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Posted Date: 27 February 2025

doi: 10.20944/preprints202502.2225.v1

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Article

Molecular Characterization and Pathogenicity Analysis of *Alternaria alternata* Associated with Leaf Spot Disease of *Toona sinensis* in China

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Abstract: The tree species of *Toona sinensis* has been cultivated in China for over 2,000 years with multiple purposes. In 2022, leaf spots were detected on the leaves of *T. sinensis* in a germplasm resource nursery in Zhejiang Province, China. Symptomatic leaves were collected and fungal isolation was conducted. Four fungal isolates resembling *Alternaria* were isolated from the diseased leaves. These isolates were identified as *Alternaria alternata*, combining the morphological characteristics and multigene phylogenetic analysis. To fulfill Koch's postulates, pathogenicity of the selected isolate YKHH was tested on 2-year-old container seedlings of *T. sinensis*. Disease symptoms basically consistent with those observed in the field developed 12 days after inoculation (DAI) when either conidia or mycelia were used as the inoculum. Nevertheless, no visible symptoms developed on the leaves of the control groups. The fungi were reisolated from the diseased leaves, and both the morphological characteristics as well as the three gene sequences (*GAPDH*, *RPB2*, and *TEF1-α*) of the reisolated fungi were consistent with those of the original YKHH isolate. Susceptibility trial results showed that family 55 of *T. sinensis* (F55) had the highest susceptibility to *A. alternata*, while family 28 of *T. sinensis* (F28), family 52 of *T. sinensis* (F52), and *Toona ciliata* var. *pubescens* (TCP) exhibited equal susceptibility to *A. alternata*. To the best of our knowledge, this is the first report of leaf spot disease caused by *A. alternata* on *T. sinensis* in China.

Keywords: *Toona sinensis*; *Alternaria alternata*; germplasm resources; leaf spot disease; phylogenetic tree; Koch's postulates

Introduction

Toona sinensis (A. Juss) Roem (family Meliaceae), also known as Chinese toon or Chinese mahogany, is a multi-purpose tree species that has been cultivated in China for over 2,000 years [1, 2]. The timber of *T. sinensis* is regarded as a valuable mahogany that is used for high-value furniture [2-4]. The young buds of *T. sinensis* are rich in protein, fat, vitamins, trace elements, and other nutrients, and they are non-toxic to humans [5-7]. The stems and leaves of *T. sinensis* can be used as traditional Chinese medicine for treating dysentery, enteritis, carminative and itchiness [8-10]. Till now, various bioactive compounds such as flavonoids, gallic acid, anthrones, terpenoids, phenylpropanoids, and alkaloids have been extracted and identified from its stems and leaves [8-12]. These compounds exhibit diverse pharmacological actions, including anti-cancer, anti-viral, anti-fatigue, anti-gout, anti-inflammatory, glucoregulatory, immune-enhancing, and anti-aging properties [8-10, 12-15]. Additionally, *T. sinensis* has a beautiful crown, upright trunk, and brightly colored foliage, making it suitable for planting along roadsides and in ornamental gardens [4,16].

Alternaria Nees is a ubiquitous fungal genus comprising over 300 described species [17]. Many species of *Alternaria* are pathogens of forest trees, crops, and vegetables that cause stem cankers, leaf blight, and leaf spots on various host plants [18-20]. More than 4,000 *Alternaria*–host associations are recorded in the United States Department of Agriculture Fungal Host Index, and *Alternaria* is ranked

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10th among fungal genera in terms of entire host plant records [21,22]. *Alternaria alternata* (Fr.) Keissl. is the type and most prevalent species within the genus, infecting more than 380 plant species globally [23]. In China, the pathogen reportedly causes destructive disease in *Camellia sinensis* [23], *Cannabis sativa* [24], *Citrullus lanatus* [25], *Glycine max* [26], *Malus domestica* [27,28], *Nicotiana tabacum* [29], and *Pyrus bretschneideri* [30], leading to significant economic losses. *Alternaria* species produce more than 70 phytotoxins during their infection process [31-33]. *Alternaria* phytotoxins have destructive effects on host plants, causing DNA damage, apoptosis, interrupting plant physiology through mitochondrial oxidative phosphorylation and affect membrane permeability [32,34]. In addition, these phytotoxins can cause detrimental effects on animals and humans, including fetotoxicity, cytotoxicity, and teratogenicity [32-34].

