Neodeightonia phoenicum, a new species for the funga of Iran

Azadeh Habibi✉: Assistant Prof., Department of Biodiversity, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran (a.habibi@kgut.ac.ir)

Fariba Ghaderi: Associate Prof., Department of Plant Protection, College of Agriculture, Yasouj University, Iran

Abstract

Coconut fruits showing rot symptoms were collected from market in Kerman (Iran). The aim of this study was to identify the causal agent of the observed symptom. To isolate the pathogen, tissue pieces were taken from the interface of the symptomatic and healthy areas of the inner parts of the fruits and placed on potato dextrose agar (PDA) medium. A filamentous fungus was consistently isolated from the symptomatic tissues. Colonies on PDA were white with fluffy aerial mycelium gradually turning olivaceous in the center. Conidiomata formed on pine needles, pycnidial and dark brown to black Conidiogenous cells were hyaline, cylindrical, smooth and swollen at base. Immature conidia were single-celled, ovoid to ellipsoid and hyaline and mature conidia were septate, dark brown, measuring 21.1 ± 1.2 × 10.5 ± 0.8 µm. Based on morphological characterization coupled with molecular data of ITS and *tef1*, the fungus was identified as *Neodeightonia phoenicum*. This is the first report of *N. phoenicum* on *Cocos nucifera* worldwide and the first report of the occurrence of *N. phoenicum* in Iran.

Keywords: *Botryosphaeriaceae*, *Cocos nucifera*, fruit rot, Kerman, palm, tropical crop

Neodeightonia phoenicum، گونه جدیدی برای قارچ‌های ایران

آزاده حبیبی✉: استادیار گروه تنوع زیستی، پژوهشگاه علوم و تکنولوژی پیشرفته و علوم محیطی، دانشگاه تحصیلات تکمیلی صنعتی و فناوری پیشرفته، کرمان، ایران (a.habibi@kgut.ac.ir)

فریبا قادری: دانشیار گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه یاسوج، یاسوج، ایران

خلاصه

طی نمونه‌برداری‌های انجام شده از نارگیل‌های موجود در بازار در سطح استان کرمان نمونه‌های دارای پوسیدگی جمع‌آوری شد. این پژوهش به منظور شناسایی عوامل ایجاد کننده علایم انجام شد. برای جداسازی قارچ عامل بیماری، قطعات بافت از محل بین ناحیه دارای علائم و سالم از میوه نارگیل برداشته و روی محیط کشت PDA کشت داده شد. جدایه‌های قارچی به دست آمده، روی محیط کشت PDA، پرگنه‌های سفید با مرکز زیتونی رنگ تولید کرد. یاخته‌های کنیدیوم‌زا شفاف، سیلندری شکل با دیواره صاف و تورم اندکی در پایه بودند. کنیدیوم‌های نابالغ تک‌یاخته‌ای، تخم‌مرغی تا بیضوی و شفاف و کنیدیوم‌های بالغ دویاخته‌ای، قهوه‌ای تیره و با ابعاد ۲۱/۱ ± ۱/۲ × ۱۰/۵ ± ۰/۸ میکرومتر بودند. با استفاده از تلفیق داده‌های ریخت‌شناختی و داده‌های مولکولی نواحی ITS و *tef1* تحت عنوان گونه *Neodeightonia phoenicum* شناسایی شدند. براساس اطلاعات موجود، این نخستین گزارش از این گونه برای قارچ‌های ایران و نخستین گزارش از این گونه روی میزبان *Cocos nucifera* در دنیا است.

واژه‌های کلیدی: پوسیدگی میوه، کرمان، محصول گرمسیری، نخل، *Cocos nucifera*، *Botryosphaeriaceae*

Introduction

Cocos nucifera L. (*Arecaceae*) is an important crop grown worldwide, especially in tropical areas. Coconuts are mainly produced in Asia region and the top producers are Indonesia, Philippines and India (Dheepa *et al*. 2018). This plant species is grown in southern regions of Iran mainly in Hormozgan Province (Iran) (Goudarzi *et al*. 2019). Most of

the coconut fruits in Iran market is imported from India and Sri-Lanka (FAO 2021). Palm trees are susceptible to a large number of fungal species. More than 1500 fungal species are described from palms representing a taxonomically diverse group of almost all major fungal classes (Taylor & Hyde 2003, Xiong *et al*. 2022). During 2022–23, Coconut fruits showing rot symptoms were observed in fruits collected from local market in Kerman, Iran. The aim of the present study was to determine the causal agent of the observed symptoms.

