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## SEVERITY, ETIOLOGY, AND DISTRIBUTION OF EARLY BLIGHT DISEASE ON POTATO IN GILGIT-BALTISTAN REGION, PAKISTAN

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### ABSTRACT

Potato, one of the world's most important crops, is susceptible to various pathogens, including *Alternaria alternata* and *Alternaria solani*, responsible for early blight disease (EB). This study aimed to assess the prevalence and incidence of EB in nine valleys (Gouro, Nalter, Nomal, Jutal, Chilmish, Bagrote, Danyore, Jalalabad, and Oshikhandas) of Gilgit Baltistan (GB), Pakistan, and identify the dominant *Alternaria* spp. causing the disease. A comprehensive field survey was conducted in these valleys, encompassing 45 fields. The survey revealed variations in the prevalence, incidence, and severity of early blight across different locations. Nalter exhibited the highest prevalence at 58%, with an incidence of 29% and disease severity of 9.67%, indicating a substantial impact on potato crops. Bagrot and Gouro also reported substantial prevalence rates of 53% and 49.33%, respectively, with notable incidence and severity. In contrast, the Oshikhandas and Danyore regions had the lowest disease severity, signifying less extensive damage. To understand the pathogens responsible for early blight, 60 isolates were collected and identified. *A. alternata* was the dominant species, with 60 isolates, while *A. solani* was identified in only 5 isolates. *A. alternata* exhibited characteristic dense and multi-colored growth, particularly when cultured on PDA media. Microscopic examination confirmed its identity, with short chains of conidia showing a distinctive beak-like projection. Notably, the GIS maps show that the northwestern section exhibits higher disease incidence than the northeastern part, emphasizing the non-uniform distribution of EB. In conclusion, this research is important to understand the disease's dynamics and can inform targeted management strategies to mitigate its impact on potato crops, contributing to enhanced food security and agricultural sustainability.

**Keywords:** Gilgit-Baltistan, Potato, Early blight, Distribution, Severity, Incidence, Prevalence.

### INTRODUCTION

Potato (*Solanum tuberosum* L.), a globally cultivated food crop renowned for its high nutritional and economic value, outpaces cereals like rice and wheat in calorie production per hectare (Xue *et al.*, 2018; FAO, 2020). Belonging to the *Solanaceae* family alongside chili, eggplant, tobacco, and tomato, its origin in the Andes

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region expanded to the subcontinent via Portuguese traders, integrating into various dishes across Pakistan due to its adaptability to unique weather conditions (Meno *et al.*, 2020). Recently, Gilgit Baltistan (GB), Pakistan, has witnessed an upswing in potato production attributed to expanded acreage and the introduction of new cultivars (Abbas *et al.*, 2016; Abbas and Madadi, 2016). Serving as a cash crop, potatoes have become a primary income source for farmers, with an approximate yield of 20 bags (70-80 Kg each) per Kanal in 2017 (Abbas *et al.*, 2023). Accurate estimation of GB's total annual potato production proves challenging due to its mountainous terrain, resulting in diverse

maturation dates based on altitude (Abbas *et al.*, 2023). The shift from disease-resistant traditional varieties to high-yielding yet susceptible types exposes the potato crop to various threats, with early blight (EB), caused by *Alternaria* spp. — primarily *A. solani* (Soraeur) and *A. alternata* (Fr.) Keissl—emerging as a paramount concern (Meno *et al.*, 2021a). Currently, EB stands as the most destructive disease in GB due to its expanding distribution potential. Symptoms manifest as small brown or black spots on older leaves, enlarging with a yellow halo, resembling a bullseye with concentric rings. As the disease progresses, leaves become chlorotic, ultimately defoliating. Stem lesions, oval with a grey or light center, and sunken, asymmetrically shaped tubers with a pink or purple line indicate the severity of infection. The pathogens of EB survive as conidia and mycelium in plant residues, volunteers, alternative hosts, and in several solanaceous weeds (Tymon *et al.*, 2016). Environmental conditions have been reported to play an important role in the development of EB. Favorable temperature (20 to 26°C) and long leaf wetness duration or high relative humidity can lead to severe EB epidemics (Holley *et al.*, 1985; Rotem, 1994; Gold *et al.*, 2020). GB's elevated temperatures and alternating dry and humid cycles, coupled with light-textured, sandy, and low-organic-matter-content soil in irrigated areas, create

