

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 al.*  
60 *et 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 MgCl<sub>2</sub>, 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 GGTCCTGTTTCAAGACGG 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.

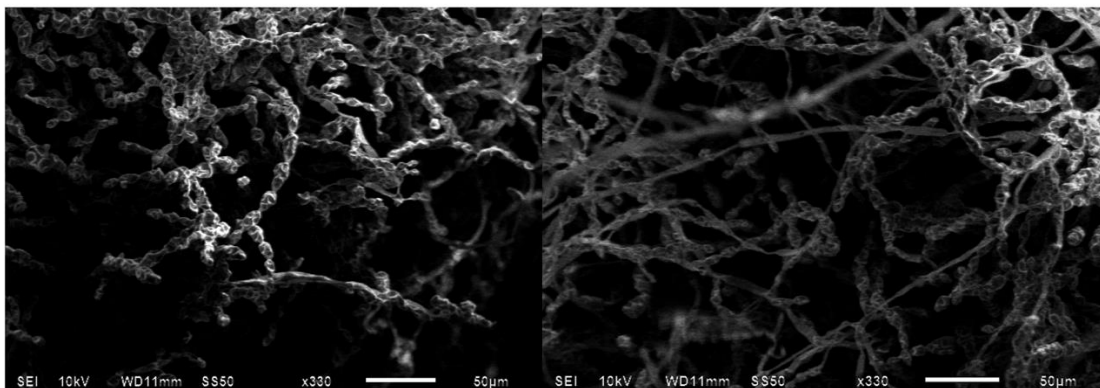
185 Lesion Area Measurement: The total lesion area on leaves was measured using ImageJ  
186 software.

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.  
197

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 GGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCAATCCCGACACG  
213 GGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGA  
214 ATGAGTACAATTTAAACCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCC  
215 AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAGTTGTTGCAGTTAAA  
216 AAGCTCGTAGTTGAAACTTGGGCCTGGCTGGCGGGTCCGCCTCACCGCGTGCACT  
217 CGTCCGGCCGGGCCTTCCTTCTGAAGAACCTCATGCCCTTCACTGGGCGTGCTGG  
218 GGAATCAGGACTTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTC  
219 GAATACGTTAGCATGGAATAATAAAATAGGGCGTGCGTTTCTATTTTGTGGTTTC  
220 TAGAGACGCCGCAATGATTAACAGGAACAGTCGGGGGCATCAGTATTCCGTTGTC  
221 AGAGGTGAAATTCTTGGATTTACSGAAGACYMACTACTGCGAAGCATTGCCAGG