Materials and Methods

Coconut fruits showing rot symptoms were collected from market in Kerman County during 2022–23. To isolate the pathogen, tissue pieces were taken from the interface of the symptomatic and healthy areas of the inner parts of the fruits. The pieces were surface sterilized in 96% ethanol for 15 s, then in 1% sodium hypochlorite for 1 min, rinsed in sterile distilled water and dried on sterile filter paper and placed on potato dextrose agar (PDA) medium. Plates were incubated for 5–7 days at 25 °C in the dark. A filamentous fungus was consistently isolated. The isolates were sub-cultured onto PDA for subsequent identification of species. Pine-needle-agar (2% tap water agar, with sterile pine needles) (Crous *et al.* 2006) was used to induce fungal sporulation. Digital images of fungal structures were made using a Dino-eye microscope camera USB lens (The Microscope Store, LLC, USA). Morphological characteristics were compared with species descriptions given in the literature. Living culture was deposited in Fungal Culture Collection of the Iranian Research Institute of Plant Protection (Tehran, Iran).

For molecular identification of the pathogen, genomic DNA was extracted from fresh mycelium using a CTAB extraction procedure. The cells were lysed using CTAB solution, and the DNA extraction was performed using DNG™-Plus solution (Sinaclon, Iran) according to the manufacturer's instructions. Partial sequences of the ITS-rDNA region and translation elongation factor (*tef1*) were amplified with primers ITS1/ITS4 (White *et al*. 1990) and EF-688F/EF-986R (Carbone & Kohn 1999), respectively. Amplifications were performed in a Biometra TAdvanced Thermal Cycler (Biometra, Göttingen, Germany). Sequencing was performed by Pishgam (Pishgam Biotech Co., Iran).

DNA sequences were checked and manually edited with Geneious software (Biomatters Inc., USA). A BLAST search was used to compare the obtained sequences with those in NCBI/GenBank database to find the closest matching taxa. To further clarify the phylogenetic relationships of the pathogen, a phylogenetic analysis using the combined sequences of ITS and *tef1* was performed. Maximum parsimony (MP) analysis was done with PAUP Ver. 4.0a133 (Swofford 2002) with bootstrap analysis of 1,000 replicates to test robustness of the branches.

Specimen examined: IRAN: Kerman Province, Kerman, on rot lesions of coconut fruit (*Cocos nucifera*), Feb. 2022, A. Habibi, (IRAN 5132C)

Results and Discussion

The symptoms of the collected coconuts included rotting lesions that in most cases were covered with white-gray fungal mycelium in the shells (Fig. 1 a-b). In many fruits, the rot had progressed to the entire fruit. The coconut water tasted bitter and was not suitable to consume. Isolations from naturally infected fruits resulted in a filamentous fungus. Colonies on PDA at 25 °C were white with fluffy aerial mycelium gradually turning olivaceous in the center (Fig. 1, b-c). Conidiomata formed on pine needles, pycnidial, dark brown to black, 210-360 µm × 150-250 µm, n = 10 (Fig. 1, d-e). Conidiogenous cells were hyaline, cylindrical, smooth and swollen at base, 8-21 × 4-6 µm, n = 15 µm (Fig. 1, f).

Immature conidia were single-celled, ovoid to ellipsoid and hyaline. Mature conidia had one median septum and the color was dark brown measuring 21.1 ± 1.2 × 10.5 ± 0.8 µm, n = 30 (Fig. 1, g-h). These features were consistent with the description of *Neodeightonia phoenicum* A.J.L. Phillips & Crous (Phillips *et al*. 2008). The conidia were shorter and narrower than the conidia of the closely related similar fungus, *N. palmicola* J.K. Liu, Phook. & K.D. Hyde, described by Liu *et al.* (2010). Teleomorph formation of *N. subglobosa* Booth in culture have been reported (Punithalingam 1969). However, our isolates of *N. phoenicum* failed to form teleomorph in culture even after long periods of incubation (Three months).