an environment conducive to EB development (Abbas *et al.*, 2016; Abbas and Madadi, 2016; Abbas *et al.*, 2023). Managing early blight is challenging due to its capacity to generate vast secondary inoculum in the form of conidia (El-Batal *et al.*, 2016). Fungicide spraying is extensively used to mitigate losses, supplemented by cultural controls like removing debris and volunteer potatoes and employing proper harvesting and storage practices (Horsfield *et al.*, 2010). Traditional strategies involve applying protective fungicides during warm-hot weather, yet variations in determining the optimal timing for initial fungicide applications result in unnecessary sprays (Jindo *et al.*, 2021). Considering the gravity of the impact of EB on potato production in Gilgit Baltistan and the current lack of information regarding its prevalence, incidence, and etiology, this research aims to investigate the causes, severity, prevalence, and incidence of early blight disease in potatoes within the Gilgit Baltistan region.

**Materials and methods**

**Study Location:** The research was conducted in the nine valleys (Gouro, Nalter, Nomal, Jutal, Chilmish, Bagrote, Danyore, Jalalabad and Oshikhandas) of Gilgit as shown in Figure 1. The climate in this region is defined by arid and semi-arid conditions, showcasing varied average temperatures and precipitation levels.

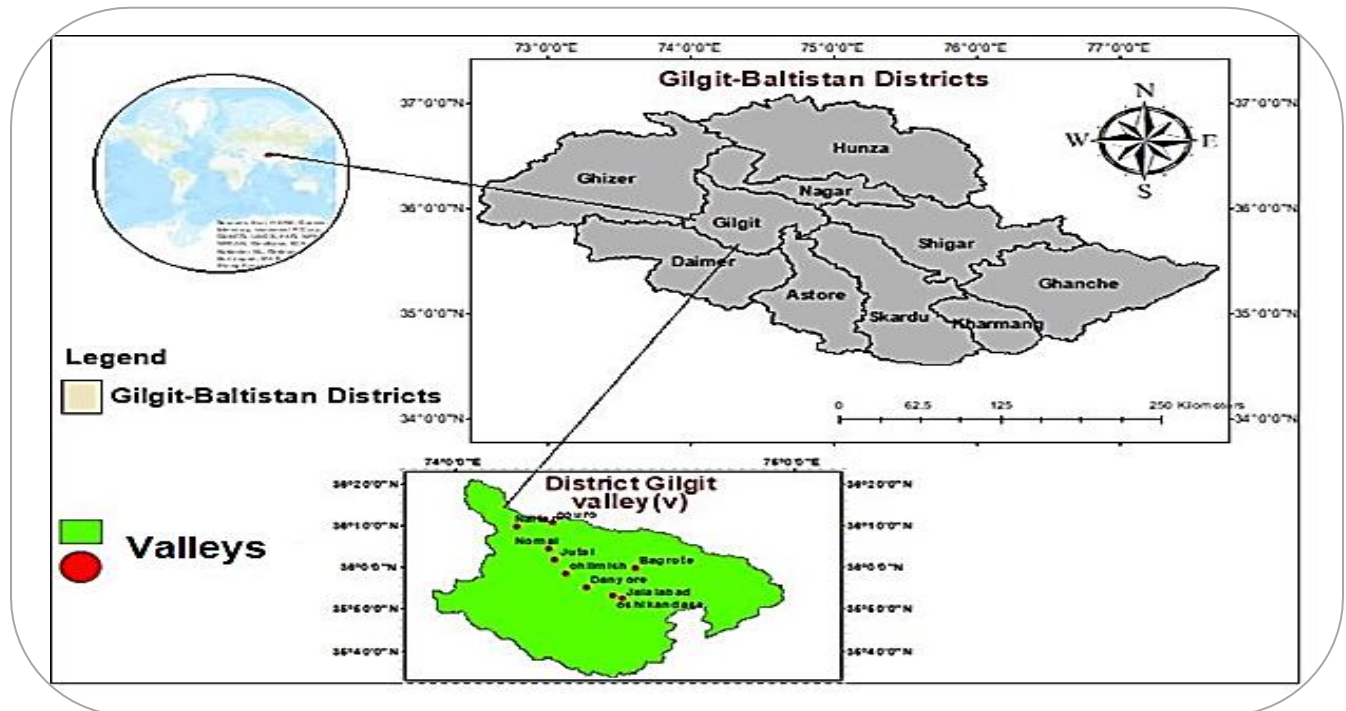


Figure 1. Map of valleys where the study has been carried.

**Incidence, severity, and prevalence of EB: Disease**

**incidence:** A comprehensive survey was undertaken during May 2023 in the nine prominent potato cultivation valleys of GB to assess the incidence and severity of EB. Five fields with consistent potato crop production were

randomly chosen in each valley. About 45 fields in 9 different valleys were surveyed (Table 1). About 30 plants within each field were randomly assessed for EB. Disease incidence was quantified using the following equation:

$$\text{Percent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plant examined}} \times 100$$

Table 1. Valleys and the number of field samples were collected.

Valleys	Number of fields
Nalter	5
Bagrot	5
Goro	5
Nomal	5
Jutal	5
Chilmis	5
