Research Article

Epidemiological and Taxonomic Insights into the Tomato Mycobiota of Bajaur, Pakistan

Rashid Ahmad, Muhammad Iqbal, Hazrat Bilal, Muhammad Abdul Haq* and Aminul Haq

Department of Botany, Govt. Post Graduate College Khar, District Bajaur, Pakistan.

Abstract | Tomato (*Solanum lycopersicum* L.), a globally significant vegetable crop, faces significant productivity constraints due to fungal pathogens. This study investigated the diversity, prevalence, disease incidence, and yield losses of tomato crops in Bajaur District, Khyber Pakhtunkhwa, Pakistan, during the years 2023 (August to November) and 2024 (April to July). At eight sites in four tehsils, fifty-nine symptomatic plant samples and eight rhizosphere soil samples were gathered. From rhizosphere soils, morphological and microscopic analyses revealed three Rhizopus species (*R. homothallicus*, *R. arrhizus* var. *delemar*, and *R. stolonifera*) and seven pathogenic fungi: *Fusarium oxysporum*, two unidentified *Fusarium* spp., *Alternaria alternata*, *A. solani*, *A. arborescens*, and *Curvularia pallescens*. Notably, *A. arborescens*, *C. pallescens*, *R. homothallicus*, and *R. arrhizus* var. *delemar* are reported for the first time in Pakistan, with *C. pallescens* being newly associated with tomato globally. Disease incidence averaged 33.5%. Among the isolated taxa, *A. alternata* is the most prevalent pathogen (87.5% site occurrence). Farmer-reported yields averaged 11.9 t/ha just 6.9% of potential yield indicating catastrophic losses of 93.1%. This study provides taxonomic descriptions of newly recorded taxa, underscores the critical need for integrated disease management strategies, and expands the mycological knowledge of tomato-associated fungi in Pakistan.

Received | July 10, 2025; **Accepted** | Sep 9, 2025; **Published** | December 08, 2025
***Correspondence** | Muhammad Abdul Haq, Department of Botany, Govt. Post Graduate College Khar, District Bajaur, Pakistan; **Email:** muhammad.abdulhaq@gmail.com
Citation | Ahmad, R., M. Iqbal, H. Bilal, M. Abdul Haq and A. Haq. 2025. Epidemiological and taxonomic insights into the tomato mycobiota of bajaur, Pakistan. *Sarhad Journal of Agriculture,* 41(5): 14-23.
DOI | https://dx.doi.org/10.17582/journal.sja/2025/41.5.14.23
Keywords | Incidence, Mycoflora, New records, Pakistan, Prevalence, Tomato.

Introduction

The tomato (*Solanum lycopersicum* L.) is one of the most widely produced vegetables in the world. It ranks as the second most cultivated vegetable crop globally, following potatoes, with a production of 186 million tons from 5 million hectares (Lata *et al*., 2024). During the agricultural year 2022-2023, tomatoes were cultivated on 68862 hectares in Pakistan, yielding a total production of 762736 tons (Ministry of National Food Security & Research, 2024).

Tomato cultivation in the Bajaur district of Khyber Pakhtunkhwa province, has become an essential agricultural activity, contributing significantly to both the economy and food security for local communities. The region's climate and soil conditions are well-suited for growing tomatoes. In the district, tomatoes were cultivated on 313 hectares in 2022-23 growing season (Ministry of National Food Security & Research, 2024). Indeterminate varieties are predominantly grown due to their long growing seasons, lower susceptibility to diseases, and higher profitability. Popular varieties include ANNA, SAHIL F1, SEHAR F1, T-1359, TO-1057 F1, and Advanta F1 (personal communication). Tomatoes are primarily cultivated in two seasons within the district. The summer season (February to Auust) is especially favourable in the plains, with cultivation concentrated in the tehsils of Khar, Salarzai, and Utman Khel. Additionally, a second crop can be grown in the fall (August to December) in frost-free zones, including tehsil Barang, parts of tehsil Mamund, as well as the Arang and Alizo regions (personal communication).

Diseases are thought to be responsible for 14.1% of the 36.5% average of total losses, which equates to a cost of roughly $220 billion (Agrios, 2005). Of these diseases, 70-80% are caused by pathogenic fungi (Peng *et al*., 2021). The cultivation of tomatoes faces significant challenges due to various fungal diseases that can reduce crop productivity and quality. The major fungal diseases affecting tomatoes include early blight, *Septoria* leaf spot, grey mold, *Fusarium* wilt, crown and root rot, *Verticillium* wilt, and late blight (Panno *et al*., 2021). In Pakistan, several fungal species have been identified and reported from tomato plants (Bashir *et al*., 2014; Chohan *et al*., 2016; Anwar *et al*., 2017; Gondal *et al*., 2019; Safi *et al*., 2020; Riaz *et al*., 2021; Abdul Haq *et al*., 2023; Abdul Haq *et al*., 2024).

