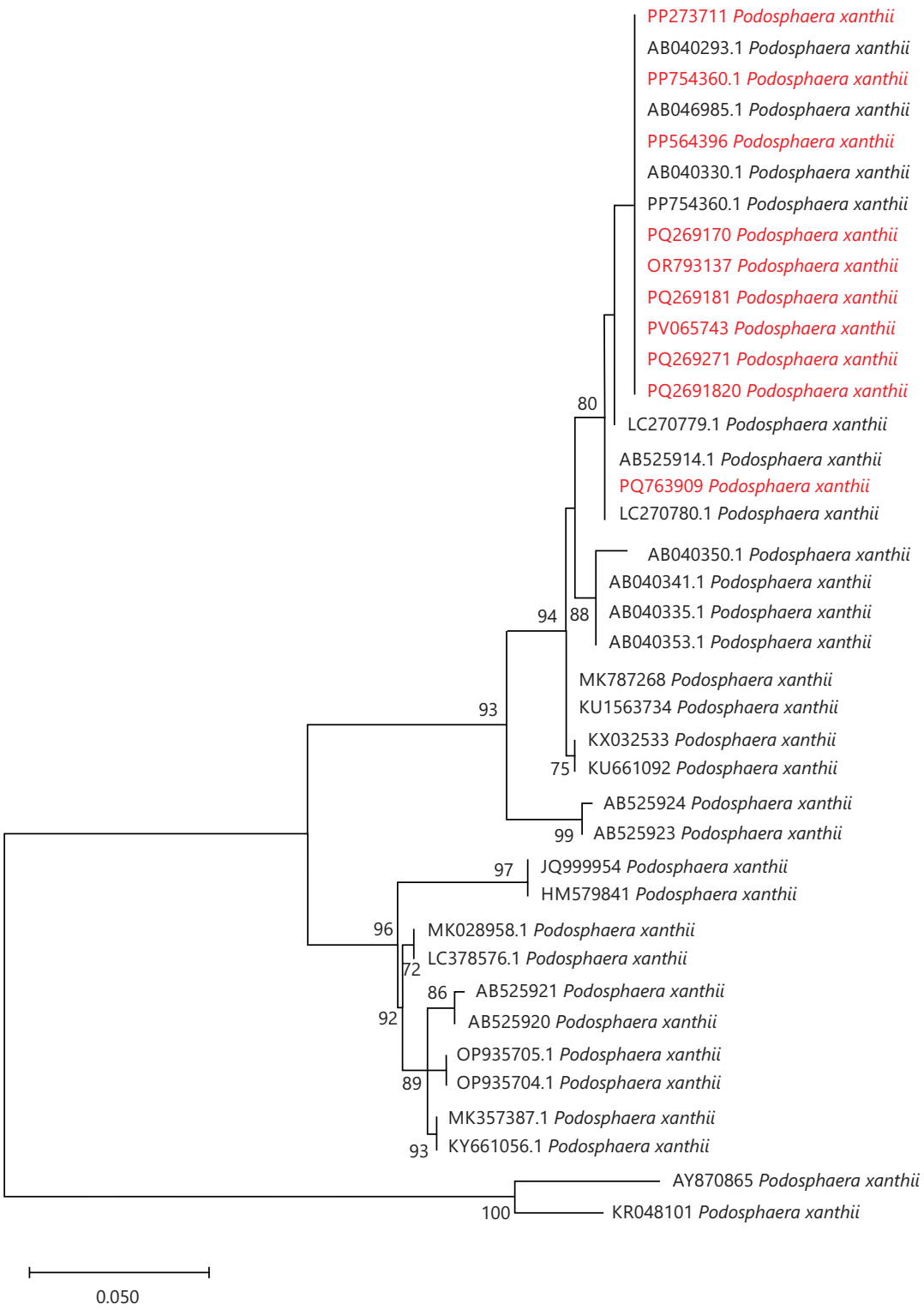

Fig. 2: Phylogenetic tree based on the ITS region of species of *Podosphaera* exhibiting *Phyllactinia guttata, Ph. populi* as an outgroup selected in phylogram

The tree was constructed based on 27 different strains of *Podosphaera* sequences collected from GenBank

broadly ellipsoid-ovoid to subglobose, 15-23×12-17 µm, maturing late, but occasionally larger when fully mature, colourless (Fig. 3f); conidiophores arising from the upper surface of hyphal mother cells, mostly towards one end of the cell but sometimes±centrally, erect, foot cells cylindrical, 30-100×10-13 µm, sometimes slightly constricted at the basal septum or slightly swollen at the very base, followed by 1-3(-4) shorter cells, forming catenescent conidia, in long chains (Fig. 3g); conidia ellipsoid-ovoid to doliiform, 25-45×14-22 µm (Fig 3h-i).