JalalAbad	5
Oshikhandas	5
Danyore	5
Total fields	45

**Disease severity:** The severity of the disease in each field was evaluated using a scale ranging from 0 to 5 (Pandey *et al.*, 2003). In this scale, a score of 0 indicates the

absence of visible symptoms on foliage/fruits, while a rating of 5 signifies more than 76 % infection as shown in Table 2.

Table 2. Disease severity scale

S.No	Grade/Numerical value	Category	Description
1	0	Disease Free	Healthy
2	1	1-10%	One to ten percent area of foliage are covered with the disease
3	2	11-25%	Few spots have covered foliage
4	3	26-50%	Many spots coalesced on the foliage,
5	4	51-75%	Besides, foliage, stems, and fruits were also infected; Sunken lesions with concentric rings formed on foliage, stems, and fruits.
6	5	> 76%	Severe lesions on foliage, stem, and fruit rotting

The percent disease severity was subsequently calculated, according to the following equation

$$\text{Percent disease severity} = \frac{\text{Sum of individual rating}}{\text{Number of plants examined} \times \text{Maximum disease scale}} \times 100$$

**Disease prevalence:** Disease prevalence was calculated as either present or not present in the potato fields by using the formula shown below:

$$\text{Percent disease prevalence} = \frac{\text{Number of infected potato fields}}{\text{Total number of potato fields}} \times 100$$

**Spatial distribution of early blight:** The spatial arrangement of early blight was analyzed by creating a database with X and Y coordinates for the study area. This involved opening the shapefile of the study area in GIS software (ArcGIS 10.7). Within this project, three fields— X, Y, and Z—were employed. The x-field represented X-coordinates, the Y-field denoted Y-coordinates, and the Z-field stored disease-related data. The ArcView Spatial Analyst "Interpolate grid option" was employed, configuring the grid extension in the output to align with that of District Nagar. The chosen interpolation method was inverse distance weight (IDW). The spatial

distribution of the disease variable could be characterized using the semivariogram function, and its calculation could be based on equations suggested by previous researchers (Hussain *et al.*, 2023).

