MOLECULAR PHYLOGENY OF *LASIODIPLODIA THEOBROMAE* ASSOCIATED WITH *NELUMBO NUCIFERA* IN THUA THIEN HUE, VIETNAM AND THEIR SENSITIVITY TO SILVER NANOPARTICLES

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SUMMARY

In Vietnam, lotus is a plant with cultural and spiritual significance, representing purity, spiritual growth, and enlightenment. *Lasiodiplodia* fungi are associated with a wide range variety of host worldwide along with endophytes, pathogens, and saprobes. In this study, symptomatic leaves of *Nelumbo nucifera* were collected from Thua Thien Hue Provinces and *Lasiodiplodia theobromae* are characterized, identified based on combined sequence data analyses (multi locus) of internal transcribed spacer (ITS), beta tubulin (*tub2*), and translation elongation factor 1-alpha (*tef-1α*) coupled with morphological characteristics. Furthermore, AgNPs showed potent activity against *L. theobromae* at a concentration of 30 μg mL^{-1}, a 73.72% inhibition of mycelial growth was observed for the pathogen *L. theobromae* VNHUCC.NEL38 at 3 days post-inoculation. Thus, the present study indicates AgNPs may have considerable antifungal activity, these findings will contribute to the development of effective management strategies to control these diseases and improve lotus production

Keywords: Lasiodiplodia theobromae, *Nelumbo nucifera*, phylogeny, Vietnam, silver nanoparticles.

INTRODUCTION

Lasiodiplodia genus (Botryosphaeriaceae family, Botryosphaeriales) was first described by Clendenin in 1896 and is represented by *L. tubericola*, which is now recognized as *L. theobromae* (Phillips *et al*., 2013; Dou *et al*., 2017). This genus with characterized by large, ovoid to oblong, usually hyaline, aseptate ascospores and hyaline or pigmented, aseptate, one or rarely multi-septate, conidia usually with longitudinal striations. Both sexual and asexual morphs have been recorded for *Lasiodiplodia* genus. The family Botryosphaeriaceae consists of a group (monophyletic lineage) of 22 genera that are distinguished based on their ascospores and conidia, as well as their phylogenetic relationships. Conidiophores are typically reduced to conidiogenous cells, which, when present, are characterized by hyaline, cylindrical conidiophores that are sometimes septate and rarely branched *Lasiodiplodia* species have subglobose or oval smooth, thick-walled conidia that are initially hyaline and become dark brown and striated when mature

Distinguishing between *Lasiodiplodia* species based on morphological features alone is challenging and unreliable, leading to inaccurate identification because of their similar cultural and conidial characteristics. Hence, a DNA sequencing approach is recommended for accurate species identification, as well as clear phylogenetic boundaries. Recent research has advanced the recognition of various *Lasiodiplodia* species using sequence data of multi-locus with the internal transcribed spacers (ITS), partial translation elongation factor-1α (t*ef1*), and partial β-tubulin (*tub2*) with high phylogenetic support. *Lasiodiplodia* species are wide distribution, commonly found in tropical and subtropical regions and exhibit different lifestyles as endophytes, pathogens, and saprobes. These species have been known to infect various crops, including almonds (*Prunus amygdalus*), blueberries (*Vaccinium corymbosum*), baobabs, citrus fruits (*Citrus* spp.), cocoa (*Theobroma cacao*), coconuts (*Cocos nucifera*), grapevines (*Vitis* spp.), durians (*Durio* spp.), dragon fruits (*Hylocereus polyrhizus*), groundnuts (*Arachis hypogaea*), jackfruits (*Artocarpus heterophyllus*), mango (*Mangifera indica*), mulberries (*Morus* spp.), olives (*Olea europaea*), pines (*Pinus* spp.), strawberries (*Fragaria* spp.), and rice (*Oryza* spp.) (Berraf-Tebbal *et al*. 2020; El-Ganainy *et al*. 2022; Ko *et al*. 2023). There are few studies on *Lasiodiplodia*-associated leaf disease in *Nelumbo nucifera*. Therefore, the current study aimed to characterize *Lasiodiplodia* isolates from *Nelumbo nucifera* in Thua Thien Hue Province, Vietnam, using morphological and phylogenetic analyses based on ITS, *tef-1α,* and *tub2* sequence data. In addition, assessing the sensitivity of fungal reactions to AgNPs will provide essential knowledge regarding their potential use as plant protection products. This information is crucial for sustainable breeding and the design of management strategies against fungicide-resistant strains.