In 2022, leaf spot disease symptoms were detected on the leaves of *T. sinensis* seedlings in a germplasm resource nursery in Zhejiang Province, China. The infected leaves produced brown spots surrounded by yellow halos. However, it was unclear whether leaf spot disease on *T. sinensis* was associated with *A. alternata*. In this study, we isolated pathogenic fungus from infected leaves of *T. sinensis*, and characterized it to species level using a combination of morphological and molecular methods. Its pathogenicity was tested according to Koch's postulates. The pathogen was inoculated on the leaves of four *Toona* germplasm resources to determine their susceptibility. The aim of the study was to isolate and identify the causal agent of leaf spot disease on *T. sinensis* in Zhejiang Province. Our results provide a theoretical basis for controlling leaf spot disease in this valuable plant species.

2. Materials and Methods

2.1. Sample collection

The study was conducted in the Chinese toon germplasm resource nursery (119° 57'16"E，30° 3'43"N), located in Huzhou city, Zhejiang Province, China. The location has a typical subtropical monsoon climate, characterized by hot, humid summers and cold, windy winters, with an average annual temperature of 16.70℃. Annual precipitation of 1477.90 mm is mainly concentrated from March to September. The area of the germplasm resource nursery is about 0.67 ha, which preserves 87 germplasm resources of *Toona* with ages ranging from 1 to 5 years.

Five samples were collected from naturally infected leaves exhibiting leaf spot symptoms in the germplasm resource nursery in June 2022. Samples, identified by Prof. Jun Liu (the co-author of this paper), were placed into separate polyethylene bags and immediately transported to laboratory in a cooler box after sampling.

2.2. Fungal isolation and morphology

Fungi were isolated from the collected leaf samples using a tissue isolation approach as described by Geng et al. [35], with modifications. Briefly, the samples were washed with running water for 2 h, and the margins of leaf lesions were cut into small pieces using a sterilised scalpel. The pieces were immersed in 70% ethanol for 30 s, followed by 10% sodium hypochlorite for 5 min, rinsed five times in sterile distilled water, and blotted dry with sterile filter paper. The dry pieces were planted on potato dextrose agar (PDA) medium supplemented with streptomycin (200 mg/L) and incubated at 25℃.

Single-mycelium tip cultures of the isolates were established to ensure their genetic uniformity [36]. The purified isolates were transferred to PDA medium in Petri plates and incubated at 25°C for 4 days. The colony sizes of the isolated fungi were determined using the cross method. The cultural and morphological characteristics of isolated fungi were observed by Eclipse Ni-E microscope (Nikon France, Champigny-sur-Marne, France), and conidia (n=30) were measured using NIS-Elements D software. Morphological identification of the fungi obtained in this study was based on the descriptions of morphological and cultural characteristics of *Alternaria* species by Woudenberg et al. [37] and Saharan et al. [38].

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2.3. Phylogenetic analysis

The mycelia of the fungal isolates were scraped from PDA plates and grounded into powder using a tissue lyser at 60 Hz for 2 min. Genomic DNA was extracted using the MiniBEST Universal Genomic DNA Extraction Kit (Takara Biotech (Beijing) Co., Beijing, China), in accordance with the manufacturer's instructions.

Polymerase chain reaction (PCR) amplification was conducted using the primer pairs gpd1/gpd2 [39], RPB2-5F2/fRPB2-7Cr [40,41], and EF1-728F/EF1-986R [42], which targeted the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), RNA polymerase second-largest subunit (*RPB2*), and translation elongation factor 1 alpha (*TEF1-α*), respectively (Table S1). The PCR mixture for all genes consisted of 10.00 μL of 2 × PCR Mix (Tiangen Biotech CO., Beijing, China), 1.00 μL of genomic DNA, 0.50 μL of each primer (concentration 10 pmol/μL), and 8.00 μL of double-distilled sterilised water. PCR conditions for all genes were performed as described by Geng et al. [43], but the denaturing temperature was adjusted to 60℃ for *RPB2*. PCR products were separated on 2% agarose gel and purified for bi-directional sequencing. The obtained sequences were checked using Chromas v2.23 software, and representative sequences were deposited in GenBank.