**Isolation and identification:** Samples with early blight symptoms (concentric rings) were randomly collected from all the nine valleys (Figure 2). A total of 100 leaves were gathered from potato fields in the nine valleys mentioned earlier. Five fields were randomly selected from each valley. The collected leaves were carefully placed in sealed plastic bags to prevent contamination and transported to the laboratory of Integrated Pest and

Disease Management in Gilgit. Subsequently, stored at 4°C until further processing. Leaves-bearing lesions were disinfected by immersing them in 70% ethanol for a duration of 3 to 5 seconds. After disinfection, the leaflets were gently blotted with a paper towel and allowed to air dry. One to three lesions per leaf (equating to one lesion per leaflet) were systematically sampled. The margins of these lesions were excised for further analysis. The excised lesion margins were plated on a potato dextrose agar (PDA). This provided a conducive environment for the growth of the fungal isolates. Single-conidial isolates were obtained from individual lesions on single leaflets. These isolates were cultivated on PDA at temperatures ranging from 21 to 23°C under constant light conditions. To achieve purification, peripheral hyphal tips of the growing culture were excised and aseptically placed on the PDA. The Petri plates were sealed, and incubated at 25 ± 2°C, and the pathogen was identified using the mycological keys (Ellis, 1971; Woudenberg *et al.*, 2015). The identified pathogen cultures were maintained at 4°C for subsequent studies.

**Pathogenicity tests:** For the detached leaf assay, fully developed and healthy leaves from the "Desiree" susceptible variety were obtained, ensuring they were of uniform size. Leaves were carefully detached from the base of the petiole from 25-day-old plants. They were then disinfected using a 1% sodium hypochlorite solution for 2 minutes, followed by four thorough rinses with sterile distilled water. After disinfection, the leaves were placed on paper towels to air-dry inside a laminar airflow cabinet. Once dried, the leaves were arranged abaxial side up on filter paper within sterile 90 mm Petri plates. For the assay, each leaf was punctured at the center on the abaxial side and inoculated with mycelial agar plugs. These plugs, measuring 6 mm in diameter, were sourced from the margin area of a 7-day-old culture that had been cultivated on PDA media. Petri plates were maintained in a humid environment by adding 2 ml of water and were subsequently placed inside an incubator, initially in complete darkness for 48 hours at a temperature of 22 °C. Following this dark incubation, the plates were relocated under cool-white fluorescent lighting, replicating a diurnal light cycle of 12 hours. The duration of exposure under these conditions was 7 days, with occasional moisture addition as needed. Each fungal isolate was subjected to three separate replications to ensure the reliability of the results. The assessment of disease severity was conducted following the previously mentioned rating scale.

## STATISTICAL ANALYSIS

The data obtained during the field survey and the pathogenicity assessment were analyzed using Statistix 8.1 analytical software. Graphs and figures were generated using Microsoft Excel 2019.

## RESULTS

**Incidence, severity, and disease prevalence of early blight:** In the field survey conducted across various locations, the prevalence, incidence, and severity of the observed early blight disease were recorded (Figure 1). The highest prevalence was observed in the Nalter region, with a prevalence rate of 58%. This indicates that a significant portion of the plant population in Nalter was affected by the disease during the survey. In Bagrot, the prevalence was slightly lower but still substantial, at 53%. The Goro region also showed a relatively high prevalence of 49.33%. Incidence, which measures the rate of new disease cases, followed a similar pattern. Nalter had the highest incidence at 29%, indicating a relatively high rate of new disease cases during the survey period. Bagrot and Goro also had notable incidence rates of 25.67% and 22.67%, respectively. The severity of the disease, which assesses the extent of damage caused by the pathogen, was calculated as a percentage. Nalter exhibited the highest disease severity, with a score of 9.67%. This suggests that in areas with a high disease prevalence and incidence, the severity of the disease was also notably elevated. In comparison, the Oshikhandas and Danyore regions had the lowest severity, with values of 3% and 2%, respectively, indicating less extensive damage caused by the disease in these areas.