Tomato yields in Bajaur, Khyber Pakhtunkhwa, and Pakistan are reported at 6.97, 8.90, and 11.10 tons per hectare, respectively (Ministry of National Food Security & Research, 2024), well below the global average of 36.97 tons per hectare (Lata *et al*., 2024). This yield gap is largely influenced by biotic and abiotic constraints. Recent studies have documented several fungal taxa from the tomato rhizosphere, including *Globisporangium schmitthenneri*, *Pythium oligandrum*, *P. aristosporum, and Phytophthora capsici* (Abdul Haq *et al*., 2023; Abdul Haq *et al*., 2024). However, a comprehensive account of the diversity, prevalence, and impact of the broader fungal complex affecting tomatoes in the agriculturally important yet understudied Bajaur district is lacking. The present study was therefore designed to (i) investigate the prevalence and diversity of fungi associated with tomato crops in Bajaur; (ii) assess disease incidence and associated yield losses; and (iii) provide taxonomic descriptions and illustrations of newly identified fungal taxa. Disease incidence and yield losses were assessed through farmer questionnaires to capture local agricultural practices and outcomes.

Materials and Methods

Study area and agronomic practices

In this study, four out of the eight tehsils in the district were selected (see Table 1). All locations are situated in the Northwestern Mountains of the Hindukush Region, characterized by a highland climate featuring warm summers and cold winters. The soils of Bajaur vary significantly in texture, ranging from sandy loams to clay loams, with a range of fertility levels. The area is primarily cultivated with various food crops such as wheat, maize, and mustard. Additionally, solanaceous crops like tomatoes, peppers, and eggplants are also grown. Water from tube wells is used for irrigating the vegetables throughout the region. Farmers apply chemical fertilizers like Urea, DAP, and SOP to provide essential nutrients (N-P-K). Some farmers combine them with bio-stimulants to enhance nutrient uptake, promote growth, and improve stress resistance.

Isolation and identification of fungi

Two fields/tehsils were surveyed. Eight samples of rhizosphere soil and fifty-nine samples of symptomatic and asymptomatic plant parts were collected in sterile polythene bags and transferred to the botanical lab at GPGC Khar Bajaur for analysis. The diseased samples were rinsed with running tap water to remove surface impurities. A small section (3-5 mm^2) of the symptomatic tissue and adjacent healthy tissue was then excised using a sterile blade. After a 30-second surface sterilization with 70% ethanol, these fragments were washed 3-4 times with sterile tap water and dried with sterile blotting paper. They were then aseptically transferred onto Petri dishes containing potato dextrose agar (PDA) supplemented with penicillin and streptomycin. The plates were incubated at room temperature for three to seven days, with regular checks for fungal mycelial growth. Additionally, foliar fungi, especially biotrophs, were examined and characterized using the scotch tape method (Langvad, 1980). The isolation from soil samples was done using surface-sterilized tar apples as bait (Erwin and Ribeiro, 1996; Abdul Haq *et al*., 2024). Morphological features were documented using a light microscope after staining the mycelium with lactophenol cotton blue. Key characteristics of the taxa were captured using a mobile camera. Species-level identification was conducted using morphological characteristics. For members of the Ascomycota, we primarily relied on the keys of Ellis (1965, 1971, 1976) and Watanabe (2018). For *Fusarium* species, we followed the taxonomic framework of Leslie and Summerell (2008), while for general fungi and less common taxa, we consulted the keys of Sutton (1961), Zheng *et al*. (2007), and Guarro *et al*. (2012).

Collection and analysis of data

Data on cultivated varieties, yields, and agronomic practices were collected from 8 tomato farms using a semi-structured questionnaire. Field sampling was carried out at each location to evaluate fungal disease incidence by examining all plants within a randomly placed 10' x 10' quadrat. A plant was classified as diseased if it showed visible symptoms. The prevalence of each fungal species was determined by the number of sites from which it was isolated. To evaluate crop losses, the actual yield (kg per hectare) was recorded and compared with the potential yield for the respective variety. Venn diagrams were generated using the Venny 2.1 online tool (https://bioinfogp.cnb.csic.es/tools/venny/) to visualize the distribution and overlap of fungal taxa across the study areas. All data were analyzed using descriptive statistics (means, percentages) in Microsoft Excel, employing the following formulae:

$$\text{Prevalence (\%)} = \frac{\text{Number of sites in which the fungus was found}}{\text{Total number of sites studied}} \times 100$$

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Number of observed plants}} \times 100$$

$$\text{Losses (\%)} = \frac{\text{Potential Yield} - \text{Actual Yield}}{\text{Potential Yield}} \times 100$$

Results

Isolation and identification of fungi

To investigate the fungal diversity associated with tomato plants in the study area, a total of 59 plant specimens and eight rhizosphere soil samples were collected from eight locations across four tehsils of District Bajaur (Table 1). Fungal pathogens were identified based on the symptomatology of infected tomato plants and microscopic examination of fungal isolates, with reference to established taxonomic literature.

