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IN VITRO EFFICACY OF SELECTED FUNGICIDES AGAINST *Pestalotiopsis telopeae* ISOLATE 2A6 ASSOCIATED WITH LEAF SPOT DISEASE ON *Photinia* × *fraseri* 'RED ROBIN'

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Abstract: In the present study, fungicide sensitivity of *Pestalotiopsis telopeae* isolate 2A6, identified as the causal agent of leaf disease on *Photinia* × *fraseri* 'Red Robin' in outdoor ornamental plant nurseries in Sakarya, Türkiye, was evaluated under *in vitro* conditions. The active ingredients azoxystrobin, captan, difenoconazole, tebuconazole, and thiophanate methyl were evaluated at concentrations of 1, 3, 5, 10, 30, 50, and 100 µg mL^{-1} on potato dextrose agar medium. The highest inhibition rates of mycelial growth of *P. telopeae* isolate 2A6 were 99.23%, 91.83%, and 81.20% for fungicides tebuconazole, difenoconazole, and captan, respectively, at 100 µg mL^{-1}. Lower inhibition rates were observed for the active ingredients azoxystrobin and thiophanate methyl compared to the other fungicides tested. Consistent with these findings, the EC_{50} values obtained from Probit analysis confirmed that difenoconazole and tebuconazole exhibited the highest antifungal activity among the tested active ingredients. The results of the present study provide preliminary insights for future research on the control of leaf spot diseases caused by *P. telopeae* in outdoor ornamental plants. This study serves as a preliminary *in vitro* screening to establish baseline fungicide sensitivity for *P. telopeae* in Türkiye.

Keywords: Fungicide efficacy, Outdoor ornamental plants, *Pestalotiopsis telopeae*, *Photinia* × *fraseri*

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1. Introduction

With its natural resources and ecological advantages, Sakarya province holds a strong position in the ornamental plant production sector in Türkiye. Today, in addition to this, it has developed the capacity to export ornamental plants abroad, thereby contributing to the national economy (Dellal et al., 2018). Also, the sector provides a source of income for many people; its contribution to employment continues to grow each year. A comprehensive analysis of ornamental plant production reveals that the foreign trade volume has increased, reaching $190 million in 2024 (SÜSBİR, 2025). An analysis of current data shows that outdoor ornamental plants account for the largest share among all ornamental plants grown in Türkiye. The total area where outdoor ornamental plants are produced is 41.702.916 m^2. When looking at the total ornamental plants production areas across Türkiye, Sakarya ranks second after İzmir (TÜİK, 2024). Ornamental plant nurseries in Sakarya are mostly located in Adapazarı, Akyazı, Arifiye, Erenler, Hendek, Pamukova, Sapanca, and Serdivan.

Photinia × *fraseri* 'Red Robin' is a plant belonging to the Rosaceae family and classified as an ornamental shrub, a hybrid of *P. glabra* and *P. serrulata*. The plant is evergreen, and its flowers, which bloom in spring, are white and grow in clusters. *P.* × *fraseri* has a woody structure, grows rapidly, and can reach a height of 3 to 5 m. Its oval-shaped, stiff leaves are green when mature but turn red when young (Çetiner and Zencirkıran, 2020; Özel et al., 2023). It is frequently used in landscape architecture for its striking leaf colour design, and it is also extensively cultivated in ornamental nurseries in Sakarya because of its high economic value.

Species of *Pestalotiopsis* may occur as plant pathogens or endophytes on a wide range of hosts. In addition to having a wide host range, *Pestalotiopsis* spp. also exhibit species diversity (Maharachchikumbura et al., 2011, 2014). A limited number of studies worldwide have identified some *Pestalotiopsis* species as leaf pathogens in *P.* × *fraseri* (Xu et al., 2022; Zhong et al., 2024).

In this study, surveys conducted in Sakarya province based on grower complaints identified *Pestalotiopsis* spp. in areas where *P.* × *fraseri* was cultivated. The pathogen caused severe leaf spot symptoms, initially appearing as chlorotic spots that later developed into brown necrotic lesions with dark margins and grey centers. In some nurseries, disease incidence reached 35-40%, leading to significant decline in the aesthetic and economic value of the plants, thereby negatively impacting their

marketability. Based on detailed morphological and molecular studies, the pathogen was identified as *Pestalotiopsis telopeae* (Bal et al., 2026). To our knowledge, this represents the first report of *P. telopeae* causing leaf spot on *P.* × *fraseri* in Türkiye and worldwide.