**Pathogens associated with the disease:** About 65 isolates of *Alternaria* were collected from early blight lesions on potato plants (Table 3). The majority of these isolates were identified as *A. alternata*. The colony of *A. alternata* was dense and colored in shades of black, grey, and brown. Interestingly, the growth was found to be particularly dense when these isolates were cultured on PDA media. Under microscopic examination, the isolates of *Alternaria* with specific characteristics were identified as *A. alternata*. These characteristics included short chains of two to three conidia attached to the hyphae, and these conidia had a short beak-like projection. This distinct morphology of the conidia and their arrangement on the hyphae allowed for the differentiation and identification of *A. alternata*, a common species associated with early blight lesions on potato plants.

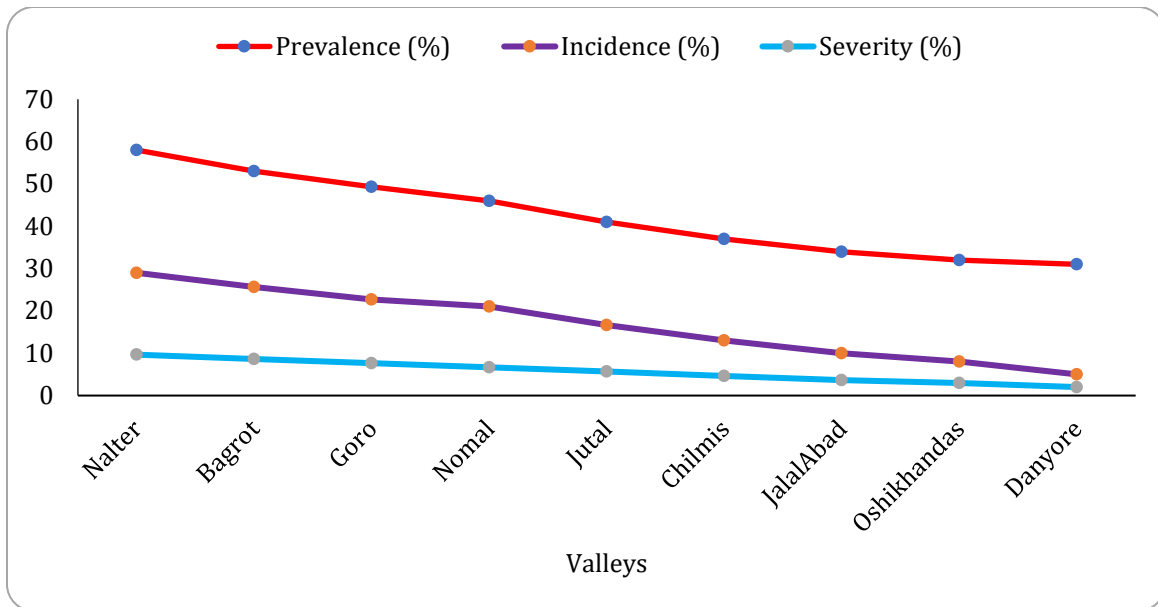


Figure 1. Incidence, prevalence, and severity of the early blight of potato in nine valleys of Gilgit.



Figure 2. Isolation and Morphological Features of Early Blight Fungus from Affected Potatoes. (a) Brownish lesions visible on the surface of infected potato leaves; (b) Concentric lesions present on the leaves; (c) Culture of *Alternaria* spp. fungus on PDA medium after 5 days, showing the front view; (d) Detailed view of the reverse side of the culture; (e) Hyaline, septate, smooth-walled hyphae and mycelia with developing conidia; (f) Brown, fusiform, smooth-walled conidia with transverse and oblique septa.



