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Alternaria Leaf Spot of Lentil in the Steppe Zone of North Kazakhstan: Species Complex Identification and Field Efficacy of Chemical and Biological Fungicides

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ABSTRACT

Lentils (*Lens culinaris* Medik.) are a valuable high-protein leguminous crop. However, the productivity of lentils in North Kazakhstan is significantly undermined by the spread of fungal diseases, including alternariosis. The study focused on the detection and molecular identification of phytopathogenic fungi of the genus *Alternaria* in lentils in the steppe zone of North Kazakhstan, and testing the efficacy of chemical and biological fungicides to control their development. Studies were carried out in 2023-2025 on lentil crops in the Akmola Region of Kazakhstan. Fungi from affected plant organs were isolated and cultured. Morphological diagnostics were supplemented with molecular genetic identification based on Internal Transcribed Spacer region of ribosomal DNA (*ITS* rDNA) nucleotide sequence analysis, followed by Basic Local Alignment Search Tool (BLAST) analysis and the construction of a phylogenetic tree. Field experiments were conducted to investigate the development and spread of the disease and test the biological efficacy of fungicides Kolosal Pro and Biograno Forte compared to untreated controls. The studied isolates were found to belong primarily to *Alternaria alternata*. BLAST analysis showed 100% similarity to *A. alternata* sequences and high similarity to *A. tenuissima*, whereas phylogenetic analysis confirmed the predominant isolates belonged to *A. alternata*. Field studies showed that the development and spread of alternariosis depended significantly on weather conditions. At 15 days after treatment at the bud development stage, the chemical fungicide Kolosal Pro showed the highest biological efficacy against disease prevalence (P), ranging from 65.8% to 76.9% across the 2023–2025 growing seasons. The biofungicide Biograno Forte had a more moderate effect, with efficacy ranging from 55.6% to 69.4% over the same assessment period. The findings can be applied in the development of evidence-based systems to protect the crop against alternariosis.

1. INTRODUCTION

Lentils are one of the most important leguminous crops of food and feed value. From a nutritional standpoint, lentils hold one of the leading places among leguminous crops and present the most valuable source of complete plant protein [1-3]. Lentils do not accumulate nitrates, toxic elements, and radionuclides, and can be considered an environmentally friendly product. Furthermore, lentils are considered one of the best leguminous crops in terms of taste and nutrition [4, 5].

Lentils are a widely cultivated legume crop grown across diverse agroecological zones worldwide. According to Food and Agriculture Organization Corporate Statistical Database (FAOSTAT), major producers include Australia, Canada, India, and Turkey, while in Kazakhstan, lentil production is concentrated in the Kostanay, North Kazakhstan, and Akmola regions, with a cultivated area of about 225 thousand ha and a gross yield of 192 thousand tons in 2023 [6, 7]. However, lentil production in Kazakhstan is characterized by unstable yield dynamics, reflecting the combined influence of abiotic stress and phytosanitary constraints (see Figure 1). Among these, plant diseases represent a major limiting factor affecting both yield and seed quality. Globally, the most economically significant diseases of lentil include ascochyta blight, fusarium wilt, rust, and *Alternaria* leaf spot. While ascochyta blight has been extensively studied and is known to cause substantial yield losses (up to 35–40% in foliage and over 70% through seed infection in Canada), other diseases, particularly those caused by the *Alternaria* species complex, remain less well characterized in many production regions [8, 9].

While diseases such as ascochitosis and fusariosis are widely recognized as major threats to lentil production, the role of the *Alternaria* species complex is increasingly significant [9, 10]. In Kazakhstan, recent studies using molecular and morphological tools have identified *A. alternata*, *A. tenuissima*, and *A. infectoria* as key pathogens in diverse crops ranging from wheat and oilseeds to vegetables [10-12]. For instance, *A. alternata* has been documented as a highly aggressive pathogen on tomatoes in the Akmola and Pavlodar regions, and both *A. alternata* and *A. tenuissima* were identified as primary causes of leaf spot in spring camelina within the dry steppe zone of North Kazakhstan [10, 12].

However, despite the economic importance of lentils and the widespread presence of *Alternaria* in other regional crops, there is a distinct lack of research focusing on the molecular identification of the specific *Alternaria* species complex infecting lentils in the unique steppe zone of North Kazakhstan. Most previous regional research has prioritized more established pathogens such as *Ascochyta lentis*, leaving a critical void in our understanding of *Alternaria* diversity and its specific impact on lentil productivity [9].

