

Article

Occurrence of Black Spot Caused by *Alternaria alternata* on *Celosia cristata* L. in Henan Province, China and In Vitro Screening of Control Agents

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Abstract

Celosia cristata is an important ornamental and medicinal plant widely cultivated in China. In July 2025, a severe black spot disease was observed on *C. cristata* in the Zhang Zhongjing Medicinal Plant Garden, Nanyang, Henan Province, China, resulting in substantial defoliation and yield loss. To identify the causal agent and provide a scientific basis for disease management, the pathogen was isolated and purified from diseased tissues using the tissue isolation method. The pathogen was identified based on morphological characteristics, multi-gene sequence analysis (ITS, *RPB2*, and *TEF1- α*), and pathogenicity tests conducted in accordance with Koch's postulates. Seven isolates with uniform morphological features were obtained. Sequence analysis and multilocus phylogenetic reconstruction indicated that the isolates shared 99–100% identity with *Alternaria alternata*. Pathogenicity tests demonstrated that the isolates induced typical black spot symptoms on healthy plants. To our knowledge, this is the first report of *A. alternata* causing black spot disease on *C. cristata* in Henan Province. In vitro effective fungicides were screened, among which mancozeb demonstrated the strongest inhibitory activity against *A. alternata*, exhibiting an EC₅₀ value of 97.72 mg/L. Additionally, the biocontrol agent *Trichoderma harzianum* achieved a mycelial growth inhibition rate of 69.95% against the pathogen. These findings provide a scientific foundation for the future prevention and control of black spot disease in *C. cristata*.

Keywords: *Celosia cristata*; black spot disease; *Alternaria alternata*; isolation and identification; fungicide screening



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1. Introduction

Celosia cristata L. is an annual herbaceous plant belonging to the genus *Celosia* in the family Amaranthaceae, renowned for its distinctive flattened, fleshy, spicate inflorescences. Native to tropical regions of Africa and Asia, it is now widely cultivated throughout both northern and southern China and is predominantly distributed in tropical and subtropical areas [1]. In addition to its ornamental value in landscape enhancement and aesthetic appeal, it also exhibits significant medicinal potential. In China, *C. cristata* has been used as a medicinal plant for more than a thousand years, exhibiting pharmacological activities such as hemostatic, anti-inflammatory, antioxidant, antiviral, and immunomodulatory effects [2,3]. *C. cristata* prefers environments with abundant sunlight and warm, humid conditions; therefore, sufficient light is required throughout its entire growth period. The optimal growth temperature ranges from 15 to 30 °C. The species is not highly demanding

with respect to soil type and can be cultivated in most soils; however, loose, fertile, and well-drained soils are most suitable [4,5]. Henan Province is located in a temperate monsoon climate zone, characterized by distinct seasons, hot and rainy summers, clear and dry autumns, and ample annual sunshine. Consequently, *C. cristata* is widely cultivated throughout the province [6]. However, particularly during the transition from spring to summer, these climatic conditions are highly conducive to the occurrence of various foliar diseases. Previous studies have confirmed that both *Alternaria alternantherae* [7] and begomoviruses [8] can cause foliar lesions in *C. cristata*. In July 2025, black spot symptoms were observed on the leaves of *C. cristata* in the Zhang Zhongjing Medicinal Plant Garden in Nanyang City. At the early stage of disease development, nearly circular, oval, or irregular lesions appeared on the leaves, exhibiting dark brown to brown coloration. As the disease progressed, the centers of the lesions became paler and concentric rings formed, accompanied by the growth of a light black mold layer on the lesion surface. Under severe infection, lesions coalesced, resulting in leaf abscission, with a disease incidence of 100% among plants examined ($n = 30$ plants surveyed). This disease severely impairs photosynthesis and normal growth of *C. cristata*, thereby reducing its ornamental value and medicinal properties.

Accurate identification of the pathogen responsible for *C. cristata* black spot disease is crucial for achieving precise disease management. Only by determining the specific pathogen species can we gain a comprehensive understanding of its biological characteristics, epidemiology, and transmission routes, which in turn facilitates the formulation of targeted, scientific, and effective control measures. However, no information is currently available regarding the etiology of black spot disease affecting *C. cristata* in Henan Province. Consequently, in this study, the pathogen was isolated from symptomatic tissues using the tissue isolation method, and its pathogenicity was verified according to Koch's postulates. Subsequently, the pathogen was identified using morphological and molecular techniques, as species delimitation within the genus *Alternaria* often requires multilocus phylogenetic analyses due to overlapping morphological characters. Furthermore, potential fungicides and biological control strategies were screened in vitro for disease control. This research aims to provide a solid theoretical foundation for the integrated management of *C. cristata* black spot, thereby ensuring the healthy cultivation of *C. cristata* and supporting the sustainable development of its related industry.

