

1 **Pathogenicity and Phylogenetics of *Alternaria alternata* Affecting *Tulipa L.***
2 **in Greenhouse Conditions of the Botanical Garden**

3
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5
6 **ABSTRACT**

7 The study aimed to assess the pathogenicity of the fungus *Alternaria alternata* on various
8 *Tulipa L.* species and elucidate its phylogenetic position. The research focused on five specific
9 tulip varieties: *T. Albatros*, *T. Tarda*, *T. Delta Storm*, *T. Biflora*, and *T. Biebersteiniana*.
10 Methodologies included molecular analysis, microscopic examinations, cultivation of fungi on
11 PDA, and sequencing of the 18S and 5.8S rRNA genes, as well as the D1/D2 region of the 26S
12 rRNA gene. Results revealed variable pathogenicity across tulip species, with *T. Albatros*
13 showing complete leaf damage and extensive conidium formation, while *T. Biebersteiniana*
14 exhibited minimal damage. Factors influencing infection severity included plant variety,
15 conidium formation, and environmental conditions. Sequencing confirmed the fungus's
16 affiliation with the *Alternaria* genus and highlighted its close relation to other species. The
17 findings underscore the importance of molecular methods for accurate pathogen identification
18 and phylogenetic classification. These results are crucial for developing targeted disease
19 management strategies and enhancing plant resilience in agriculture. The application of the
20 findings is feasible within agriculture to develop resilient varieties and methods for managing
21 the dissemination of *A. alternata*. Plant diseases involve complex interactions between
22 pathogens and hosts, where fungi like *Alternaria alternata* disrupt plant physiology through
23 toxin production and enzyme secretion, making effective management crucial.

24 **Keywords:** ecosystem, fungal species, sequencing, *Alternaria alternata* (Fr.) Keissl.

25
26 **INTRODUCTION**

27 Plant disease management involves understanding and controlling the complex interactions
28 between plants and their phytopathogenic agents, including fungi. Effective strategies for
29 managing plant diseases focus on preventing infection, minimizing disease spread, and
30 mitigating the impact on plant health and yield. This encompasses practices such as regular
31 monitoring of plant health, implementing resistant plant varieties, and employing integrated

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32 pest management (IPM) techniques. For example, in the case of fungi like **Alternaria*
33 *alternata**, management practices may include the use of fungicides, crop rotation, and removal
34 of infected plant material to reduce pathogen load and prevent future outbreaks (Khmelnitskaya
35 *et al.*, 2003). Types of diseases caused by *Alternaria* spp. are widespread, and the symptoms
36 are abundant. *Alternaria* belongs to cosmopolitans, affects all plant organs, and, most
37 importantly, severely damages the quality and yield of products (Chacón *et al.*, 2022; Ospanov
38 *et al.*, 2018).

39 The distribution of fungi of the genus *Alternaria* was studied by Abiev (2002), Hannibal
40 (2011), Kuprienko (2005), Maksimov *et al.* (2020), Salybekova *et al.* (2019). Along with
41 saprophytic lesions of various plants, these species lead a parasitic lifestyle under favourable
42 conditions (Abiev, 2002; Gannibal, 2011; Kuprienko, 2005; Maksimov *et al.*, 2020; Ospanov
43 *et al.*, 2020; Salybekova *et al.*, 2019).

44 *Alternaria* leaf blight causes mold of fruits and seeds, contaminating agricultural products
45 with toxins, allergens, or enzymes (Bauer *et al.*, 2023; Hannibal, 2011; Karabassov *et al.*, 2018).
46 It induces foliar lesions, impairing photosynthesis and leading to yield reductions. In India,
47 tomato yield losses reached 78% (Mehmood *et al.*, 2020), while winter rape seed losses in
48 Germany were up to 50% (Zhu *et al.*, 2021). Small-spore *Alternaria* species are major allergens,
49 affecting about 3% of the European population (Bavbek *et al.*, 2006) and causing severe allergic
50 reactions and infections (Bush & Prochnau, 2004; Fung *et al.*, 2000; Robertshaw & Higgins,
51 2005).