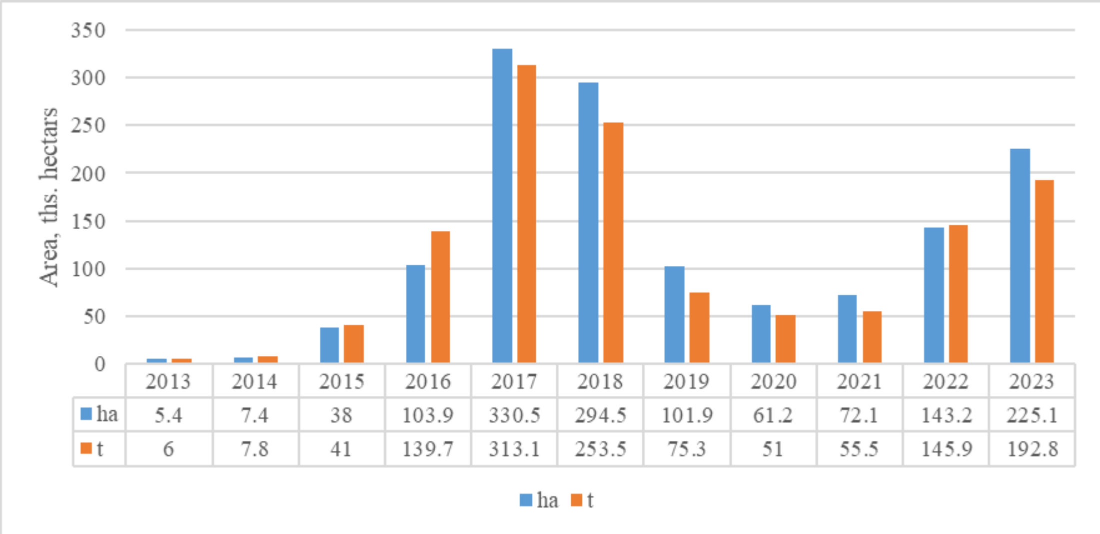


Figure 1. Dynamics of lentil cultivated area in the Republic of Kazakhstan from 2013 to 2023 (thousand hectares)
Source: Food and Agriculture Organization Corporate Statistical Database (FAOSTAT)

This knowledge gap extends to disease management strategies. While fungicides containing active ingredients like tebuconazole and propiconazole (such as Kolosal Pro) have shown high biological efficacy (80–83%) against *Alternaria* in mustard and walnut crops, there is a notable absence of localized field data regarding their performance on lentils under the unique environmental pressures of North Kazakhstan [13, 14]. Furthermore, the comparative potential of biological control options remains largely unexplored for this crop in the region. Without specific data on pathogen identity and fungicide efficacy, growers lack the evidence-based tools necessary for effective disease control.

Consequently, this study was designed to detect and perform molecular identification of the *Alternaria* pathogens affecting lentils in North Kazakhstan and to evaluate the field efficacy of chemical and biological fungicides across the 2023–2025 growing seasons. These findings aim to provide a targeted foundation for improving regional crop protection strategies and supporting the transition to more science-based agricultural systems.

2. METHODS AND MATERIALS

Field studies were conducted in 2023-2025 with the aim of optimizing the phytosanitary state of lentil crops in the steppe zone of North Kazakhstan (Akmola region, Shortandy district, SPCGF named after A. I. Barayev LLP). In the course of research, the phytopathogenic fungus *Alternaria alternata*, the causative agent of alternariosis, was detected.

The materials for the research were samples of mycelium of *Alternaria* fungi isolated from the affected organs of lentil plants. Laboratory phytopathological studies were carried out in specialized laboratories of the Department of Plant Protection and Quarantine of S. Seifullin Kazakh Agro-Technical University and in the plant protection laboratories of the SPCGF named after A. I. Barayev, LLP.

Samples were collected from one experimental location by three replicated field blocks, at three growth stages: bud development, flowering, and seed maturation. The sampling dates were June 28, July 17, and August 15 in 2023; June 27, July 16, and August 14 in 2024; and June 30, July 18, and August 18 in 2025.

On each sampling date, 30 plants per plot were examined, and for laboratory isolation, 15 to 20 symptomatic plants were collected from each treatment on diagonal transects. Sampled plant organs with signs of *Alternaria* infection were leaves (approximately 45%), stems (25%), pods (20%), and seeds (10%).

The symptomatic pieces were washed, cut into 5 to 7 mm long pieces, and sterilized by immersion in 70% ethanol for 30 s, and 1% sodium hypochlorite for 1 to 2 min. They were then rinsed three times in sterile distilled water, dried on sterile filter paper, placed in potato-glucose agar medium, and incubated at 24 to 25 °C for 7 days and a light/dark cycle of 12 h/12 h. Putatively *Alternaria*-like colonies were cultured on fresh media, establishing pure cultures that were identified using morphological and molecular techniques.

Molecular identification of the *Alternaria alternata* fungus on the basis of *ITS* nucleotide sequences was carried out in the

National Center of Biotechnology of the Ministry of Education and Science of the Republic of Kazakhstan.

DNA was isolated from fungal isolates using the hexadecyltrimethylammonium bromide (CTAB) method. 500 µl of TES buffer was added to the samples (0.1-0.2 g) and left to incubate at 37 °C overnight. After the incubation, 100 µl of 5 M NaCl was added and mixed, followed by adding 65 µl of 10% CTAB with NaCl preheated to 65 °C, mixing thoroughly, and incubating for 10 minutes at 65 °C. After 3 minutes of centrifugation at 13.000 g (to prevent CTAB from cooling), the supernatant was transferred to new tubes. An equal volume of chloroform: isoamyl alcohol (24:1) was added to the supernatant, mixed, and left at 4 °C for 20 minutes. Centrifugation was performed for 5 min at 13.000g, after which the aqueous (upper) phase was collected without touching the precipitate and transferred to a clean tube. An equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed thoroughly until a uniform emulsion was formed. Centrifugation was performed for another 3 minutes, and the aqueous phase was transferred to a clean tube. Cooled isopropanol (0.6 of the volume) was added, mixed by tipping, and left for 20 min at -20 °C. After 5 min of centrifugation at 13.000 g, the supernatant was removed. The DNA precipitate was washed with 70% ethanol, dried, dissolved in 50 µl TE buffer, and stored at -20 °C.

DNA concentration was measured by spectrophotometry with a NanoDrop 1000 spectrophotometer at 260 nm (UV region) and 280 nm (DNA protein component).