A total of seven fungal pathogens were isolated from various plant tissues, including leaves, fruits, stems, and roots. The identified pathogens included *Fusarium oxysporum* (associated with fruit rot (Figure 2 A-B)), two unidentified *Fusarium* species (designated as *Fusarium* sp. 1 and *Fusarium* sp. 2, both associated with wilt (Figure 2 C-F)), *Alternaria alternata* (causing stem canker (Figure 3 A-B)), *A. solani* (causing early blight (Figure 3 C-D)), *A. arborescens* (Figure 3 E-J), and *C. pallescens* (Figure 4 A-E) (both associated with leaf spots. Additionally, three fungal species belonging to the genus *Rhizopus*: *R. homothallicus* (Figure 6 A-J), *R. arrhizus* (Figure 5 A-F), and *R. stolonifera* were isolated from the rhizosphere soil samples. Notably, *A. arborescens* , *C. pallescens*, *R. homothallicus*, and *R. arrhizus* are reported here as new records for Pakistan. Furthermore, to the best of our knowledge, this is the first report of *C. pallescens* and *A. arborescens* associated with tomato plants.

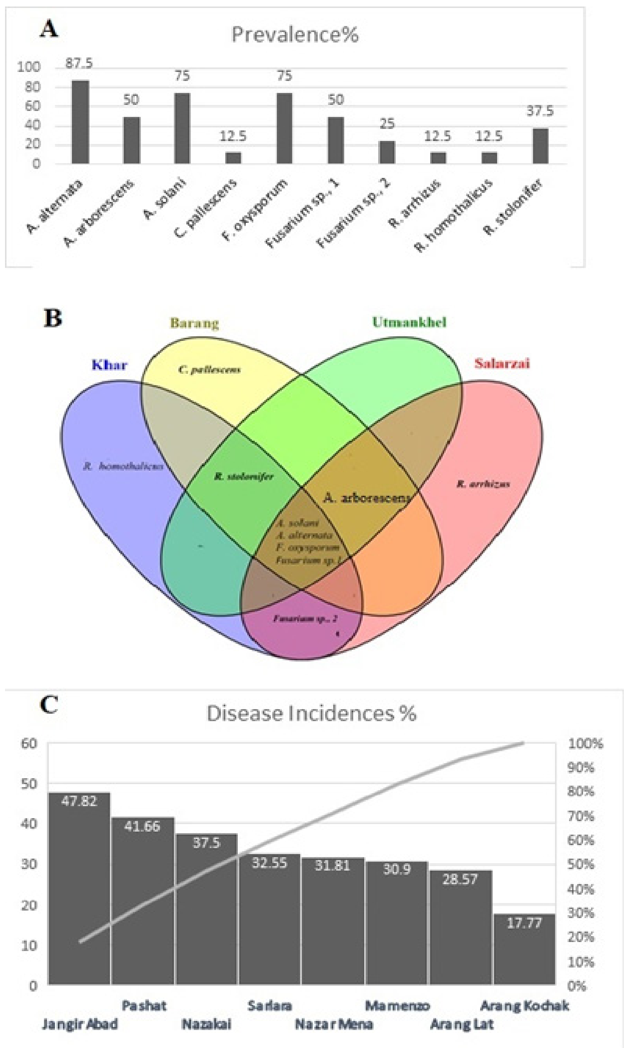

Figure 1: *A. Bar chart showing Prevalence (%) of isolated taxa B. Venn diagram showing the distribution of the isolated taxa in the four tehsils C. Disease incidence at all study sites*

Table 1: *Showing details of study areas, varieties, fungicides used, yield and fungal distribution*

Tehsil	Location	GPS value	Variety	Fungicides used	Disease incidence (%)	Actual yield (t/h)	Fungal distribution
Khar	Jangir abad	34.7123427, 71.5158607	Anna	Mancozeb, Azoxystrobin	47.82	12.4	A, C, E, G, J
	Mamenzo	34.7573868, 71.5380134	Anna	Chlorothalonil, Mancozeb	30.9	12.1	A, C, E, F, I
Barang	Nazar mena	34.6707875, 71.6157344	T-1359	Metalaxyl, Mancozeb	31.81	10.4	A, B, C, E, F, J
	Sarlara	34.6354375, 71.6185625	Anna	Chloratin, Mancozeb	32.55	13.1	A, D
Utman-Khel	Arang lat	34.7426667, 71.7138333	Anna	Captan, Mancozeb	28.57	11.6	A, B C E, J
	Arang kochak	34.7497222, 71.7116389	Anna	Mancozeb	17.77	11.1	A, B, C, F
Salarzai	Pashat	34.8628223, 71.5331908	Anna	Strobilurins	41.66	11.1	A, E, F, G
	Nazakai	34.7935000, 71.5606944	Anna	Mancozeb	37.5	13.3	B, C, E, H

A. A. alternata B. A. arborescens, C. A. solani D. C. pallascens E. F. oxysporum F. Fusarium sp.1 G. Fusarium sp.2 H. R. arrhizus I. R. homothalicus J. R. stolonifer

Prevalence and distribution of fungal taxa

Among the fungal taxa identified, *A. alternata* was the most prevalent, being detected at 87.5% of the surveyed sites. This was followed by *A. solani* and *F. oxysporum*, each of which was present at 75% of the sites. *Alternaria arborescens* and an unidentified *Fusarium* species were each observed at 50% of the sites. The remaining taxa were detected at lower frequencies (Figure 1A). Analysis of spatial distribution revealed that four taxa were present in all four tehsils, two taxa were identified in three tehsils, while three taxa were restricted to a single tehsil (Figure.1B).