52 Research on *Alternaria* in greenhouse-grown *Tulipa L.* highlights the impact of temperature
53 on disease development (Iqbal *et al.*, 2019; Kuroyanagi *et al.*, 2022; Otero-Blanca *et al.*, 2021;
54 Pandit *et al.*, 2022; Srivastava *et al.*, 2021; Stauder *et al.*, 2020; Xu, 2023). At 35°C, 75% of
55 *Tulipa L.* plants were affected by *Alternaria alternata* within 7 days, compared to 45% at 25°C
56 and 60% at 30°C (Otero-Blanca *et al.*, 2021). Photosynthesis decreased by 40% in infected
57 plants (Xu, 2023). The "Red Velvet" cultivar showed 85% infection, while "Golden Sunrise"
58 had 30% (Stauder *et al.*, 2020). Infected plants had 25% less chlorophyll, 40% increased
59 antioxidant enzyme activity, and reduced magnesium and iron concentrations (Kuroyanagi *et*
60 *al.*, 2022). Elevated soil humidity increased infection intensity by 15% (Srivastava *et al.*, 2021).
61 Infection during leaf formation reduced growth by 20% and leaf length by 15%, with increased
62 antioxidant content (Kaur, 2023; Pandit *et al.*, 2022). Plants also showed 20% less carbon
63 allocation to leaves and 25% shorter roots with structural changes (Jin *et al.*, 2021; Wang *et al.*,
64 2019a).

65 Studies on *Alternaria alternata* and *Tulipa L.* under greenhouse conditions highlight the
66 importance of managing alternariosis for optimal crop yield and quality. However, aspects of
67 phytopathogenic fungi remain underexplored, such as cultivar resistance, temperature effects,
68 and bioagent efficacy. Accurate pathogen identification is crucial for understanding species-
69 specific characteristics and developing effective disease control measures. The taxonomy of
70 *Alternaria* is complex due to high variability and similar morphologies among species,
71 complicating precise classification. Despite advancements, more research is needed to clarify
72 its taxonomy. The economic relevance of these studies is significant, as *Alternaria* can severely
73 impact crop yields and quality, affecting food security and agricultural economics.

74 *Alternaria* leaf spot disease on *Tulipa L.* plants represents a significant economic threat due
75 to its impact on crop yields and quality. Infected tulips exhibit reduced aesthetic appeal and
76 shorter shelf life, leading to substantial losses in both commercial and ornamental sectors. For
77 instance, a 30% reduction in flower production and a 40% decrease in market value have been
78 reported in severe cases. The disease also threatens other cultivated crops by potentially
79 spreading to different plant species, exacerbating economic losses in agriculture. This research
80 is crucial for understanding the broader implications of *Alternaria alternata* and developing
81 strategies to mitigate its impact.

82 This study aims to fill several critical gaps in the scientific understanding of *Alternaria* in
83 *Tulipa L.* plants. Despite previous research on fungal pathogens, there is a lack of detailed
84 morphological and genetic characterization of *Alternaria* species affecting tulips. Specifically,
85 the relationship between the genetic variations of *A. alternata* and its pathogenic mechanisms
86 has not been thoroughly explored. Additionally, the impact of specific fungal strains on
87 different tulip varieties and their physiological responses remains under-researched. This study
88 will provide new insights into these aspects, enhancing our understanding of fungal
89 pathogenesis and resistance mechanisms.

90 The practical significance of this research lies in its potential to inform the development of
91 targeted disease management strategies for tulips, enhancing crop resilience and yield.
92 Understanding the pathogenicity of *Alternaria alternata* can help in breeding more resistant
93 tulip varieties and optimizing agricultural practices.

94 **Objective:**

95 The objective of this study is to analyze the pathogenicity and phylogenetic relationships of
96 *Alternaria alternata* affecting *Tulipa L.* plants under greenhouse conditions.

97 **Research tasks:**

98 a) Analyze phylogenetic relationships by sequencing multiple rRNA gene segments.

99 b) Analyze the influence of *A. alternata* isolates on different varieties of *Tulipa L.*

100 We hypothesize that *Alternaria alternata* exhibits varying levels of pathogenicity across
101 different *Tulipa L.* cultivars, influenced by factors such as temperature and genetic variation,
102 which affect the plant's physiological responses and overall resilience.

103

104 METHODS AND MATERIALS

105 The study was conducted over a six-month period in 2022 at the greenhouse facilities of the
106 Botanical Garden (Almaty, Kazakhstan). All laboratory analyses were carried out at the
107 facilities of Khoja Akhmet Yassawi International Kazakh-Turkish University. Isolation of the
108 *Alternaria* leaf spot pathogen was conducted on *Tulipa gesneriana* plants grown in the
109 greenhouse facilities of the Botanical Garden (Almaty, Kazakhstan).