2.1 Amplification of the Internal Transcribed Spacer fragment

The polymerase chain reaction (PCR) reaction was performed with universal primers *ITS*5 - ggaagtaaaagtcgtaacaagg and *ITS*4 - tcctccgcttattgatatgc in a total volume of 30 µL per reaction according to the following mix: 5 µL DNA template, 1 U Taq DNA polymerase (ThermoFisher), 0.2 mM each dNTP, 10 × KCl buffer (ThermoFisher), 2.5 mM $MgCl_2$ and 10 pmol each primer. The PCR amplification program was as follows: 94 °C for 5 min followed by 30 cycles of 95 °C for 30 sec, 55 °C for 40 sec and 72 °C for 50 sec, followed by a final elongation step at 72 °C for 7 min. PCR was performed on an Applied Biosystems Simpli Amp Thermal Cycler.

PCR product cleanup was performed by the enzymatic method using Exonuclease I (Fermentas) and alkaline phosphatase (Shrimp Alkaline Phosphatase, Fermentas). The sequencing reaction was performed using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) as per the manufacturer's instructions, followed by fragment separation using an automatic genetic analyzer 3730xl DNA Analyzer (Applied Biosystems).

MEGA 11 software was used for phylogenetic analysis. The nucleotide sequence alignment was done using ClustalW, and the phylogenetic tree was constructed using the NJ method. The distances between the taxa were calculated using the Kimura 2-parameter model, while gaps and missing data were treated as pairwise deletion. A bootstrap analysis of 1000 replicates was also conducted to assess the robustness of the tree.

Reference *ITS* sequences of closely related *Alternaria* species were retrieved from GenBank and included in the analysis. The reference sequences used for tree construction were MZ066739.1 for *Alternaria alternata* and MN593338.1 for *Alternaria tenuissima*. Additional reference sequences of closely related *Alternaria* species were also included to clarify the phylogenetic position of the studied isolate.

The electrophoresis of the amplified products was performed in 1.5% agarose gel in 1× TAE buffer.

The gel filling procedure consisted of adding 1.5 grams of agarose and 8 µL of ethidium bromide to 100 mL of 1× TAE buffer. Electrophoresis was performed under 120 V for 40 minutes [15, 16].

2.2 Determining the development and spread of diseases

Throughout the growing season, lentils were regularly monitored in the field for the development of diseases. At each stage of vegetation, symptoms of plant damage were recorded, and samples with signs of infection with fungi of the genus *Alternaria* were collected.

Disease monitoring in the field relied on two parameters: disease prevalence, or the number of affected plants in crops, and disease progression, or the degree of organ damage. The prevalence of diseases (P) was calculated by formula (1):

$$P = \frac{n \times 100}{N} \qquad (1)$$

where,

P – disease prevalence, %; n – number of organs with evidence of disease in the sample; N – total number of analyzed organs in the sample.

Disease progression (R) and average damage to individual organs in percent were determined by formula (2):

$$R = \frac{\sum(a \times b)}{N \times K} \times 100 \qquad (2)$$

where,

R – disease progression, %; $\sum(a \times b)$ – sum of the products of the number of affected organs and their corresponding severity scores; N – the total number of analyzed organs in the sample; K – the highest scale score. To ensure better accuracy, researchers used specialized scales characterizing the intensity of development of a particular disease.

Disease severity was assessed using a visual scale from 0 to 5. It was based on the proportion of the affected organ surface area of the lentil:

0 – no visible symptoms;

1 – single small necrotic or chlorotic spots, up to 5% of the organ surface affected;

2 – several distinct spots, 6–10% of the organ surface affected;

3 – numerous spots with partial coalescence, 11–25% of the organ surface affected;

4 – large coalesced lesions, 26–50% of the organ surface affected;

5 – severe damage with extensive necrosis, deformation, drying of the organ, or more than 50% of the organ surface affected.

In the calculation of disease progression (R), K was therefore equal to 5. The same scale was applied to leaves, stems, pods, and seeds, with visual assessment adjusted to the affected organ.

The study determined the biological efficacy of fungicides during the vegetative stage of the crop.

The biological efficacy of fungicides was calculated using the modified Abbott's formula, comparing the prevalence of the disease before and after treatment:

$$C = \frac{100(P - p)}{P} \quad (3)$$

where,

C – biological efficacy of the fungicide, %;

P – disease prevalence in the control variant;

p – disease prevalence in the experimental variant.

Following the identification and determination of lentil diseases, field studies were conducted at the experimental site of the Scientific and Production Center of Grain Farming named after A. I. Barayev, LLP, to identify effective drugs. The study tested the effects of the fungicide Kolosal Pro, microemulsion concentrate (m.e.c.) with the active substances propiconazole, 300 g/L + tebuconazole, 200 g/L, and the biofungicide Biograno Forte with active microorganisms *Bacillus* spp., *Trichoderma* spp., in comparison with a treatment-free variant (control).

The treatments were applied once at the bud development stage of the lentil. The application dates were June 28, 2023, June 27, 2024, and June 30, 2025. Spraying was carried out using a hand-held plot sprayer equipped with flat-fan nozzles. The spray volume was 300 L/ha, the working pressure was 0.25–0.30 MPa, and the travel speed was approximately 4–5 km/h. No adjuvants were used. In this experiment, Biograno Forte was applied once, at the same growth stage as Kolosal Pro, to ensure direct comparison between the biological and chemical treatments.