Disease incidence, yield losses, and control measures

Disease incidence was evaluated across eight surveyed sites by recording the proportion of tomato plants exhibiting visible symptoms of fungal infection. Incidence rates varied considerably among sites, with the lowest observed at Arang Kochak (17.5%) and the highest at Jangir Abad (47.8%). The mean disease incidence across all sites was 33.53%, indicating that approximately one-third of the tomato crop was affected by fungal pathogens (Table 1, Figure 1C).

Farmer survey data indicated that two tomato varieties were cultivated in the study areas: 'Anna' (grown at seven sites) and 'T-1359' (grown at one site). Recorded yields ranged from 10.4 to 13.3 tons per hectare, with a mean yield of 11.9 tons per hectare. This yield corresponds to only 6.9% of the estimated potential yield for these varieties, resulting in an average crop loss of 93.1% (Table 1, Figure 3). In terms of disease management practices, mancozeb was the most commonly used fungicide, applied at seven of the eight sites. Additional fungicides used by the farmers included azoxystrobin, chlorothalonil, metalaxyl, chloratin, captan, and strobilurins (Table 1).

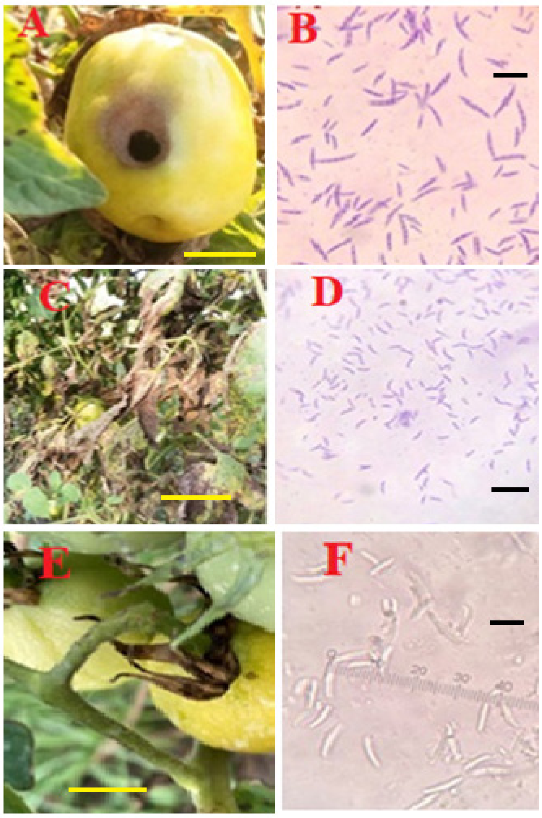

Figure 2: *(A, B) F. oxysporum A. infected fruit (B) conidia (C, D) Fusarium sp., 1 C. infected plant D. conidia (E, F) Fusarium sp.2 E. infected Calyx (F) conidia (scale bar A, C, & E=1 cm B ,D, F=20 um*

Taxonomy

Among the isolated taxa, several have previously been reported and described from Pakistan. In this study, we provide re-descriptions of four taxa that represent new records for the country. Detailed morphological characteristics of these newly recorded taxa are presented below, expanding the known fungal diversity in Pakistan.

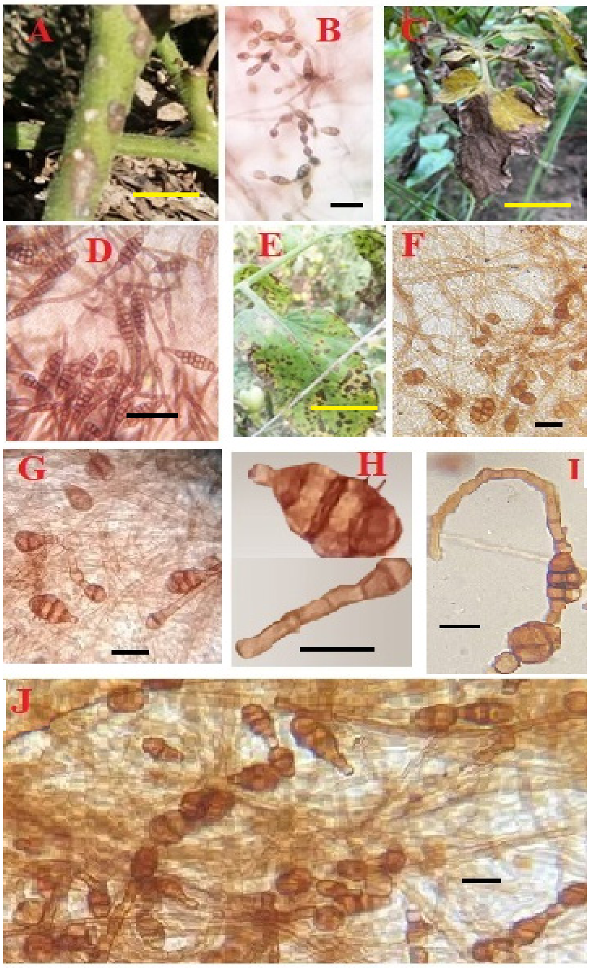

Figure 3: *(A, B) A. alternata A. infected plant B. conidia (C, D) A. solani C. infected plant D. conidia (E-J) A. arborescens E. infected leaves F-J Conidia with false beak (scale bar A, C, & E=1 cm B ,D, F-J=20 um).*