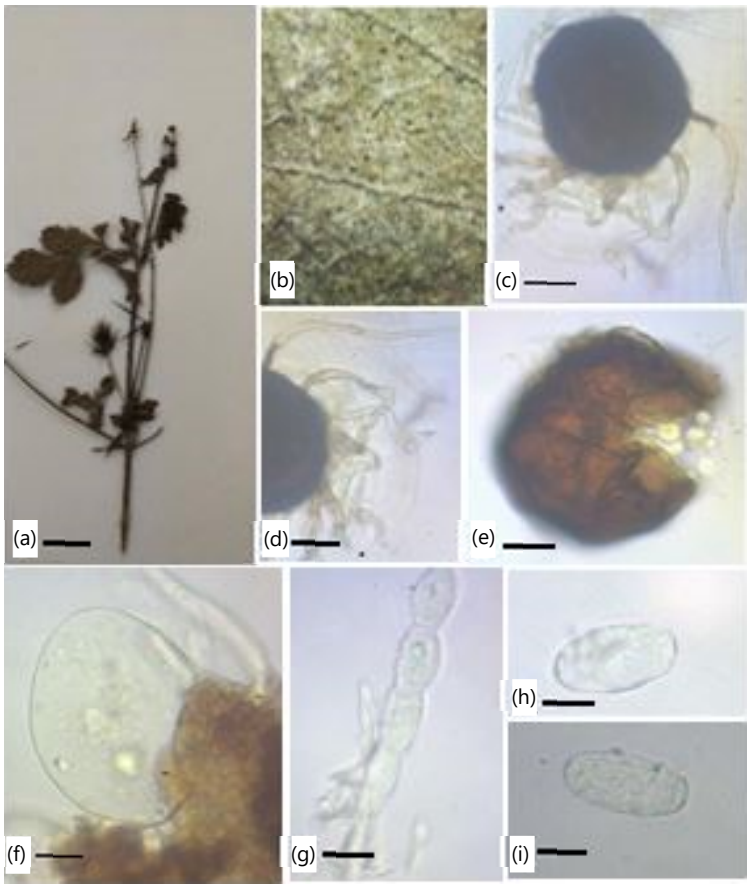

Fig. 3(a-i): Morphological features of *Podosphaera xanthii* associated with powdery mildew infection on Bidens alata, (a) Bidens alata showing powdery mildew symptoms on leaf surfaces, (b) Teleomorphic and anamorphic stages of *Podosphaera xanthii* observed under a stereomicroscope, (c-e) Microscopic view of the fruiting body (chasmothecium) of *P. xanthii*, (d) Filamentous appendages emerging from the chasmothecium, (f) Ascus sac-like structure inside the chasmothecium containing ascospores, (g) Conidiophore bearing asexual conidia and (h-i) Asexual spores (conidia) produced by *P. xanthii*

Scale bars: 3A = 2 cm, 3B = 4 cm, 3C & 3E = 22 µm, 3D = 18 µm, 3F = 19 µm, 3G = 31 µm, 3H–3I = 21 µm

The ITS region showed 99.38% similarity with *P. xanthii* reported from Japan[12]. Previously, *P. xanthii* was recorded on *Bidens* L. plant in Korea, Thailand and Taiwan[8,13,14]. *Bidens* plant was also infected by other powdery mildew species, including *P. bidenticola* U. Braun on the morphological basis[3]. *Bidens alata* is a new host record for *P. xanthii* from Pakistan.

First record on *Cirsium arvense* (L.) All. (Asteraceae) in Pakistan (Fig. 4a-h).

Material examined: On *Cirsium arvense* (L.) All. (Asteraceae) with Anamorph & Teleomorph, Mansehra, Hazara, Khyber Pakhtunkhwa, Pakistan, 3140 m.a.s.l., 21 August, 2023, MK 100, Voucher No. LAH 38450, Genbank Accession No. PP754360.1 (ITS).

Mycelium amphigenous, on stem and inflorescences, irregular patches, thin to dense; hyphal walls thin walled, smooth, 3-6 µm wide; hyphal appressoria slightly lobed; conidiophores centrally erect, septate, 40-90 µm long, with constricted cylindrical foot cells, with 2-4 following cells, forming catenescent, doliiform, conidia; 10-13 (12)×27-39 (33) µm (Fig. 4c); chasmothecia scattered to gregarious, 75-105 (90) µm diameter (Fig. 4e); peridium cells large, daedaleoid, 20-45 µm in diameter (Fig. 4f);