This study investigated the *in vitro* control possibilities of *P. telopeae*. The fungicides used in the *in vitro* assays were selected based on information obtained from ornamental plant producers regarding fungicides commonly used in *P.* × *fraseri* production nurseries, their registration against different fungal pathogen groups in Türkiye, and previous studies investigating fungicide efficacy against *Pestalotiopsis* spp. (Zhang et al., 2012; Amrutha and Vijayaraghavan, 2018; Zhang et al., 2022; Baggio et al., 2023; Olivares-Rodriguez et al., 2024). Additionally, fungicides with different modes of action were included in the study based on the Fungicide Resistance Action Committee (FRAC, 2025) list in order to compare their efficacy against *P. telopeae* under *in vitro* conditions.

Given the recent identification of *P. telopeae* on *P.* × *fraseri* in Sakarya, establishing a preliminary *in vitro* screening of fungicide efficacy is essential to provide immediate guidance for nursery management and a foundation for future large-scale population studies.

2. Materials and Methods

In this study, surveys were conducted in outdoor ornamental plant nurseries in Sakarya, Türkiye. Ç2, Ç3, D4 and 2A6 isolates were obtained from symptomatic *P.* × *fraseri* leaf tissues. Pathogenicity tests were performed to evaluate the virulence of these isolates. The isolate 2A6 was selected as a representative isolate for fungicide sensitivity assays because it demonstrated the highest virulence among the tested isolates. Therefore, fungicide assays were conducted using the isolate 2A6, in order to represent the highest disease pressure under *in vitro* conditions. Based on morphological similarity, isolate 2A6 was identified as *P. telopeae* by multilocus sequence analysis of the internal transcribed spacer (ITS), *β*-tubulin (*tub2*), and translation elongation factor 1-alpha (*tef1*) regions, as described by Bal et al. (2026). The obtained sequences were deposited in GenBank under accession numbers PX829063 (ITS), PX833978 (*tub2*), and PX833979 (*tef1*). Phylogenetic analyses confirmed that the isolate clustered with *P. telopeae*.

In vitro assays were conducted to evaluate the efficacy of different fungicides against *P. telopeae* (Table 1). The mode of action and FRAC classification data of the fungicides used in this study are presented in Table 2. The mycelial growth of the pathogen was evaluated using concentrations of 1, 3, 5, 10, 30, 50, and 100 µg mL^{-1}, including an untreated control. Fungicide concentrations were prepared based on the active ingredient equivalent of each commercial formulation. In this study, commercial formulations were utilized rather than technical-grade active ingredients. This approach was adopted to better simulate the actual conditions encountered in nursery practices, thereby accounting for the potential synergistic or additive effects of surfactants and adjuvants present in the final products used by growers. Stock solutions were prepared using sterile distilled water, and no additional solvent was used during fungicide preparation. Appropriate dilutions were made to obtain the final concentrations in Potato Dextrose Agar (PDA, Merck, Darmstadt, Germany) medium. The PDA medium was autoclaved at 121 °C and allowed to cool to 40–45 °C. Once cooled, the medium was amended with streptomycin sulfate (100 mg L^{-1}), followed by the addition of fungicides as described by Delen et al. (1988). The mixture was homogenized using a magnetic stirrer to ensure uniform distribution of the fungicides. The PDA medium containing fungicides was poured into 9 cm diameter Petri dishes. A 5 mm diameter agar disc from 14-day-old *P. telopeae* culture was placed at the center of these solidified PDA media. PDA media without fungicide were used as control Petri dishes. All treated and untreated control Petri dishes were incubated at 24 °C (Maharachchikumbura et al., 2014). The experiment was conducted in a completely randomized design with five Petri dishes (replicates) per fungicide concentration. Colony diameter measurements were performed when the mycelial growth in the untreated control Petri dishes approached the edge of the plate. Colony diameter was determined as the mean of two perpendicular measurements, excluding the initial 5 mm mycelial agar disc. Fungicide efficacy against isolate 2A6 of *P. telopeae* was calculated as the percentage of mycelial growth inhibition using Abbott's formula (Abbott, 1925). Prior to ANOVA the assumptions of normality and homogeneity of variance were evaluated to ensure the suitability of the analysis. The inhibition data obtained from different fungicide concentrations were analyzed using two-way analysis of variance (ANOVA) with the SAS statistical program to evaluate the effects of fungicide, concentration, and fungicide × concentration interaction on mycelial growth of *P. telopeae*. Comparisons of means among concentrations were performed using Tukey's HSD test ($P<0.01$) (Genç and Soysal, 2018). EC_{50} values were calculated using probit analysis in SAS based on dose-response inhibition data.