The obtained sequences were aligned using DNAMAN 9.0 software to analyze the homology between each locus of the four isolates, and then the sequences of YKHH isolate were submitted to the National Center for Biotechnology Information (NCBI) database for BLASTN search and online alignment analysis. GenBank sequences with high similarity to the target sequences were downloaded to generate three gene matrices (*GAPDH*, *RPB2*, and *TEF1-α*) (Table S2). Sequence alignment was performed for each matrix using MAFFT v7.313 [44]. The aligned matrices were checked and manually adjusted using PhyloSuite v1.2.2 [45]. Redundant sequences at both ends and sequences with low matching were eliminated, and the three matrices were concatenated into a single dataset. Nucleotide substitution models for each locus were chosen using PartitionFinder v2 [46], and a Bayesian phylogenetic tree was obtained using MrBayes v3.2.6 [47]. The parameters for Bayesian phylogenetic analysis were implemented as described by Geng et al. [43]. *Stemphylium vesicarium* was used as an outgroup to construct the phylogenetic tree.

2.4. Pathogenicity test

The pathogenicity of mycelia and conidia from isolate YKHH was tested on healthy leaves of 2-year-old container seedlings of *T. sinensis*. The fungus was incubated on PDA plates at 25°C for 9 days, and mycelial plugs (0.50 cm in diameter) and conidia were harvested from the edge and the center of actively growing cultures, respectively. For mycelial inoculation, a mycelial plug was placed on a leaf wound made with a sterile dissecting needle, and the inoculation region was covered with aluminum foil to conserve moisture. Control leaves were inoculated with a sterile PDA plug. To prepare for conidial inoculation, the concentration of the conidial suspension was adjusted to 7.50×10^5 spores/mL using sterilized water containing 0.10% Triton X-100. Subsequently, 200 μL of the conidial suspension was injected to a leaf, and the inoculation region was covered with an appropriate amount of sterile cotton and aluminum foil to maintain moisture. In contrast, the control leaf was injected with 200 μL of sterilized water containing 0.10% Triton X-100. Each inoculation treatment groups and the control groups consisted of four plants, and the experiments were repeated five times (20 leaves per group).

The inoculated seedlings were grown in a climate-controlled chamber, maintained at a temperature of 25°C and a relative humidity of 90%, with a photoperiod of 12 hours. At 5 days after inoculation (DAI), all coverings were removed from the treated leaves, and the seedlings were allowed to continue to grow in the climate-controlled chamber. At 5, 12, and 30 DAI, disease symptoms and lesion progression were observed; the size of lesions were measured using the cross method. Statistical analyses were conducted using SPSS 26 software for Windows, and histogram with error bars was constructed using GraphPad Prism software v8.

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After symptoms appearance, reisolation was performed as described above to confirm the presence of the pathogenic fungi. The reisolates were identified via morphological characterization and the three gene sequences (*GAPDH*, *RPB2*, and *TEF1-α*).

2.5. Toona germplasm resources susceptibility evaluation

This study assessed the susceptibility of four *Toona* germplasm resources, including family 28 of *T. sinensis* (F28), family 52 of *T. sinensis* (F52), family 55 of *T. sinensis* (F55), and *Toona ciliata* var. *pubescens* (TCP), to *A. alternata*. Two-year-old container seedlings of those germplasm resources, provided by Prof. Jun Liu, were used to inoculate mycelia of the isolate YKHH and sterile PDA. The inoculation method followed the description detailed in the pathogenicity test section. At 12 DAI, the size of lesions was measured using the cross method. The susceptibility of *Toona* germplasm resources to *A. alternata* were assessed by incidence rate and lesion size; higher incidence rate and larger lesion size indicated higher susceptibility. Statistical analyses were performed using SPSS 26 software for Windows, while histograms with error bars were generated using GraphPad Prism software v8.

2.6. Statistical analysis

The data were evaluated for normality and equal variance to meet the statistical assumptions prior to statistical analysis. Independent sample t-test was used to evaluate the differences in lesion sizes between the inoculation treatment group and the control group, as well as between the mycelial inoculation group and the conidia inoculation group. One-way analysis of variance (ANOVA) was performed to evaluate the effects of days after inoculation and germplasm resources on lesion sizes, with statistical significance determined through Fisher's least significant difference (LSD) post hoc test ($p \leqslant 0.05$).

3. Results

3.1. Fungal isolation and morphology

In June 2022, leaf spot disease symptoms were detected on the lower crown leaves of *T. sinensis* seedlings grown in a germplasm resource nursery in Zhejiang Province, China. The infected leaves produced brown spots surrounded by yellow halos (Figure 1A-B).