MATERIALS AND METHODS

Sample Collection and Fungal Isolation

Symptomatic leaf samples were collected, and stored in plastic at Gene Laboratory, Institute of Biotechnology, Vietnam, where isolation was subsequently conducted. Fungal isolation was performed using the tissue

transplantation method. Pieces of symptom-containing healthy parts of 5 × 5 mm were cut and surface disinfected using 70% ethanol and 1% a.i sodium hypochlorite (NaOCl). Then, washed 3 times with sterile distilled water (DW), and dried on sterile filter paper. The samples were placed directly on water agar (WA) and incubated at ambient temperature (28 ± 2 °C) for 2-3 days. Hyphal tips recovered from the infected tissues were cut and directly placed on potato dextrose agar (PDA; HiMedia, India) for purification. The obtained fungal samples were transferred to PDA slant/PDA-glycerol and preserved at -40 °C for further analyses. To induce sporulation, isolates were transferred to 2 % water agar with sterilized pine needles on the agar surface (PNA).

DNA Extraction, PCR Amplification, and Sequencing

Each fungal isolate was cultured on PDA for 5 days to obtain young mycelia, which were subjected to DNA extraction using the Genomic DNA Extraction Kit following the manufacturer's instructions (ABT, Vietnam). Portions of internal-transcribed spacer (ITS), translation elongation factor 1-α (*tef1-α*), and β-tubulin (*tub2*) regions were amplified using the primer pairs ITS1/ITS4 (White *et al*., 1990), EF1-728F/ EF-986R (Carbone and Kohn, 1999), and Bt2a/Bt2b (Glass and Donaldson, 1995), respectively (Table 1). PCR amplification was performed in 60 uL reaction volume containing 30 µL of Go Taq Green 2x Master Mix (Promega, USA), 5 µL of the DNA template (50 ng), 5 µL of each primer (10 pmol), and 15 µL of ddH_2O. PCR profile was denatured at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, annealing at 52 °C (ITS), 55°C (*tef-1α,* and *tub2)* for 30 s, elongation at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were observed via 1% agarose electrophoresis gel using SafeView staining.

Table 1. Gene regions and respective primer pairs were used in the study.

Locus	Gene region	Primer	Sequence (5'-3')
internal transcribed spacer regions with intervening 5.8S nrRNA gene	ITS	ITS1	TCC GTA GGT GAA CCT GCGG
		ITS4	TCC TCC GCT TAT TGA TAT GC
Partial beta-tubulin gene	*tub2*	Bt2a	GGT AAC CAA ATC GGT GCT GCT TTC
		Bt2b	ACC CTC AGT GTA GTG ACC CTT GGC
Partial translation elongation factor 1-alpha	*tef*	EF1-728F	CAT CGA GAA GTT CGA GAA GG
		EF1-986R	TAC TTG AAG GAA CCC TTA CC

Phylogenetic analyses

A portion of the PCR product was sequenced by the sequencing service provider First BASE Laboratories (Seri Kembangan, Malaysia). A BLASTn search was used to compare the sequences found in our study with other sequences deposited for similarity analysis in those of Genebank - NCBI (National Center for Biotechnology Information, https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 15 December 2023) database. Sequences for each locus were constructed and aligned in MAFFT v7.487 using default settings and trimmed using trimAl 2.rev0 b g. Gaps were deemed to be missing data, and ambiguously aligned areas were discarded. The alignment was further manually adjusted using BioEdit v. 7.0.9.0 if necessary.To establish the identity of the isolates at species level, phylogenetic analyses were conducted individually for individual loci and then as combined analyses of three loci (ITS, TEF, and TUB2) based on maximum likelihood (ML) in IQ-TREE. The ModelFinder was used to determine the best-fit model (Kalyaanamoorthy *et al*., 2017). Branch support was determined using 10,000 ulfabootstraps, a Bayesian posterior probabilities support and 10,000 SH-aLRT bootstrap replicates. The resulting trees were plotted using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree), and Interactive Tree of Life (iTOL) v.5 (Letunic and Bork, 2021), and further edited using PowerPoint (Microsoft, CA, USA) and Adobe Illustrator CC 2021 (Adobe Systems, San Jose, CA, USA).