Table 1. Active ingredients used in *in vitro* assays

Active ingredient	Trade name/Manufacturer	Formulation	Active ingredient content
Azoxystrobin	Heros/Agrisciences	SC	250 g L^{-1}
Captan	AgriCaptan/Safa Agriculture	WP	50%
Difenoconazole	Scorpion/Safa Agriculture	EC	250 g L^{-1}
Tebuconazole	Pivot 25 WP/Cansa Chemistry	WP	25%
Thiophanate methyl	Sarpa 70 WP/AgroTez	WP	70%

Table 2. FRAC classification and mode of action of fungicides used in this study

Active ingredient	FRAC code	Mode of action	Target site
Azoxystrobin	11	Respiration	C3 complex III: cytochrome bc1 (ubiquinol oxidase) at Qo site (*cyt b gene*)
Captan	M 04	Chemicals with multi-site activity	multi-site contact activity
Difenoconazole	3	Sterol biosynthesis in membranes	G1 C14-demethylase in sterol biosynthesis (*erg11/cyp51*)
Tebuconazole	3	Sterol biosynthesis in membranes	G1 C14-demethylase in sterol biosynthesis (*erg11/cyp51*)
Thiophanate-methyl	1	Cytoskeleton and motor protein	B1 tubulin polymerization

3. Results

The fungicidal activity of the selected fungicides against the representative isolate 2A6 of *P. telopeae*, which was isolated from outdoor ornamental shrubs grown in Sakarya, was evaluated under *in vitro* conditions. The efficacy of the active ingredients was evaluated at concentrations of 1, 3, 5, 10, 30, 50, 100 µg mL^{-1}, and the results were compared. All five fungicides used in the assays exhibited varying degrees of mycelial growth inhibition depending on fungicide concentration (Figure 1).

Azoxystrobin exhibited relatively low inhibitory activity against isolate 2A6 under the present in vitro assay conditions, with an inhibition rate of 20.50% at 100 µg mL^{-1}. The inhibitory effect of captan showed a significant increase in its ability to inhibit colony growth starting at 30 µg mL^{-1}, achieving 57.10% inhibition, and further increased to 81.20% at 100 µg mL^{-1}. Difenoconazole, a triazole group fungicide, showed a high efficacy even at 1 µg mL^{-1}, with an inhibition rate of 52.05%. Similarly, tebuconazole demonstrated 44.41% antifungal activity at the lowest dose, and showed high inhibitory activity against mycelial growth. The inhibition rates of both triazole group fungicides against tested isolate growth were 91.83% and 99.23% at 100 µg mL^{-1}, respectively. Accordingly, these two fungicides exhibited the highest antifungal efficacy against the tested isolate 2A6. However, thiophanate-methyl exhibited relatively low inhibitory activity against the tested isolate. In addition, EC_{50} values obtained from probit analysis revealed differences in fungicide sensitivity of the tested isolate. Among the evaluated active ingredients, difenoconazole and tebuconazole showed the lowest EC_{50} values, indicating relatively high antifungal efficacy against the tested isolate. While captan showed moderate activity, azoxystrobin and thiophanate-methyl showed weak inhibitory effects and did not achieve 50.00% inhibition at the tested concentrations.

Table 3. Effects of fungicide concentrations on the mycelial growth and EC_{50} values of isolate 2A6 under *in vitro* conditions

Fungicide	Concentrations (µg mL^{-1})									95% CI	Slope
	Control (0)	1	3	5	10	30	50	100	EC_{50}		
A	7.90±0.00^{a}	7.26±0.28^{b}	7.24±0.31^{b}	7.10±0.22^{b}	6.98±0.24^{b}	6.64±0.27^{c}	6.64±0.25^{c}	6.28±0.19^{d}	>100	ND	-
B	7.80±0.00^{a}	7.36±0.18^{b}	7.34±0.16^{b}	7.26±0.14^{b}	6.84±0.21^{c}	3.34±0.20^{d}	2.90±0.15^{e}	1.46±0.12^{f}	18.45	15.6–21.7	0.88
C	7.84±0.00^{a}	3.78±0.25^{b}	3.14±0.18^{c}	3.10±0.16^{c}	2.66±0.14^{d}	1.10±0.10^{e}	0.86±0.09^{ef}	0.64±0.05^{f}	1.38	1.1–1.7	1.42
D	7.88±0.00^{a}	4.38±0.24^{b}	3.78±0.20^{c}	3.28±0.18^{d}	2.58±0.15^{e}	1.10±0.08^{f}	0.44±0.05^{g}	0.06±0.01^{g}	2.32	2.0–2.7	1.35
E	7.84±0.00^{a}	7.50±0.21^{ab}	7.48±0.20^{ab}	7.46±0.19^{ab}	7.40±0.18^{ac}	7.26±0.17^{bc}	7.00±0.16^{cd}	6.66±0.14^{d}	>100	ND	-