110 To isolate the causative agent of *Alternaria* leaf blight in tulips taken from the cultivated
111 greenhouse system of the Botanical Garden at the university, molecular analysis was made to
112 accurately determine the location of the phytopathogen in the taxonomic system along with
113 morphological features. Light microscopes (Micros Austria Camera 519 Cu 5 Otcmos with a
114 video camera MCX100, microscope eyepiece EW10X/20, lens PLAN 40X/0.65) and scanning
115 microscopes (JSM-6510LA Analytical Scanning Electron Microscope, JEOL, Japan) were used
116 in microscopic studies. Microscopic studies included an assessment of the fungal pathogen's
117 morphological characteristics, such as conidia shape, size, cell structure, and arrangement.

118 Pure fungi were grown in potato-dextrose agar (PDA) at the temperature of 27 °C. After the
119 colonies were separated on the 10th day, and the biomass was taken for the analysis of 18S
120 RNA, DNA was isolated by the protocol of the CTAB method (Mishra *et al.*, 2003); one strain
121 of pure culture was studied and compared with other species of the genus *Alternaria* from the
122 GenBank database and other fungal isolates for phylogenetic analysis.

123 For further use, DNA samples were stored at the temperature of 4 °C. The DNA concentration
124 of 900 ng/μl (OD260) was measured using a spectrophotometer (Nanodrop Thermo ND-1000,
125 Thermo Scientific, Massachusetts, USA). Each PCR reaction was carried out in the final
126 volume of 50 μl and contained a 10x Taq buffer with 5.0 mcl - KCl (Thermo Scientific,
127 Massachusetts, USA), 3.0 mcl - 2.5 mM MgCl₂, 100 mM 8.0 mcl dNTP, 1 mcl for each primer,
128 0.25 mcl -5U/ mcl Taq DNA polymerase recombinant (Thermo Scientific, Massachusetts,
129 USA), 27.8 mcl – sterile distilled water, 4 mcl – suspensions (100 ng) of fungal DNA, used as
130 samples.

131 DNA samples according to the PCR amplification program primers were used to create a
132 sequence of coding 5.8S RNA genes and internal transcribing spacers ITS1 –
133 TCCGTAGGTGAACCTGCGC and ITS4 – TCCTCCCGCTATTGATGC.

134 Denaturation was provided at the temperature of 95 °C for 3 minutes, as well as 35 cycles
135 lasting 30 seconds at 95 °C, 50 seconds at 57 °C and 30 seconds at 72 °C; the stage of the last
136 elongation was 72 °C for 5 minutes.

137 To amplify the D1/D2 domain of the 26S rRNA gene, a PCR program for primers NL-1
138 GCATATCAATAAGCGGAGGAAAG and NL-4 GGTCCGTGTTTCAAGACGG provided
139 denaturation for 3 minutes at the temperature of 95 °C, 35 cycles of 30 seconds at 95 °C, 50
140 seconds at 52 °C and 30 seconds at 72 °C, the stage of the last elongation was 5 minutes at 72
141 °C (De Clerck *et al.*, 2004).

142 Amplified PCR products (10 µl) and 100 bp DNA (Thermo Scientific, Massachusetts, USA)
143 were separated at 0.5x TAE in 1h 30 min in the buffer of 80 V/cm, 1.5% agarose gel using gel
144 electrophoresis. The agarose gel of ethidium bromide was applied for 10 minutes (0.5 µg/ml).
145 The gel was photographed under ultraviolet rays using a special photographing system.

146 Sequencing of 18S rRNA and 5.8S rRNA genes, comparison of sequences and construction
147 of a generic tree was carried out on an automatic sequencer AE3000, and a specialized computer
148 program BLAST was used to analyze the sequencer. Sequences sufficient to assign the strain
149 to a certain taxonomic group of microorganisms were determined for the reliability of results.

150 The conditions of PCR electrophoresis of the studied samples were 1.0% agarose gel and an
151 electric field strength of 5 V/cm.

152 According to the method of V.N. Vasilevsky, small pieces of pure culture (inoculum) were
153 applied to growing sprouts and vegetative sections of 23-day-old vegetables were isolated and
154 applied to 14 places under the leaves, and 7 places on the surface of the leaves. The stem and
155 roots were also tested: they were kept in wet chambers for 3 days at the temperature of 23-25
156 °C, and then left in the open. Daily monitoring was conducted.

157 The analysis of phylogenetic relationships, constructed using strains of closely related
158 microorganisms, showed that the species closest to the studied strain is *Alternaria alternata*.

159 To perform a thorough phylogenetic analysis, we used both single-gene and multi-gene
160 approaches.

161 Single-gene analysis:

162 Sequencing of 18S rRNA gene: We sequenced the 18S rRNA gene segment using specific
163 primers ITS1 and ITS4.

164 Sequence alignment: The obtained sequences were aligned with reference sequences from the
165 GenBank database using the ClustalW algorithm.