Alternaria arborescens E.G. Simmons, Mycotaxon 70: 356 (1999) (Figure 3 E-J)

Growth rate on PDA was ~7mm/day at room temperature (20-30°C). Hyphae were light brown, septate, and 3-5 µm wide. Conidiophores were solitary, dark brown, straight or curved, 2–8 septa, and variable in length, 30-90 µm. Conidia were oval or obclavate, brown to dark brown, with 1–4 transverse septa and 1–4 longitudinal or oblique septa, (19.7-)23.6 - 28.4(-34.3) × (9.8-)11.0 - 12.8(-13.7) µm (mean ± SD = 26.0 ± 2.4 × 11.9 ± 0.9 µm, n = 30). Conidia ellipsoid, pale brown to brown and smooth, with 3–8 transverse septa and 0–4 longitudinal septa in chains , measuring 18–53 × 8-18 (mean=34×13) µm in dimension,. Conidial beak 3-6 µm long. Teleomorph was not observed on medium.

Alternaria arborescens produces larger (20-60 × 9-18 µm), short-beaked conidia compared to *A. alternata* ller, beakless) and *A. tenuissima* (narrower). It differs from *A. solani* (larger, long-beaked) in conidial size and branched conidiophores. Colonies are gray-green and velvety, distinct from related species.

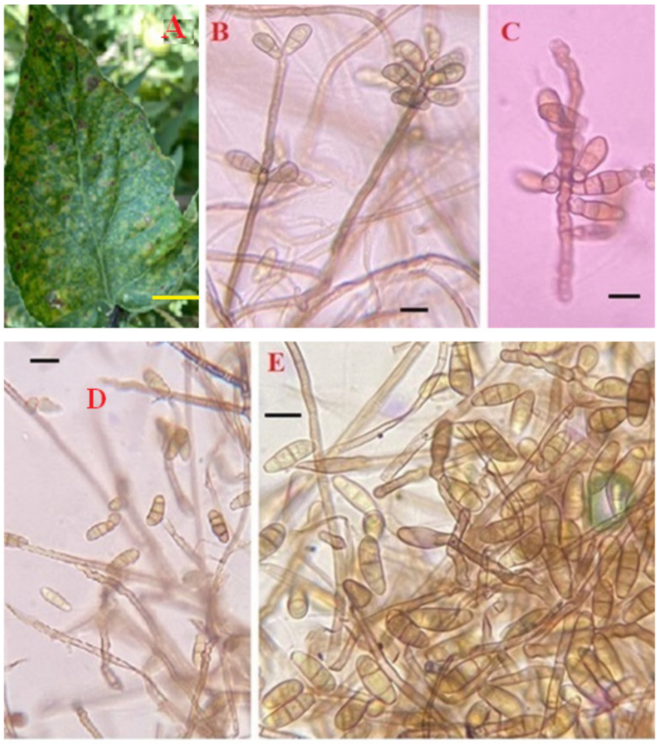

Figure 4: *C. pallescens A. infected leaf B-E conidia with conidiophores beak (scale bar A =1 cm B-E=20 um).*

Curvularia pallescens Boedijn, Bull. Jard. bot. Buitenz. 3(13): 127 (1933) (Figure 4 B-E)

Synonyms:

Cochliobolus pallescens (Tsuda & Ueyama) Sivan.

Curvularia leonensis M.B. Ellis

Pseudocochliobolus pallescens Tsuda & Ueyam

Colonies exhibited concentric zones shifting from pale gray-brown to dark brown or black with age. *Conidiophores* were solitary, unbranched, brown, up to 150-400 µm long and 5–6 µm wide, straight or geniculate near the apex. Conidia were smooth, typically 3-septate, ellipsoidal to fusiform, slightly curved, 15–25 (x=20.1) × 5–8 (x=6.6) µm, often with disproportionate third cell enlargement which is sometime geniculate.

The genus *Curvularia* includes several morphologically similar species requiring careful differentiation. *Curvularia lunata* is characterized by three-septate conidia (21-31 × 9-13 µm) with a distinctively enlarged and pigmented third cell (Shivas and Sivanesan, 1987). *Curvularia pallescens* shares similar septation but exhibits paler, more strongly curved conidia (Ellis, 1965). *Curvularia spicifera* produces uniformly pigmented, cylindrical conidia: 20-40 × 9-14 µm in dimention (Revankar and Sutton, 2010). *Curvularia australiensis* displays straight, oblong conidia without cell enlargement (Madrid *et al*., 2014). Colony morphology varies from lanate (*C. lunata, C. pallescens*) to glassy (*C. spicifera*).