Pathogenicity assay

Young and healthy leaves of *Nelumbo nucifera* were collected, washed with tap water, and surface-disinfected with 1% sodium hypochlorite. They were then submerged in 70% ethanol for 1 min and rinsed twice with sterilized water. One piercing wound was made in the mid-region of each leaf using a sterilized needle to form a tiny dot. Mycelium plugs (6 × 6 mm) of ½ PDA were cut from an actively growing colony, placed at the inoculation points, and wrapped in parafilm. The plants were kept under high humidity conditions (>85% relative humidity) in a covered plastic container for 1–3 days at 28 °C. Sterile PDA plugs were used to inoculate the control plants. The non-wounding experiment was conducted similarly without causing any damage to the leaves. The petri dishes were placed inside a plastic box, and the leaves were incubated at 25 °C with humidity and a 12/12-hour fluorescent light/dark cycle. After five days, the leaves were examined for symptom development, and the diameter of the diseased spot was measured.

Antifungal effects of AgNPs against *L. theobromae*

An *in vitro* assay was carried out on PDA with 0.1, 1, 10, 20, and 30 mg/L of silver nanoparticles (AgNPs). Stock nanoparticle solution was transferred into PDA to obtain different initial AgNPs concentrations (0.1, 1, 10, 20 and

30 mgL^{-1}). Medium containing AgNPs was poured into 90 × 15 mm petri dishes and incubated at room temperature. After 48 h of incubation, an agar plug of 6x6 mm diameter containing fungi was inoculated simultaneously at the center of each Petri dish and incubated at 28 ± 2°C. Culture medium without AgNPs was inoculated and cultured under the same conditions as the control treatment. The size of the colonies was measured after 1, 2,3, 7 days and each treatment was replicated. The mycelial growth inhibition rate – MGIR (%) was calculated using the following formula: where R is the radial growth of fungi in the control plate, and r is the radial growth of fungi in silver nanoparticle treated plates.

$$MGRI(\%) = \frac{R_{control} - R_{treated}}{R_{control}}$$

RESULTS & DICCUSSION

Symptom Recognition and Fungal Isolates

The disease appeared as small irregular brown spots on the lotus leaves (only recorded as aerial/standing leaves), originating from the outer leaf margins or damaged leaf areas. Subsequently, the spots enlarged and coalesced into regular or irregular brown necrotic lesions with dark-brown margins. Lesions on leaf petioles were characterized by brown areas, separating the color between infected and non-diseased areas. On PDA, the fungal isolates produced dense fast-growing mycelia. Colonies with abundant aerial mycelia reached the lid, covering the surface of the Petri plate after 3-4 days. Initially, the aerial mycelium was white to pale gray or smoke-gray colonies, gradually becoming dark grayish on the surface and reversing with age. (Fig. 1E). The conidia were immature and mature. Both immature and mature conidia were ovoid to ellipsoid in shape and 1-septate, with longitudinal striations, a broadly rounded apex, and tapering to the truncated base. Based on the morphological characteristics of the fungal isolates, we identified the recorded isolates as *Lasiodiplodia* sp., which was consistent with the morphology described by Alves và đồng tác giả (2008).

Five strains of *Lasiodiplodia* isolated from leaves (aerial leaf) and petioles of *Nelumbo nucifera* in Thua Thien Hue Province, Vietnam, were grown in culture. From the five isolates, two representative isolates were selected for molecular analysis based on a combined analysis of ITS, *TUB2*, and *TEF* gene sequences. The final dataset comprised of 79 taxa of representative isolates of *Lasidodiplodia* genus, including two isolates obtain in this study. *Diplodia multila* (CMW7060) is used as the outgroup taxon. Phylogenetic analysis revealed that the topologies of the ML trees generated from individual and concatenated genes (ITS, *tef1-α*, and *tub2*) were similar.