166 Phylogenetic tree construction: A phylogenetic tree was constructed using the Neighbor-
167 Joining method with 1000 bootstrap replications to ensure the reliability of the branching.

168 Multi-gene analysis:

169 Selection of multiple genes: In addition to 18S rRNA, we selected other informative genetic
170 markers such as ITS and D1/D2 domains of the 26S rRNA gene for a comprehensive analysis.

171 Sequencing and alignment: Each gene segment was sequenced and aligned with sequences
172 from closely related species available in the GenBank database.

173 Concatenated sequence analysis: The aligned sequences were concatenated to form a multi-
174 gene dataset.

175 Phylogenetic tree construction: The concatenated sequences were used to construct a
176 phylogenetic tree using the Maximum Likelihood method with 1000 bootstrap replications to
177 provide a robust analysis of phylogenetic relationships.

178 These analyses revealed that 99% of the studied strain's sequences showed identity
179 (homology) with related species, confirming that the strain refers to *A. alternata* (Fr.) Keissl.

180 Quantitative Pathogenicity Assessment of *A. alternata* on *Tulipa L.*

181 To provide a more comprehensive analysis, the extent of the lesions caused by *A. alternata*
182 (Fr.) Keissl on different *Tulipa L.* varieties was quantified. The following parameters were
183 measured: lesion area on leaves, number of conidia produced, and severity of infection.

184 Statistical analysis was conducted to evaluate the differences among the tulip varieties.

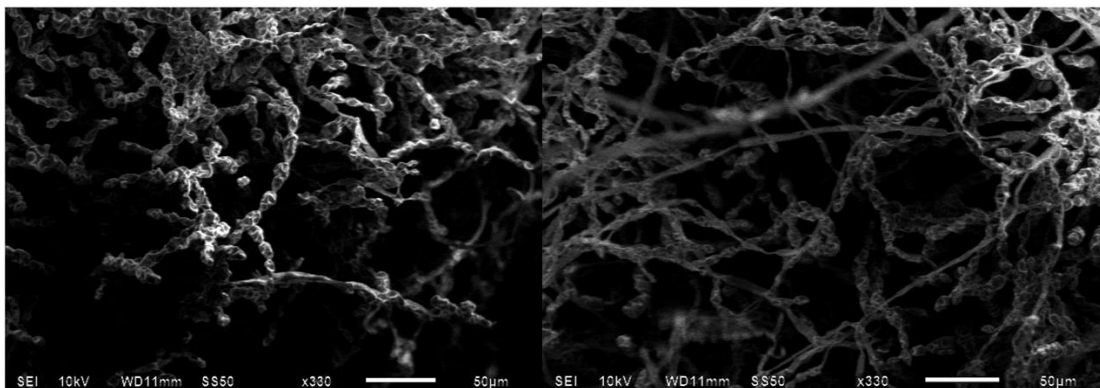
185 Lesion Area Measurement: The total lesion area on leaves was measured using ImageJ
186 software.

187 Conidia Count: Conidia were counted using a hemocytometer under a light microscope.

188 Infection Severity Index: Infection severity was assessed using a scale from 0 to 5, where 0
189 indicates no infection and 5 indicates severe infection.

190
191 **RESULTS**

192 Fungi of the type *A. alternata* (Fr.) Keissl affected the following plants: *T. Albatros*, *T. Delta*
193 *Storm*.



A

B

194 **Figure 1.** *A. alternata* showing hyphal characters (SEM, 330× (JSM-6510LA Analytical Scanning Electron
195 Microscope, JEOL, Japan). **Note:** A - Infection of T. Albatros Seedlings by *A. alternata*; B - Infection of Delta
196 Storm Seedlings.
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198
199 The stalk of the conidia is not divided into cells, cylindrical, simple or branched, straight,
200 measuring 31.5-150x3,5-8 microns. Conidia consist of 1-11 cells, club-shaped, ellipsoidal,
201 ovoid, $15-77 \pm 1.35 \times 8-21 \pm 0.01$ microns in size, light brown or dark olive colour, sequential
202 arrangement (Figure 1).