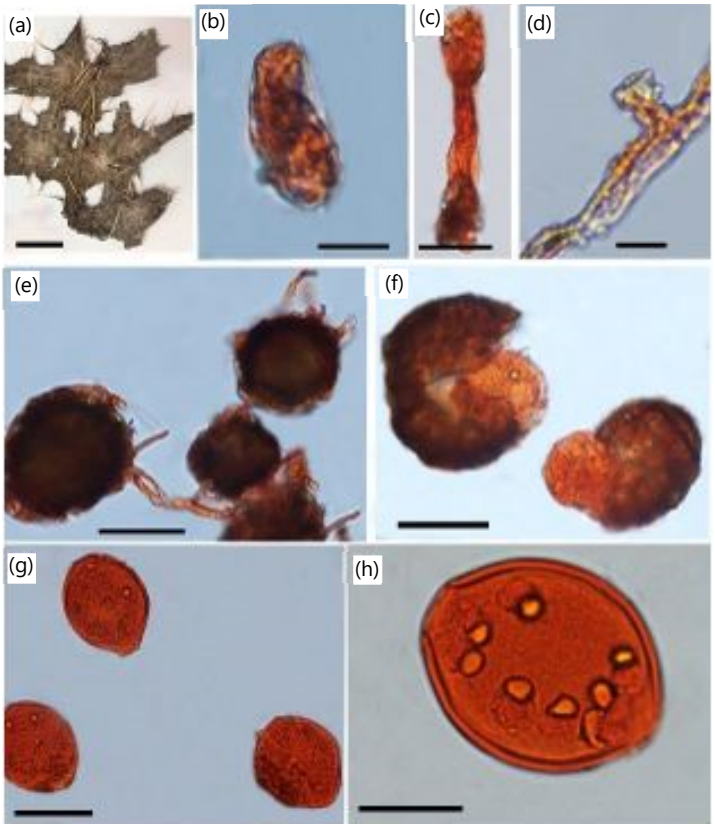

Fig. 4(a-h): Morphological structures of *Podosphaera xanthii* associated with powdery mildew infection on Cirsium arvense, (a) Cirsium arvense leaves exhibiting white powdery mildew symptoms, (b) Individual conidium an asexual spore of *Podosphaera xanthii*, (c) Conidiophore bearing a conidium, (d) Basal cell (foot cell) of the conidiophore, (e) Globose chasmothecia, (f) Open chasmothecium containing a single ascus, (g) Multiple asci bearing ascospores and (h) A single ascus containing ascospores

Scale bars: a = 2 cm, b = 15 µm, c = 10 µm, d = 10µm, e,f = 30 µm, g = 30 µm and h = 20 µm

appendages mycelioid, few to abundant, smooth, simple, irregularly branched, dense at base, paler towards tip, short dense appendage interwoven with longer and pale appendages, 4-8 (7.5)×30-50 (42.5) µm; ASCI ellipsoid-ovoid, subglobose, sessile or very short stalked saccate, 7-8 spored, 52-66 (65)×57-79 (77) µm (Fig. 4f).

The ITS sequence was up to 100 % identical to published ones AB046985.1 in GenBank[15]. *Cirsium arvense* for *P. xanthii* is a new host record for Pakistan. *Cirsium* was recorded for *P. xanthii* in Korea[16]. Powdery mildew is frequently observed on this host in Pakistan. *Cirsium* has been reported as a host plant for *Erysiphe mayorii* S. Blumer, *Golovinomyces biocellatus* Ehrenb., *G. depressus* Wallr., *G. montagnei* U. braun, *Leveillula duriaei* Lev., *L. lappae* Castagne, whereas *Cirsium* species in New Zealand are infected by *P. xanthii*[3].

First record on *Cucurbita maxima* L. (Asteraceae) in Pakistan (Fig. 5a-k).

Material examined: On leaves of *Cucurbita maxima* L. (Cucurbataceae), with only anamorphic stage, Fairy Meadows, Diamir, Gilgit Baltistan, Pakistan, 3,300 m.a.s.l., August 06, 2023, FM 12, Voucher No. LAH38326 GenBank accession number PQ763909 (ITS); on leaves of *Cucurbita pepo* L. (Cucurbataceae), with only anamorphic stage, Khanaspur, KPK, Pakistan, October 10, 2024, RA24, Voucher No. LAH38560 GenBank accession number PV065743 (ITS).

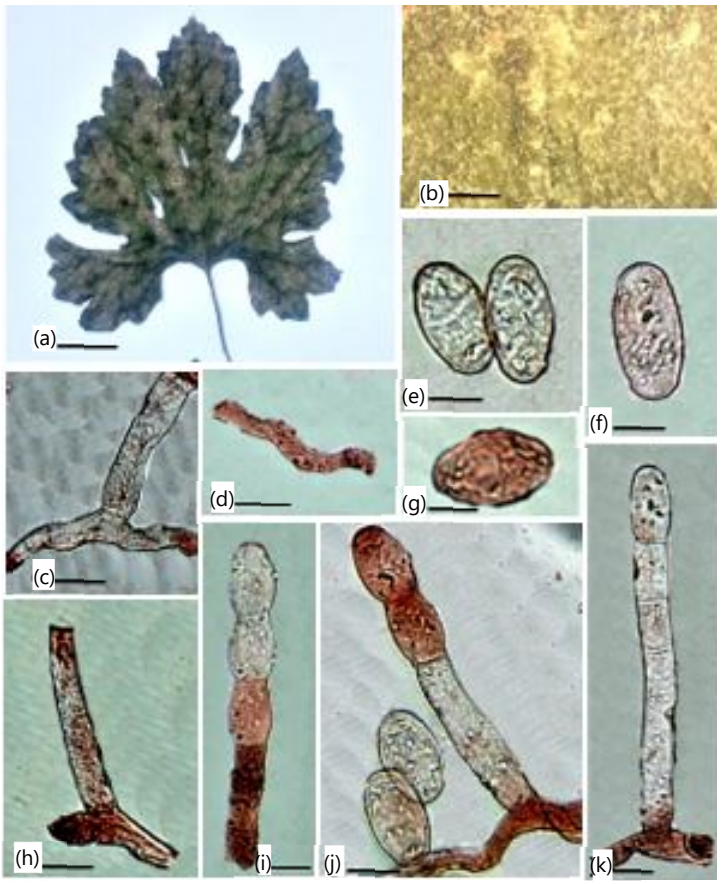

Fig. 5(a-k): (a) Cucurbita maxima leaf, displaying characteristic powdery mildew symptoms such as white fungal growth on the leaf surface, (b) Stereomicroscopic view of the infected area reveals dense mycelial growth and the presence of both conidiophores and conidia, (c-h). Basal cell of conidiophore, foot cell, (d) An appressorium, specialized infection structure that facilitates fungal penetration into the host tissue, (e-g) Asexual spores, conidia which are typically ellipsoid to cylindrical, hyaline, and smooth-walled and (i-k). Conidiophores are erect, unbranched, and terminate in developing conidia
Scale bars: a = 1 cm, c,h = 10 µm, d = 5 µm, e,g = 37 µm and i-k = 17 µm