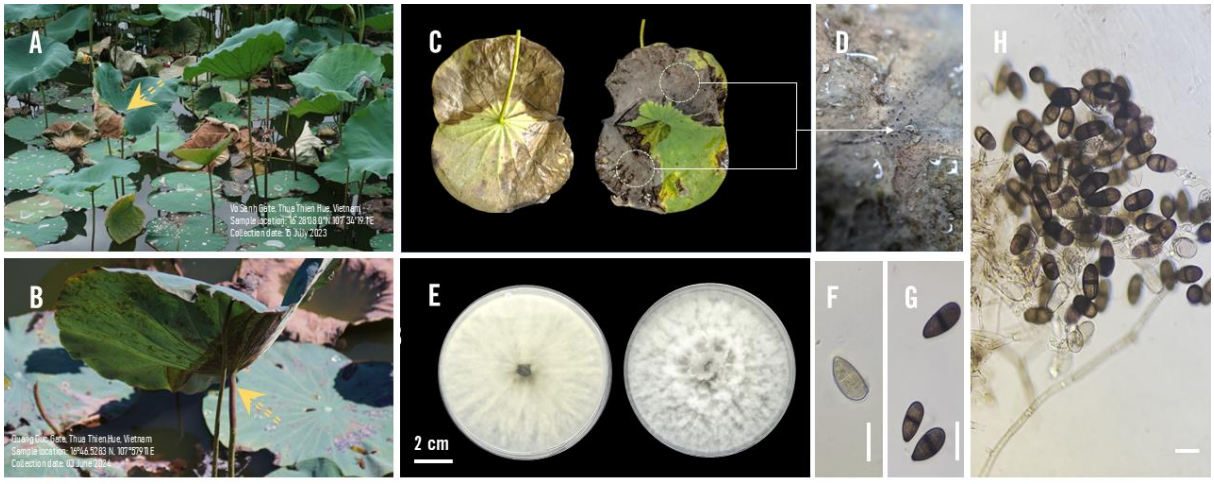
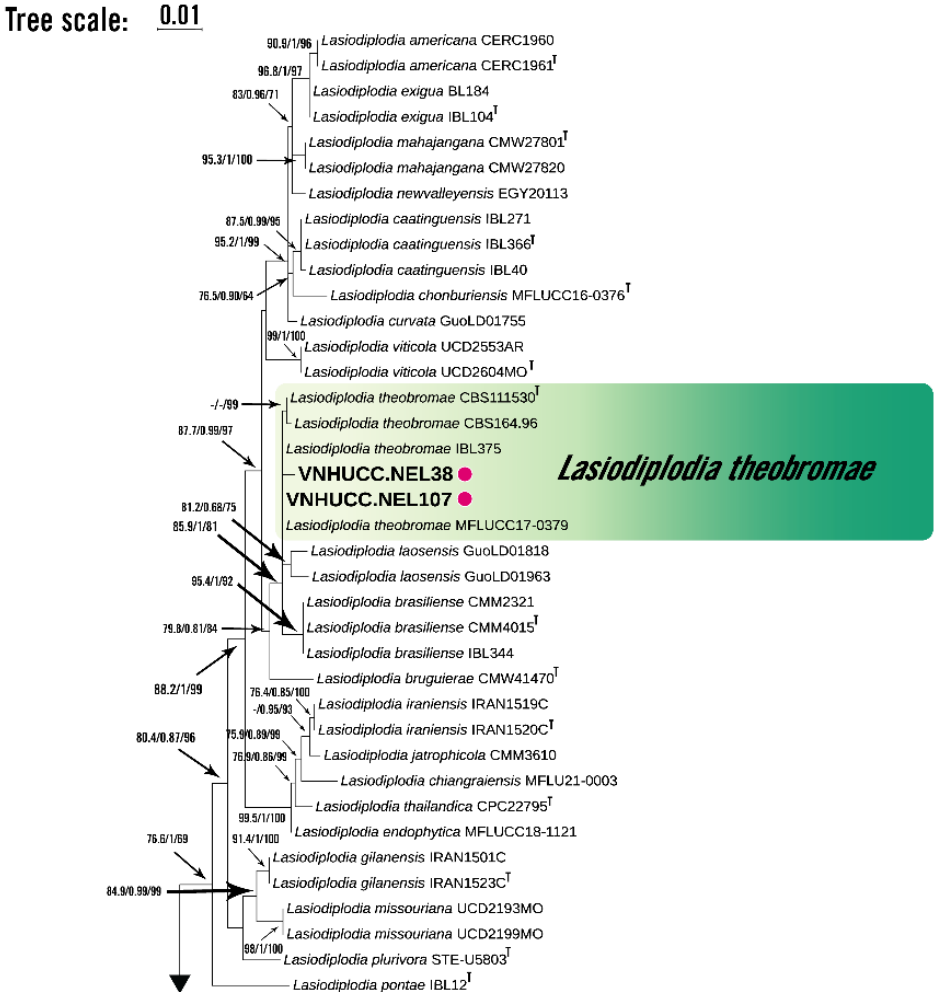


Figure 1. A, B. Symptomatic leaf samples observed in the lotus (N. nucifera) field. C, D. Zoomed in view of lesions with black pycnidia; E. Top and bottom view of VNHUCC.NEL38 colony on PDA; F: immature conidia; G: mature conidia with longitudinal striations; H: Conidia and fungal mycelia. Scale: E: 2cm; F-G: 15µm; H: 10µm.