The field experiment was arranged as a randomized complete block design (RCBD) with three replications. Each replication represented one block, and the three treatments were randomly allocated within each block to reduce the influence of spatial heterogeneity, including possible soil fertility and moisture gradients across the experimental field. The size of the plot was 4.2 m × 25 m = 105 m² (see Table 1). The total number of experimental plots was nine. Buffer strips of at least 1.0 m were maintained between plots and blocks to reduce spray drift and cross-contamination between treatments. The sowing date was May 20. The seeds were sown at a rate of 2.5 million germinating seeds per hectare and embedded at a depth of 4-5 cm. The preceding crop was wheat on fallow. In spring, once the soil reached its physical ripeness, it was harrowed with a Catros disk harrow to a depth of 4-5 cm. Harvesting was carried out using standard agricultural combines when the lentils ripened, depending on weather conditions.

Statistical analysis was performed to evaluate the effects of year, treatment, and their interaction on disease progression (R) and disease prevalence (P). The data were analyzed using two-way analysis of variance (ANOVA), with year and treatment as fixed factors. When significant differences were detected, means were compared using Tukey's test at $p < 0.05$. The results are presented as mean values based on three replications.

Table 1. Experiment design

Variant	Product	Repetition		
		I	**II**	**III**
Control	-	1	4	7
Biological background	Biograno Forte (*Bacillus* spp., *Trichoderma* spp.), application rate – 1.5 L/ha	2	5	8
Chemical background	Kolosal Pro, m.e.c. (propiconazole, 300 g/L + tebuconazole, 200 g/L), application rate – 0.4 L/ha	3	6	9

2.3 Soil and climatic conditions during crop vegetation

The site of the Scientific and Production Center of Grain Farming named after A. I. Barayev, LLP is located on the territory of the Shortandy district of Akmola region of Kazakhstan. The climate of the region is characterized by an average annual amount of precipitation of 250-280 mm and a growing season of 110-120 days. It has a sharply continental arid climate, with hot summers and cold winters, with an important temperature difference between day and night. Sunny weather is common, and the summer insolation is nearly as high as in the tropics; cloud cover is very low. Annual precipitation decreases from north to south, with rainfall peaking in July and at its lowest in February.

The soil-climatic zone of the study area is steppe, with southern carbonate chernozems, medium loamy in grain size composition. Humus content is 3.4-3.6%, and soil pH is 7.0-7.2.

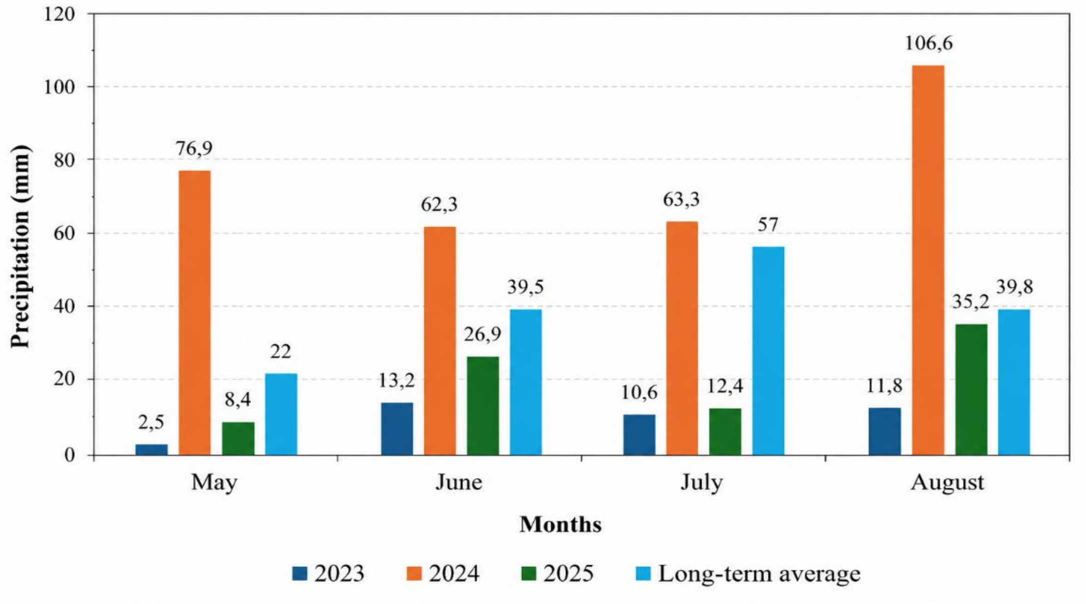


Figure 2. Average monthly precipitation in 2023-2025 compared with long-term averages, mm