Mycelium present on both upper and lower surfaces and on inflorescences and stems, in effuse or irregular patches, dense to thin, persistent to evanescent; hyphae smooth, thin-walled; appressoria slightly lobed shaped to indistinct, solitary (Fig. 5d); conidiophores erect, emerging from the top surface of hyphal mother cells, towards one end of the cell mostly (Fig. 5b); foot cells cylindrical, 30-100×10-13 µm, swollen at the very base, having 1-3(-4) following shorter cells, forming canescent conidia, in long chains (Fig. 5c-h); CONIDIA doliiform to ellipsoid-ovoid, 25-45×14-22 µm (Fig. 5e-g); GERM TUBES are of Fibroidium type.

The ITS sequence was 100% identical to *P. xanthii* (OQ729760) from Turkey in GenBank. *Cucurbita maxima* has been reported here as a new host plant for *P. xanthii* from Pakistan. Previously, *Cucurbita* was recorded as a host plant for *Golovinomyces cucurbitacearum*, *Leveillula taurica* and *Podosphaera xanthii* on a morphological basis[3].

First record on *Chrysanthemum coronarium* L. (Asteraceae) in Pakistan (Fig. 6a-f).

Material examined: On *Chrysanthemum coronarium* L. (Asteraceae), with anamorph, Swat, Khyber Pakhtunkhwa, Pakistan, 2409 m.a.s.l, 10 September 2022, DS–19, Voucher No. LAH38202, GenBank accession number PP564396 (ITS).

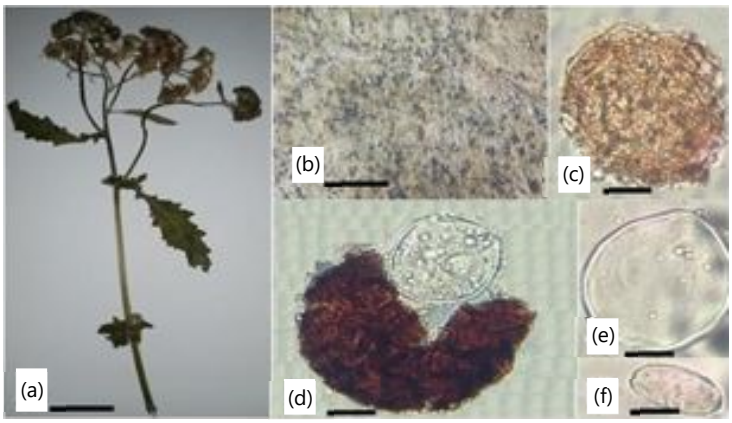

Fig. 6(a-f): (a) *Chrysanthemum coronarium* showing powdery mildew symptoms, (b) Infection under stereomicroscope, (c) Chasmothecium, (d) Chasmothecium releasing an ascus (e) An Ascus and and (f) Ascospore
Scale bars: (a) 20 cm, (c-d) 30 µm, (e) 20 µm and (f) 15 µm

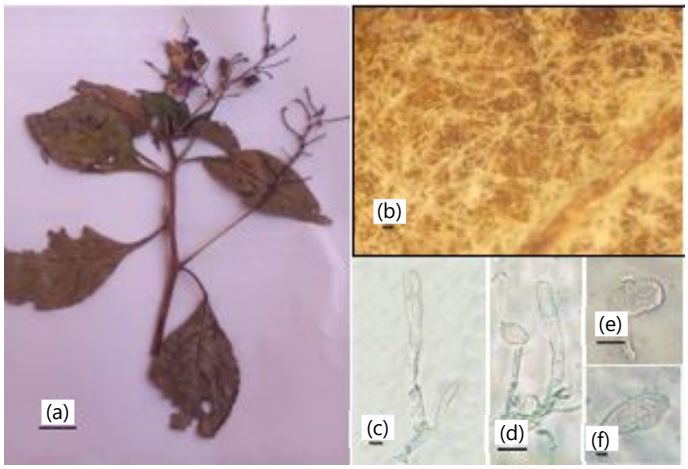

Fig. 7(a-f): (a) *Impatiens bicolor* showing powdery mildew symptoms, (b) Infection under stereomicroscope, (c-d) Conidiophores and (e-f) Condia
Scale bar: (a) 5 cm (b) 1 mm, (c-d) 42 µm and (e-f) 20 µm

Mycelium dense, persistent, amphigenous, forming white patches; chasmothecia scattered, globose to sub-globose, brown, 70-115 µm diam (Fig. 6c); peridial cells irregularly shaped, 15-50 µm diam (Fig. 6c); ascus sessile, globose to ellipsoid, immature, 60-90×60-80 µm, 6-8 spored (Fig. 6e); ascospores hyaline, ellipsoid to ovoid, 15-22×13-18 µm (Fig. 6f).

About 904 species of powdery mildews belonging to 16 genera are reported worldwide[17], while from Pakistan, 52 species of powdery mildews are known[18-26]. The genus *Podosphaera* Kunze comprises 146 species that cause disease on 1,056 plant species of 41 families[22]. The combination of ITS-based phylogeny and morpho-anatomical description suggested that the identity of our collection is *Podosphaera xanthii* (PP564396). *Chrysanthemum coronarium* is being reported here as a new host plant for *P. xanthii* from Pakistan and worldwide.