Note: A) azoxystrobin, B) captan, C) difenoconazole, D) tebuconazole, E) thiophanate-methyl. Values represent mean colony diameter (cm) ± standard deviation from five replicates. Different letters within each fungicide column/row indicate statistically significant differences among concentrations according to Tukey's HSD test (P<0.01). EC50: Effective concentration required to achieve 50% inhibition of mycelial growth. Values >100 µg mL–1 indicate that 50% inhibition was not reached at the maximum tested concentration. 95% CI: 95% Confidence Interval for the EC50 value. Slope: Slope of dose-response curve derived from Probit analysis (± SE). ND: Not Determined due to low inhibitory activity.

Table 4. Two-way analysis of variance (ANOVA) of the effects of fungicide, concentration, and fungicide × concentration interaction on the mycelial growth inhibition of isolate 2A6 under *in vitro* conditions

Source of variation	*df*	*F* value	*P* value
Fungicide	4	2215.04	< 0.0001
Concentration	7	398.31	< 0.0001
Fungicide × Concentration	28	50.71	< 0.0001

Df= degrees of freedom, *F*= F-statistic, *P*= probability value. Significant effects were considered at P<0.01.

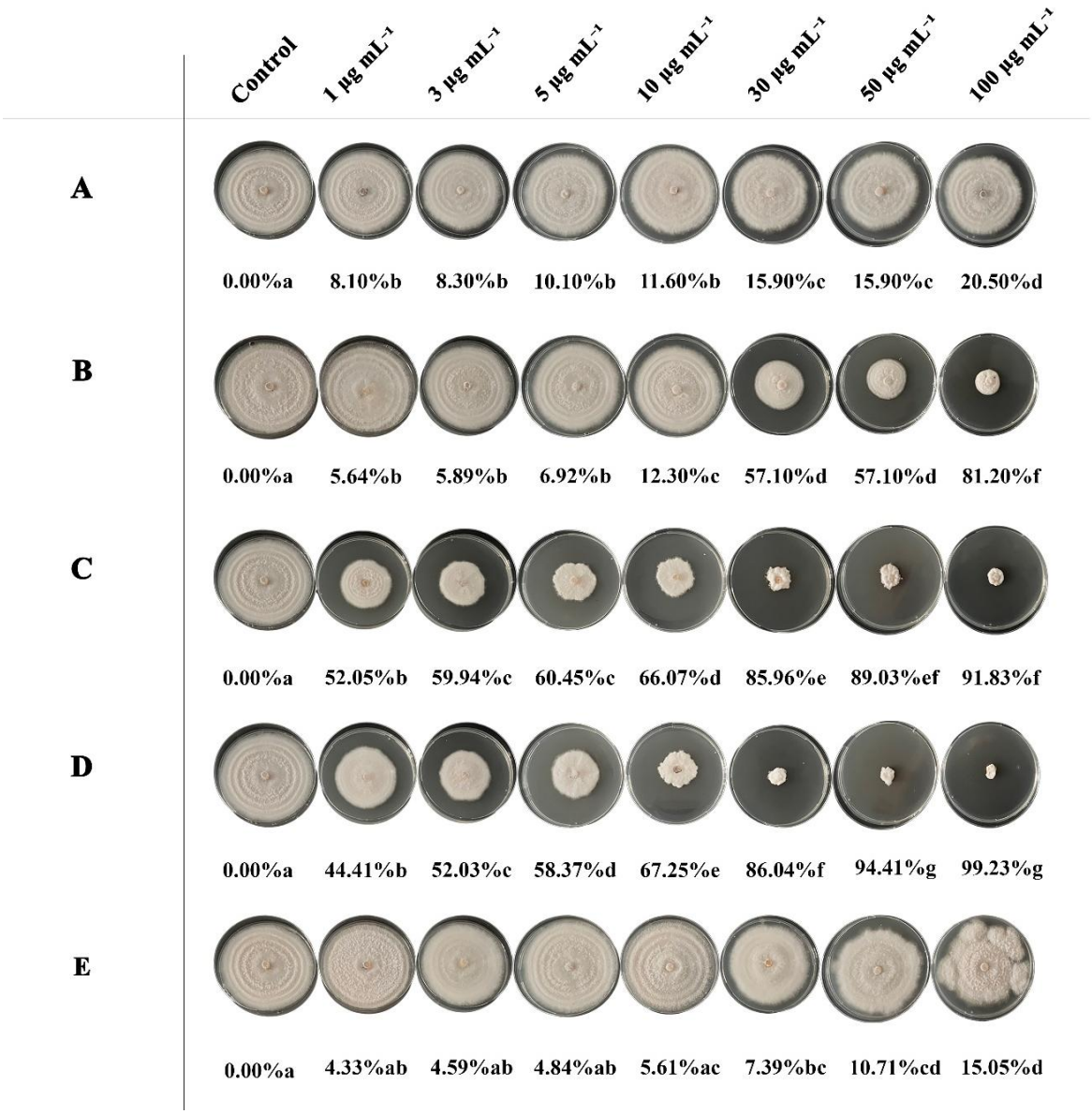

Figure 1. Effects of different fungicide concentrations on mycelial growth inhibition of isolate 2A6 under *in vitro* conditions. Values represent mean percentage inhibition ± standard deviation of five replicates. Different letters within each fungicide indicate statistically significant differences among concentrations according to Tukey's HSD test (P < 0.01). Representative Petri dish images showing mycelial growth at different fungicide concentrations: A) azoxystrobin, B) captan, C) difenoconazole, D) tebuconazole, E) thiophanate-methyl. Fungicide concentrations were arranged from left to right as control, 1, 3, 5, 10, 30, 50, 100 µg mL^{-1}.