2. Materials and Methods

2.1. Pathogen Isolation and Purification

In August 2025, ten *C. cristata* plants infected with black spot disease were randomly collected from five sampling sites within the Zhang Zhongjing Medicinal Plant Garden in Nanyang City (32°58'0" N, 112°32'50" E; altitude 116 m). The samples were immediately transported to the laboratory, where the pathogen causing black spot disease was isolated using the tissue isolation method. From each diseased leaf, tissues at the junction between healthy and diseased areas were selected and cut into approximately 5 mm × 5 mm pieces using sterilized scissors. The tissue pieces were sequentially immersed in 75% ethanol for 30 s and in 0.1% mercuric chloride solution (following institutional biosafety regulations) for 2 min for surface sterilization to eliminate epiphytic microorganisms. After sterilization, the tissues were rinsed 3–5 times with sterile distilled water to remove residual disinfectants. The treated tissue pieces were then placed onto potato dextrose agar (PDA) plates, with 3–4 pieces per plate evenly distributed. The plates were then incubated at 25 °C in a constant-temperature incubator and monitored daily for colony development. Once fungal colonies emerged, hyphal tips from the colony margins were transferred to fresh PDA plates for purification. This purification procedure was repeated three times until pure

single-colony isolates were obtained. The purified isolates were finally transferred onto PDA slants and stored at 4 °C for subsequent use.

2.2. Morphological Identification

The purified isolates were put onto the PDA plates, with one mycelial plug of 5 mm in diameter placed on each plate. The inoculated plates were incubated in a constant-temperature incubator at 25 °C. Colony growth was monitored periodically, and macroscopic characteristics such as colony morphology, color, and texture were recorded. After 10 days of cultivation, the micro-morphological characteristics of the pathogen were examined under a light microscope (DM1000, Leica, Germany Wetzlar) using the slide preparation method. Specifically, a small amount of vigorously growing mycelia and conidia was collected and placed on a glass slide, to which a drop of distilled water was added. The conidia were evenly dispersed using a dissecting needle, and a coverslip was gently applied to prepare a temporary mount. Under the light microscope, the morphology, color, and mode of attachment of conidiophores, as well as the shape, size, color, and septation of conidia, were observed. Simultaneously, 50 conidia were randomly selected for size measurement. The observed morphological features were compared with existing morphological descriptions in the literature on *Alternaria* species to preliminarily determine the taxonomic identity of the pathogen.

2.3. DNA Extraction, Amplification and Sequencing

Five randomly selected isolates were subjected to DNA extraction and sequencing. The selected isolates were first cultured on PDA medium, and mycelia were harvested after 7 days of incubation. Genomic DNA was then extracted from the mycelia using a fungal DNA extraction kit (Beijing Tsingke Biotech Co., Ltd., Beijing, China) in accordance with the manufacturer's instructions. DNA concentration and purity were assessed spectrophotometrically. Polymerase chain reaction (PCR) amplification was performed using the universal primer pairs ITS1/ITS4 [9] for the internal transcribed spacer (ITS) region of ribosomal DNA, rpb2-5F/rpb2-7CR [10] for the second largest subunit of RNA polymerase II (RPB2) region, and EF1-728F/EF1-986R [10] for the translation elongation factor 1- α (TEF1- α) region. All amplification reactions were conducted using a Bio-Rad T100 thermal cycler (Bio-Rad California, Hercules, USA). Each PCR was carried out in a total volume of 50 μ L, containing 45 μ L of 1 $\times$ TSE102 PCR mix, 2 μ L each of forward and reverse primers (10 pM), and 1 μ L of template DNA. The primer sequences and PCR cycling conditions are listed in Table 1.

Table 1. Gene loci and primers used in this study.

Gene	Primer	Sequence (5'–3')	PCR Conditions
ITS	ITS1 (F)	5'-TCCGTAGGTGAACCTGCGG-3'	98 °C 2 min, (98 °C 10 s, 54 °C 10 s, 72 °C 10 s) 35 cycles, 72 °C 5 min
	ITS4 (R)	5'-TCCTCCGCTTATTGATATGC-3'	
TEF1- α	EF1-728 (F)	5'-CATCGAGAAGTTCGAGAAGG-3'	98 °C 2 min, (98 °C 10 s, 54 °C 10 s, 72 °C 10 s) 35 cycles, 72 °C 5 min
	EF1-986R (R)	5'-TACTTGAAGGAACCCTTACC-3'	
RPB2	rpb2-5 (F)	5'-GAYGAYMGWGATCAYTTYGG-3'	98 °C 2 min, (98 °C 10 s, 55 °C 10 s, 72 °C 10 s) 35 cycles, 72 °C 5 min
	rpb2-7C (R)	5'-CCCATRGCTTGYTTRCCCAT-3'	

After verification by 1% agarose gel electrophoresis, qualified PCR products were submitted to Beijing Tsingke Biotechnology Co., Ltd. Beijing, China for bidirectional Sanger sequencing. The resulting sequences were deposited in the GenBank database (<http://www.ncbi.nlm.nih.gov>, accessed on 6 February 2026). Homology analyses were then performed using the nucleotide BLAST program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 6 February 2026). Sequences of closely related strains, together with those of

type strains, were downloaded and imported into MEGA-X (version X) [11] for alignment using the Clustal W algorithm, and saved in MEGA format. Subsequently, a multigene concatenated phylogenetic tree (ITS-*RPB2*-*TEF1-α* gene sequences) was constructed using the maximum likelihood method, with bootstrap analysis based on 1000 replicates. The taxonomic position of the isolated strains within fungal classification was determined by analyzing the topology of the phylogenetic tree.