Notes: Data from the Shortandy weather station

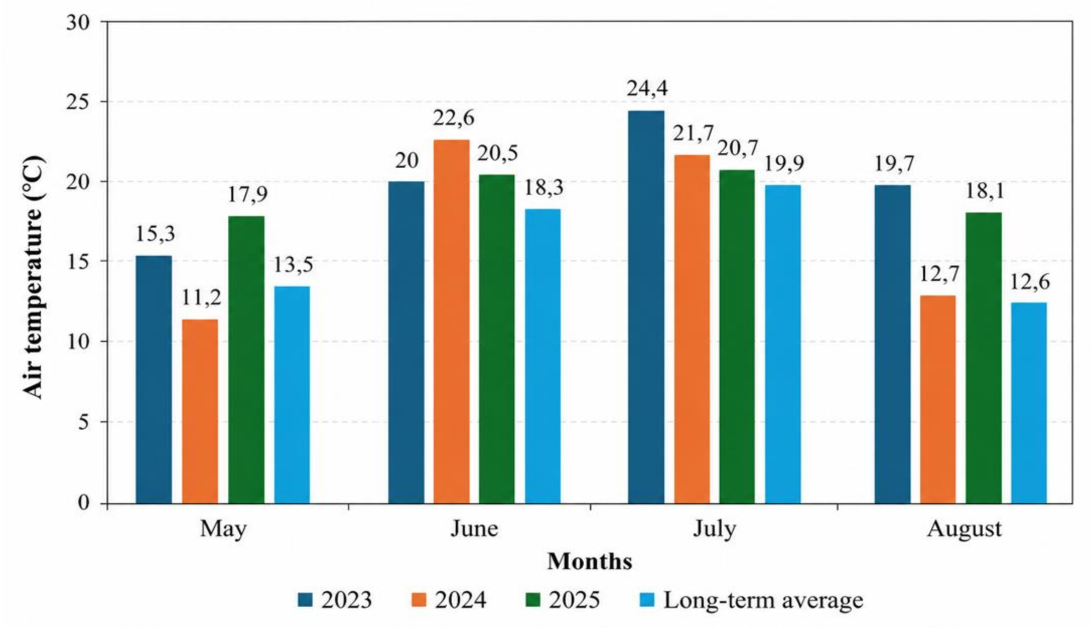


Figure 3. Average monthly air temperatures in 2023-2025 compared with long-term averages, ℃
Notes: Data from the Shortandy weather station

In the 2023 growing season, the weather conditions differed from the long-term average in the key meteorological parameters, primarily the amount of precipitation and temperatures. Total precipitation over the crop growing season from May to August inclusive was 38.1 mm, which is 120.2 mm below the long-term average for the same period. According to the hydrothermal coefficient, this growing season can be categorized as dry (Hydrothermal Coefficient (HTC) = 0.2).

The 2024 growing season experienced 309.1 mm of precipitation from May to August, which exceeds the long-term average value by 150.8 mm. In terms of moisture content, the year was favorable for plant growth and development and the formation of the lentil vegetative mass. The main bulk of precipitation fell in May (76.9 mm), July (63.3 mm), and especially August (106.6 mm), when the maximum monthly precipitation was recorded.

The 2025 growing season had a total of 82.9 mm of precipitation from May to August, which is 75.4 mm less than the long-term average for the same period. Based on the hydrothermal coefficient, the growing season was dry (HTC = 0.3) (see Figure 2).

The growing season of 2023 was characterized by elevated average monthly air temperatures compared with the long-term average. The average air temperature was 15.3 ℃ in May, 20 ℃ in June, 24.4 ℃ in July, and 19.7 ℃ in August. The greatest deviations were recorded in July and August, when the temperature exceeded the long-term average by 4.5 ℃ and 7.1 ℃, respectively. Combined with the precipitation deficit shown in Figure 2, these conditions indicate a dry and warm growing season.

In 2024, the highest average monthly air temperatures were recorded in June and July, reaching 22.6 ℃ and 21.7 ℃, respectively. May and August were cooler, with average temperatures of 11.2 ℃ and 12.7 ℃. Compared with the long-term average, June and July were warmer, whereas May and August were slightly cooler. Thus, the 2024 growing season was characterized by contrasting temperature conditions combined with increased precipitation (see Figure 3).

Thus, the prevailing weather conditions contributed to the rapid spread and development of diseases. Overall, the weather conditions during the crop growing season aggravated the phytosanitary condition of crops.

3. RESULTS

3.1 Identification of common disease pathogens

During phytoexamination, isolates of the fungus of the genus *Alternaria* were isolated from lentil seeds and stems.

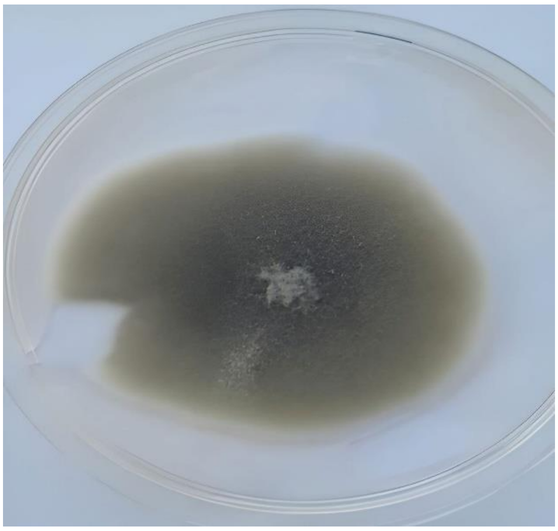
Pure fungal cultures were isolated and cultured on potato-glucose agar (PGA). Fungal colonies appearing on the fragments were viewed under a microscope. One *Alternaria* species was identified in advance*: Alternaria alternata.*

Alternaria alternata colonies are dark gray in color, round, with a rhizoid edge. The edge of colonies is wavy, the diameter of colonies is 89 × 90 mm on day 7, with branched conidia, 3-6 conidia in a chain. The shape of conidia is ovoid, club-shaped (see Figure 4).

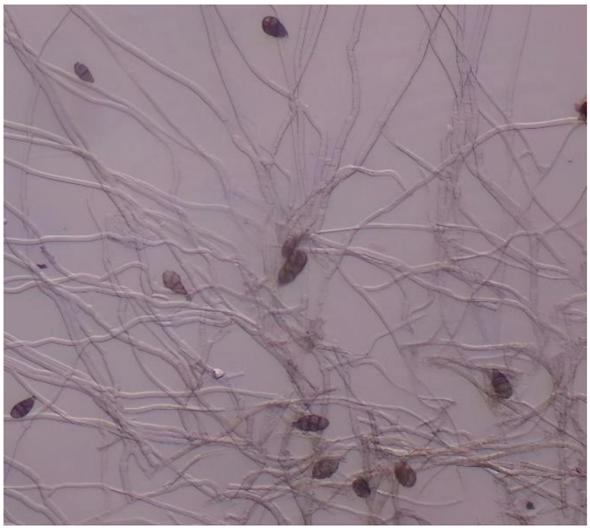
The strain of the fungus was identified by determining the direct *ITS* nucleotide sequence and subsequently identifying nucleotide identity using sequences from the international GenBank database and constructing phylogenetic trees with the nucleotide sequences of reference strains.