Table 3. Number of fields infected with early blight disease pathogens in different valleys

Valleys	Number of fields	<i>A.alternata</i>	<i>A. solani</i>
Nalter	5	8	5
Bagrot	5	8	
Goro	5	5	
Nomal	5	9	
Jutal	5	6	
Chilmis	5	6	
JalalAbad	5	7	
Oshikhandas	5	7	
Danyore	5	7	
Total fields	45	60	5

**Distribution of EB:** There is a wide range of disease prevalence, which ranges from 31% to 58%. The spatial distribution of the disease, as illustrated in Figure 1 and Figure 3, emphasizes that the impact of early blight is not uniform and varies significantly across the valleys. The concentration of the highest rates of prevalence, incidence, and severity in the northwestern and northeastern regions is a noteworthy observation. Additionally, the spatial distribution patterns, as depicted in Figure 3, further emphasize the non-random distribution of the disease, which may be influenced by environmental and geographical factors. The variation in disease incidence, ranging from 5% to 29%, is another significant outcome. The notably higher incidence values

in the northwestern section of the Gilgit region, in comparison to the northeastern part, suggest a non-uniform distribution of newly occurring disease cases. This distribution pattern, as revealed by the GIS maps, may be indicative of underlying environmental factors or variations in agricultural practices. The semivariogram analysis provided insights into the prevalence ratios of different disease parameters. Shot hole disease exhibited the highest prevalence ratio, followed by disease index, mean severity, and incidence. Understanding the varying impact and prevalence of these disease parameters is essential for tailored disease management strategies. Lastly, the trend analysis graph indicated lower values in the southern portion of the Gilgit region.