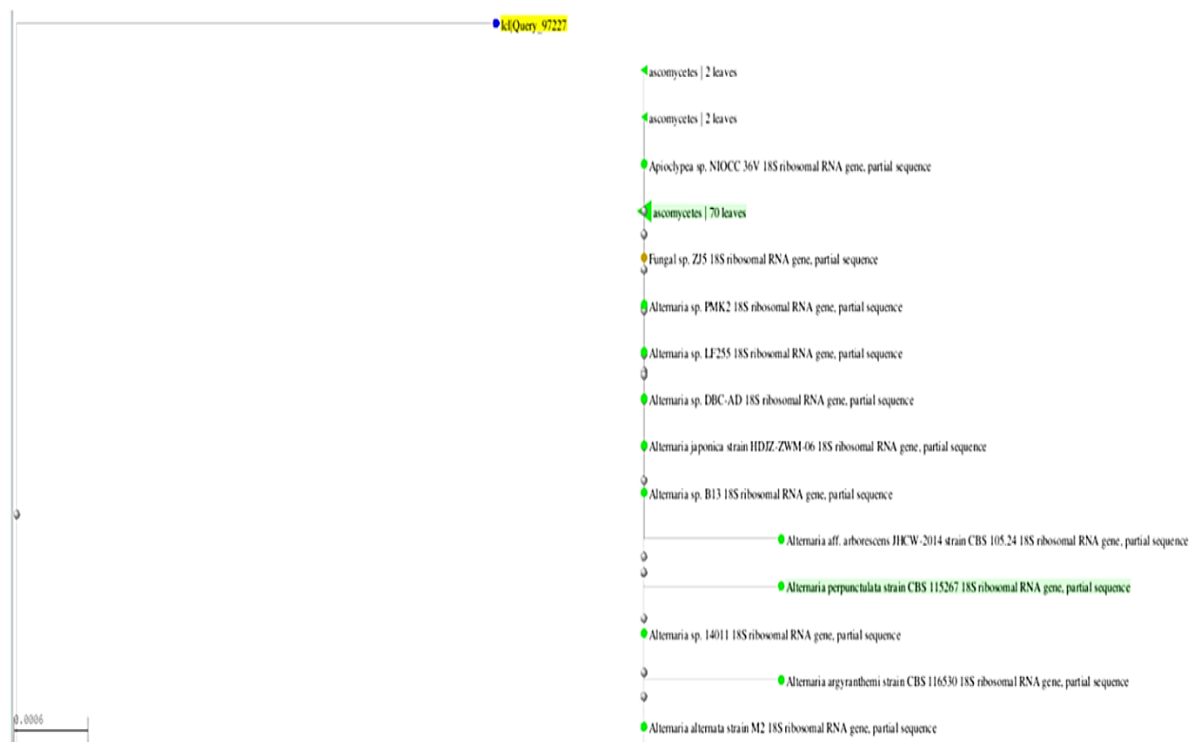


222 GATGTTTCATTAATCAGTKGACGAAGTTAGGGGA

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).

230



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 GTTTATGGTTTGTCTATGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAA  
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 <sup>2</sup> ) ± SD	Conidia Count (×10 <sup>3</sup> /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 cm<sup>2</sup> and a conidia count of 450 × 10<sup>3</sup>/ml. In contrast, *T. Biebersteiniana* exhibited  
367 minimal damage with a lesion area of only 6.5 cm<sup>2</sup> and a conidia count of 130 × 10<sup>3</sup>/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

## 385 REFERENCES

- 386 Abiev, S. A. 2002. *Rust fungi of Kazakhstan*. Gylym, Almaty.
- 387 Ansari, A. A., Siddiqui, Z. H., Alatawi, F. A., Alharbi, B. M. and Alotaibi, A. S. 2022. An  
388 assessment of biodiversity in Tabuk Region of Saudi Arabia: A comprehensive review.  
389 *Sustainability*, **14**(17): 10564. <https://doi.org/10.3390/su141710564>

- 390 Banchi, E., Ametrano, C. G., Greco, S., Stanković, D., Muggia, L. and Pallavicini, A. 2020a.  
391 PLANiTS: A curated sequence reference dataset for plant ITS DNA metabarcoding.  
392 *Database*, **2020**: baz155. <https://doi.org/10.1093/database/baz155>
- 393 Banchi, E., Ametrano, C. G., Tordoni, E., Stanković, D., Ongaro, S., Tretiach, M., Pallavicini,  
394 A., Muggia, L. and ARPA Working Group. 2020b. Environmental DNA assessment of  
395 airborne plant and fungal seasonal diversity. *Sci. Total Environ.*, **738**: 140249.  
396 <https://doi.org/10.1016/j.scitotenv.2020.140249>
- 397 Baturó-Ciesniewska, A., Pusz, W. and Patejuk, K. 2020. Problems, limitations, and challenges  
398 in species identification of Ascomycota members on the basis of ITS regions. *Acta Mycol.*,  
399 **55(1)**: 5512. <https://doi.org/10.5586/am.5512>
- 400 Bauer, M., Mukhametov, A. and Trifonov, P. 2023. Relationship between the state of the  
401 countrys logistics and perishable goods output: dairy industry. *The TQM Journal*, **35 (7)**:  
402 1799-1814. <https://doi.org/10.1108/TQM-04-2022-0131>
- 403 Bavbek, S., Erkeköl, F. O., Ceter, T., Mungan, D., Ozer, F., Pinar, M. and Misirligil, Z. 2006.  
404 Sensitization to *Alternaria* and *Cladosporium* in patients with respiratory allergy and  
405 outdoor counts of mold spores in Ankara atmosphere, Turkey. *J. Asthma*, **43(6)**: 421–  
406 426. <https://doi.org/10.1080/02770900600710706>
- 407 Bush, R. K. and Prochnau, J. J. 2004. *Alternaria*-induced asthma. *J. Allergy Clin. Immunol.*,  
408 **113(2)**: 227–234. <https://doi.org/10.1016/j.jaci.2003.11.023>
- 409 Chacón, F. I., Sineli, P. E., Mansilla, F. I., Pereyra, M. M., Diaz, M. A., Volentini, S. I.,  
410 Poehlein, A., Meinhardt, F., Daniel, R. and Dib, J. R. 2022. Native cultivable bacteria  
411 from the blueberry microbiome as novel potential biocontrol agents. *Microorganisms*,  
412 **10(5)**: 969. <https://doi.org/10.3390/microorganisms10050969>
- 413 De Clerck, E., Vanhoutte, T., Hebb, T., Geerinck, J., Devos, J. and De Vos, P. 2004. Isolation,  
414 characterization, and identification of bacterial contaminants in semifinal gelatin extracts.  
415 *AEM*, **70(6)**: 3664-3672. <https://doi.org/10.1128/AEM.70.6.3664-3672.2004>
- 416 Didelon, M., Khafif, M., Godiard, L., Barbacci, A. and Raffaele, S. 2020. Patterns of sequence  
417 and expression diversification associate members of the *PADRE* gene family with  
418 response to fungal pathogens. *Front. Genet.*, **11**: 491.  
419 <https://doi.org/10.3389/fgene.2020.00491>
- 420 Fung, F., Tappen, D. and Wood, G. 2000. *Alternaria*-associated asthma. *Appl. Occup. Environ.*  
421 *Hyg.*, **15(12)**: 924–927. <https://doi.org/10.1080/104732200750051157>

