

Occurrence of *Pestalotiopsis* sp. on Ornamental Plants *Camellia japonica* L. in Romanian Public Gardens

Andreea-Mihaela Florea ¹, Andrei-Mihai Gafencu ¹, Florin-Daniel Lipşa ², Iulian Gabur ^{1,*} and Eugen Ulea ^{1,*}

¹ Department of Plant Science, Faculty of Agriculture, “Ion Ionescu de la Brad” Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700489 Iasi, Romania; andreea.florea@iuls.ro (A.-M.F.); andrei.gafencu@iuls.ro (A.-M.G.)

² Department of Food Technologies, Faculty of Agriculture, “Ion Ionescu de la Brad” Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700489 Iasi, Romania; florin.lipsa@iuls.ro

* Correspondence: iulian.gabur@iuls.ro (I.G.); eugen.ulea@iuls.ro (E.U.)

Abstract: *Camellias* are evergreen shrubs native to the eastern and southern regions of Asia. In Romania, camellias are popular ornamental plants grown in pots. *Pestalotiopsis* species have a worldwide distribution and are pathogenic to a wide range of hosts. They also represent an important group of endophytes capable of producing a variety of bioactive secondary metabolites. This paper reports the occurrence of grey blight and twig or branch dieback caused by *Pestalotiopsis* sp. on *Camellia japonica* L. grown in pots and used in the landscaping of a public garden located in Iasi city, Romania (GPS coordinates: 47°09′24.0″ N 27°35′15.6″ E). *Pestalotiopsis* species were identified based on morphological characteristics and Sanger sequencing. Genomic analysis of the fungal isolate coded P_CJ_24, obtained from living plants, was based on ribosomal internal transcribed spacer region amplification using the LSU primers. This first report of *Pestalotiopsis* species infection of ornamental plants in Romania suggests that new plant pathogens can be introduced in new environmental conditions through plant pods and their incidence increases with the widespread of plant material among gardens.

Keywords: *Pestalotiopsis*; ornamental camellia; new infected areas; wide pathogen incidence



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1. Introduction

Camellias are evergreen plants that can grow into large shrubs or medium-sized trees, native to the temperate and subtropical forests of southeastern Asia. Some species, such as *Camellia sinensis* Kuntze, are widely used for tea production due to their diverse applications and medicinal benefits [1]. Other species in the *Camellia* genus, like *C. japonica*, are valued for their ornamental blooms and play a significant role in landscaping, while *C. oleifera* is cultivated for its edible oil [2,3].

Camellias were introduced to Europe in the 17th century, where their ornamental beauty quickly gain recognition. During the colonial period, these plants became highly sought after by wealthy colonists, who collected them prominently in their expansive gardens and large conservatories. The most popular ornamental species, *C. japonica* L., is known for its stunning flowers and has been the subject of several studies focused on its antioxidant and anti-inflammatory potential [4–11].

In landscaping, camellias are often used to form hedges and borders, or pruned for growth as small trees, even in pots. The most commercially important ornamental species include *C. sasanqua* Thunb., *C. japonica* L., and *C. reticulata* Lindl. Additionally, there are

over 20,000 registered hybrids of camellias, most of which are known to be susceptible to petal blight and twig dieback [12–15].

The most common fungal pathogens affecting camellias, causing brown leaf blotches, premature leaf loss, and twig or branch dieback, are species of *Pestalotiopsis* [16,17]. The most recent classification of *Pestalotiopsis*-like species places them in the order *Xylariales*, within the family *Sporocadaceae*. These fungi are primarily phytopathogenic and are commonly found in tropical regions, where they cause blight diseases in many crops [18,19].

The genus *Pestalotiopsis* encompasses fungi with a variety of ecological roles, especially as plant pathogens, endophytes, or saprobes. These fungi are distributed widely across tropical and temperate regions [20]. Some species are known to cause diseases in plant foliage, twigs, or roots of plants, resulting in considerable losses in commercial production, particularly in southern India and Japan. For instance, gray blight disease caused by *Pestalotiopsis* spp. has led to yield reductions exceeding 17% in tea plantations in these regions [21–23].