2.4. Pathogenicity Assays

The pathogenicity of the isolates was evaluated according to the method described by Yang et al. (2022) [12], with minor modifications. Uniformly growing and healthy *C. cristata* seedlings were selected. Leaf surfaces were sterilized with 75% ethanol, rinsed thoroughly with sterile water, and air-dried. The purified isolates (JGHB-2 to JGHB-3) were situated onto PDA medium and incubated at 25 °C for 10 days. Conidia were then harvested to prepare a conidial suspension, and the concentration was adjusted to 1×10^6 conidia m^{-1} using a hemocytometer. The conidial suspension was applied onto the leaf surfaces using a micropipette. Each leaf received two drops ($10 \mu L \text{ drop}^{-1}$) of the suspension, and three leaves per plant were inoculated. Control plants were mock-inoculated with sterile water using the same procedure. Five plants were used for each treatment. After inoculation, the plants were maintained in an artificial climate chamber at 25 °C, with relative humidity $\geq 90\%$ and a 12 h light/12 h dark photoperiod. Disease symptoms were observed daily, and the onset of disease, lesion morphology, and lesion expansion were recorded. After symptom development, the pathogen was re-isolated from diseased tissues following the standard procedures for pathogen isolation and purification, and the isolates were cultured and identified. The pathogenicity of the isolate was confirmed when the re-isolated pathogen exhibited morphological characteristics identical to those of the originally inoculated strain.

2.5. Effects of Control Agents on Pathogens

The sensitivity of the *C. cristata* black spot pathogen to 80% mancozeb and 75% chlorothalonil (Syngenta [Suzhou] Crop Protection Co., Ltd. Suzhou, China) was determined using the mycelial growth rate method [13]. First, PDA plates containing different concentrations of the fungicides were prepared. Specifically, five concentration gradients were established for 80% mancozeb (15.63, 31.25, 62.5, 125, and 250 mg/L) and 75% chlorothalonil (312.5, 625, 1250, 2500, and 5000 mg/L) and set aside for use. The test strain was pre-cultured on sterile PDA plates at 25 °C under light for 7 days. Mycelial plugs (5 mm in diameter) were then cut from the margins of the colonies and placed individually onto the center of the fungicide-amended PDA plates. PDA plates without fungicides served as the control, and each treatment was performed in triplicate. After incubation at 25 °C under light for 7 days, colony diameters were measured using the cross-measurement method, and the mean value for each treatment was calculated. The inhibition rate of mycelial growth under different fungicide treatments was calculated using the following formula (where D1 represents the control colony diameter, D2 represents the treated colony diameter, and D0 represents the mycelial plug diameter):

$$\text{Antifungal rate (\%)} = (D1 - D2)/(D1 - D0) \times 100 \quad (1)$$

The toxicity regression equations (where x represents the logarithm of the fungicide concentration and y represents the inhibition rate) and correlation coefficients for each fungicide were calculated using Microsoft Excel 2019. The median effective concentration (EC₅₀) values of the fungicides and their 95% confidence intervals were determined using DPS software (version 9.01).

To evaluate the antifungal activity of *Trichoderma harzianum* T22 (Zhengzhou Zhongke Chemical Products Co., Ltd. Zhengzhou, China) against the black spot pathogen of *C. cristata*, a dual-culture assay was employed. Actively growing mycelial plugs (0.5 cm in diameter) of both *T. harzianum* (cultured for 3 days) and the pathogen strain (cultured for 5 days) were placed on PDA plates at a distance of 4.5 cm from each other. A control group, having solely the pathogen plug, was established. Each treatment was performed in triplicate. Experiments were conducted under a completely randomized design. The inoculated Petri dishes were incubated in a constant-temperature incubator at 25 °C. Once the control colony had nearly covered one side of the Petri dish, the radius of the control colony (Rc) and the radius of the pathogen colony growing toward the *T. harzianum* strain (Rp) were measured. The inhibition rate was calculated using the following formula, with three replicates performed for each strain:

$$\text{Inhibition rate (\%)} = (R_c - R_p) / (R_c - 0.25) \times 100 \quad (2)$$

2.6. Statistical Analysis

The inhibition rate data were analyzed by one-way analysis of variance (ANOVA) and Tukey's multiple range test ($p < 0.05$) using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Description of Symptoms

A survey conducted in the Zhang Zhongjing Medicinal Plant Garden of Nanyang Institute of Technology revealed that black spot disease of *C. cristata* primarily affects the leaves, with a disease incidence reaching 100% ($n = 30$). At the early stage of infection, nearly circular, elliptical, or irregular lesions appear on the leaf surface. The lesions are dark brown to brown in color and are clearly demarcated from healthy tissue. As the disease progresses, the lesions gradually enlarge; at later stages, the central areas become paler, turning grayish white or light brown, and conspicuous concentric rings develop (Figure 1). Under humid conditions, a light black mold layer forms on the lesion surface, consisting of the pathogen's conidiophores and conidia. In severely affected areas, multiple lesions coalesce, resulting in extensive leaf chlorosis and necrosis, eventually leading to leaf abscission. This disease potentially impairs the growth and ornamental value of *C. cristata*.