The dataset combined alignment included 80 sequences (taxa) with 1261 characters, including gaps. ML analysis indicated 355 distinct patterns, 165 parsimony-informative, 98 singleton sites, 998 constant sites. The ML tree was contrusted with optimal log-likelihood value of -4925.922. Estimated base frequencies were as follows: A: 0.207, C: 0.306, G: 0.257, T: 0.231, rate parameters: A-C: 1.00000; A-G: 4.03082; A-T: 1.00000; C-G: 1.00000; C-T: 4.03082; G-T: 1.00000. Proportion of invariable sites: 0.614. Gamma distribution shape alpha: 0.776. Best-fit model: HKY+F+I+G4 chosen according to Bayesian Information Criterion (BIC), TIM3+F+I+G4 according Akaike Information Criterion (AIC). Two isolates VNHUCC.NEL38, VNHUCC.NEL107 developed a cluster with *L. theobromae*, (Fig. 2). Notably, within the same cluster, our two isolates developed distinct evolutionary lengths with these *L. theobromae* isolates CBS 164.96 (TYPE strain), CBS 11530, IBL375 (Pitomba - *Talisia esculenta*,

Brazil), MFLUCC 17-0379 (stem of *Hevea brasiliensis,* Thailand). The combined ITS, tef1 and tub2 phylogeny showed that *Lasiodiplodia theobromae* (including VNHUCC.NEL38, VNHUCC.NEL107) clades sister to *L. laosensis, L. brasiliense*, and *Lasiodiplodia bruguierae* with high support (85.9/1/91). As a result, all the present two isolates (VNHUCC.NEL38, VNHUCC.NEL107) were verified as *L. theobromae* by virtue of molecular identification and phylogenetic analysis.

Genus *Lasiodiplodia* is cosmopolitan and most species can be found primarily in tropics and subtropics. Many phytopathogenic fungal species with widespread distribution can be found within this genus, responsible for causing over 500 plant diseases such as fruit rot, root rot, collar rot, stem-end rot, dieback, canker, and leaf necrosis. In Vietnam, *Lasiodiplodia* species have been associated with various destructive diseases, such as fruit rot of jackfruit (*Artocarpus heterophyllus)* in DakLak*,* stem end rot disease in pomelo (*Citrus maxima*) and mango in Ben Tre; gummosis on Thanh Tra pomelo *Citrus grandis* in Thua Thien Hue (Khuong *et al*., 2023; Thao *et al*., 2023; Trai *et al*., 2024). Recently, Cuong và đồng tác giả (2023) also reported *L. theobromae* as the causative agent of blight disease in lotus plants (Cuong *et al*., 2023). Notably, all these reports indicated that *L. theobromae* is the causative agent of the disease. To date, there have been no instances of any other species within the *Lasiodiplodia* genus.



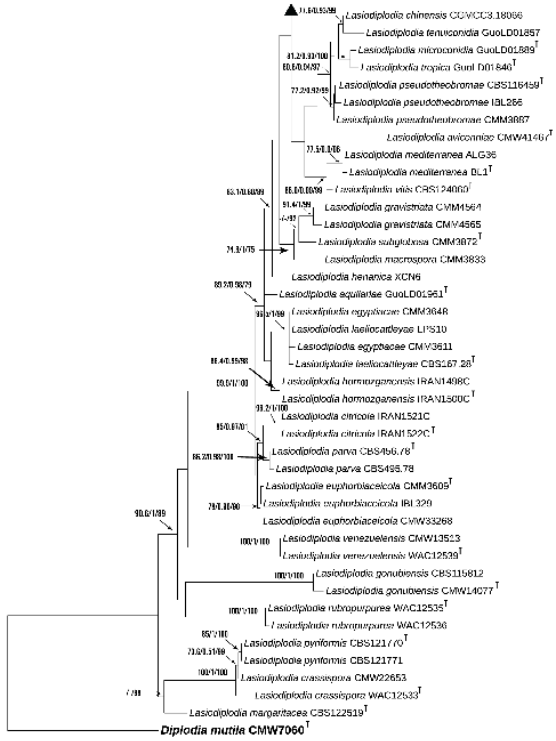


Figure 2. Maximum likelihood (ML) tree of the *Lasiodiolodia* species. *Diplodia mutila* CMW7060 was selected as the outgroup. At the nodes, bootstrap support values for ML (≥ 70%) and BYPP (≥ 0.5), SH-aLRT (≥ 75%) are displayed (SH-aLRT /BYPP/ML). Ex-type isolates are marked with "T", and our isolates obtained from this study are in bold. The scale bar indicates 0.01 nucleotide changes per site