In recent years, *Pestalotiopsis* species have garnered attention not only for their role as plant pathogens but also for their ability to produce diverse secondary metabolites. For example, *Pestalotiopsis microspora* produces E2712A, an enzyme capable of degrading Impranil DLN [24,25], while *Pestalotiopsis chamaeropsis* synthesizes a biologically active exopolysaccharide [26]. Additionally, a prenylated resveratrol derivative, 2-C-prenyl resveratrol, identified in *Pestalotiopsis fici*, exhibits enhanced antioxidant and neuroprotective properties compared to resveratrol [27,28]. Endophytic strains such as *P. microspora* and *P. mangiferae*, are also known to produce secondary metabolites involved in many biological activities [29].

A study by Jiang et al. [30] reported the identification of 307 new compounds derived from the *Pestalotiopsis* genus, including 52 terpenoids, 15 coumarins, 40 lactones, 189 polyketides, and 11 alkaloids. Remarkably, about 62% of the metabolites identified from *Pestalotiopsis* species are polyketides [31].

Research on *Pestalotiopsis* species as fungal pathogens of camellias is relatively new in Romania. In this country, camellias are cultivated exclusively as ornamental plants and are not grown for tea production. While local production in this sector remains limited, the increasing popularity of ornamental horticulture has led to a significant rise in the importation of ornamental plants in recent years. This trend reflects a growing demand that domestic production cannot fully satisfy. Consequently, understanding the epidemiology of fungal pathogens like *Pestalotiopsis* spp., which can severely impact ornamental plants growth, has become essential.

This study documents the first confirmed case of *Pestalotiopsis* sp. infecting ornamental *Camellia japonica* L. plants in Romania. The infection was detected in spring 2024 on potted camellias used in landscaping at a public garden in Iasi city, Romania (GPS Coordinates: 47°09'24.0" N 27°35'15.6" E). The article describes the identification of *Pestalotiopsis* sp. based on both morphological analysis and molecular techniques, including PCR and Sanger sequencing. Genomic DNA was amplified with primers specific to the ribosomal internal transcribed spacer (ITS) region, and the resulting sequences were analyzed to confirm the pathogen.

2. Results

2.1. Symptomatology, Isolation and Identification of the Pathogen

In spring 2024, potted plants of *Camellia japonica* L. displaying symptoms of gray blight were examined to identify the pathogen causing the infection. Further analysis were conducted on diseased twigs and branches collected from plants used in the landscaping of a public garden located in Iasi city, Romania (Figure 1).

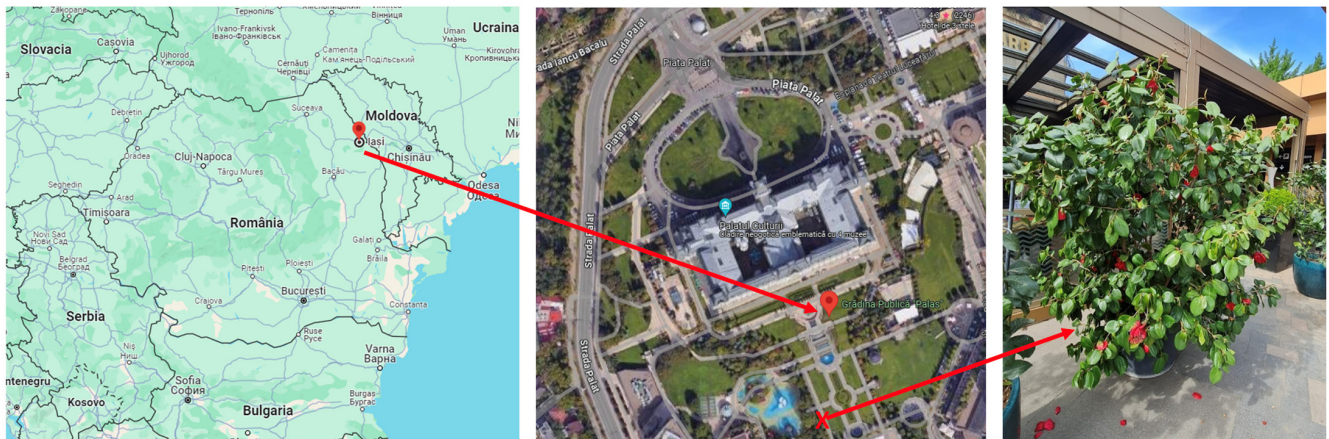


Figure 1. *Pestalotiopsis* sp. on camellia plants grown in pots as landscaping of a public garden located in Iasi city, Romania (GPS coordinate: www.google.com/maps, accessed on 4 July 2024, 47°09′24.0″ N 27°35′15.6″ E). Red arrows represent the exact location of the plant material investigated. Red x marks the physical location of the ornamental plant pots.