Figure 1. Field symptoms of black spot disease on *C. cristata*.

3.2. Morphological Characterization of the Pathogen

From the collected diseased leaf tissues, seven isolates with similar colony morphologies were successfully obtained through repeated isolation and purification. When cultured on PDA medium, colonies were initially white and floccose, gradually turning grayish

black to black with prolonged incubation. The colony margins were entire and circular, with well-developed aerial mycelia and a loose texture (Figure 2A–D). Light microscopic observations revealed distinctive morphological characteristics of the conidiophores and conidia. Conidiophores were solitary or in clusters, dark brown in color, and bore conidia that were often arranged in chains. The conidia were morphologically diverse, predominantly clavate, but also ovoid or obpyriform, brown in color, and possessed multiple transverse, longitudinal, or oblique septa, with conspicuous constrictions at the transverse septa (Figure 2E–H). Conidia measured $19.11\text{--}43.11$ (mean 29.12 ± 5.46) $\times$ $10.22\text{--}17.33$ (mean 13.77 ± 1.63) μm ($n = 50$). Based on colony characteristics and conidial morphology, the isolates were preliminarily identified as belonging to the genus *Alternaria* [10,14].

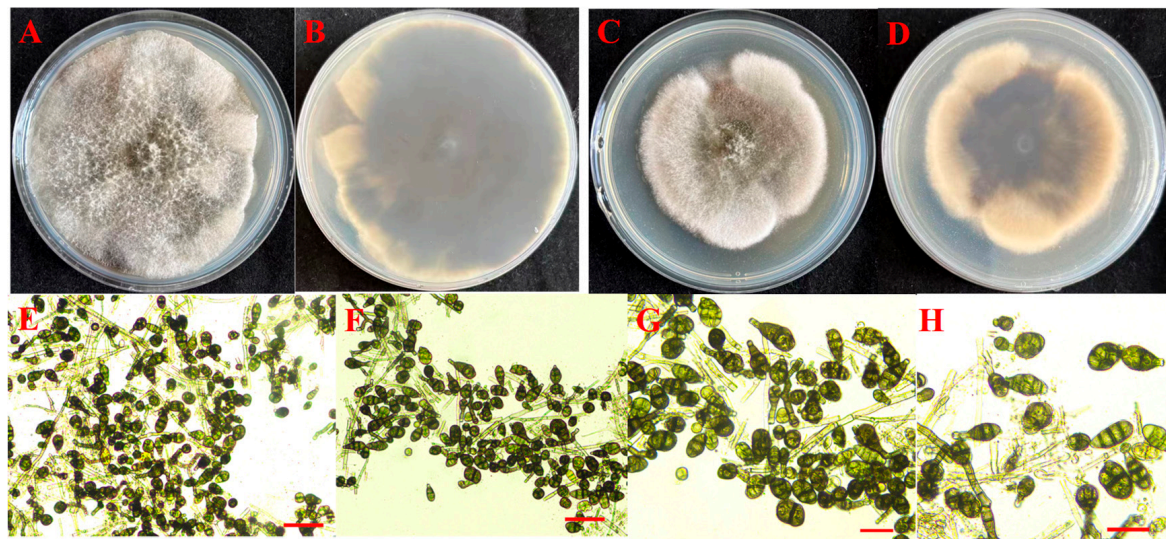


Figure 2. Colony and conidial morphological characteristics of *A. alternata*. (A–C). Frontal (A,C) and reverse (B,D) views of cultural features of *A. alternata* on PDA. (E–H). Conidia. Scale bars = 50 μm in (E,F). Scale bars = 25 μm in (G,H).

3.3. Molecular Characterization and Phylogenetic Analysis

To further precisely determine the taxonomic identity of the isolates causing black spot disease of *C. cristata*, five representative isolates (JGHB-1 to JGHB-5) were randomly selected for additional gene sequencing and comparative analysis. The resulting sequences were deposited in GenBank under the following accession numbers: ITS (PX945268–PX945269, PZ203761–PZ203763), *RPB2* (PX960074–PX960075, PZ225211–PZ225213), and *TEF1- α* (PX960072–PX960073, PZ225208–PZ225210). BLAST analyses were performed using the NCBI database. The results showed that all obtained sequences shared 99–100% identity with corresponding sequences of *Alternaria alternata*. Furthermore, the corresponding ITS, *RPB2*, and *TEF1- α* gene sequences were aligned with the homologous loci of 14 related type strains (Table S1) within the same genus. A phylogenetic tree based on ITS, *RPB2*, and *TEF1- α* sequences were constructed using the maximum likelihood method. As shown in Figure 3, isolates JGHB-1 to JGHB-5 clustered within the *Alternaria alternata* species complex with a bootstrap value of 97%, indicating strong phylogenetic support. Based on the combined results of molecular and morphological identification, the pathogenic isolates JGHB-1 to JGHB-5 were identified as *A. alternata*.