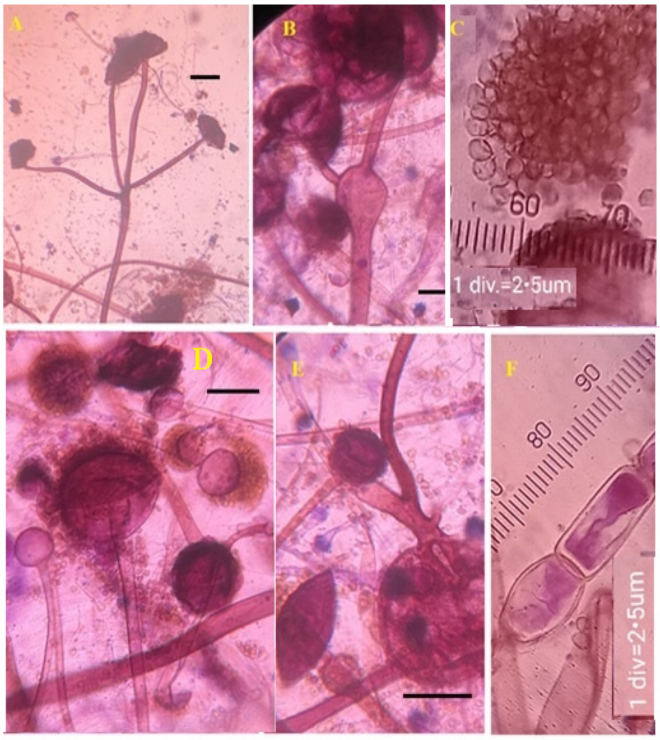

Figure 5: *R. arrhizus var. delemar A. tetrafurcate sporangiophore B. Sporangiophore with apical swelling C. Sporangiospores D. Columella E. rhizoids F. Chlamydospores (bar=25 um)*

*Rhizopus arrhizus var. delemar (Wehmer & Hanzawa) J. J. Ellis, Mycologia 77: 247. 1985 (*Figure 5 *A-J)*

Daily growth rate on PDA at room temperature (20-30 °C) attaining 25 mm. Colonies were at first white, soon becoming deep gray to black. Stolons were subhyaline to brown and aseptate. Rhizoids were rare, finger-like or branched 1-several times when present, short to long, grayish-brown to brown. Sporangiophores mostly arising directly from mycelia and without rhizoids, or sometimes from stolons with opposite rhizoids or sometimes, solitary or 2-5 in groups, mostly straight, rarely curved, simple, sometimes forked or 3-4 furcate at the apex, (500-)1000-3000 (-4000) um long, equal throughout or enlarging at the uppermost part., brownish, non-septate, swellings common, mostly at the upper half. Sporangia globose to subglobose, 62-120 (mean=90.5) um diam. Columellae roundish to subglobose 25-38 um diam. Sporangiospores irregular in shape and size, ovoid when regular in shape,5-8 (mean=6.1) um. Chlamydospores on mycelia are rare, usually catenulate. Zygospores were not observed, probably heterothallic. *Rhizopus arrhizus* var. *delemar* differs morphologically from closely related varieties primarily in sporangiophore structure and spore regularity. Its sporangiophores (1000–4000 µm) often arise from stolons with opposite rhizoids, exhibit apical swellings near the apophysis, and may fork, while *var. arrhizus* sporangiophores are more variable, frequently emerging from aerial mycelia, and less branched. *Var. tonkinensis* shares stolon-borne sporangiophores but lacks prominent swellings and has strictly ovoid columellae with narrow bases. Sporangiospores in *var. delemar* are irregular (5–8 µm), contrasting with the regular, smaller spores of *var. arrhizus* (4–9 µm) and *var. tonkinensis* (4–10 µm). Rhizoids are sparse in *var. delemar*, poorly developed in *var. arrhizus*, and elongated/branched in *var. tonkinensis*. These distinctions, alongside columella shape (roundish in *delemar*, strictly ovoid in *tonkinensis*), support their taxonomic separation despite genetic similarity.

*Rhizopus homothallicus Hesselt. & J.J. Ellis, Mycologia 53(4): 419 (1962) [1961] (*Figure 6 *A-F)*

Colonies exhibit a drab gray to drab coloration, with poorly developed or absent rhizoids that are non-septate. Sporangiophores primarily arise from hyphae in an indeterminate manner, occasionally from stolons, and may or may not feature opposite rhizoids. Typically, they occur as solitary structures, although they can be found in pairs. These sporangiophores widen towards the apex. The sporangia were 50 - 72.5 (mean = 61.75) µm in diameter. The columellae are generally smooth, though they may be very rarely subverrucose at the upper portion, measuring 35-47.5 (mean = 39.75) µm in diameter. Sporangiospores are relatively consistent in shape and size, displaying very faint striations, 320-660 (mean = 485) µm. Zygospores are characteristically tuberculate; 30-87.5 (mean= 58.3) µm. The two suspensors are equal in shape but differ in size and are often constricted at the base, 25x28-37x45 um. Sexual reproduction in this species is homothallic.