First record on *Impatiens bicolor* Royle. (Boraginaceae) from Pakistan (Fig. 7a-f).

Material examined: On *Impatiens bicolor* Royle. (Boraginaceae), with anamorphic stage, Battagram, Hazara, Khyber Pakhtunkhwa, Pakistan, 1430 m.a.s.l., 13th September, 2021, AFE021, Voucher No. HUP 16733, Gen Bank Accession number (PQ269181).

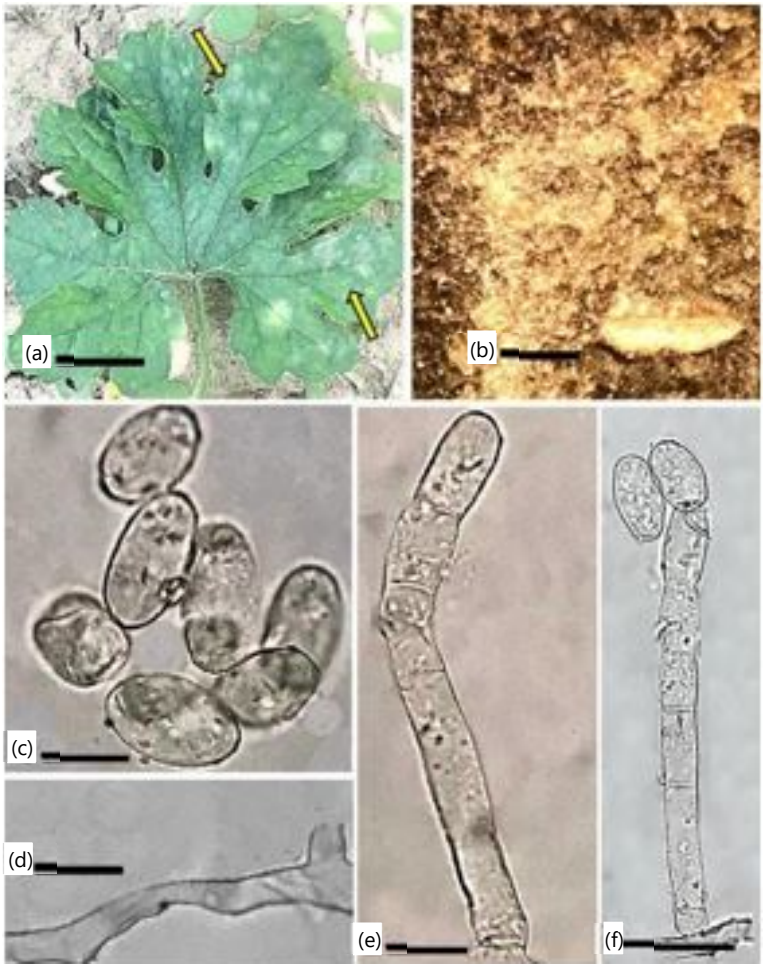

Fig. 8(a-f): (a) Visible signs of powdery mildew infection on the leaf surface of M. charantia, appearing as white powdery patches, indicating the presence of fungal mycelium and conidia, (b) Magnified view of the infected leaf surface using a stereomicroscope, revealing superficial fungal growth, (c) Conidia, asexual spores produced by the powdery mildew fungus, (d) An appressorium helps in the penetration of fungal spores into host tissue and (e-f) Conidiophore, the specialized hyphal structure that bears conidia
Scale bars: (a) 5 cm, (b) 1 cm, (c) 15 µm, (d) 5 µm and (e-f) 20 µm

Mycelium amphigenous and on stems and inflorescences, in irregular patches or effuse, thin to dense, evanescent to±persistent; conidiophores arising from the upper surface of hyphal mother cells, mostly towards one end of the cell but sometimes±centrally, erect, footcells cylindrical, 30-100×10-13 µm, sometimes slightly constricted at the basal septum or slightly swollen at the very base, followed by 1-3(-4) shorter cells, forming catenescent conidia, in long chains (Fig. 7c-d); CONIDIA ellipsoid ovoid to doliiform, 25-45×14-22 µm (Fig. 7e-f).

Impatiens plant was recorded as a host plant for a wide range of powdery mildews, including *Fibroidium abelmoschi* Thum, *Podosphaera balsaminae* Wallr., *P. bidenticola* U. Braun, and *P. xanthii* Castagne. *Impatiens bicolor* has been reported here as a new host plant for *P. xanthii* from Pakistan.

First record on *Momordica charantia* L. (Cucurbitaceae) in Pakistan (Fig. 8a-f).

Material examined: On *Momordica charantia* L. (Cucurbitaceae), with anamorph, Pakistan, Khyber Pakhtunkhwa, Shogran, 2,362 m.a.s.l., September 29, 2021, SA-01, Voucher No. LAH38037, GenBank accession numbers OR793137 (ITS).