Pathogenicity test

All isolates of *Lasiodiplodia* were pathogenic to detached leaves of lotus, resulting in visible lesions 5 days after inoculation. The symptoms observed both on the surface and internally were dark brown necrotic lesions that extended upward and downward from the point of inoculation on both wounded and unwounded samples. Lesion development in wounded samples was found to be more rapid and severe than in unwounded samples. No visible symptoms were observed in control leaves. The lotus cultivars differed significantly in the severity of foliar symptoms caused by *L. theobromae*. Two lotus varieties in Hue showed that *L. thebromae* only causes disease in the Cao san lotus variety, while the white lotus variety has no symptoms. Notably, when tested using the isolated wound method, the leaves had larger diameter lesions (114 mm), leading to visible lesions five days after infection, whereas in the non-wounded method, the lesions progressed very slowly, were barely visible, or were small (24.34 mm). Symptoms observed both on the surface and internally were dark brown necrotic lesions extending up and down (light brown) from the inoculation site in both the wounded and non-wounded methods.

Antifungal effects of AgNPs against *L. theobromae*

To determine the sensitivity of *L. theobromae* (VNHUCC.NEL38) to silver nanoparticles (AgNPs), different concentrations of were used to generate fungitoxicity curves and determine the concentration of AgNPs needed to inhibit colony growth by 50% (EC50 values). Mycelial plugs of the VNHUCC.NEL38 isolates were inoculated on PDA plates containing various concentrations of AgNPs (0, 0.1, 1, 10, 20, and 30 µg mL^{-1}) and PC (broad spectrum fungicide - hexaconazole 500 µg mL^{-1})

The effectiveness of the silver nanomaterials in inhibiting the growth of *L. theobromae* VNHUCC.NEL38 was assessed. One day after inoculation (1dpi), all concentrations exhibited a certain level of inhibition compared wih the control. However, after 2 dpi, the rapid mycelial growth of *Lasiodiplodia* species resulted in no significant differences between the control and low concentration (0.1 - 1 µg mL^{-1}) PDA plates. At higher concentrations (10 µg mL^{-1} and 20 µg mL^{-1}), the inhibition rate was 13.76%-16.27%. The concentration of 30 mg/L resulted in a high level of inhibition (73.72%). This level of inhibition was maintained at 30 µg mL^{-1} after 72 h (72.39%), whereas the remaining concentrations were no longer different from that of the control. Additionally, an inhibitory effect of up to 54.51%.was observed at a concentration of 30 µg mL^{-1} even 7 days after inoculation. VNHUCC.NEL38 also exhibited high sensitivity to PC-hexaconazole (500 µg mL^{-1}), with a relatively high inhibition rate ranging from 46.31% to 57.33% on the first and second day after inoculation. However, the inhibitory efficacy of hexaconazole against *L. theobromae* decreased rapidly by the third day of exposure, reaching 15.37%.

In conclusion, the inhibitory efficacy of AgNPs is highly dependent on the concentration and duration of exposure. In our experiment, at low concentrations (0.1 – 1 µg mL^{-1}), AgNPs have very little inhibitory effect. At high concentrations (30 mL^{-1}), AgNPs exhibit strong inhibition of mycelial growth, but this effect may decrease over time. This suggests that the effectiveness of silver compounds also depends on the duration of their expose. Tarazona (2019) demonstrated complete inhibition of the mycelium growth of *F. graminearum, F. culmorum, F. sporotrichioides, F. langsethiae, F. poae, F. proliferatum* and *F. verticillioides* after the longest exposure (20–30 h) to citrate-stabilized AgNP at concentarions of 30 and 45 mg L^{-1} (Tarazona *et al*., 2019). The antifungal activity of AgNPs is attributed to their ability to modify fungal colony morphology and disrupt cell membranes. In a study, Linh và đồng tác giả (2021) identified pathogen-caused stem-end rot mango fruits in Vietnam as *L. theobromae* XB1. In addition, nano-Cu at 4000 ppm inhibited fungal *L. theobromae* XB1 growth by 52.4% after seven days. Silver nanoparticles had a limited effect, with the highest inhibition of 80% at 400 ppm, and no growth at 25 ppm. Previous studies have reported that silver nanoparticles damage the transport system, leading to an outflow of intracellular ions and disruption of cellular processes such as metabolism and respiration. Previous studies by Xia và đồng tác giả have indicated that *Trichosporon asahii* organelles, including mitochondria, chromatin, and ribosomes, sustained significant damage when exposed to AgNPs (Xia *et al*., 2016)