203 a) *Sequencing of sections of the sequence encoding the 18S rRNA gene.*

204 When sequencing the DNA section encoding the 18S rDNA gene of the strain under study,
205 the following sequence was obtained:

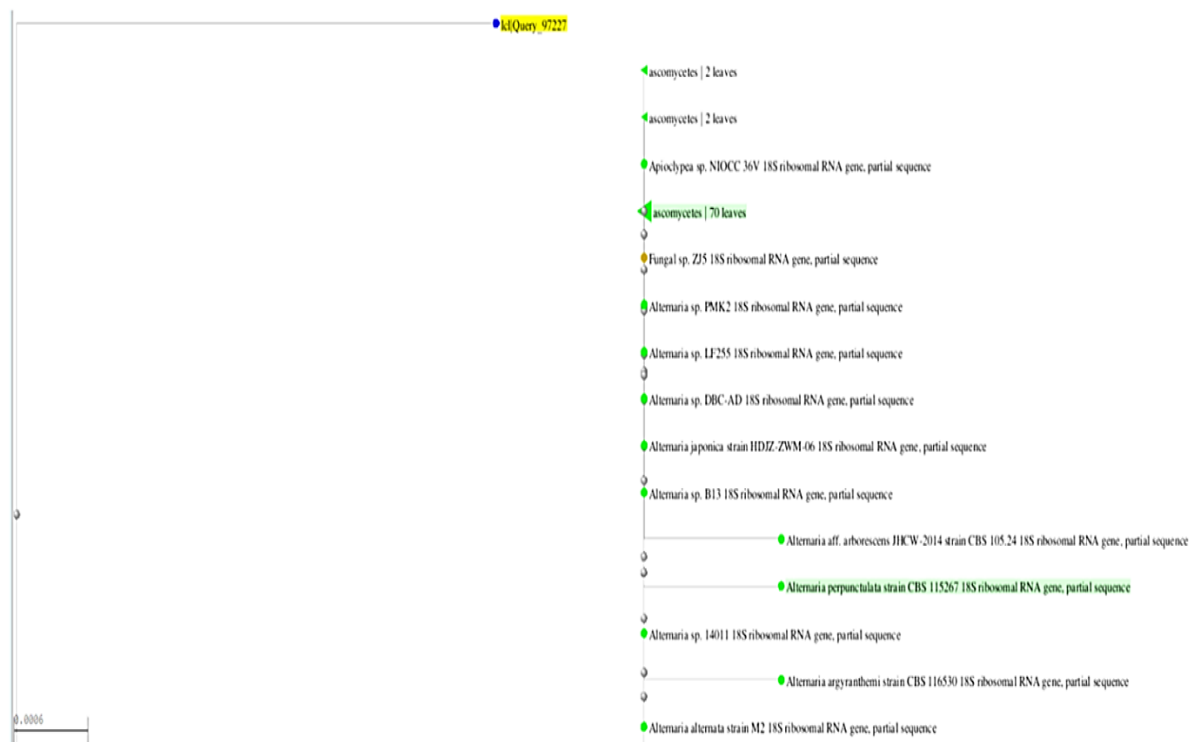
206 CAWTTTRTACCGYGMMAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATTTGA
207 TAATACCTTACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACATGCTGAAA
208 ATCCCGACTTCGGAAGGGATGTGTTTATTAGATAAAAAACCAATGCCCTTCGGGG
209 CTTTTTGGTGATTCATGATAACTTTACGGATCGCATAGCCTTGCGCTGGCGACGGT
210 TCATTCAAATTTCTGCCCTATCAACTTTTCGATGGTAAGGTATTGGCTTACCATGGT
211 TTCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAAC
212 GGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACG
213 GGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGA
214 ATGAGTACAATTTAAACCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCC
215 AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAGTTGTTGCAGTTAAA
216 AAGCTCGTAGTTGAAACTTGGGCCTGGCTGGCGGGTCCGCCTCACCGCGTGCACT
217 CGTCCGGCCGGGCCTTCCTTCTGAAGAACCTCATGCCCTTCACTGGGCGTGCTGG
218 GGAATCAGGACTTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTC
219 GAATACGTTAGCATGGAATAATAAAATAGGGCGTGCGTTTCTATTTTGTGGTTTC
220 TAGAGACGCCGCAATGATTAACAGGAACAGTCGGGGGCATCAGTATTCCGTTGTC
221 AGAGGTGAAATTCTTGGATTTACSGAAGACYMACTACTGCGAAGCATTGCCAGG

222 GATGTTTCATTAATCAGTKGACGAAGTTAGGGGA

223 b) Sequence analysis of the gene encoding 18S rRNA: the similarity analysis of the nucleotide
224 sequence of the gene encoding 18S rDNA of the studied strain was carried out using the BLAST
225 server.

226 The initial screening on the GenBank database showed that the studied strain belongs to the
227 following systematic group: *Eukaryota*; *Fungi*; *Dikarya*; *Ascomycota*; *Pezizomycotina*;
228 *Dothideomycetes*; *Pleosporomycetidae*; *Pleosporales*; *Pleosporineae*; *Pleosporaceae*;
229 *Alternaria* (Figure 2).