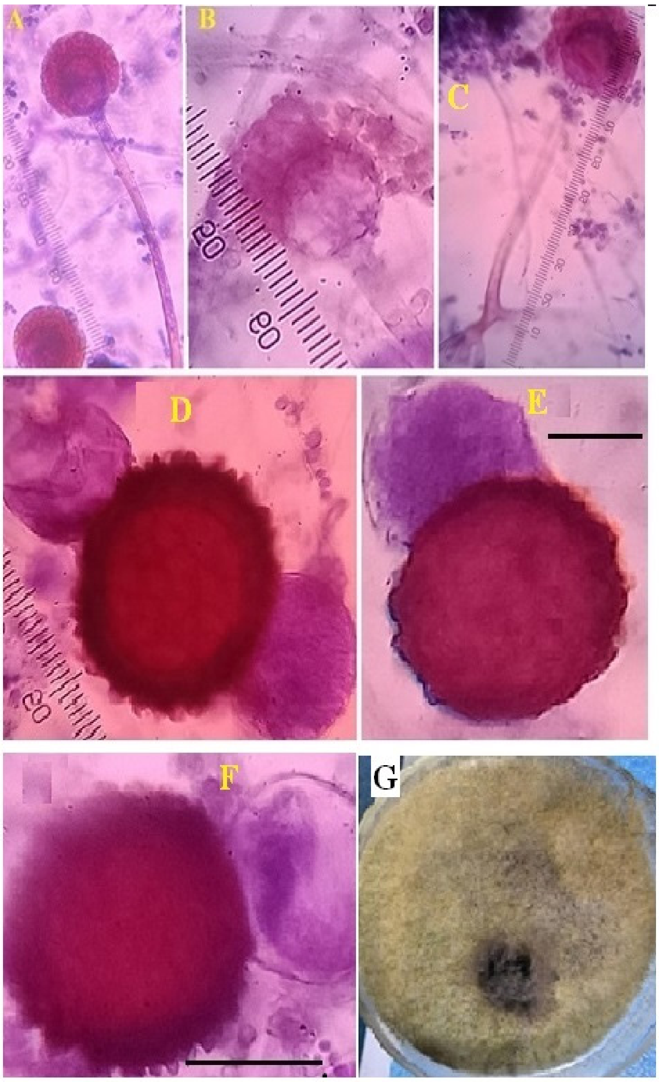

Figure 6: *R. homothalicus A-C Sporangiophore, sporangia, sporangiospores and columella D-F Zygospores with suspensors G colony on PDA (1 div.=2.5 um in A-E, bar=25 um in F-I)*

There are three homothallic species of the genus *Rhizopus: R. homothallicus, R. sexualis,* and *R. americanus.* Suspensors of equal shape but unequal size, verrucose zygospores, and sparse, finger-like rhizoids are characteristics of *R. homothallicus*. On the other hand, *R. sexualis* exhibits dense, branched rhizoids, smooth pale brown zygospores with equal globose suspensors, and produces abundant azygospores along with taller colonies (4-7mm). *Rhizopus americanus* shows well-developed rhizoids, pale greenish zygospores with abruptly globose suspensors, and forms the smallest colonies (1-2mm) among the three. All three species share moderate thermotolerance with a maximum growth temperature around 33°C, distinguishing them from more thermotolerant heterothallic species like *R. microsporus*. This is the first report of this taxon from Pakistan.

Discussion

This study presents the first phytopathological and taxonomic assessment of fungi associated with tomato crops in the Bajaur district, a significant yet understudied agricultural region in Khyber Pakhtunkhwa, Pakistan. Our results elucidate a mycbiome of concerning complexity, characterized by high prevalence of key pathogens, catastrophic yield losses, and the presence of previously unreported taxa, underscoring a severe threat to tomato production in the region.

The isolation of seven pathogenic fungi from symptomatic tomato tissues including *F. oxysporum*, two unidentified *Fusarium* spp., *A. alternata, A. solani, A. arborescens*, and *C. pallescens* demonstrates the multifaceted disease pressure faced by local growers. The additional recovery of three *Rhizopus* species from the rhizosphere further reveals a rich and largely unexplored fungal diversity within these agroecosystems. The most prevalent pathogens *A. alternata* (87.5% of sites), *A. solani*, and *F. oxysporum* (both 75%) are consistent with the major fungal constraints on tomato production globally (Panno *et al.*, 2021) and align with reports from other regions of Pakistan (Safi *et al.*, 2020; Riaz *et al.*, 2021). The mean disease incidence of 33.5% is substantial, but the economic impact is truly revealed by the yield data. The average yield of 11.9 t/ha represents a mere 6.9% of the potential yield, culminating in catastrophic losses of 93.1%. These losses are among the most severe documented for open-field tomato production and signify a regional agricultural crisis.