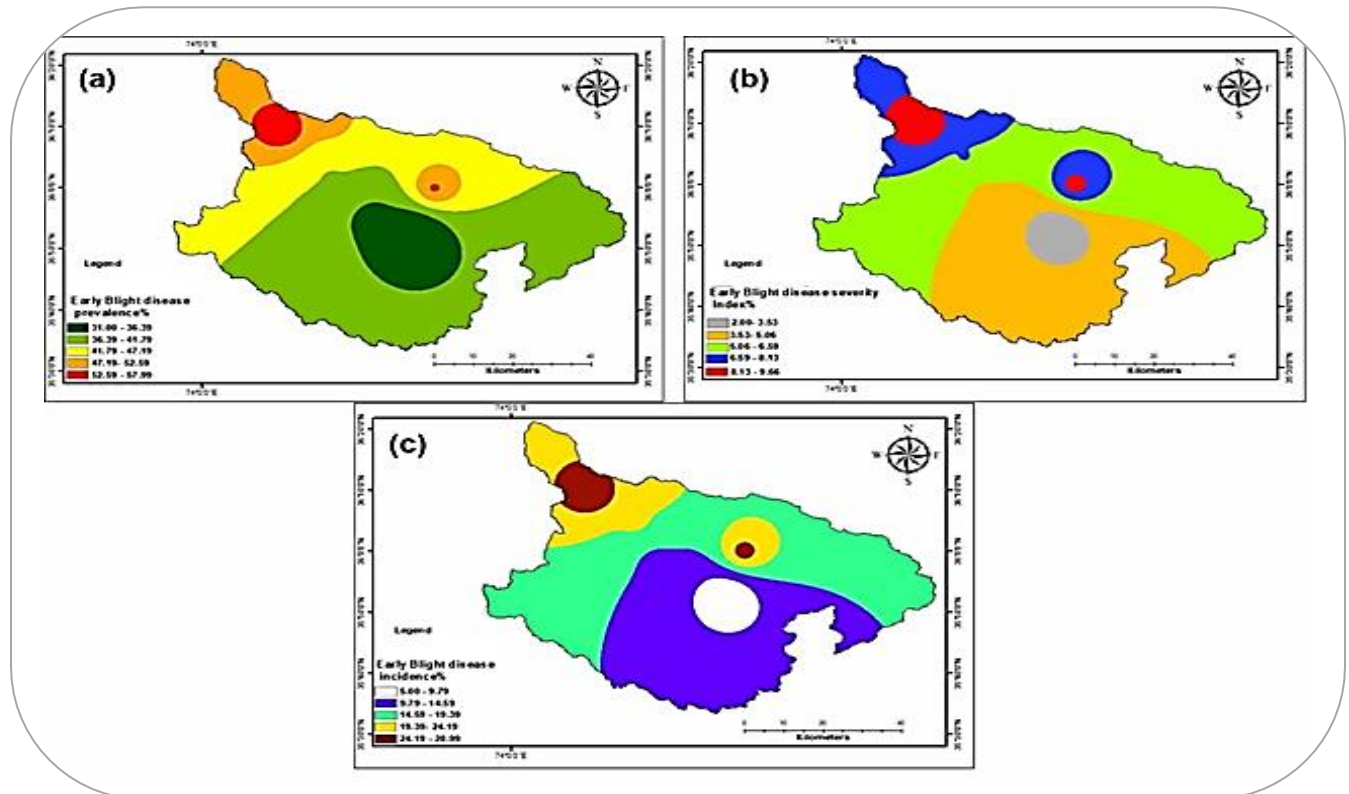


Figure 3. Spatial distribution map of EB. (a) Disease prevalence, (b) Disease severity, (c) Disease incidence

## DISCUSSION

This study highlights the prevalence, incidence, and severity of early blight disease in various valleys of GB and the pathogens associated with this disease. The field survey conducted in different locations revealed significant variations in the incidence, severity, and prevalence of early blight disease. The Nalter region exhibited the highest disease prevalence, indicating a substantial portion of the plant population was affected. Similarly, in Bagrot, the prevalence was slightly lower than Nalter but was higher than other valleys but still notable. Similarly, the Goro region displayed a relatively high prevalence followed by Nalter and Bagrot. Incidence, representing the rate of new disease cases, followed a similar pattern. Similarly, Nalter followed by Bagrot and Goro also reported notable disease severity and incidence rate as compared to other valleys. This indicates that areas with high prevalence and incidence also experienced significantly elevated disease severity. Both Bagrote and Nalter, located at high altitudes, experience greater rainfall and higher humidity levels compared to other valleys. Consequently, the prevalence, severity, and incidence of Early Blight (EB) are elevated in these valleys. On the contrary, the Oshikhandas and Danyore regions exhibit the lowest severity, indicating less extensive damage. These valleys are characterized by drier conditions and lower humidity levels in comparison to other areas, resulting in less severe EB. Previous research has similarly reported a positive correlation between disease incidence and high relative humidity, as well as a negative correlation with maximum temperature (Ivanović *et al.*, 2022). Further, the valleys with high altitudes such as Nalter, Bagrote and Goro, experience more influence of wind, rain and irrigation water. Furthermore, the variability of disease can be attributed to various factors, including the emergence of new pathogen strains, different culture methods employed, the host plants' susceptibility, the presence of inoculum from each pathogen, and the prevailing environmental conditions. Environmental factors can significantly influence the development and spread of the disease. For instance, higher temperatures may create conditions favorable for pathogen growth and disease progression, while increased humidity can provide moisture essential for pathogen propagation. Likewise, wind can facilitate the dispersal of pathogen spores, contributing to disease spread. Overall, the interplay of these factors can result in variable disease outcomes