In this study, four fungal isolates, designated as YKHH, YK51, YK76, and YK77, were isolated from the diseased leaves. These isolates growing on PDA were initially gray-white, then became olive-colored (Figure 1D); they produced colonies with a diameter of 4.25 ± 0.35 cm after 4 days of incubation at 25℃. All isolates produced dark-colored, obovoid, narrowly ellipsoid, or obclavate conidia with transverse and longitudinal septa (Figure 1E). The conidia were 22.48 ± 4.69 μm in length and 10.70 ± 2.47 μm in width. These characteristics indicate that the four fungal isolates resemble those of *Alternaria* species.

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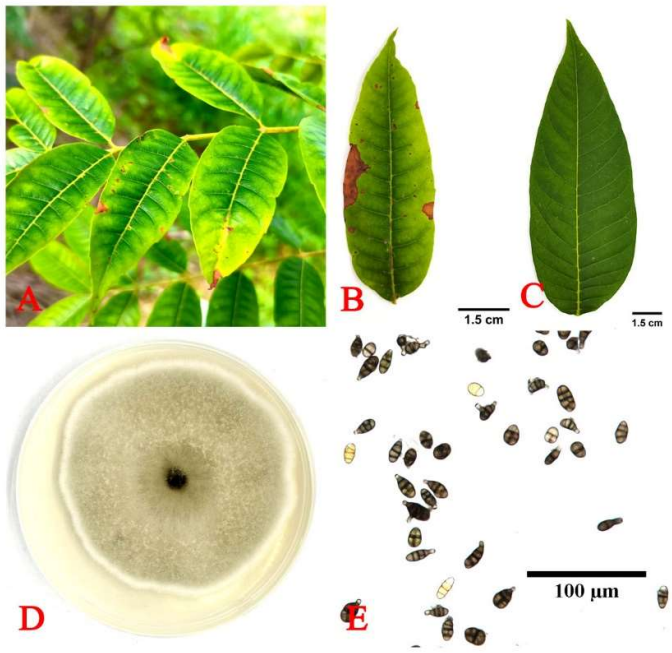

Figure 1. Disease symptoms on leaves of *Toona sinensis* and fungal morphology. (A-B) Symptoms observed on leaves of *T. sinensis* in the field; (C) Healthy leaf of *T. sinensis*; (D) Upper side of colony on PDA; (E) Conidia.

3.2. Phylogenetic analysis

The PCR products of the four isolates were purified, sequenced, and the resulting sequences were deposited in GenBank. The homology of *GAPDH*, *RPB2*, and *TEF1-α* fragments among the four isolates was 100.00%, 99.80%, and 100.00%, respectively. Based on the alignment results, it can be inferred that these four isolates belong to the same species. BLASTN analysis indicated that the *GAPDH*, *RPB2*, and *TEF1-α* sequences of isolate YKHH showed 100.00% similarity to those of three strains of *A. alternata* (OQ992206, MK605900, and LC132710).

The *GAPDH*, *RPB2*, and *TEF1-α* sequences of 30 strains/isolates of *Alternaria* and *Stemphylium* were concatenated to construct a multigene phylogenetic tree using Bayesian inference. According to the Bayesian information criterion, the best nucleotide substitution models chosen were GTR + I + G for *GAPDH* locus, SYM + I + G for *RPB2* locus, and GTR + G for *TEF1-α* locus. Phylogenetic analysis revealed that the four isolates obtained in this study clustered with *A. alternata* (CBS 916.96) with high Bayesian posterior probability, whereas these isolates were not clustered with the strain of *A. tenuissima* (Figure 2). DNA sequence alignment and multigene phylogenetic analysis demonstrated that these four isolates represent *A. alternata*.