Initial symptoms of the disease were observed on twigs and branches, presenting as expanded gray lesions with black fungal spots on the affected areas. Subsequently, leaves exhibited small dark brown spots that gradually enlarged into grayish-brown circles surrounded by a dark brown border. These symptoms align with those caused by species of the *Pestalotiopsis* genus, as documented previously by other researchers [32,33].

The suspected pathogen was isolated from infected twigs onto PDA (potato dextrose agar) plates. Within three to five days of culturing, hyphae began emerging from the margins of the twig tissue segments. Upon transferring to fresh PDA medium, the hyphae formed whitish colonies that darkened with age, starting from the edges and progressing toward the center. Around ten days after incubation, black acervuli developed in roughly concentric rings. The conidia were fusiform, mostly straight but occasionally slightly curved, with four septa dividing the conidium into five cells. The basal and apical cells were hyaline, with transparent, thin, smooth walls, while the middle cells were dark tan (Figure 2). These morphological characteristics are consistent with those of the *Pestalotiopsis* genus, as also described by Maharachchikumbura et al. (2013) [19].

2.2. Molecular Validation of *Pestalotiopsis* sp. in *Camellia japonica* L. Infected Tissues

Genomic DNA was extracted from the purified *Camellia japonica* L. isolate, designated P_CJ_24, and analysed using ITS primers via PCR to determine the fungal species responsible for the symptoms. The PCR amplification results showed an amplicon of 564 bp, consistent with expectations. The resulting PCR product was subjected to Sanger sequencing, and the sequence was analysed using the GenBank database [34] through BLAST searches (blastn) for local alignment, simple neighbor joining clustering and uncorrected p-distances [35].

BLAST analysis revealed that the PCR amplicon from isolate P_CJ_24 shared 100% identity with over 100 sequences from the NIH library (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 26 July 2024), all identified as belonging to the *Pestalotiopsis* sp. (Figure 3 and Supplementary File S1).



Figure 2. *Pestalotiopsis* sp. causing twigs gray blight on *Camellia japonica* L. (A) Infected camellia twigs; (B) Infected camellia leaf spot; (C) Colony surface on PDA medium; (D) Conidia.

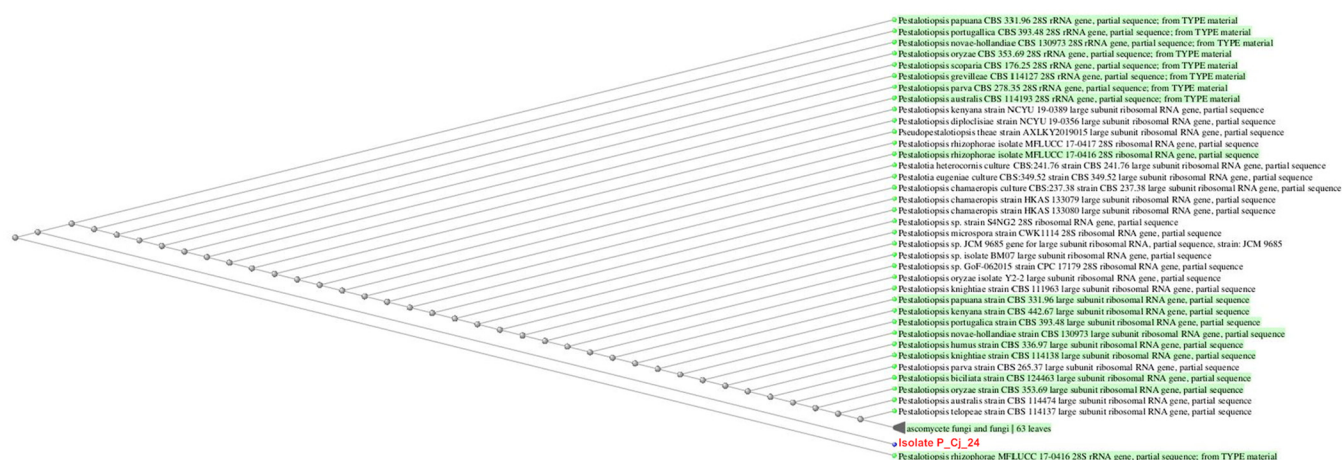


Figure 3. Genetic distance tree of isolate P_CJ_24 (marked in red) and the NCBI-BLAST results, using “neighbor joining” method, max seq difference = 0.5. Sample sequence has a length of 564 bp and was amplified using PCR specific nuclear ribosomal internal transcribed spacer (ITS) primers.