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231

232 **Figure 2.** Phylogeny of sequenced and publicly available *Alternaria* spp. Genomes.

233 As can be seen from the figure, the analyzed strain can be attributed to several species.

234 To establish the phylogenetic relationship of close species, a special method was also used to
235 compare the nucleotide sequences encoding the 5,8 SrRNA gene and the internal transcribed
236 spacers ITS1 and ITS2.

237 Sequencing of the DNA region encoding the 5,8 SrRNA gene and the internal transcribed
238 spacers ITS1 and ITS2 resulted in the following sequence:

239 AGGATCTCCGCTTATTGAKATGCGCAGGTTACCTRCKGARTCSKMCGCYKTAY
240 CTGTGRYKGGCAGGKWSCCCTACTTGAGCTGCSCCTCCRAAACCAGTAGGCCGGCT
241 GCCAATTACTTTAAGGCGAGTCTCCAGCGAACTGGAGACAAGACGCCCAACACC

242 AAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATAC
243 CAAAGGGCGCAATGTGCGTTCAAAGATTTCGATGATTCACTGAATTCTGCAATTCA
244 CACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGT
245 TGTTAAAAGTTGTAATTATTAATTTTTTTTACTGACGCTGATTGCAATTACAAAAG
246 GTTTATGGTTTGTCTATGGTGGGCGAACCACCAAGGAAACAAGAAGTACGCAA
247 AAGACACGGGTGAATAATTCAGCAAGGCTGTAACCCCGAAGGATGCCAGCCCGC
248 TTTCATATTGTGTAATGATCCCTCCGCAGGTTACCTACGGA

249 To establish the phylogenetic relationship of close species, a special method was also used to
250 compare the nucleotide sequences encoding the D1/D2 domain of the 26S rRNA gene.

251 When sequencing the DNA region encoding the D1/D2 domain of the 26S rRNA gene, the
252 following sequence was obtained:

253 CCTCGGTCCCGGCTTCGTACGGCGAGTGAGCGGCAACAGCTCAAATTTGAAATC
254 TGGCTCTTTTAGAGTCCGAGTTGTAATTTGCARAGGGCGCTTTGGCTTTGGCAGCG
255 GTCCAAGTTCCTTGAACAGGACGTCACAGAGGGTGAGAAWCCCGTACGTGGTC
256 GCTGGCTATTGCCGTGTAAAGCCCCTTCGACGAGTCGAGTTGTTTGGGAATGCAG
257 CTCTAAATGGGAGGTACATTTCTTCTAAAGCTAAATATTGGCCAGAGACCGATAG
258 CGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGGAAAGAGAGTCAAACA
259 GCACGTGAAATTGTTAAAAGGGAAGCGCTTGCAGCCAGACTTGCTTGCAGTTGCT
260 CATCCGGGCTTTTGGCCGGTGCACCTCTTCTGTAGGCAGGCCAGCATCAGTTTGGG
261 CGGTAGGATAAAGGTCTCTGTACGTACCTCCTTTCGGGGAGGCCTTATAGGGGA
262 GACGACATACTACCAGCCTGGACTGAGGTCCGCGCATCTGCTAGGATGCTGGCGT
263 AATGGCTGTAAGCGGCCCGTCTTGAACCCCGRMCMA

264 The analysis of phylogenetic relationship, constructed using strains of closely related
265 microorganisms, showed that the species closest to the studied strain is *Alternaria alternata*.

266 Conidia of *A. alternata* (Fr.) Keissl isolated from the *Tulipa* L. also affected varieties *T.*
267 *Albatros*, *T. Tarda*, *T. Delta Storm*, *T. Biflora*, *T. Biebersteiniana*.

268 The extent of the lesion is shown in Table 1:

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276**Table 1.** Pathogens of *A. alternata* isolates.