This devastating situation is likely multifactorial, arising from a confluence of agronomic practices and ecological factors. The high disease pressure is exacerbated by the region's agricultural landscape, which is dominated by solanaceous crops. Notably, chilli pepper (*Capsicum annuum* L.) is a major and extensively cultivated crop in the district, often grown in adjacent fields or in rotation with tomato. Both tomato and chilli are members of the Solanaceae family and share susceptibility to a common suite of fungal pathogens, including *F. oxysporum*, *A. alternata*, *A. solani*, and several *Rhizopus* species (Agrios, 2005; Shafique *et al*., 2022). This practice creates a continuous "green bridge," allowing pathogens to persist, multiply, and disseminate between these two host crops throughout the year. The lack of effective crop rotation with non-host plants enables the build-up of inoculum in the soil, a problem compounded by the conducive subtropical climate of Bajaur, the imbalanced use of nitrogenous fertilizers which can increase plant susceptibility to fungal diseases (Dordas, 2008), and the observed reliance on a limited range of fungicides like mancozeb. This creates a perfect storm for pathogen proliferation and the consequent astronomical losses.

Beyond the epidemiological findings, the primary mycological novelty of this work lies in the taxonomic documentation of four new records for Pakistan: *Alternaria arborescens*, *Curvularia pallescens*, *Rhizopus homothallicus*, and *Rhizopus arrhizus* var. *delemar* (Ahmad *et al*., 1997). Crucially, to our knowledge, this study also reports the first global isolation of *Curvularia pallescens* from tomato plants. The presence of *A. arborescens* is particularly concerning. This species is a known producer of host-specific toxins (Akimitsu *et al*., 2003) and has established virulence on solanaceous crops worldwide (Gou *et al*., 2023). Its emergence in Pakistani tomato fields highlights a shifting pathogen landscape and necessitates enhanced surveillance, especially given its potential for developing fungicide resistance (Ma *et al*., 2023). Similarly, the isolation of *R. homothallicus* from the rhizosphere is significant. While this species is increasingly recognized as an emerging agent of human mucormycosis, particularly in neighbouring India (Rudramurthy *et al*., 2023; Kaur *et al*., 2021), its ecological role in the soil and its potential as a plant pathogen or opportunistic endophyte remain poorly understood and warrant further investigation.

Our findings on the *Fusarium* community align with previous work in Pakistan, confirming *F. oxysporum* as a prevalent pathogen (Ahmad *et al*., 2021) and suggesting a greater diversity of Fusarium spp. affecting tomatoes than previously recognized, as indicated by the two unidentified species isolated in this study. The reporting of *C. pallescens* adds a new dimension to the leaf spot complex affecting tomatoes in the region. While its cousin *C. lunata* has been reported on tomato in Pakistan (Iftikhar *et al*., 2016), *C. pallescens* has a broader known host range including cereals and ornamentals (Farr and Rossman, 2024), and its pathogenicity on tomato now requires confirmation.

This study has certain limitations. The identifications were based solely on morphological characteristics due to financial constraints. Furthermore, Koch's postulates were not fulfilled for the newly associated pathogens due to time limitations. Consequently, future studies must integrate molecular phylogenetics (e.g., multi-locus sequence analysis) to confirm the identity of the taxa reported here, especially the unidentified *Fusarium* species and the new records. Pathogenicity assays are also essential to confirm the virulence of *C. pallescens* and *A. arborescens* on tomato.

In conclusion, this research provides a critical baseline for understanding the fungal diseases plaguing tomato production in Bajaur. The extremely high yield losses demand immediate intervention through integrated disease management (IDM) strategies. These should include: (1) the introduction and promotion of resistant tomato varieties; (2) education for farmers on the importance of crop rotation with non-solanaceous hosts to break the disease cycle; (3) sanitation practices to remove crop debris; and (4) the judicious use of effective, targeted fungicides in rotation to mitigate resistance development. The discovery of new fungal records underscores that the phytopathological landscape of Pakistan is dynamic and underexplored, highlighting the need for continued surveillance and modern diagnostic techniques to safeguard the country's food security.

Acknowledgments

The authors sincerely thank the principal of Government Post Graduate College, Khar Bajaur for their support and encouragement, and the laboratory staff for their valuable assistance throughout the study. We are also grateful to the farmers who willingly participated and shared their field-level observations.

Novelty Statement

The study presents the first comprehensive survey of fungal biota associated with tomato crops in the Bajaur district of Pakistan. It reports the discovery of several fungal species not previously known to be associated with tomatoes in the country, including one which is a first-ever global record. The research also documents catastrophic yield losses, establishing a critical baseline for addressing a major threat to regional tomato production and food security.

Author's Contribution

Rashid Ahmad, Muhammad Iqbal, Hazrat Bilal: Conducted the field and laboratory work.
Muhammad Abdul Haq: Supervised the study, identified and described the isolated taxa, and wrote the manuscript.
Aminul Haq: Reviewed the manuscript and edited the figures, tables, and references.

Disclaimer

Information obtained from farmers through questionnaires was based on personal recollection and may be subject to recall bias, particularly in estimating produce quantities and describing past farming practices.

Generative AI or AI assisted technology statement
During the preparation of this work, the authors used Grammarly in order to improve readability and language. After using this online tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Conflict of interest:
The authors declare that there is no conflict of interest.

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