The DNA of phytopathogenic fungi of the genus *Alternaria* was isolated.

Electrophoresis further proved the presence of fungi of this genus in the samples. A 1.5% agarose gel with the intercalating agent ethidium bromide was used to separate the increased DNA fragments and visualize them for the identification of increased DNA. All samples were run using a PowerPac horizontal electrophoresis chamber, the Bio-Rad Electrophoretic Bath power supply, and 1× TAE buffer as the electrode buffer. No products were observed in the negative PCR control, suggesting no contamination (Figure 5).



(A)



(B)

Figure 4. (A) The color of colonies on potato-glucose agar (PGA) and conidiophores emerging on the surface of the agar; (B) *Alternaria* spores in 1000× magnification

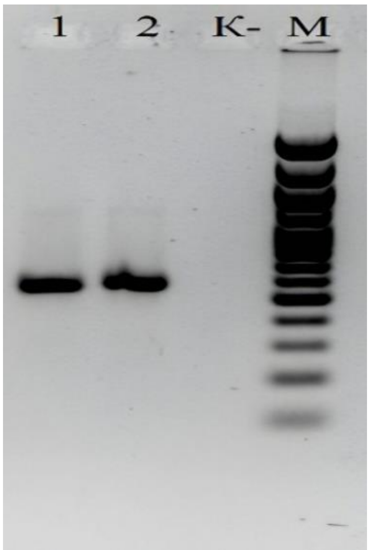


Figure 5. Electropherogram of the polymerase chain reaction (PCR) products of the Internal Transcribed Spacer (*ITS*) gene fragment amplification

Notes: 1, 2 – test samples according to Table 1; (M) molecular weight marker (Biolabmix) (100-3000 bp, in increments of 100 bp), (K-) negative control sample.

3.2 Nucleotide sequence analysis

Alignments and assembly of the nucleotide sequences were performed in SeqMan software (DNA Star) to produce a single sequence. The final sequences were trimmed of the nucleotide sequences corresponding to the primers and the lowest quality scored fragments. *ITS* rDNA sequence obtained from the studied *Alternaria* isolate was subjected to BLAST analysis against reference sequences stored in GenBank. Since the sequence has not yet been assigned an individual GenBank accession number, the identification was based on comparison with available reference sequences and phylogenetic analysis. The results of the BLAST analysis and identification parameters are summarized in Table 2.

The results of the BLAST nucleotide analysis indicate a strong similarity of the tested sample with several species of the genus *Alternaria*. However, simple sequence matching does not always identify the species precisely, since closely related species can show a high degree of conservatism in certain genome regions.

To determine species affiliation more accurately, we carried out phylogenetic analysis, building an evolutionary "tree of kinship." This approach takes into account not only nucleotide similarity but also the evolutionary relationships between sequences, which enables more reliable identification of phylogenetically related species. Thus, the phylogenetic analysis has clarified the results of BLAST and allowed us to determine the evolutionary position of the studied strain within the genus *Alternaria*.

As can be seen in Figure 6, sample 1 is located on the same branch as *Alternaria arborescens, Alternaria alternata, Alternaria daucifolii, Alternaria cerealis, Alternaria senecionicola, Alternaria tenuissima,* and *Alternaria destruens.*

Figure 6 shows that the tested sample shares its branch with *Alternaria tenuissima* and *Alternaria alternata*.

The analysis reveals that the studied sample is located on the same branch as the *Alternaria alternata* species. This indicates a high degree of evolutionary similarity with our specimen, thus suggesting that *Alternaria alternata* is the most likely species to which our strain belongs (see Table 3).

In this way, the phylogenetic analysis refines the results of BLAST, indicating that the studied sample belongs to the *Alternaria alternata* species, judging by its evolutionary position among closely related species [17].

Table 2. Results of genetic identification of fungal isolates based on *ITS* rDNA sequence analysis

Isolate	Sequence Length (bp)	Accession Number	Matched Strain	Match (%)
1	558	MZ066739.1	*Alternaria alternata*	100.00%
		MN593338.1	*Alternaria tenuissima*	99.82%