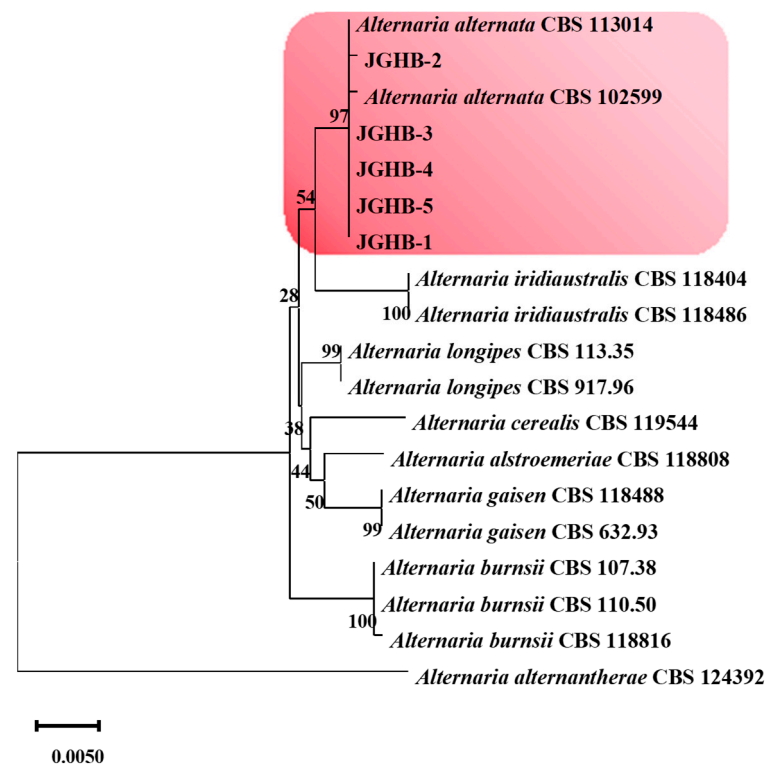


Figure 3. Phylogenetic tree generated based on the combined ITS, *RPB2* and *TEF1- α* gene sequences.

3.4. Pathogenicity Test

Two randomly selected isolates (JGHB-2 and JGHB-3) were evaluated for pathogenicity. A prepared spore suspension of the isolate was inoculated onto healthy *C. cristata*. Under moist incubation conditions, inoculated leaves began to exhibit symptoms after 3 days. At the early stage, pinpoint-sized brown spots appeared on the leaf surface; these lesions gradually expanded and developed into black spot symptoms consistent with those observed in the field. The lesions were nearly circular, elliptical, or irregular in shape and ranged in color from dark brown to brown (Figure 4A–D). In contrast, control leaves inoculated with sterile water showed no disease symptoms (Figure 4E). The pathogen was re-isolated from the diseased leaves, and subsequent culturing and morphological observations demonstrated that the re-isolated organism exhibited morphological characteristics identical to those of the original inoculated strain. Through these procedures, Koch's postulates were fulfilled, confirming that this isolate is the causal pathogen of black spot disease in *C. cristata*.

3.5. Inhibitory Effects of Fungicides and *T. harzianum* on the Pathogen Causing Black Spot Disease on *C. cristata*

This study evaluated the toxicity of two fungicides, 80% mancozeb and 75% chlorothalonil, against the mycelial growth of *A. alternata*, the causal agent of black spot disease on *C. cristata*. The inhibitory effects of both fungicides on the mycelium exhibited a concentration-dependent relationship, with the inhibition rate progressively increasing as the fungicide concentration rose (Tables 2 and 3, Figure 5). Based on the measured inhibition rates, toxicity regression equations for the two fungicides were established, and their EC_{50} values were calculated. The correlation coefficient (R^2) of the toxicity regression equations for the two fungicides were 0.9824 and 0.9962, respectively (Table 4). Both values approached 1, indicating a high degree of linear fit between fungicide concentration and the inhibition rate, thereby confirming the reliability of the experimental data. A substantial difference was observed in the EC_{50} between the two fungicides: the EC_{50} of 80% mancozeb

was only 97.72 mg/L, whereas that of 75% chlorothalonil reached 2469.64 mg/L. These results demonstrate that mancozeb exhibits stronger inhibitory activity against this black spot pathogen, with a toxicity significantly superior to that of chlorothalonil.