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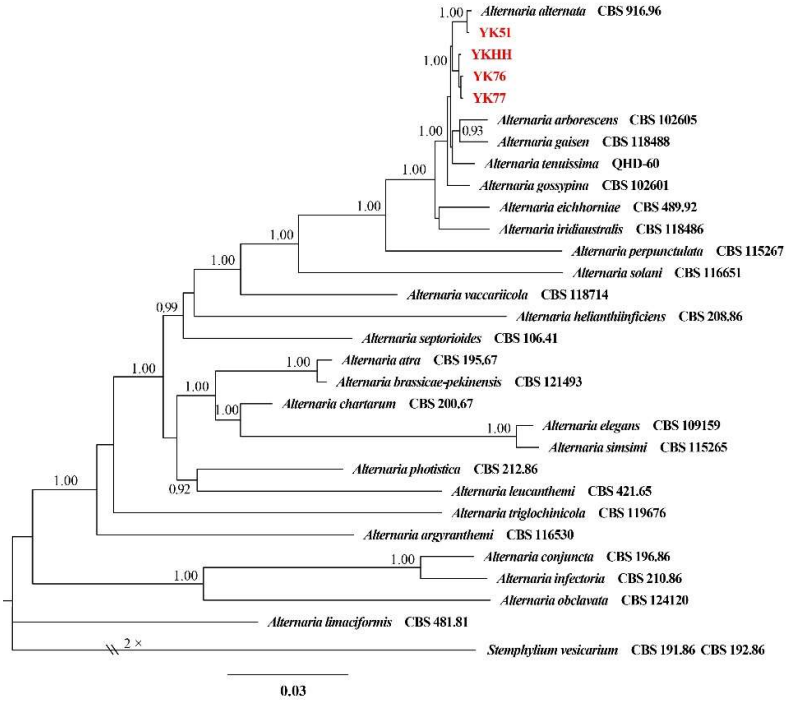

Figure 2. Phylogenetic tree generated from Bayesian inference (BI) analysis based on *GAPDH, RPB2,* and *TEF1-α* concatenated datasets. *Stemphylium vesicarium* was used as outgroup. Posterior probability values above 0.80 for BI analysis were indicated at the nodes. The isolates obtained in this study were highlighted in red.

3.3. Pathogenicity test

The pathogenicity of mycelia and conidia from isolate YKHH was tested on leaves of 2-year-old container seedlings of *T. sinensis*. At 12 DAI, both mycelia and conidia of the YKHH isolate produced symptoms on healthy leaves (Figure 3C-D), while sterile PDA plugs and sterilized water containing 0.10% Triton X-100 did not produce visible symptoms on healthy leaves. Necrotic lesions observed on the diseased leaves were grey-brown and surrounded by yellow halos (Figure 3C-F), basically consistent with leaf spot disease symptoms observed in the field. At all three time points, the size of lesions produced by mycelia were significantly larger than those produced by conidia (p<0.05) (Figure 3A-G). In the initial 5 days after mycelial inoculation, the lesions expanded rapidly, reaching a diameter of 0.64 ± 0.20 cm at 5 DAI (Figure 3A, 3G). Subsequently, the lesions expanded slowly, reaching 0.75 ± 0.20 cm at 12 DAI and 0.84 ± 0.18 cm at 30 DAI (Figure 3C, 3E, 3G). Further ANOVA analysis showed that the lesion size at 30 DAI was significantly larger than that at 5 DAI (p<0.05), while the difference in lesion size at other time points was not significant (p>0.05).

The fungi were reisolated from diseased leaves produced by two inoculation methods, and both their morphological characteristics and three gene sequences consistent with those of the original isolate YKHH of *A. alternata*. In contrast, *A. alternata* could not be reisolate from asymptomatic leaves of the control groups. These results indicate that *A. alternata* is the causal agent of leaf spot disease on *T. sinensis* in China.

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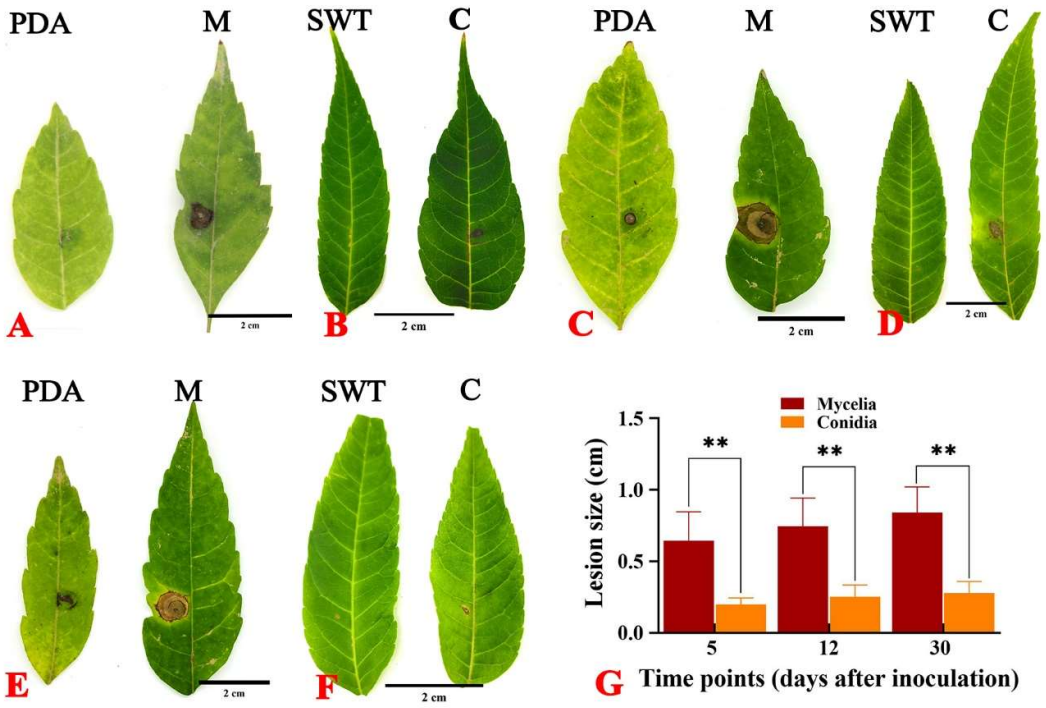