- 422 Gannibal, Ph. B. 2011. Understanding the phylogeny of the alternarioid hyphomycetes: What  
423 can the consequences be in taxonomy? In: *Systematics and evolution of Fungi*, (Eds.):  
424 Misra, J. K., Tewari, J. P. and Deshmukh, S. K. CRC Press, Boca Raton, PP. 305–333.
- 425 Hannibal, F. B. 2011. *Monitoring of crop alternariosis and identification of fungi of the genus*  
426 *Alternaria*. GNU VIZR Russian Agricultural Academy, Saint Petersburg.
- 427 Hashimoto, S., Tanaka, E., Ueyama, M., Terada, S., Inao, T., Kaji, Y., Yasuda, T., Hajiro, T.,  
428 Nakagawa, T., Noma, S., Honjo, G., Kobashi, Y., Abe, N., Kamei, K. and Taguchi, Y.  
429 2019. A case report of pulmonary *Botrytis* sp. infection in an apparently healthy  
430 individual. *BMC Infect. Dis.*, **19(1)**: 684. <https://doi.org/10.1186/s12879-019-4319-2>
- 431 Iqbal, A., Khan, R. S., Shehryar, K., Imran, A., Ali, F., Attia, S., Shah, S. and Mii, M. 2019.  
432 Antimicrobial peptides as effective tools for enhanced disease resistance in plants.  
433 *PCTOC*, **139**: 1–15. <https://doi.org/10.1007/s11240-019-01668-6>
- 434 Jin, G. Q., Mao, G. Y., Li, D. W., Wan, Y. and Zhu, L. H. 2021. First report of *Alternaria*  
435 *alternata* causing leaf spots of *Liriodendron chinense* × *tulipifera* in China. *J. Plant*  
436 *Pathol.*, **103**: 689–690. <https://doi.org/10.1007/s42161-021-00775-8>
- 437 Jitjak, W., Chairap, W. and Sanoamuang, N. 2021. Molecular Identification of fungal species  
438 causing brown circular leaf spot disease in seedlings of Siamese Rosewood (*Dalbergia*  
439 *cochinchinensis* Pierre ex Laness). *Sci. Technol. Asia*, **26(3)**: 156–166.
- 440 Karabassov R., Bauer M., Mogilnyy S., Mauyanova A., Mikhnova S. 2018. Development of  
441 recommendations to create the conditions for attraction of highly-qualified specialists to  
442 the farming sector of Kazakhstan (based on the materials of the Akmola region). *Revista*  
443 *Espacios*, **39(12)**: 20-22.
- 444 Karimzadeh, S. and Fotouhifar, K. B. 2021. Report of some fungi of Pleosporaceae family  
445 associated with leaf spot symptoms of plants in Chaharmahal and Bakhtiari province,  
446 Iran. *J. Crop Prot.*, **10(2)**: 319–340.
- 447 Kaur, S. 2023. An overview on fungal diseases in angiospermic plants. *Asian Plant Res. J.*,  
448 **11(2)**: 24–33. <https://doi.org/10.9734/aprj/2023/v11i2207>
- 449 Khmel'nitskaya, I. I., Vepri'tskaya, I. G., Arinbasarov, M. U. and Velikanov, L. L. 2003. Soil  
450 Deuteromycetes of central and eastern areas of Samara Oblast. *Mycol. Phytopathol.*,  
451 **37(3)**: 58–63.
- 452 Kuprienko, N. P. 2005. *Diseases of onions in Belarus*. Belprim, Minsk.
- 453 Kuroyanagi, T., Bulasag, A. S., Fukushima, K., Ashida, A., Suzuki, T., Tanaka, A., Camagna,  
454 M., Sato, I., Chiba, S., Ojika, M. and Takemoto, D. 2022. *Botrytis cinerea* identifies host