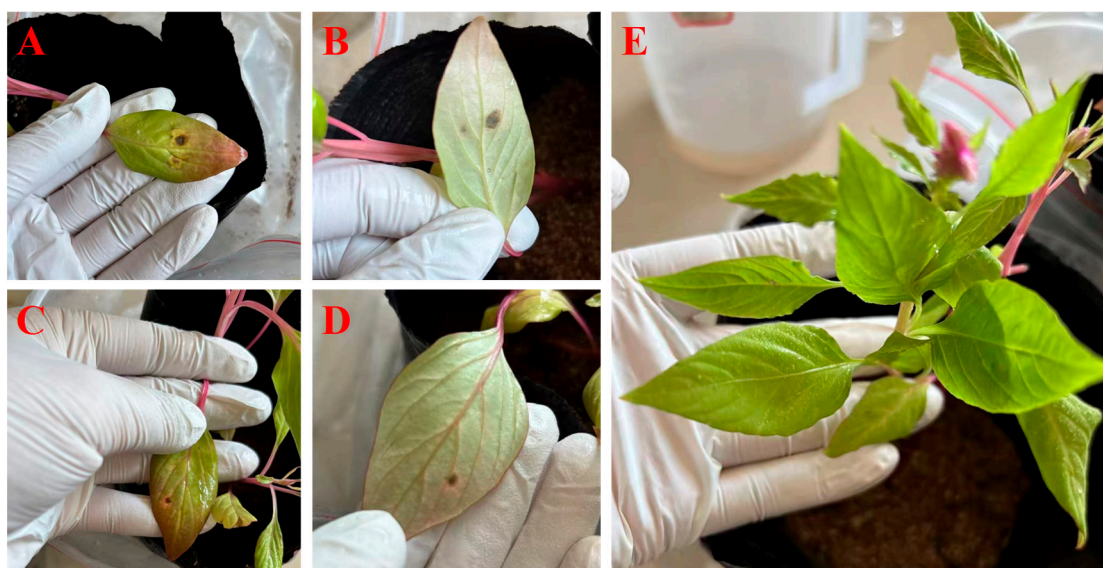


Figure 4. Symptoms on *C. cristata* leaves after inoculation of a conidial suspension. (A–C). Frontal (A,C) and reverse (B,D) views of symptoms on *C. cristata* leaves. (E). *C. cristata* leaves remained healthy for seven days when being mock-inoculated with sterile water.

Table 2. Inhibitory rate of 80% mancozeb at different concentrations against the mycelial growth of *A. alternata*.

Fungicide Concentration (mg/L)	Average Inhibition Rate (%)
15.63	17.79 ± 3.78 ^d
31.25	34.05 ± 8.10 ^{cd}
62.5	45.40 ± 2.30 ^{bc}
125	52.76 ± 0.87 ^{ab}
250	64.11 ± 5.96 ^a

Data for the average inhibition rate are presented as mean ± SD (n = 3). Letters to the right of the values indicate significant differences between treatments ($p < 0.05$).

Table 3. Inhibitory rate of 75% chlorothalonil at different concentrations against the mycelial growth of *A. alternata*.

Fungicide Concentration (mg/L)	Average Inhibition Rate (%)
312.5	26.35 ± 4.05 ^d
625	33.53 ± 2.76 ^{cd}
1250	41.35 ± 3.47 ^{bc}
2500	49.36 ± 4.84 ^{ab}
5000	59.29 ± 2.94 ^a

Data for the average inhibition rate are presented as mean ± SD (n = 3). Letters to the right of the values indicate significant differences between treatments ($p < 0.05$).

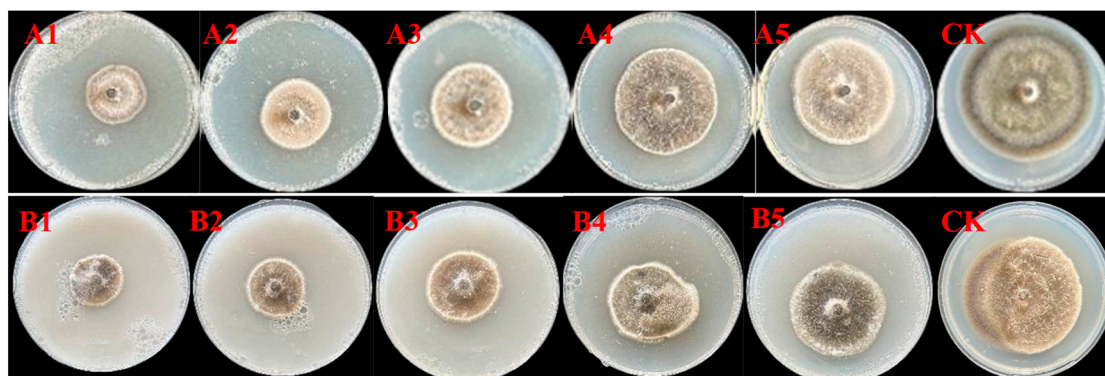


Figure 5. Effects of different fungicides on mycelial growth. (A) Mancozeb. The concentrations corresponding to (A1–A5) are 250, 125, 62.5, 31.25, and 15.63 mg/L, respectively. (B) Chlorothalonil. The concentrations corresponding to (B1–B5) are 5000, 2500, 1250, 625, and 312.5 mg/L, respectively. CK: blank control.