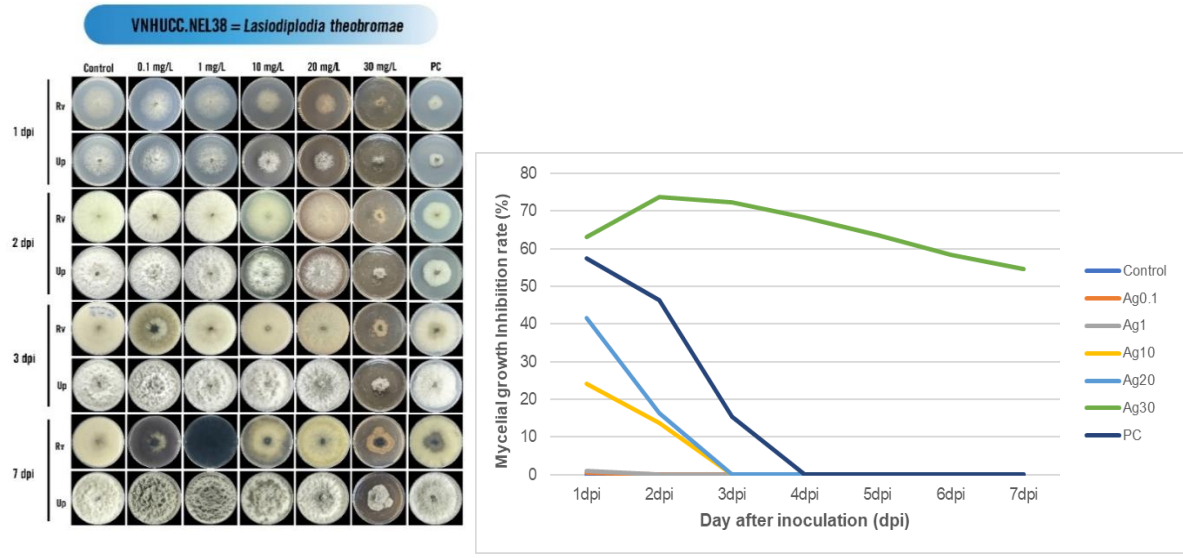


Figure 3. Mycelial growth inhibition (MGIR, %) of *L. theobromae* VNHUCC.NEL38 after application of AgNPs at different concentrations

CONCLUSION

In the current study, pathogens causing leaf blight, dieback petioles of *Nelumbo nucifera* in Thua Thien Hue, Vietnam were identified as *L. theobromae* based on morphological and molecular studies of multiple DNA sequences. This study has expanded the knowledge for disease management of *L. theobromae* in vitro by using silver nanoparticles.

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REFERENCES

Alves A, Crous PW, Correia A, Phillips AJL. (2008). Morphological and molecular data reveal cryptic speciation in Lasiodiplodia theobromae. *Fungal Divers*. 28.

Berraf-Tebbal A, Mahamedi AE, Aigoun-Mouhous W, Špetík M, Čechová J, Pokluda R, Baránek M, Eichmeier A, Alves A. (2020). Lasiodiplodia mitidjana sp. nov. And other Botryosphaeriaceae species causing branch canker and dieback of Citrus sinensis in Algeria. *PLoS One*. 15(5). https://doi.org/10.1371/journal.pone.0232448

Dou ZP, He W, Zhang Y. (2017). Does morphology matter in taxonomy of Lasiodiplodia? An answer from Lasiodiplodia hyalina sp. nov. *Mycosphere*. 8(2). https://doi.org/10.5943/mycosphere/8/2/5

El-Ganainy SM, Ismail AM, Iqbal Z, Elshewy ES, Alhudaib KA, Almaghasla MI, Magistà D. (2022). Diversity among Lasiodiplodia Species Causing Dieback, Root Rot and Leaf Spot on Fruit Trees in Egypt, and a Description of Lasiodiplodia newvalleyensis sp. nov. *Journal of Fungi*. 8(11). https://doi.org/10.3390/jof8111203

Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*. 14(6). https://doi.org/10.1038/nmeth.4285

Khuong NQ, Nhien DB, Thu LTM, Trong ND, Hiep PC, Thuan VM, Quang LT, Thuc LV, Xuan DT. (2023). Using *Trichoderma asperellum* to Antagonize Lasiodiplodia theobromae Causing Stem-End Rot Disease on Pomelo (Citrus maxima). *Journal of Fungi*. 9(10). https://doi.org/10.3390/jof9100981

Ko YZ, Liyanage WK, Shih HC, Tseng MN, Shiao MS, Chiang YC. (2023). Unveiling Cryptic Species Diversity and Genetic Variation of Lasiodiplodia (Botryosphaeriaceae, Botryosphaeriales) Infecting Fruit Crops in Taiwan. *Journal of Fungi*. 9(9). https://doi.org/10.3390/jof9090950