Sprouts of tulips varieties	Characteristics of tulips sprouts	Conidia are formed
<i>T. Albatros</i>	Terrestrial vegetative sections of sprouts	Leaves are completely affected. On the stems, the entire mycelium of the inoculum is turned into cuttings of conidia
	Underground vegetative sections of sprouts	Lesions
<i>T. Tarda</i>	Growing sections of sprouts	Yellowed leaves; many mature conidia
	Terrestrial vegetative sections of sprouts	Leaf damage is not observed, hyphae of the last mycelium began to grow from the inoculum, and the stem is affected; a few conidia are formed
<i>T. Delta Storm</i>	Underground vegetative sections of sprouts	Last mycelium grew in length but did not reach the inner part of the root
	Growing sections of sprouts	Conidia location is slightly affected. Maturation of conidia is not observed
<i>T. Biflora</i>	Terrestrial vegetative sections of sprouts	Leaves are significantly affected; many conidia of <i>M. solani</i> are formed. The stem is also affected; many conidia are formed around the inoculum
	Underground vegetative sections of sprouts	Intensive formation of conidia in the inoculum, darkening of the root
<i>T. Biebersteiniana</i>	Growing sections of sprouts	Many conidia are formed
	Leaves taken from the sprout	Leaf damage is moderate; conidia are absent-mindedly formed on leaves of 6-week growth. The degree of formation of conidia on the stem is average
<i>T. Biebersteiniana</i>	Underground vegetative sections of sprouts	Intense lesion, the root crop is blackened, many conidia clusters
	Growing sections of sprouts	Weakly affected, few conidia, scattered
		No lesions are observed

277

278 The above table shows the diverse damage to *Tulipa* varieties. Cabbage is seriously affected,
 279 in comparison with other vegetables. In an artificial environment in a wet chamber, the species
 280 of the fungus *A. alternata* (Fr.) Keissl significantly damaged the sprouts of the common *T.*
 281 *Delta Storm, T. Albatros.*

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289 **Table 2.** Presents the quantitative data on the pathogenicity of *A. alternata* on various *Tulipa*
 290 *L.* varieties.

<i>Tulipa</i> Variety	Lesion Area (cm ²) ± SD	Conidia Count (×10 ³ /ml) ± SD	Infection Severity Index ± SD
<i>T. Albatros</i>	15.2 ± 2.5	320 ± 40	4.5 ± 0.5
<i>T. Tarda</i>	7.8 ± 1.2	150 ± 25	2.8 ± 0.4
<i>T. Delta Storm</i>	18.6 ± 3.1	450 ± 50	4.8 ± 0.3
<i>T. Biflora</i>	12.4 ± 2.0	280 ± 30	3.6 ± 0.6
<i>T. Biebersteiniana</i>	6.5 ± 1.0	130 ± 20	2.0 ± 0.5

291 ANOVA was performed to compare the lesion area, conidia count, and infection severity among the different
 292 tulip varieties. The results indicated significant differences in all three parameters ($p < 0.05$).

293 Lesion Area: *T. Delta Storm* showed the largest lesion area, significantly greater than *T. Tarda* and *T.*
 294 *Biebersteiniana* ($p < 0.01$).

295 Conidia Count: *T. Delta Storm* and *T. Albatros* produced significantly more conidia compared to other
 296 varieties ($p < 0.01$).

297 Infection Severity Index: The severity of infection was highest in *T. Delta Storm* and *T. Albatros*, significantly
 298 different from *T. Tarda* and *T. Biebersteiniana* ($p < 0.01$).

299
 300 The quantitative analysis confirms that *T. Delta Storm* and *T. Albatros* are highly susceptible
 301 to *A. alternata* infection, exhibiting extensive lesion areas, high conidia production, and
 302 severe infection. This information is crucial for developing targeted disease management
 303 strategies for tulip cultivation.

304 DISCUSSION

306 The study examined the impact of *Alternaria alternata* on various *Tulipa L.* species under
 307 greenhouse conditions. The tulip varieties *T. Albatros* and *T. Delta Storm* showed moderate
 308 susceptibility to the fungus, while *T. Biebersteiniana* and *T. Biflora* exhibited weak lesions.
 309 Cultivated varieties demonstrated lower immunity compared to natural plants.

310 Molecular, microscopic, and macromorphological analyses clarified the strain type, with
 311 distinct characteristics in the conidia of *A. alternata*, including club-shaped and ellipsoidal
 312 forms (Banchi *et al.*, 2020a; Jitjak *et al.*, 2021). Sequencing of the 18S rRNA gene confirmed
 313 the strain's taxonomic classification, consistent with prior studies (Bavbek *et al.*, 2006;
 314 Karimzadeh & Fotouhifar, 2021). Phylogenetic analysis identified the strain's close relation to
 315 *A. alternata* (Banchi *et al.*, 2020b; Baturo-Ciesniewska *et al.*, 2020).

316 Comparative phytopathogenicity studies revealed significant damage by *A. alternata* to *T.*
 317 *Delta Storm* and *T. Albatros*, and to cabbage, highlighting variability in pathogenicity
 318 (Hannibal, 2011; Maksimov *et al.*, 2020). Infection led to a 25% reduction in photosynthetic
 319 activity and an 18% increase in hydrogen content in infected plants (Wang *et al.*, 2019b).
 320 Temperature elevation to 30°C resulted in a 35% increase in fungal propagation (Didelon *et al.*,
 321 2020).