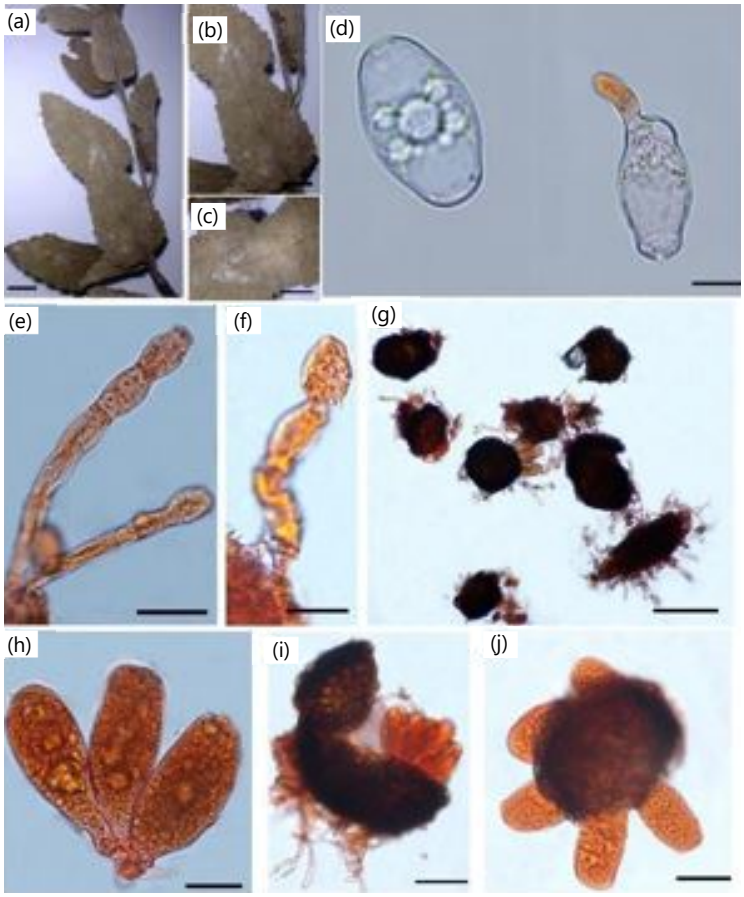

Fig. 9(a-j): (a.b) External symptoms of powdery mildew on Salvia officinalis, characterized by white, powdery fungal growth on the upper leaf surfaces, (c) View of infected leaf with powdery mildew symptoms under a stereomicroscope, (d) Conidium, an asexual spore that detaches from the conidiophore to spread the infection. and germinating conidium showing the emergence of a germ tube, indicating the initiation of infection on a new host surface, (e,f) Conidiophores bearing conidia, (g) Chasmothecia are the closed, spherical sexual fruiting bodies of powdery mildew fungi, (h) Asci bearing Ascospores and (i-j) Open chasmothecium bearing Ascospores

Scale bars: a = 30 µm, b = 15µm, c = 15µm and d = 15µm

Mycelium amphigenous, patchy, thin, forming irregular white, evanescent. Hyphal walls branched, septate, hyaline, thin-walled, smooth, sub-straight to sinuous, 5-8 µm wide. Hyphal appressoria solitary, slightly nipple-shaped, 3-5 µm in diameter (Fig. 8d). Conidiophores hyaline, two to four immature conidia produced in chains with a sinuate outline, erect, straight, 93.7-142.2 (110.2) µm long, followed by one to three cells (Fig. 8e-f). Foot cells are clear, usually straight, sometimes slightly constricted at the basal septum or slightly swollen at the very base, cylindrical or subcylindrical, elongated, 55.2-75.5 (66.3) µm long, forming canescent conidia (Fig. 8e-f). Conidia hyaline, vacuolated, ellipsoid–ovoid, doliiform, smooth, primary conidia were rounded at the apex and subtruncate at the base, 29.0-38.0 (27.0)×16.0-20.0 (29.0) µm (Fig. 8c). Fibrosin bodies present. Chasmothecium was not observed.

Podosphaera xanthii has been infecting *Momordica charantia* L., a new host record for Pakistan. Previously, the fungus has been reported on *Ageratum conyzoides* L. and *Tarlmounia elliptica* (DC.) H. Rob., S.C. Keeley, Skvarla & R. Chan in Lahore[18], from the country. Another powdery mildew, *Oidium xanthii* Bhatn & Kothari, has been found on *Xanthium strumarium* L. in India. It is morphologically similar to the *P. xanthii* anamorph, but has conidia with fibrosin bodies, distinguishing it from the former[3].

First record on *Salvia officinalis* (L.) All., (Lamiaceae) in Pakistan (Fig. 9a-j).