(Thomson, 2000). The observed results can also be attributed to the continuous availability of inoculum throughout the year in alternative host plants. In addition to this, the prevalence of virulent isolates of the pathogen may have played a significant role in shaping these outcomes (Meno *et al.*, 2021b). In essence, the disease-causing pathogen likely had a constant source of inoculum from other host plants, which served as a reservoir for the pathogen, allowing it to persist and thrive. These alternative hosts provided a continuous supply of the pathogen, ensuring that it was readily available for infection, even during unfavorable conditions for the primary host. Furthermore, the presence of virulent isolates of the pathogen, which are highly capable of causing disease, would have intensified the overall impact of the disease on the primary host. The combination of these factors, including year-round inoculum availability and the prevalence of aggressive pathogen strains, likely contributed to the observed results and the persistence of the disease. Furthermore, in this study, about 65 isolates of *Alternaria* were collected from early blight lesions on potato plants. The majority of these isolates were identified as *A. alternata*. *A. alternata* colonies displayed dense growth with shades of black, grey, and brown as reported in earlier studies. Interestingly, their growth density was most pronounced when cultured on PDA media. Microscopic examination revealed specific characteristics allowing the identification of *A. alternata*. These characteristics included short chains of two to three conidia attached to hyphae, with conidia exhibiting a distinctive beak-like projection. The unique morphology of these conidia and their arrangement on the hyphae facilitated the differentiation and identification of *A. alternata*, a common species associated with early blight lesions on potato plants. The information available presents a complex scenario regarding the etiology of *Alternaria* spp. responsible for early blight. This complexity arises from a lack of comprehensive data on the origin and establishment of these pathogens. The presence of *Alternaria* spp. could be attributed to multiple factors. First, it remains uncertain whether these pathogens are native to the Gilgit region or were introduced through potato seed from other parts of Pakistan. The latter possibility raises concerns, particularly in cases where seed material's health status is uncontrolled and uncertain. Introducing pathogenic strains via seed material can significantly impact disease dynamics in

potato crops. Furthermore, the distribution of various large-spored *Alternaria* spp. causing early blight displays regional variability in different potato-growing areas. This variation can lead to the presence of diverse *Alternaria* species on potato crops, with *A. alternata* emerging as the predominant species. This observation aligns with earlier research findings and highlights the ecological dynamics of *Alternaria* spp. in different agricultural settings. It underscores the importance of conducting more extensive research to elucidate the origins and distribution patterns of these pathogens in the Gilgit region. Such research efforts will be critical for implementing effective disease management and prevention strategies tailored to the specific ecological context of this potato-growing area. Furthermore, this study reveals a striking spatial distribution of early blight disease prevalence, with a broad range spanning from 31% to 58% across the Gilgit district. The most significant prevalence, incidence, and severity of the disease are concentrated in the northwestern and northeastern regions. Notably, the GIS maps show that the northwestern section exhibits higher disease incidence compared to the northeastern part, emphasizing the non-uniform distribution of newly occurring cases. This spatial non-randomness suggests potential influences from environmental and geographical factors on the disease's prevalence and spread (Kaur *et al.*, 2019). The findings underscore the importance of considering the spatial dynamics of early blight disease for tailored management and control strategies in the Gilgit region. Further research is needed to unravel the environmental and agricultural variables contributing to these spatial disparities.

## CONCLUSION

In conclusion, this study sheds light on the significant threat posed by early blight disease to potato crops in the GB region of Pakistan. Variations in disease prevalence, incidence, and severity were observed across different valleys, with Nalter, Bagrot, and Goro experiencing substantial impacts on crop health. *A. alternata* emerged as the dominant pathogen responsible for early blight, exhibiting distinct characteristics and morphology. The non-uniform distribution of disease, particularly with a higher incidence in the northwestern section, highlights the importance of spatial analysis in understanding disease dynamics. These findings are crucial for devising targeted disease management strategies, ultimately contributing to enhanced food security and the

sustainability of agriculture in the region.

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**Contribution of Authors:**

Riaz Ahmad	: Collected samples and conducted lab experiments
Alamdar Ali	: Performed the statistical and GIS analysis
Aqleem Abbas	: Designed and supervised the study and reviewed the manuscript
Zakir Hussain	: Technical assistance
Iqbal Hussain	: Lab resources
Abdul Razaq	: Technical assistance and guidance
Faiza Akbar	: Conducted lab experiments
Syeda S. Zehra	: Conducted lab experiments