Figure 3. Symptoms on leaves of *T. sinensis* caused by the isolate YKHH of *Alternaria alternate* at different time points. (A-B) 5 DAI; (C-D) 12 DAI; (E-F) 30 DAI. (G) Lesion size on leaves of *T. sinensis*. C, conidia of isolate YKHH; M, mycelia of isolate YKHH; SWT, sterilized water containing 0.10% Triton X-100; PDA, sterile PDA plug. Significance levels of the lesion size are indicated by double asterisks for $p<0.05$.

3.4. Toona germplasm resources susceptibility evaluation

After 12 days post-inoculation, all leaves of four *Toona* germplasm resources produced disease symptoms when inoculated with the isolate YKHH of *A. alternata* (Figure 4A-D). The lesion sizes on leaves of TCP, F28, F52, and F55 inoculated with isolate YKHH of *A. alternata* were 0.46 ± 0.25, 0.52 ± 0.27, 0.55 ± 0.23, and 0.91 ± 0.08 cm, respectively (Figure 4E-H). In contrast, the lesion sizes on leaves of the corresponding control groups were 0.19 ± 0.05, 0.16 ± 0.03, 0.19 ± 0.06, and 0.17 ± 0.06 cm (Figure 4E-H). Results of the independent samples t-test demonstrated that all four *Toona* germplasm resources inoculated with isolate YKHH of *A. alternata* had significantly larger lesion sizes than those of the corresponding control groups (Figure 4E-H) ($p<0.05$). In all four germplasm resources, the lesion size on each leaf of the inoculation treatment group was greater than the average lesion size assayed in the corresponding control group. In conclusion, the disease incidence rate of all four *Toona* germplasm resources were 100.00% when inoculated with isolate YKHH of *A. alternata*.

The lesion sizes in the inoculation group of four *Toona* germplasm resources were subjected to ANOVA analysis. The germplasm F55 displayed a significantly larger lesion size than the other three *Toona* germplasm resources (Figure 5) ($p<00.5$). However, there were no significant differences observed among the inoculation groups of germplasm TCP, F28, and F52 (Figure 5) ($p>00.5$). Combining the results of the statistical analyses of incidence rate and lesion size, germplasm F55 showed the highest susceptibility to *A. alternata*, while germplasm TCP, F28, and F52 showed equal susceptibility to *A. alternata*.

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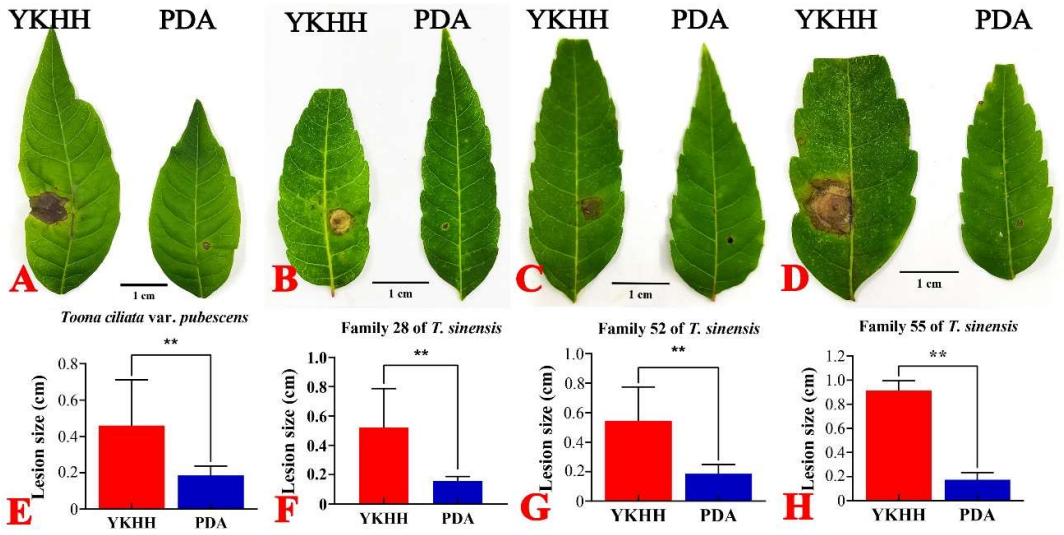