- 455 plants via the recognition of antifungal capsidiol to induce expression of a specific  
456 detoxification gene. *PNAS Nexus*, **1(5)**: pgac274.  
457 <https://doi.org/10.1093/pnasnexus/pgac274>
- 458 Maksimov, I. V., Singh, B. P., Cherepanova, E. A., Burkhanova, G. F. and Khairullin, R. M.  
459 2020. Prospects and applications of lipopeptide-producing bacteria for plant protection.  
460 *Appl. Biochem. Microbiol.*, **56**: 15–28. <https://doi.org/10.1134/S0003683820010135>
- 461 Mehmood, M. A., Zhao, H., Cheng, J., Xie, J., Jiang, D. and Fu, Y. 2020. Sclerotia of a  
462 phytopathogenic fungus restrict microbial diversity and improve soil health by  
463 suppressing other pathogens and enriching beneficial microorganisms. *J. Environ.*  
464 *Manage.*, **259**: 109857. <https://doi.org/10.1016/j.jenvman.2019.109857>
- 465 Mekapogu, M., Jung, J. A., Kwon, O. K., Ahn, M. S., Song, H. Y. and Jang, S. 2021. Recent  
466 progress in enhancing fungal disease resistance in ornamental plants. *Int. J. Mol. Sci.*,  
467 **22(15)**: 7956. <https://doi.org/10.3390/ijms22157956>
- 468 Mishra, P. K., Fox, R. T. and Culham, A. 2003. Development of a PCR-based assay for rapid  
469 and reliable identification of pathogenic Fusaria. *FEMS Microbiol. Lett.*, **218(2)**: 329–  
470 332. <https://doi.org/10.1111/j.1574-6968.2003.tb11537.x>
- 471 Ospanov A. A, Muslimov N. Z, Timurbekova A. K, Mamayeva L. A, Jumabekova G. B. 2020.  
472 The Effect of Various Dosages of Poly-Cereal Raw Materials on the Drying Speed and  
473 Quality of Cooked Pasta During Storage. *Curr Res Nutr Food Sci*, **8(2)**: 1-10. doi:  
474 <http://dx.doi.org/10.12944/CRNFSJ.8.2.11>
- 475 Ospanov, A.A., Popescu, C.V., Muslimov, N.Z., Gaceu, L., Timurbekova, A.K., Stefan, M.,  
476 Dune, A., Popescu, C., & Jumabekova, G.B. 2018. Study of the food safety and nutritional  
477 value of the buckwheat grains of Kazakhstani selection. *Journal of Hygienic Engineering*  
478 *and Design*, **22**: 33-38.
- 479 Otero-Blanca, A., Pérez-Llano, Y., Reboledo-Blanco, G., Lira-Ruan, V., Padilla-Chacon, D.,  
480 Folch-Mallol, J. L., Sánchez-Carbente. M. d. R., Ponce De León, I. and Batista-García,  
481 R. A. 2021. *Physcomitrium patens* infection by *Colletotrichum gloeosporioides*:  
482 Understanding the fungal–bryophyte interaction by microscopy, phenomics and RNA  
483 sequencing. *J. Fungi.*, **7(8)**: 677. <https://doi.org/10.3390/jof7080677>
- 484 Pandit, M. A., Kumar, J., Gulati, S., Bhandari, N., Mehta, P., Katyal, R., Rawat, C. D., Mishra,  
485 V. and Kaur, J. 2022. Major biological control strategies for plant pathogens. *Pathogens*,  
486 **11(2)**: 273. <https://doi.org/10.3390/pathogens11020273>