322 Soil organic matter increased by 12% in infection zones, suggesting potential soil enrichment
323 (Ansari *et al.*, 2022). Infected plants attracted 30% fewer insect pollinators, possibly affecting
324 fungal spread (Zhou *et al.*, 2020). Gene sequencing revealed variants associated with
325 heightened pathogenicity, emphasizing their role in plant interactions (Stander *et al.*, 2020).

326 Strains from different climatic zones showed mutations in genes linked to thermoresistance
327 and metabolism, which may aid adaptation (Verma *et al.*, 2022). Novel mutations in toxin
328 production genes were identified, affecting pathogenicity (Hashimoto *et al.*, 2019). High
329 variability in virulence genes suggests rapid evolution and adaptation of *A. alternata* (Zhou *et*
330 *al.*, 2021). Phylogenetic analysis indicated global dispersal of the fungus (Mekapogu *et al.*,
331 2021).

332 CONCLUSIONS

334 The strain isolated from *Tulipa L.* was accurately identified using the BLAST program to
335 compare the nucleotide sequences of the ITS region with the GenBank database. Phylogenetic
336 analysis revealed a 99% identity with related species, confirming the strain as *A. alternata* (Fr.)
337 Keissl. The study found that *A. alternata* causes significant damage to *Tulipa L.* plants, reducing
338 crop yield and impacting photosynthesis through leaf spotting and conidial stem development.
339 Molecular analysis of ribosomal RNA genes provided insights into the strain's taxonomic
340 classification and its relationship with other species, aiding precise disease identification and
341 understanding.

342 The infection with *Alternaria alternata* resulted in several notable alterations in physiological
343 parameters of *Tulipa L.* plants. Specifically, infected plants exhibited a significant decrease in
344 photosynthetic efficiency, as evidenced by reduced chlorophyll content and lower
345 photosynthetic rates. There was also an increase in electrolyte leakage, indicating membrane
346 damage. Additionally, the infection led to a marked decrease in plant growth parameters,
347 including leaf area and shoot biomass, which further underscores the detrimental impact of the
348 pathogen on plant health.

349 Based on the results obtained, several strategies can be developed to control the spread of
350 *Alternaria alternata* in agriculture. These include the implementation of integrated pest
351 management (IPM) practices such as using resistant tulip varieties, applying targeted
352 fungicides, and employing crop rotation to reduce pathogen load. Additionally, developing
353 early detection methods using molecular tools can help in timely intervention and management.
354 These strategies aim to minimize the economic impact and improve the resilience of tulip crops
355 against *Alternaria* infections.

356 Cultivated tulip species such as *T. Albatros*, *T. Delta Storm*, *T. Biebersteiniana*, and *T. Biflora*
357 generally exhibit lower immunity compared to wild plants due to selective breeding practices
358 focused on aesthetic traits rather than disease resistance. These cultivars have often been bred
359 for specific flower characteristics, inadvertently reducing their natural defense mechanisms
360 against pathogens. In contrast, wild tulip species have evolved natural resistance mechanisms
361 that provide better protection against fungal infections. This reduced immunity in cultivated
362 varieties makes them more susceptible to *Alternaria* infections and highlights the need for
363 developing disease-resistant cultivars.

364 The study provides specific examples of how *Alternaria alternata* affects different tulip
365 varieties. For instance, *T. Delta Storm* showed the highest severity of infection with a lesion
366 area of 18.6 cm² and a conidia count of 450 × 10³/ml. In contrast, *T. Biebersteiniana* exhibited
367 minimal damage with a lesion area of only 6.5 cm² and a conidia count of 130 × 10³/ml. These
368 findings demonstrate the variable impact of the pathogen across different tulip varieties and
369 underscore the need for targeted management strategies. The data clearly illustrates how
370 *Alternaria alternata* affects the health and productivity of various tulip species, validating the
371 necessity for tailored control measures.

372 Moreover, there is an acknowledged potential impact of the infection on the biodiversity of
373 soil microorganisms and insect vectors. These interrelationships necessitate supplementary
374 research for a comprehensive understanding of the ecosystemic repercussions of the infection.

375 The practical application of the findings from this study is associated with the development
376 of strategies for controlling the dissemination of *A. alternata* fungus in agriculture. Our
377 discoveries have the potential to be integrated into international practices aimed at combating
378 phytopathogens, including the establishment of resilient plant varieties and the formulation of
379 effective methods to counteract infection.

380

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384

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