Figure 4. Susceptibility trials of *Toona* germplasm resources to the isolate YKHH of *A. alternata*. Disease symptoms on leaves of *Toona ciliata* var. *pubescens* (A), family 28 of *T. sinensis* (B), family 52 of *T. sinensis* (C), and family 55 of *T. sinensis* (D). Lesion size on leaves of *T. ciliata* var. *pubescens* (E), family 28 of *T. sinensis* (F), family 52 of *T. sinensis* (G), and family 55 of *T. sinensis* (H). YKHH, isolate YKHH of *A. alternata*; PDA, sterile PDA plug. Significance levels of the lesion size are indicated by double asterisks for $p<0.05$.

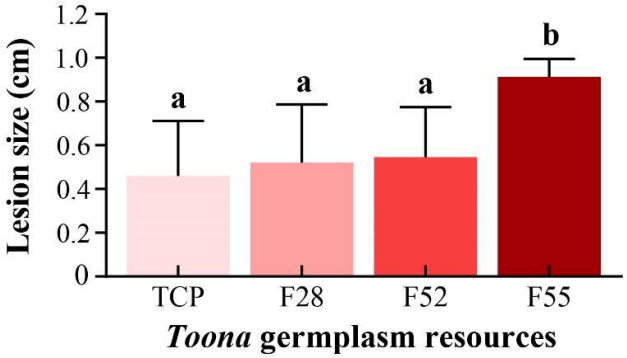

Figure 5. Lesion size on leaves of different *Toona* germplasm resources. TCP, *T. ciliata* var. *pubescens*; F28, family 28 of *T. sinensis*; F52, family 52 of *T. sinensis*; F55, family 55 of *T. sinensis*. Significance levels of the lesion size are indicated by lowercase for $p<0.05$.

4. Discussion

Alternaria is a ubiquitous fungal genus consisting of a variety of plant-associated saprophytic and pathogenic species. The two main characteristics of *Alternaria* are the production of chains of dark-colored phaeodictyospores and the presence of a beak of tapering apical cells [37,38]. In this study, four isolates (YKHH, YK51, YK76, and YK77) forming olive-colored colonies on PDA plates were isolated from the leaves of diseased *T. sinensis*. The conidia of all four isolates shared both major characteristics of *Alternaria* species. Therefore, the four isolates were preliminarily identified as *Alternaria* sp..

Initially, the taxonomy of *Alternaria* species was mainly based on their conidial morphology, cultural characteristics, and host species range [48]. However, these characteristics are variable and dependent on environmental conditions, and they also overlap among species [23,25]. Therefore, phylogenetic analysis of multiple loci has been proposed to supplement morphological characterization for the taxonomy of *Alternaria* species [23,25,49]. Woudenberg et al. [37] evaluated the efficiency of six gene loci, including small subunit (*SSU*) and large subunit (*LSU*) ribosomal RNA,

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internal transcribed spacer (*ITS*), *GAPDH*, *RPB2*, and *TEF1-α* genes for the identification of *Alternaria* species, they found that the *SSU*, *LSU*, and *ITS* loci had low resolution, whereas the *GAPDH*, *RPB2*, and *TEF1-α* loci had high resolution and could distinguish most *Alternaria* species [37]. Therefore, Woudenberg et al. [37] proposed the application of a combination of the *GAPDH*, *RPB2*, and *TEF1-α* loci to differentiate *Alternaria* species. In the present study, we selected three genes, *GAPDH*, *RPB2*, and *TEF1-α*, for molecular identification of the four isolates. Sequence analysis showed that the homology of these genes among the four isolates exceeded 99.80%, so these isolates belonged to the same species. Furthermore, multigene phylogenetic analysis grouped the four isolates with *A. alternata* (CBS 916.96), but not with *A. tenuissima*. Therefore, the four isolates was identified as *A. alternata*.