3. Discussion

The *Pestalotiopsis* genus is widely recognized as a cosmopolitan group of fungi. Known primarily for its role as a plant pathogen, this genus has garnered significant considerable attention in recent years due to its ability to produce a wide range of chemically novel and diverse metabolites. While *Pestalotiopsis* is an appendage-bearing conidial anamorphic fungus predominantly associated with plant diseases, only a few species of *Pestalotiopsis* have been linked with human infections. Notably, in immunocompromised individuals, these fungi can act as opportunistic pathogens, leading to rare conditions such as mycetoma [36].

The taxonomy of the *Pestalotiopsis* genus is highly complex and diverse. Despite numerous reports in the literature, the classification and understanding of its species remains incomplete, encompassing both well-known and lesser-studied taxa [37,38]. This study aims to enhance the understanding of *Pestalotiopsis* species associated with *Camellia japonica* L. plant infections, an ornamental plant grown in pots and used in public gardens in Romania. Since reports of *Pestalotiopsis* in Romania are limited, it is essential to study and accurately identify the species present, particularly in *Camellia* plants under these specific cultivation conditions. A thorough understanding of the species and genetic diversity of these pathogens is essential for effective disease management strategies. Given the distinct morphological traits of *Pestalotiopsis* strain, molecular techniques are indispensable for precise species identification [39].

Given the limited research of *Pestalotiopsis* spp. in Romania, the strain characterized in this study, both morphologically and molecularly, may serve as a valuable reference for future screening of resistant plant varieties and the development of highly effective biological and chemical control agents. As a result, this study could provide researchers with a foundational resource for investigating pestalotioid species that are crucial for the production of biologically active secondary metabolites.

Over the past few decades, numerous secondary metabolites have been isolated and characterized from *Pestalotiopsis* species. Recent studies highlight the identification of approximately 384 *Pestalotiopsis* species across diverse ecological habitats. Although the majority of these species remain poorly studied, many have the potential to produce secondary metabolites with unique bioactivities, which are particularly valuable in pharmacology applications and in the food industry. These bioactivities, including anticancer, antifungal, antibacterial, and nematocidal activities, continue to be the focus of intensive investigation [40–42].

The molecular identification of the isolated fungus, *Pestalotiopsis* sp., was verified through PCR using fungal-specific nuclear ribosomal internal transcribed spacer (ITS) primers. The PCR amplification produced an amplicon of approximately 564 bp, corresponding to the LSU regions, which is the targeted region of interest.

Phylogenetic analysis of the coded isolate P_CJ_24 revealed its association with several *Pestalotiopsis* species. For instance, *Pestalotiopsis portugallica* is known to cause petal disease on *Camellia japonica* L., adversely affecting its ornamental value [43]. *Pestalotiopsis eugeniae* has been identified as the causal agent of fruit rot in wax apples [44]. The role of *Pestalotiopsis microspora* remains ambiguous, as it has been reported both as a pathogen, causing various symptoms on numerous host plants, and as an endophyte that does not induce disease [45]. Furthermore, *P. microspora* has attracted significant attention for its potential to degrade synthetic plastics and for producing bioactive compounds with antimicrobial, antioxidant, and anticancer properties. However, due to the uncertainties regarding its distribution in Europe and globally, along with its endophytic nature, *P. microspora* is not listed in the Commission Implementing Regulation (EU) 2019/2072 [46].

As previously mentioned, *Pestalotiopsis* species exhibits dual behavior, functioning either as endophytes or as pathogenic fungi [47,48]. For example, *Pestalotiopsis kenyana* is

responsible for causing leaf spot disease on *Rhododendron agastum* Balf. f. & W. W. Sm. [49]. Beyond plant tissues, *Pestalotiopsis* species occupy various ecological niches, including oceans, rivers, lakes, air, and soil, as exemplified by *Pestalotiopsis papuana* [50].