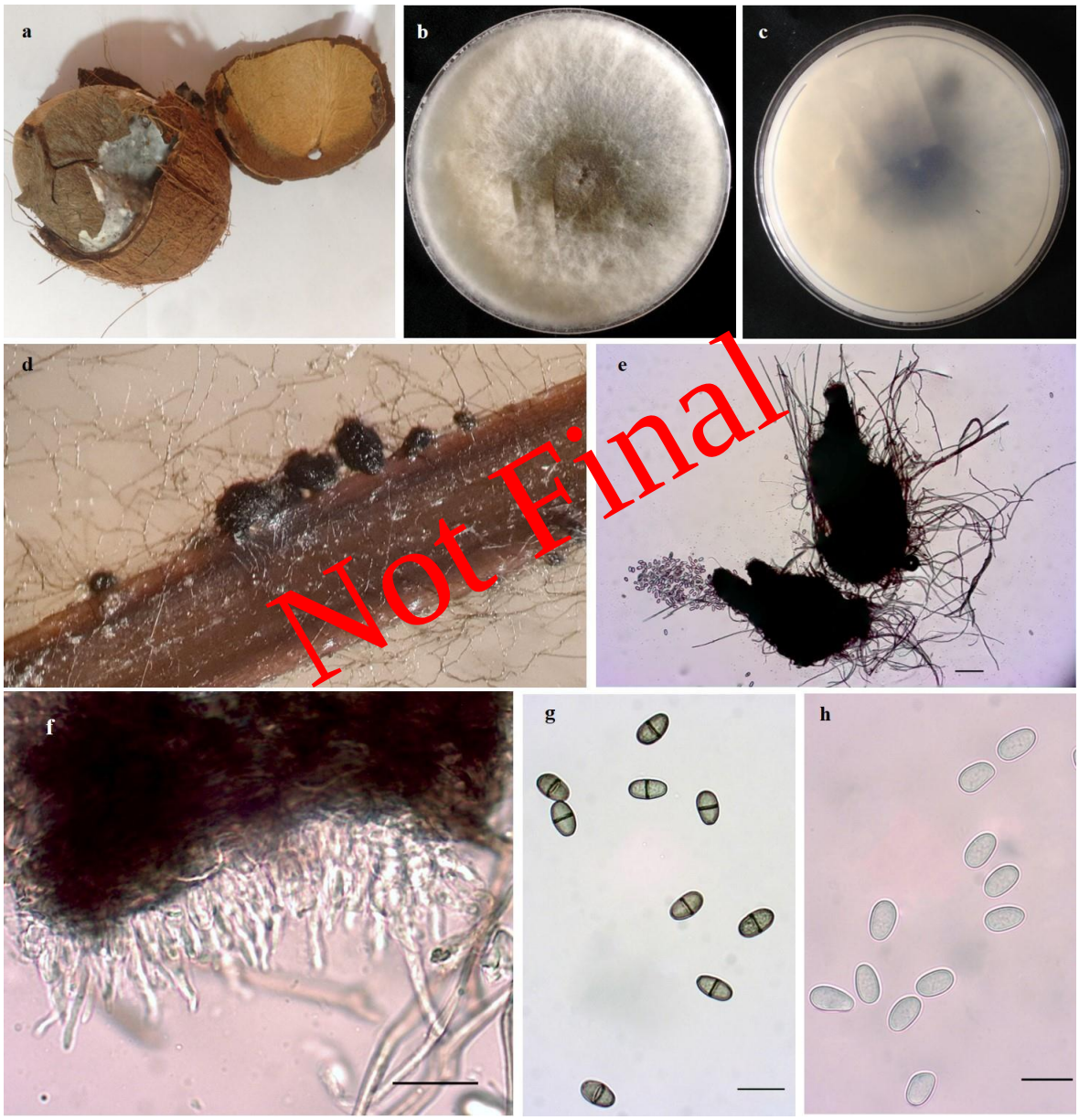

Fig. 1. Characteristics of *Neodeightonia phoenicum* isolated from coconut fruits: a. Symptoms of coconut fruit rot, b-c. Colonies on PDA after 7 d at 24 °C, d-e. Conidiomata (d, on pine needle), f, layer of Conidiogenous cells, g. Mature conidia, h. Young, hyaline conidia, (Bars: e = 80 µm, f-h=20 µm).