A. alternata is the most prevalent species in the genus *Alternaria*, infecting more than 380 plant species worldwide [23]. *A. alternata* causes disease in a wide range of economically important plants, resulting in significant economic losses. However, there have been few reports on the relationship between *A. alternata* and *Toona* species. Mall et al. [50] first reported that *A. alternata* was a foliicolous fungus of *T. ciliata*. Mehra and Upadhyaya [51] isolated *A. alternata* from the decomposing leaf of *T. ciliata*, suggesting that this fungus plays an important role in the decomposition of host leaves. Wang et al. [52] isolated *A. alternata* from diseased leaves of *T. ciliata*, and proved *A. alternata* was the pathogen of leaf spot disease by pathogenicity test. In this study, we isolated *A. alternata* from diseased leaves of *T. sinensis*. The mycelia and conidia of *A. alternata* were inoculated on healthy leaves of *T. sinensis*, and 12 days post inoculation, symptoms were produced on healthy leaves that were basically consistent with those observed in the field. Thus, *A. alternata* was confirmed as the pathogen causing leaf spot symptoms on the leaves of *T. sinensis*. In addition, we assessed the susceptibility of four *Toon* germplasm resources to *A. alternata* through susceptibility trials. The findings indicated that germplasm F55 exhibited the highest susceptibility to *A. alternata*, whereas germplasm TCP, F28, and F52 showed equal susceptibility to *A. alternata*. To the best of our knowledge, this is the first report of leaf spot disease caused by *A. alternata* on *T. sinensis* in China.

During the infection process, *Alternaria* can secrete over 70 host-specific and non-host-specific phytotoxins such as alternariol, alternariol monomethyl ether, tenuazonic acid, altenuene, and tentoxin [31-33]. These toxins interact with host plant and disrupt its normal physiological metabolism, resulting in disease on host plant [32]. Current studies have shown that *Alternaria* phytotoxins can target the plasma membrane, chloroplasts, mitochondria, Golgi complex, nucleus, and metabolic enzymes of host cells [32,33,53]. They induce massive accumulation of reactive oxygen species (ROS) in host plant cells, destroy the biofilm system, inhibit photosynthesis and respiration, suppress enzyme activity, disrupt the immune system, and cause cell death [28,32,33,53]. Moreover, these phytotoxins can induce harmful impacts on both animals and humans, such as fetotoxicity, cytotoxicity, and teratogenicity [32,34]. In recent years, *Alternaria* phytotoxins have received considerable attention from plant pathologists and food safety experts [33,34,54,55]. Further studies are necessary to investigate the effects of *A. alternata* on the yield and quality of *T. sinensis*, as well as effective strategies for the prevention and control of leaf spot disease on *T. sinensis*.

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Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1: A list of primer sequences used in this study; Table S2: Specimens used in phylogenetic analyses and their GenBank accession numbers.

Author Contributions: Conceptualization, X.G., J.L. and J.S.; methodology, X.G.; software, X.G. and H.M.; validation, X.G., K.H. and Q.M.; formal analysis, X.G.; investigation, X.G. and H.M.; resources, J.L., K.H. and Q.M.; data curation, J.S.; writing—original draft preparation, X.G.; writing—review and editing, X.G.; visualization, X.G. and H.M.; supervision, J.S.; project administration, J.S. and J.L.; funding acquisition, J.L. All authors have read and agreed to the published version of the manuscript.

Funding: The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was supported by the "Pioneer" and "Leading Goose" R&D Program of Zhejiang Province (Grant No. 2023C02034) and Fundamental Research Funds of Chinese Academy of Forestry (Grant No. CAFYBB2020SZ004).

Data Availability Statement: The *GAPDH*, *RPB2*, and *TEF1-α* sequences of isolate YKHH are available at GenBank with accession numbers OQ675825, OQ675826, and PQ855635, respectively. The *GAPDH*, *RPB2*, and *TEF1-α* sequences of the other isolates (YK51, YK76, and YK77) are available at GenBank with accession numbers PQ807220-PQ807222, PQ807217-PQ807219, and PQ855636-PQ855638, respectively.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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