4. Materials and Methods

4.1. Diseased Sample Collection and Fungus Isolation

Pestalotiopsis spp. presence was observed on April 10, 2024, to *Camellia japonica* L. plants grown in pots in a public garden located in Iasi city, Romania (GPS Co-ordinates 47°09'39.8" N 27°35'18.8" E). Multiple infected tissues of *Camellia japonica* L. host plant were collected and investigated in the research laboratory of the Phytopathology discipline within the “Ion Ionescu de la Brad” Iasi University of Life Sciences (IULS). The fruiting bodies were examined and observed first under stereomicroscopes (Motic® SMZ-168 Series, Motic, Wetzlar, Germany) then the pathogen was isolated as follows. The infected twigs were washed under running tap water and then rinsed with distilled water. Diseased twigs were surface sterilized with 0.1% mercuric chloride for 1 min, well rinsed then with distilled water for three times and blotted dry with sterilized filter papers [51]. Then were cut into small pieces of twig tissues and placed on Potato Dextrose Agar (PDA) plates. Thus, prepared plates were incubated for 10 days at 25 °C. The growing mycelial tips were aseptically transferred to new PDA plates in order to obtain pure culture [52,53]. Purified isolate was coded P_CJ_24 and used then for the molecular analysis and identification.

4.2. Pathogen Identification—Morphologic

The pathogenicity of P_CJ_24 isolate was tested on camellia leaves 2-year-old potted that were surfaced disinfected with 75% ethanol. About 20 camellia leaves were inoculated with a conidial suspension (8 µL) that was placed at wounded and no wounded sites on the middle of the camellia leaves [16]. Conidial suspension for camellia leaves inoculations was prepared according to the protocol of Chen et al. [54]. Other 20 camellia leaves inoculated with only sterile water served as controls. Inoculated camellia leaves were covered with plastic bags to maintain high relative humidity for 2 days and maintained in the greenhouse at 25 ± 2 °C until disease symptoms appeared.

4.3. DNA Extraction and Sanger Sequencing

DNA extraction was done from purified isolate P_CJ_24 using the PCR BIO Rapid Extract Lysis Kit (<https://pcrbio.com/row/products/dna-extraction/pcrbio-rapid-extract-lysis-kit/>, accessed on 26 May 2024, PCR Biosystems Ltd., London, UK). PCR amplification and purification were performed in accordance with the manufacturer's instructions, using the Platinum™ PCR SuperMix High Fidelity (<https://www.thermofisher.com/order/catalog/product/12532016>, accessed on 26 May 2024, Thermo Fisher Scientific, Waltham, MA, USA) and the ExoSap (<https://www.thermofisher.com/order/catalog/product/78205.10.ML>, accessed on 26 May 2024, Thermo Fisher Scientific, Waltham, MA, USA). PCR targets were the nuclear ribosomal internal transcribed spacer (ITS) loci, with an amplicon size of 564 bp. Sanger sequencing of the amplicon was performed by the BMR-Genomics (via Redipuglia 21/A, Padova, 35131, Italy).

4.4. Phylogenetic Analysis of DNA Sequence

DNA sequence of the PCR product was submitted to rDNA homology sequence alignment using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 6 July 2024) against the NCBI GenBank (GenBank; <http://www.ncbi.nlm.nih.gov/BLAST/incidence.html>, accessed on 6 July 2024) to verify the genetic similarity of other sequences. Phylogenetic analyses were carried out using the NCBI-BLAST tool with the method “neighbor

joining”, with a maximum seq difference of 0.5, as previously described by Florea et al. 2023 [55].

5. Conclusions

Understanding the optimal environmental conditions and life cycles of host species is essential for preventing infections caused by various pathogens. An effective integrated management program should incorporate knowledge of environmental factors, mechanisms of disease spread, and consistent monitoring and sampling practices. These measures are particularly important for minimizing the occurrence of common diseases, especially those caused by fungi.

This study represents the first report of *Pestalotiopsis* sp. isolated from *Camellia japonica* L. in Romania, shedding light on the pathogenicity of *Pestalotiopsis* species, which diminished the ornamental and economic value of these plants. Furthermore, additional research is needed to explore the germination and dormancy requirements of *Pestalotiopsis* sp., particularly in plant species of horticultural and agronomical significance.

Ultimately, a deeper understanding of the *Pestalotiopsis* genus will pave the way for the development of sustainable and effective management strategies to reduce its impact on economically and ecologically significant plant species. This study serves as a foundation for future research into the intricate relationship between *Pestalotiopsis* sp. and *Camellia japonica* L. ornamental plants.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae11010093/s1>, File S1: Sanger Sequencing results of isolate P_Cj_24.

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