Notes: *ITS*: Internal Transcribed Spacer

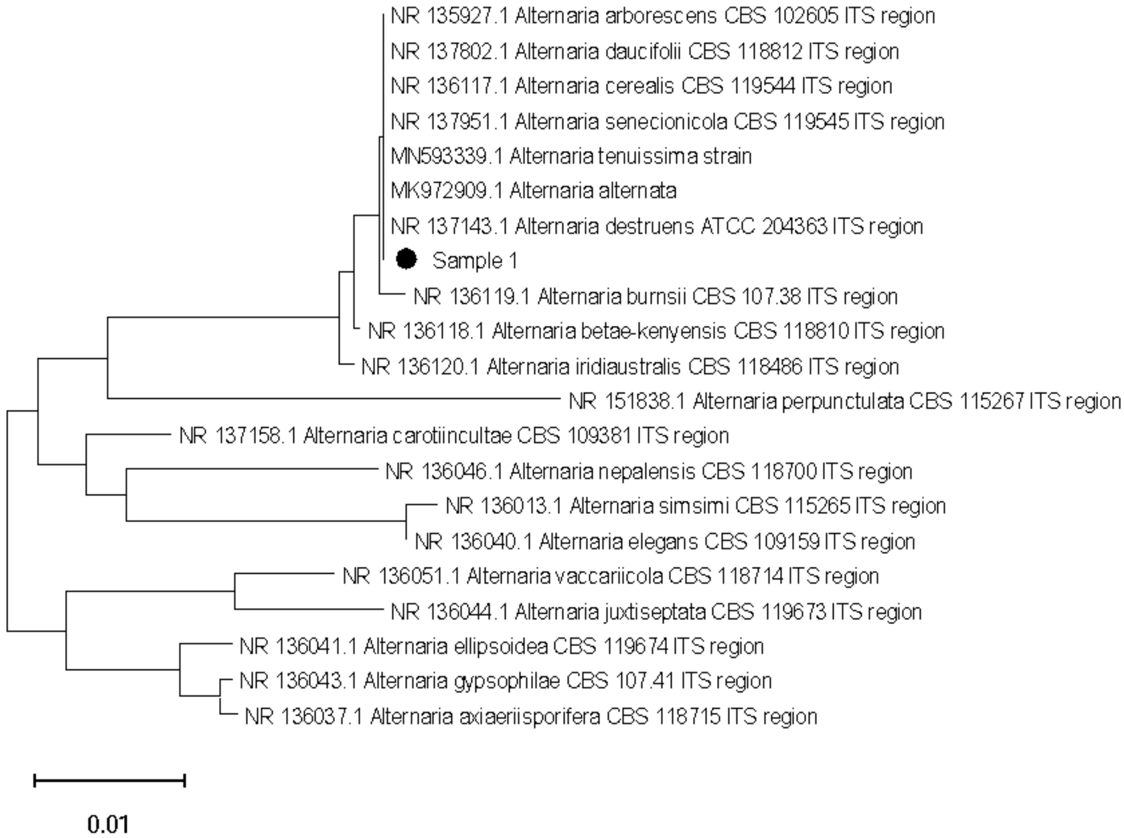


Figure 6. Phylogenetic tree based on *ITS* fragment analysis
Notes: *ITS*: Internal Transcribed Spacer

Table 3. Summary results of strain identification by the *ITS* gene fragments

No.	Identified Fungal Species	Note
1	*Alternaria alternata*	Determined on the basis of a phylogenetic tree built by the Neighbor-Joining algorithm (N-J)

Notes: *ITS*: Internal Transcribed Spacer

3.3 Progression and prevalence of identified diseases depending on fungicide treatment

Regardless of the level of agricultural technology, the phytosanitary state of crops is strongly dependent on regional climatic conditions and weather factors in the period from the emergence of seedlings to the ripening of seeds. In addition, moisture supply and thermal regimes also have a decisive influence.

The meteorological conditions in 2023-2025 significantly differed from both the long-term average and year to year, which created favorable conditions for comprehensively testing the effectiveness of various drugs for protecting crops from diseases. At the same time, weather factors had a significant impact on the phytosanitary conditions of lentil crops.

The weather conditions of 2024 were conducive to the development of fungi of the genus *Alternaria*. The processes of lentil plant development were delayed through cold temperatures and satisfactory soil moisture and precipitation in the third decade of May and June and heavy rains in the first and second decades of August. The effect of these fungicidal and biofungicidal treatments against alternariosis of crops at the bud development stage considerably reduced and delayed their development and spread until the period of seed maturation. This effect was supported by low precipitation and a temperature regime consistent with the long-term average. Early August experienced heavy rains (106.6 mm), resulting in a sharp upturn in both prevalence and progression (Alternariosis: R = 1.8-3.2%, P = 3.3-10.1%). The development and spread of diseases in the variant with fungicide Kolosal Pro, m.e.c. was considerably lower compared to the control variant (Table 4).

Statistical analysis confirmed a significant effect of year and treatment on both disease progression (R) and disease prevalence (P) ($p < 0.05$). The highest disease pressure was recorded in 2024, when the untreated control reached R = 8.6% and P = 29.7% at seed maturation. These values were significantly higher than those observed in 2023, when the corresponding values were R = 3.2% and P = 10.1%.

3.4 Biological efficacy of fungicides against diseases

The biological efficacy of fungicides is the result of their application in the field, expressed as a percentage reduction in disease prevalence or the degree of damage to the protected plants.

In this study, the biological efficacy of fungicides was determined based on the prevalence of diseases at 5, 10, and 15 days after treatment compared to the control variant.

The obtained data show that in all monitoring periods of 2023-2025, the maximum biological efficacy was demonstrated by the fungicide Kolosal Pro, m.e.c. (65.8-76.9%), which outperformed the biofungicide Biograno Forte by 7.5-10.2% (see Table 5).

Table 4. Development and spread of alternariosis during plant vegetation

Variant	Development Stage: Bud Development R, %	Bud Development P, %	Flowering R, %	Flowering P, %	Seed Maturation R, %	Seed Maturation P, %
			2023			
Control background	2.4c	8.5b	2.9c	9.5c	3.2c	10.1c
Biological background	1.3d	3.2d	1.9d	4.8e	2.1d	5.7e
Chemical background	0.9d	2.3d	1.2d	2.7f	1.8d	3.3f
			2024			
Control background	7.1a	16.4a	7.7a	24.1a	8.6a	29.7a
Biological background	2.3c	7.5b	2.7c	8.2cd	3.2c	8.9d
Chemical background	1.3d	5.9c	1.6d	7.1d	2.1d	8.4d
			2025			
Control background	5.3b	16.1a	6.0b	21.2b	7.3b	23.5b
Biological background	2.3c	7.0bc	2.8c	8.1cd	3.0c	8.6d
Chemical background	1.2d	5.2c	1.7d	5.9e	1.9d	6.3e

Notes: P – disease prevalence, %; R – disease progression, %.