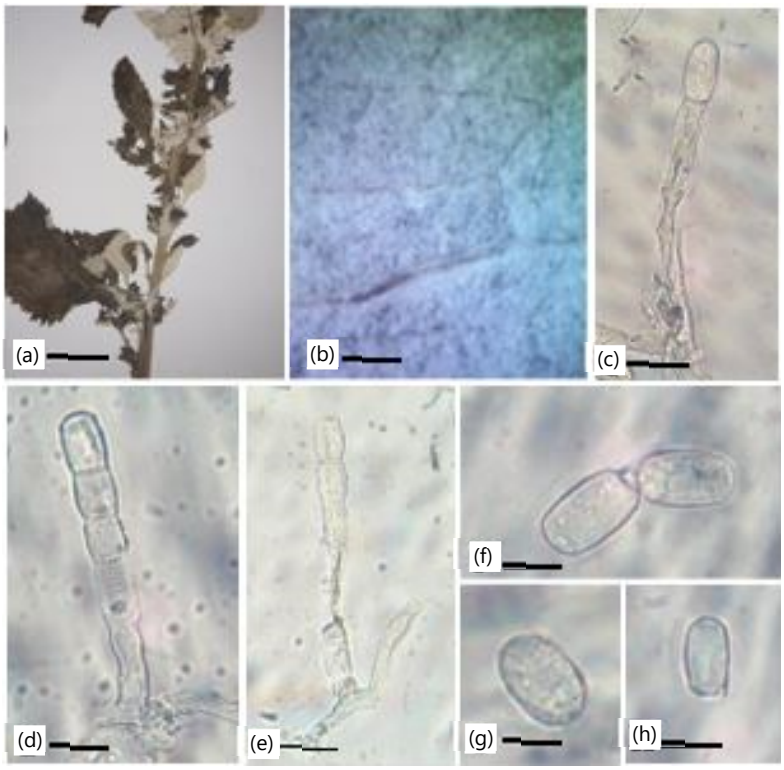

Fig. 10(a-h): (a) *Senecio chrysanthemoides* showing powdery mildew symptoms, (b) Infection under stereomicroscope, (c-e) Conidiophore and (f-h) Conidia
Scale bar: (a) 2.5 cm, (b) 4 cm, (c-e) 107.7 µm and (f-h) 21.5 µm

Material examined: On *Salvia officinale* (L.) All. (Lamiaceae), with Anamorph & Teleomorph, Pakistan. Khyber Pakhtunkhwa, Hazara, District Mansehra, Burwai, 3140 m.a.s.l., August, 2023, MK06, Voucher No. LAH38550, GenBank accession number PQ296170 (ITS).

Mycelium amphigenous, patchy, thin, forming irregular white, evanescent. Hyphal walls branched, septate, hyaline, thin-walled, smooth, sub-straight to sinuous, 4-7 µm wide. Hyphal appressoria solitary, slightly nipple-shaped, 3-5 µm in diameter. Conidiophores hyaline, two to four immature conidia produced in chains with a sinuate outline, erect, straight, 94.7-150.2 (114.2) µm long, followed by one to three cells (Fig. 9e-f). Foot cells are clear, usually straight, sometimes slightly constricted at the basal septum or slightly swollen at the very base, cylindrical or subcylindrical, elongated, 57.2-79.5 (70.3) µm long, forming canescent conidia. Conidia hyaline, vacuolated, ellipsoid-ovoid, doliiform, smooth, primary conidia were rounded at the apex and subtruncate at the base, 30.0-39.0 (28.0)×14.0-21.0 (27.0) µm (Fig. 9d).

Podosphaera xanthii has been infecting *Salvia officinale*, a new host record for Pakistan. Previously, the fungus has been reported on *Ageratum conyzoides* L. and *Tarlmounia elliptica* (DC.) H. Rob., S.C. Keeley, Skvarla & R. Chan in Lahore[27], from the country. The fungus showed a white mat of mycelium on both sides of the leaf. Black dots were also present on the leaf. Conidia and conidiophores of powdery mildew were verified by microscopic examination. Straight, unbranched conidiophores with cylindrical foot cells that measured 40-80×8-12 µm, followed by one to three smaller cells. They ranged in shape from ellipsoid to ovoid or doliform and were 20-35×14-20 µm[3].

First record on *Senecio chrysanthimoides* (Asteraceae) in Pakistan (Fig. 10a-h).

Material examined: On *Senecio chrysanthimoides* (Asteraceae) with anamorphic stage, Battagram, Hazara, Khyber Pakhtunkhwa, Pakistan, 1820 m.a.s.l., 15th September, 2022, AFE099, Voucher No. HUP16768, GenBank Accession No. PQ269271 (ITS).

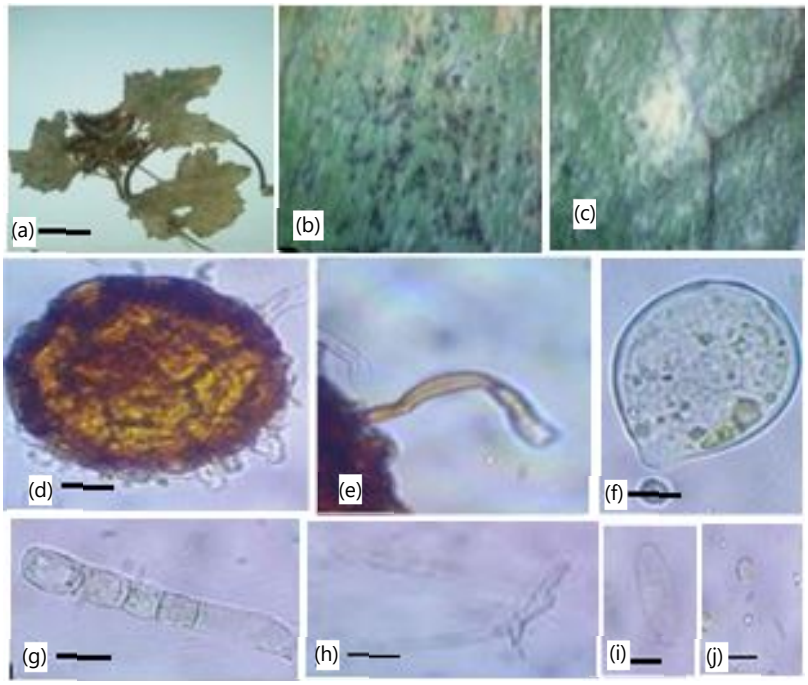

Fig. 11(a-j): (a) Infected leaves of Xanthium strumarium showing visible signs of powdery mildew colonization in the form of white, powdery patches on the surface, (b-c) Close-up views of infected leaves under a stereomicroscope, illustrating the sexual and asexual stages of *P. xanthii*, (d) Chasmothicum, spherical in shape, (e) Appendage, (f) Asci (sac-like structures) released from the chasmothecium, containing the sexual spores (ascospores), (g) An ascospore, (h) Conidiophore, (i) Foot cell and (j) Conidia
Scale bar: (a) 2.5 cm, (b) 4 cm, (c) 1.3 cm, (d) 22 µm, (e) 18.7 µm, (f) 19.6 µm, (g) 31 µm, (h) 20.25 µm, (i) 107.7 µm and (j) 21.5 µm