- 487 Robertshaw, H. and Higgins, E. 2005. Cutaneous infection with *Alternaria tenuissima* in an  
488 immunocompromised patient. *Br. J. Dermatol.*, **153(5)**: 1047–1049.  
489 <https://doi.org/10.1111/j.1365-2133.2005.06833.x>.
- 490 Salybekova, N. N., Basim, E., Basim, H. and Turmetova, G. Zh. 2019. Characterization of  
491 *Alternaria brassicae* causing black leaf spot disease of cabbage (*Brassica oleracea* var.  
492 capitata) in the southern part of Kazakhstan. *Acta Sci. Pol. Hortorum Cultus.*, **18(4)**: 3–  
493 13. <https://doi.org/10.24326/asphc.2019.4.1>
- 494 Srivastava, S., Dashora, K., Ameta, K. L., Singh, N. P., El-Enshasy, H. A., Pagano, M. C.,  
495 Hesham, A. E., Sharma, G. D., Sharma, M. and Bhargava, A. 2021. Cysteine-rich  
496 antimicrobial peptides from plants: The future of antimicrobial therapy. *Phytother. Res.*,  
497 **35(1)**: 256–277. <https://doi.org/10.1002/ptr.6823>
- 498 Stander, E. A., Williams, W., Mgwatyu, Y., Heusden, P. V., Rautenbach, F., Marnewick, J.,  
499 Roes-Hill, M. L. and Hesse, U. 2020. Transcriptomics of the rooibos (*Aspalathus linearis*)  
500 species complex. *Biotech*, **9(4)**: 19. <https://doi.org/10.3390/biotech9040019>
- 501 Stauder, C. M., Utano, N. M. and Kasson, M. T. 2020. Resolving host and species boundaries  
502 for perithecia-producing nectriaceous fungi across the central Appalachian Mountains.  
503 *Fungal Ecol.*, **47**: 100980. <https://doi.org/10.1016/j.funeco.2020.100980>
- 504 Verma, V., Kumar, A., Verma, J. and Priti, B. B. 2022. Conventional and molecular  
505 interventions for biotic stress resistance in floricultural crops. In: *Genomic designing for*  
506 *biotic stress resistant technical crops*. Springer, Cham, PP. 227–246.
- 507 Wang, C., Zhang, Y., Zhang, W., Yuan, S., Ng, T. and Ye, X. 2019a. Purification of an  
508 antifungal peptide from seeds of *Brassica oleracea* var. *gongylodes* and investigation of  
509 its antifungal activity and mechanism of action. *Molecules*, **24(7)**: 1337.  
510 <https://doi.org/10.3390/molecules24071337>
- 511 Wang, W., Chen, X., Yan, H., Hu, J. and Liu, X. 2019b. Complete genome sequence of the  
512 cyprodinil-degrading bacterium *Acinetobacter johnsonii* LXL\_C1. *Microb. Pathog.*, **127**:  
513 246–249. <https://doi.org/10.1016/j.micpath.2018.11.016>
- 514 Xu, Y. 2023. *Genetic dissection of Sclerotinia sclerotiorum biology using forward genetics*  
515 Published doctoral dissertation, University of British Columbia.
- 516 Zhou, Q., Yang, Y., Ahmed, H., Wang, Y., Zahr, K., Fu, H., Sarkes, A. and Feng, J. 2020.  
517 Diseases Diagnosed on samples submitted to the Alberta Plant Health Lab in 2019.  
518 *Canad. J. Plant Pathol.*, **42**: 11–15.

- 519 Zhou, T., Liu, H., Huang, Y., Wang, Z., Shan, Y., Yue, Y., Xia, Z., Liang, Y., An, M. and Wu,  
520 Y. 2021.  $\epsilon$ -poly-L-lysine Affects the Vegetative Growth, Pathogenicity and Expression  
521 Regulation of Necrotrophic Pathogen *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *J.*  
522 *Fungi*, **7(10)**: 821. <https://doi.org/10.3390/jof7100821>
- 523 Zhu, H. Y., Ma, Y., Ke, Y. and Li, B. 2021. Screening and identification of an antagonist against  
524 the pathogen of kiwifruit canker and its antifungal activity to the phytopathogenic fungus.  
525 *Biotechnol. Bull.*, **37(6)**: 66–72. [https://doi.org/10.13560/j.cnki.biotech.bull.1985.2020-](https://doi.org/10.13560/j.cnki.biotech.bull.1985.2020-0473)  
526 0473