Table 5. Biological efficacy of products against alternariosis

Variant	Prevalence of Alternariosis after Fungicidal Treatment at the Bud Development Stage (%)			Biological Efficacy (%)		
	5 Days Later	10 Days Later	15 Days Later	5 Days Later	10 Days Later	15 Days Later
			2023			
Control background	8.9	10.2	13.4	-	-	-
Biological background	3.4	3.7	4.1	61.8	63.7	69.4
Chemical background	2.5	2.7	3.1	71.9	73.5	76.9
			2024			
Control background	16.5	17.8	18.7	-	-	-
Biological background	7.8	8.1	8.3	52.7	54.5	55.6
Chemical background	6.2	6.3	6.4	62.4	64.6	65.8
			2025			
Control background	15.3	16.6	17.9	-	-	-
Biological background	6.9	7.2	7.4	54.9	56.6	58.7
Chemical background	5.5	5.7	5.9	64.1	65.7	67.1

4. DISCUSSION

Species of the genus *Alternaria* are ubiquitous in agricultural landscapes and are primary causes of leaf spot in leguminous crops worldwide [2]. Beyond direct tissue damage, these pathogens are known to contaminate grain with mycotoxins, posing a health risk to humans and livestock [18]. Despite their significance, the complex taxonomy of the genus has historically hindered precise research [19]. By utilizing molecular phylogenetic analysis of the *ITS* rDNA sequence, this study confirmed the dominance of *Alternaria alternata* in the North Kazakhstan lentil pathocomplex, resolving traditional morphological ambiguities [17, 20].

The progression of alternariosis in our experiments was heavily dictated by seasonal meteorological variations. The 2024 growing season was characterized by exceptional moisture, with 318 mm of precipitation, which is a 140.6 mm increase over the long-term average. This high-moisture environment, particularly the heavy rainfall in August, catalyzed a sharp increase in disease prevalence, reaching 29.7% in the control group. In contrast, the drier 2023 season (HTC = 0.2) saw a significantly lower prevalence of 10.1%. These findings align with general epidemiological models suggesting that *Alternaria* spore germination and infection are optimized under high relative humidity and frequent rainfall events [21].

A key finding of this study is the differential response of chemical and biological fungicides to these fluctuating weather conditions. The chemical fungicide Kolosal Pro (propiconazole + tebuconazole) exhibited its peak efficacy in 2023 at 76.9% but saw a notable decrease to 65.8% in 2024. This reduction in efficacy under high rainfall is likely due to the "rainfastness" threshold of triazole-based fungicides. While systemic fungicides like tebuconazole are generally more resistant to wash-off than contact protectants, intense and frequent precipitation can still dilute surface residues and diminish the overall protective barrier before the active ingredients are fully absorbed or redistributed within the plant [22, 23]. Furthermore, the extreme disease pressure in 2024 likely challenged the curative capacity of the chemical treatment [24].

In contrast, the biofungicide Biograno Forte (*Bacillus* spp., *Trichoderma* spp.) appeared relatively more stable in its performance across the different years. While its absolute efficacy was lower than that of Kolosal Pro, its consistent ability to suppress disease (ranging from 55.6% in wet years to 69.4% in dry years) suggests a robust interaction with the environment. Biological agents such as Trichoderma and Bacillus are living organisms that can actively colonize the phyllosphere [25]. In moist years like 2024, the high humidity that favors *Alternaria* also supports the growth and antagonistic activity of these beneficial microbes, potentially compensating for the dilution effects seen in chemical residues [26, 27].

Ultimately, while Kolosal Pro remains the most effective short-term chemical option for managing *Alternaria* in lentils, its sensitivity to extreme precipitation highlights the need for integrated strategies [13, 28]. The stable performance of Biograno Forte indicates that biological controls could play a vital role in maintaining baseline protection, especially during prolonged wet periods when chemical persistence is compromised. These results provide a science-based foundation for regional crop protection, emphasizing that fungicide selection should consider both the identified pathogen complex and forecasted meteorological conditions.

5. CONCLUSION

The study confirmed that the *Alternaria* species complex affecting lentils in North Kazakhstan is dominated by *Alternaria alternata* and *Alternaria tenuissima*. Quantitative monitoring over the 2023–2025 growing seasons revealed that disease dynamics were heavily influenced by seasonal moisture, with the untreated control showing a three-year prevalence (P) range of 10.1–29.7% and a disease progression (R) range of 3.2–8.6% at the seed maturation stage.

Fungicidal treatments significantly suppressed pathogen development compared to the control. The chemical fungicide Kolosal Pro achieved the highest level of control, with a three-year average biological efficacy of approximately 70% (ranging from 65.8% to 76.9%). Under this treatment, disease parameters were reduced to ranges of R = 1.8–2.1% and P = 3.3–8.4%. Notably, while the chemical treatment's efficacy fluctuated with precipitation levels, the biofungicide Biograno Forte demonstrated high environmental stability. It maintained a consistent biological efficacy range of 55.6–69.4%, effectively curbing disease development (R = 2.1–3.2%, P = 5.7–8.9%) even during high-moisture periods that typically challenge chemical persistence.

These findings provide a science-based foundation for integrated disease management in the steppe zone, suggesting that while chemical options offer maximum immediate reduction, biofungicides provide a stable and robust alternative for long-term phytosanitary control in varying climatic conditions.

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