Mycelium amphigenous and on stems and inflorescences, in irregular patches or effuse, thin to dense, evanescent to±persistent; hyphae thin-walled, smooth or almost so, (3-)5-8(-10) µm wide; Conidiophores arising from the upper surface of hyphal mother cells, mostly towards one end of the cell but sometimes±centrally, erect, foot cells cylindrical, 30-100×10-13 µm, sometimes slightly constricted at the basal septum or slightly swollen at the very base, followed by 1-3(-4) shorter cells, forming catenescent conidia, in long chains (Fig. 10c-e); conidia ellipsoid-ovoid to doliiform, 25-45×14-22 µm (Fig. 10f-h).

The ITS sequence shows identity with the sequence of *P. xanthii* AB040349 from Japan. The morphological features match the description of *P. xanthii* given in Braun & Cook[3]. *Senecio chrysanthemoides* is being reported here as a new host record for *P. xanthii* from Pakistan and worldwide.

First record on *Xanthium strumarium* L. (Asteraceae) in Pakistan (Fig. 11a-i).

Specimen on *Xanthium strumarium* L. (Asteraceae) with anamorphic stage, Battagram, Hazara, Khyber Pakhtunkhwa, Pakistan 1800 m.a.s.l., 13th September, 2022, AFE094, Voucher No. HUP 16753, GenBank Accession No. PQ273711 (ITS).

Mycelium amphigenous present on inflorescences and stems, effuse or in the form of white patches, persistent, smooth hyphae, thin-walled; chasmothecia large, scattered, an irregularly polygonal shape, blackish brown in color, 77.8×74.7 µm (Fig. 11d); appendages less in number, mycelioid, long uniformly or short, septate, varies in length, 48.7×6.5 µm; pale brown in color, rough to smooth, asci subglobose to ovoid, oculus terminal, hyaline, usually immature, 55.1×45.8µm (Fig. 11e); ascospores ellipsoid-ovoid to

round, late maturing, colorless when become fully mature, 12.5×11.3 µm (Fig. 11g); conidiophore appear from the top surface of mother cell, sometime centrally, infrequently in the middle, glossy, tubular, produce two-three conidia in chain, 95-143 µm long; foot cell cylindrical, 38×11-13 µm sometime slightly constricted at the basal septum followed by 1-3 shorter cell (Fig. 11h); conidia shaped in chain dolliform, 29-38×13-20 µm (Fig. 11g).

Previously, *Podosphaera xanthii* has been reported on *Ageratum conyzoides* L from Pakistan. *Xanthium strumarium* has been reported here as a new host record for *P. xanthii* from Pakistan.

In this study, new host plants for *P. xanthii* are being reported from different regions of Pakistan expanding the host range of this pathogen. Molecular analyses (ITS rDNA) and detailed morphological examinations of both anamorphic and teleomorphic stages provided strong evidence for the identification of *P. xanthii*. Large-sized ascomata observed in the Pakistani collections are consistent with the distinguishing features of *P. xanthii* reported in prior studies[1]. The Pakistani sequences formed a sister clade with previously published reference sequences (AB046985, AB040330, PP754360, AB040293), confirming their identity[3,8].

The findings align with reports by Zhang *et al*.[28] who documented *Podosphaera xanthii* infecting cucurbits and other economically important crops. Recent studies by Yeh *et al*.[8] demonstrated that environmental factors such as humidity and temperature strongly influence the pathogen's distribution, which corresponds with our field observations in humid temperate zones of northern Pakistan. Large-sized ascomata is the distinctive character of *P. xanthii* that differentiates it from other members of the genus *Podosphaera* as reported from Korea China[28,29]. From this research work, it is inferred that *P. xanthii* not only damages economically significant plants but also poses a threat to crops, including *Cucurbita*. These findings emphasize the need control of disease strategies against this widely distributed pathogen in Pakistan.

CONCLUSION

This study documents the expanded host range and widespread distribution of *Podosphaera xanthii* across five ecological regions in Pakistan. The confirmation of its presence on ten plant species, particularly within the Asteraceae family, highlights its adaptability and potential impact on diverse plant hosts. The combined morphological and molecular analyses enhance understanding of its taxonomy and support its persistent lifecycle in varied climates. These findings provide a foundation for improved monitoring and management strategies for this economically significant pathogen.

SIGNIFICANCE STATEMENT

Powdery mildew caused by *Podosphaera xanthii* is a major phytopathological problem affecting a wide range of economically important crops worldwide, yet its diversity, host range, and distribution remain underexplored in many regions, including Pakistan. In this study, new host records of *P. xanthii* from different ecological zones of Pakistan have been reported, confirmed through detailed morphological examination and ITS based molecular analysis.

These findings significantly expand the known geographic distribution and host spectrum of *P. xanthii*, revealing its ecological plasticity and potential threat to both cultivated and native plant species. This study contributes valuable baseline data to the global understanding of *P. xanthii* diversity and host associations.

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