These findings were consistent with the inhibition rates observed in the *in vitro* tests. The EC_{50} values obtained after probit analysis were 1.38, 2.32, and 18.45 µg mL^{-1} for difenoconazole, tebuconazole, and captan, respectively (Table 3). Azoxystrobin and thiophanate-methyl failed to achieve 50% inhibition at the tested concentrations (> 100 µg mL^{-1}). Statistical analysis using two-way ANOVA revealed that fungicide type,

concentration, and fungicide × concentration interaction had significant effects on the mycelial growth of the tested isolate ($P<0.01$; Table 4). These results demonstrated that the inhibitory efficacy was strictly dependent on both the chemical nature of the active ingredient and the applied dosage.

4. Discussion

In this study, the efficacy of several fungicidal active ingredients against the representative isolate 2A6 of *P. telopeae* was evaluated under *in vitro* conditions. Some groups of fungicidal active ingredients exhibited higher activity than others. Regarding QoI fungicides, Zhang et al. (2012) previously reported that azoxystrobin (FRAC code 11) exhibited strong inhibitory efficacy against conidial germination of *P. microspora*. In contrast, azoxystrobin demonstrated relatively low inhibitory activity against mycelial growth of isolate 2A6 of *P. telopeae* in the present study. The relatively low *in vitro* activity of azoxystrobin against isolate 2A6 may not necessarily reflect reduced efficacy under field conditions. Certain fungal species may activate an alternative oxidase (AOX) pathway under *in vitro* conditions, which can reduce sensitivity to QoI fungicides and lead to an underestimation of their antifungal activity (FRAC, 2014). This is also reflected in the latest FRAC guidelines (2025), which classify QoI fungicides as high-risk active ingredients for resistance development because of their site-specific mode of action. Therefore, the baseline susceptibility observed in this study should be further validated through greenhouse and field experiments.