Table 4. Toxicity of different fungicides on the mycelial growth of the pathogen causing black spot disease on *C. cristata*.

Fungicide Name	Toxic Regression Equation	Correlation Coefficient (R^2)	EC ₅₀ (mg/L)	95% CI
80% mancozeb	$y = 0.3699x - 0.2361$	0.9824	97.72	79.02~124.88
75% chlorothalonil	$y = 0.2715x - 0.4211$	0.9962	2469.64	2341.53~2618.62

In this study, the dual culture method was employed to systematically evaluate the inhibitory effect of *T. harzianum* on the mycelial growth of the *C. cristata* black spot pathogen. The results of the dual culture assay demonstrated that *T. harzianum* exhibited a significant antagonistic effect against the pathogen, markedly suppressing its colony growth (Figure 6). The calculated average inhibition rate reached $69.95\% \pm 5.00$ ($n = 3$), indicating that this biocontrol fungus has promising potential for the biological control of the fungus causing black spot disease on *C. cristata*.

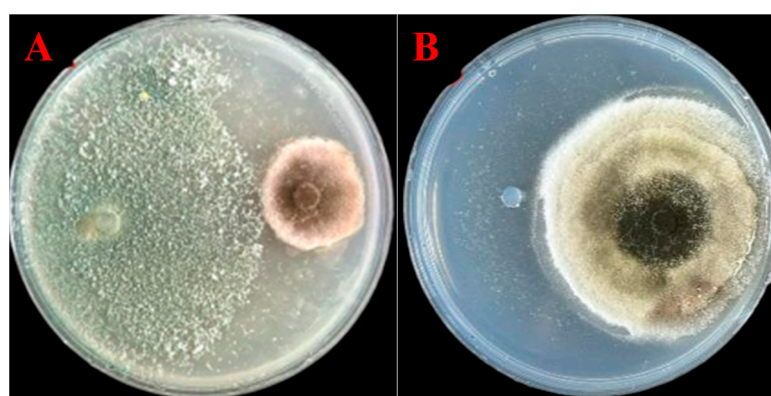


Figure 6. Inhibitory effect of *T. harzianum* on the mycelial growth of the pathogen causing black spot disease on *C. cristata*. (A) *T. harzianum* treatment. (B) Control.

4. Discussion

C. cristata is not only highly ornamental; in China, its dried inflorescences are also used as a traditional medicinal material with astringent and hemostatic properties and are included in the Chinese Pharmacopoeia [3,15]. In this study, *C. cristata* black spot disease occurring in the Zhang Zhongjing Medicinal Plant Garden in Nanyang City, Henan

Province, was investigated. Seven morphologically similar fungal isolates were obtained using the tissue isolation method. Following purification, the pathogen was identified as *A. alternata* based on colony characteristics, conidial morphology, multi-gene combined sequencing, and phylogenetic analysis, and its pathogenicity was further confirmed by Koch's postulates. This study represents the first report of black spot disease caused by *A. alternata* on *C. cristata* plants in Henan Province, providing a valuable reference for the precise prevention and control of this disease.

Alternaria spp. belong to the phylum Ascomycota, class Dothideomycetes, order Pleosporales, family Pleosporaceae, and genus *Alternaria*. Species of the genus *Alternaria* are ubiquitous plant pathogenic fungi with a broad host range and high species diversity; to date, more than 300 *Alternaria* species have been reported [16]. These pathogens can infect cereal crops, economic crops, and ornamental plants, causing various diseases such as leaf spot and black spot. However, the precise identification of *Alternaria* species remains challenging. The taxonomy of this genus has undergone extensive revision, and reliable species delimitation typically requires a combination of morphological characterization and multi-locus phylogenetic analysis [10]. As a representative species of the genus, *A. alternata* exhibits pathogenicity chiefly through the dissemination and infection of conidia. The conidia are dispersed to host surfaces via air currents and rain splash, where they germinate under favorable conditions to produce hyphae that penetrate the host epidermis, invade plant tissues, and secrete toxins that disrupt cellular structures, ultimately leading to lesion formation [17,18]. *A. alternata* has been reported to cause diseases in a wide range of crops, including tamarillo (*Solanum betaceum*) [19], honeysuckle (*Lonicera japonica*) [20], kiwiberry (*Actinidia arguta*) [21], *Menispermum dauricum* [22], kidney bean (*Phaseolus vulgaris*) [23], *Yucca gloriosa* [24], and *Pinus bungeana* [25]. These reports collectively demonstrate the remarkable ecological adaptability and host plasticity of *A. alternata*. In the present study, symptoms appeared on *C. cristata* leaves as early as 3 days after inoculation with *A. alternata*, indicating its strong infective capacity and virulence.