Letunic I, Bork P. (2021). Interactive tree of life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*. 49(W1). https://doi.org/10.1093/nar/gkab301

Phillips AJL, Alves A, Abdollahzadeh J, Slippers B, Wingfield MJ, Groenewald JZ, Crous PW. (2013). The Botryosphaeriaceae: Genera and species known from culture. *Studies in Mycology*. 76:51–167. https://doi.org/10.3114/sim0021

Tarazona A, Gómez J V., Mateo EM, Jiménez M, Mateo F. (2019). Antifungal effect of engineered silver nanoparticles on phytopathogenic and toxigenic Fusarium spp. and their impact on mycotoxin accumulation. *International Journal of Food Microbiology*. 306. https://doi.org/10.1016/j.ijfoodmicro.2019.108259

Thao LD, Hien LT, Trang TTT, Trinh PT, Tuan DK. (2023). First report of *Lasiodiplodia theobromae* causing fruit rot of jackfruit in Vietnam. *Indian Phytopatholy*. 76(4). https://doi.org/10.1007/s42360-023-00684-z

Trai VTN, Ha TTT, Hung NB. (2024). First Report of *Lasiodiplodia theobromae* Causing Gummosis on Citrus grandis (L.) Osbeck in Vietnam. *Research in Plant Disease*. 30(1):78–81. https://doi.org/10.5423/RPD.2024.30.1.78

Cuong HV, Ha TTT, Thao LQ, Thúy HTT, Vinh LV. (2023). Xác định nguyên nhân gây bệnh tàn lụi cây sen tại Thừa Thiên Huế năm 2022. *Vietnam Journal of Agricultural Sciences.* 21(5): 529-542

Xia ZK, Ma QH, Li SY, Zhang DQ, Cong L, Tian YL, Yang RY. (2016). The antifungal effect of silver nanoparticles on Trichosporon asahii. *Journal of Microbiology*, *Immunology and Infection*. 49(2). https://doi.org/10.1016/j.jmii.2014.04.013

PHÂN TÍCH PHÁT SINH LOÀI *LASIODIPLODIA THEOBROMAE* TRÊN CÂY SEN Ở THỪA THIÊN HUẾ VÀ ĐÁNH GIÁ MỨC ĐỘ NHẠY CẢM VỚI VẬT LIỆU NANO BẠC

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TÓM TẮT

Ở Việt Nam, sen là loài cây có ý nghĩa văn hóa tâm linh, tượng trưng cho sự thanh khiết, nghị lực trường tồn, đóng vai trò quan trọng vững chắc trong văn hóa tâm linh và tôn giáo. Nấm *Lasiodiplodia* đã được biết với hệ thống vật chủ đa dạng trên thế giới cùng với nhiều dạng sống từ: nội sinh, mầm bệnh gây hại và hoại sinh. Trong nghiên cứu này, các lá có triệu chứng điển hình của cây sen (*Nelumbo nucifera*) được thu thập từ các điểm trồng sen tại tỉnh Thừa Thiên Huế và phân lập. Kết quả *Lasiodiplodia theobromae* được xác định dựa trên các đặc điểm hình thái đối chiếu với phân tích dữ liệu trình tự đa locus kết hợp của 3 vùng gene ITS, beta tubulin (*tub2*) và nhân tố giãn dài dịch mã 1α (*tef-1α*). Bên cạnh đó, thử nghiệm *in vitro* với các hạt nano bạc cho thấy tiềm năng kháng nấm mạnh chống lại *L. theobromae*. Ở nồng độ 30 µg mL^{-1}, ghi nhận thấy mức độ ức chế lên đến 73,72% (3 ngày sau cấy) sự phát triển của sợi nấm đối với tác nhân gây bệnh phân lập được *L. theobromae* VNHUCC.NEL38 so với đối chứng. Do đó, nghiên cứu hiện tại chỉ ra rằng AgNP có thể có hoạt tính kháng nấm đáng kể, cần được nghiên cứu sâu hơn về quản lý bệnh sinh học, chương trình kiểm dịch. Những phát hiện này sẽ góp phần phát triển các chiến lược quản lý hiệu quả để kiểm soát các bệnh này và cải thiện quá trình canh tác, nâng cao năng suất cây sen trên địa bàn tỉnh Thừa Thiên Huế.

Từ khóa: *Lasiodiplodia theobromae, Nelumbo nucifera*, phân tích phát sinh loài, Việt Nam, nano bạc.

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