In contrast to the results obtained for QoI fungicides, tebuconazole and difenoconazole, which belong to the triazole group (FRAC code 3), and inhibit sterol biosynthesis in fungal cell membranes, exhibited significantly higher inhibitory activity than the other active ingredients tested. The high slope values observed for these DMI (Dimethylation Inhibitor) fungicides (slope>1.30) suggest that even minor increases in concentration lead to a substantial rise in fungal inhibition, indicating a high sensitivity of the isolate 2A6 to these active ingredients. These findings are consistent with previous studies investigating the efficacy of certain fungicides against *Pestalotiopsis* spp. Amrutha and Vijayaraghavan (2018) investigated the efficacy of several fungicides and biological agents against the strawberry leaf blight pathogen *Neopestalotiopsis clavispora* under both *in vitro* and *in vivo* conditions, and reported that difenoconazole exhibited high antifungal efficacy, inhibiting pathogen growth by 81.11% even at a concentration of 0.05%. Gowda et al. (2023) reported that triazole group fungicides showed high efficacy in inhibiting gray leaf blight caused by *Pestalotiopsis* sp. on coconut leaves under *in vitro* conditions. Adhikari et al. (2023) conducted biological control and fungicide assays *in vitro* against gray leaf blight caused by *P. theae* in tea plants. They reported that among the fungicides included in this study, the active ingredient hexaconazole inhibited *P. theae* by 100% regardless of concentration. Shi et al. (2024) reported that tebuconazole significantly suppressed disease development caused by *N. clavispora*, a *Pestalotiopsis*-like pathogen causing leaf spots on apple leaves. Consistent with these reports, tebuconazole and difenoconazole exhibited the lowest EC_{50} values in the present study, determined as 2.32 and 1.38 μg mL^{-1}, respectively (Table 3). In another study, *in vitro* and *in vivo* experiments on *Pestalotiopsis* and *Neopestalotiopsis* species causing fruit spot and anthracnose-like symptoms on avocado showed that the DMI fungicides hexaconazole and propiconazole were among the most effective treatments evaluated (Venkataravanappa et al., 2025).

On the other hand, the weak inhibitory effect of thiophanate-methyl observed in the present study is consistent with the widespread resistance to benzimidazole fungicides often reported in various *Pestalotiopsis*-related species. This resistance is frequently associated with specific point mutations in the *β*-tubulin gene, which significantly reduce the binding affinity of the fungicide to its target site. Kummanid et al. (2017) reported that *β*-tubulin gene mutations were associated with benzimidazole resistance in *Pestalotiopsis* isolates. Similarly, thiophanate-methyl exhibited relatively low inhibitory activity against the representative isolate 2A6 of *P. telopeae* in the present study, which may indicate reduced sensitivity to thiophanate-methyl in the tested isolate. In contrast, Abeysinghe and Ranaweera (2016) investigated the suppression of *Pestalotiopsis* spp., which causes disease in cashew tree leaves in nurseries, using fungicides and biological agents in Petri dish assays, and reported that thiophanate-methyl achieved 100% inhibition of the pathogen.

The non-systemic fungicide captan exhibited the second highest inhibitory activity against the tested isolate 2A6 of *P. telopeae*, following the triazole-group fungicides, with an EC_{50} value of 18.45 μg mL^{-1}. The relatively lower slope value of captan (0.88) compared to triazoles is characteristic of multi-site inhibitors, which typically exhibit a more gradual dose-response curve. Raj et al. (2018) identified the pathogens causing disease on *Calamus* spp. and, after determining that *P. calami* caused leaf infections, conducted control experiments. They reported that the active ingredient captan inhibited hyphal growth in Petri dishes at a concentration of 100 ppm by 55.67%, whereas in the present study, captan exhibited a relatively higher inhibitory effect against the tested isolate. In the same study, triazole-group fungicides also demonstrated high inhibitory activity.

5. Conclusion

Under *in vitro* conditions, difenoconazole and tebuconazole exhibited relatively high inhibitory activity against the tested isolate. However, since both fungicides belong to the DMI group (FRAC code 3) and share the

same mode of action, repeated and intensive use may contribute to resistance development. Therefore, integrating fungicides with different modes of action, including multi-site active ingredients such as captan, should be considered in future management strategies.

Although isolate 2A6 was selected for *in vitro* testing due to its high virulence, fungicide sensitivity may vary among isolates of the same species. Therefore, the results obtained in the present study should be interpreted as a baseline sensitivity profile for the representative isolate 2A6. The 95% confidence intervals of the EC_{50} estimates (Table 3) further support the reliability of these findings. Additional studies including a larger number of isolates, together with greenhouse and field experiments, are needed to better understand fungicide sensitivity in *P. telopeae* populations and support disease control under nursery conditions. The present study provides preliminary information for future research on the management of *P. telopeae* associated with leaf spot disease on *P. × fraseri* 'Red Robin'.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	İ.B.	M.E.G.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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