The climatic conditions of the Nanyang region provide a favorable environment for the growth, reproduction, and infection of *A. alternata*. This area is characterized by a temperate monsoon climate, with hot and rainy summers and abundant annual sunshine. The average temperature during July–August ranges from 25 to 30 °C, and relative humidity can exceed 80% [26], which closely matches the optimal temperature for *A. alternata* culture (25 °C) and the high-humidity conditions required for pathogenicity assays [27,28]. High temperature and humidity not only promote conidial germination and mycelial growth of *A. alternata*, but also prolong spore viability and enhance infection efficiency [29]. In addition, frequent summer rainfall facilitates spore dispersal, thereby accelerating disease spread and epidemic development [30,31]. In summary, the climatic characteristics and field cultivation conditions in the Nanyang region are highly consistent with the ecological adaptability of *A. alternata*, constituting a key environmental factor contributing to the occurrence of black spot disease on *C. cristata*. Accordingly, preventive fungicide applications should be implemented in advance during periods of high temperature and humidity, and rational planting density should be adopted to improve field ventilation, thereby reducing pathogen infection and dissemination.

Regarding the screening of chemical control agents, the results of this study demonstrated that 80% mancozeb exhibited significantly higher inhibitory activity against *A. alternata* than 75% chlorothalonil, with an EC₅₀ value of only 97.72 mg/L, which is substantially lower than that of chlorothalonil (2469.64 mg/L). As a broad-spectrum protective fungicide, mancozeb acts through a multi-site mechanism that inhibits the activity of sulfhydryl-containing enzymes in fungi and disrupts their metabolic processes, thereby reducing the likelihood of pathogens developing resistance [32]. Mancozeb exhibits strong

inhibitory activity against a broad spectrum of plant pathogens. Previous studies have reported that 43% mancozeb has an EC_{50} value of 202.1461 mg/L against *Colletotrichum aenigma*, the causal agent of leaf blight on *Aucuba japonica* [33], while 70% mancozeb exhibits an EC_{50} value of 447.760 mg/L against *Fusarium solani*, the pathogen responsible for white root rot in *Bletilla striata* [34]. These findings collectively demonstrate the high efficacy of mancozeb in suppressing plant pathogenic fungi. Although mancozeb and chlorothalonil have been banned by the European Union, they continue to be widely applied in numerous countries and regions due to their low toxicity and cost-effectiveness. In China, these two fungicides are commonly utilized to control foliar diseases in various crops. Nevertheless, given their potential risks to human health and environmental hazards, their practical application must strictly adhere to relevant regulatory standards to ensure compliant and safe usage. However, the long-term and exclusive application of chemical pesticides can lead to environmental pollution and pesticide residues, which demands particular caution, especially when applied to medicinal plants. Therefore, this study also evaluated the antagonistic efficacy of the biocontrol agent *T. harzianum* against *A. alternata*. Dual-culture assays showed a mean inhibition rate of 69.95%, indicating that *T. harzianum* has substantial antagonistic activity against the pathogen causing black spot in *C. cristata*, potentially mediated by competition, mycoparasitism, and antibiosis [35]. These findings clearly demonstrate that *T. harzianum* possesses outstanding potential for biocontrol development, making it a promising candidate strain for the eco-friendly management of *C. cristata* black spot disease. Future studies should conduct in vivo pot inoculation assays and field efficacy trials to further evaluate the strain's control stability, colonization capacity, and environmental adaptability against *Alternaria* pathogens under natural conditions.

By leveraging the respective advantages of chemical and biological control, we recommend adopting an integrated management strategy that combines both approaches in practical cultivation. Although this study provides a scientific basis for understanding the disease cycle and implementing precise management of *C. cristata* black spot, further research is warranted. Future studies should focus on the differentiation of pathogen pathogenicity, variations in disease resistance among different *C. cristata* cultivars, and the evaluation of fungicide efficacy under field conditions to provide more comprehensive technical support for the sustainable, long-term control of this disease.

5. Conclusions

In this study, black spot disease affecting *C. cristata* in Nanyang, Henan Province, China, was systematically investigated. The causal pathogen was identified as *A. alternata* based on morphological characteristics, multi-gene (ITS, *RPB2*, and *TEF1- α*) phylogenetic analysis, and the fulfillment of Koch's postulates. To our knowledge, this represents the first report of *A. alternata* causing black spot disease on *C. cristata* in Henan Province. Among the evaluated fungicides, 80% mancozeb exhibited the strongest inhibitory activity against *A. alternata* with an EC_{50} value of 97.72 mg/L. Additionally, the biocontrol agent *T. harzianum* inhibited mycelial growth by 69.95%, demonstrating substantial potential for biological control. These findings establish a scientific foundation for the integrated management of black spot disease on *C. cristata*.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae12081002/s1>: Table S1: Descriptions and GenBank accession numbers for the *Alternaria* species included in the phylogenetic study.

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Data Availability Statement: The nucleotide sequences generated in this study were deposited in GenBank under the following accession numbers: ITS (PX945268–PX945269, PZ203761–PZ203763), RPB2 (PX960074–PX960075, PZ225211–PZ225213), and TEF1- α (PX960072–PX960073, PZ225208–PZ225210) (<https://www.ncbi.nlm.nih.gov/genbank/>, accessed on 6 February 2026).

Conflicts of Interest: The authors declare no conflicts of interest.

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