The obtained sequences of each region were submitted to GenBank under Accessions: OR818370 and PPO94285 for ITS and *tef1* respectively. BLAST analyses of the obtained sequence data showed that, the present isolates were closest match to *N. phoenicum*. To aid accurate identification, a parsimony tree was constructed using the obtained ITS and *tef1* sequences which were combined and aligned with sequences of 13 taxa retrieved from GenBank (Fig. 2). The isolates obtained in this study grouped with *N. phoenicum*.

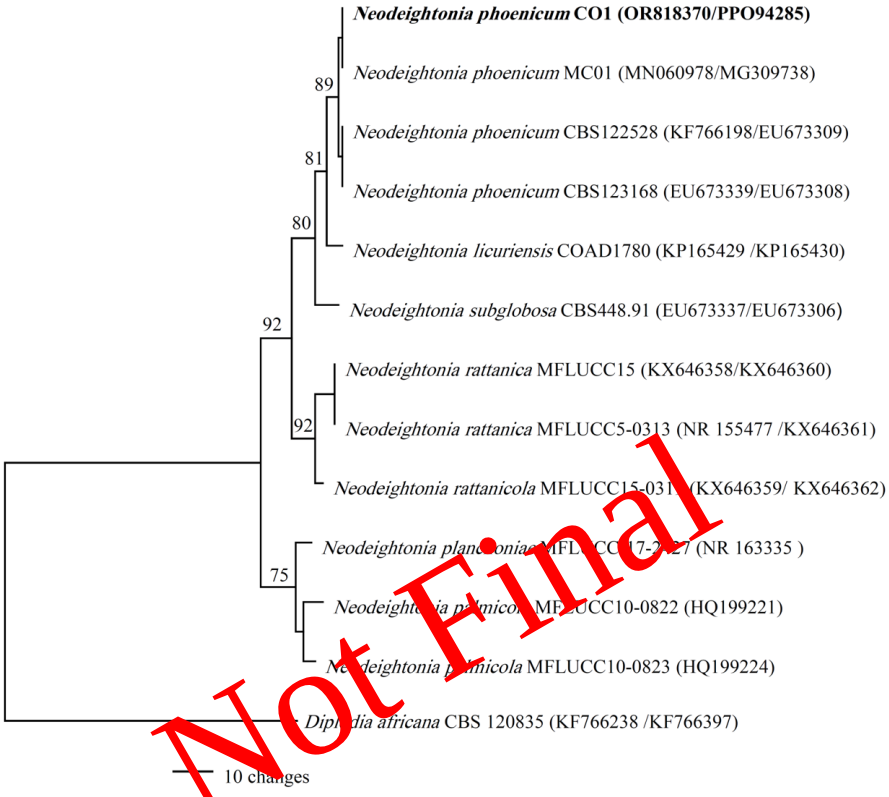

Fig. 2. Phylogram derived from maximum parsimony inference analysis of a combined ITS and *tef1* sequence dataset. Bootstrap values are presented at the nodes. The tree is rooted with *Diplodia africana*. In parentheses are the NCBI GenBank accession numbers for ITS/ *tef1*.

Botryosphaeriaceae is a family with opportunistic fungal pathogens that have both ecological and economic importance (Xiong *et al.* 2022). The genus *Neodeightonia* C. Booth was introduced by Booth (in Punithalingam 1969) for *N. subglobosa*. Later, Von Arx & Müller (1975) because of their broad concept of the genus *Botryosphaeria* reduced *Neodeightonia* to a synonymy under *Botryosphaeria*. Phillips *et al.* (2008) showed that, *N. subglobosa* was phylogenetically and morphologically distinct from the genera in the *Botryosphaeriaceae* and is distinguishable from *Botryosphaeria*. They reinstated this genus as a separate lineage in the same family and described *N. phoenicum* as a new species.

Neodeightonia species are mostly associated with palms and bamboos as saprobes, and in some cases as plant pathogens (Ligoxigakis *et al.* 2013, Dai *et al.* 2017, Nishad & Ahmed 2020). *Neodeightonia phoenicum* has been reported to cause palm rot of *Phoenix dactylifera* and *P. canariensis* in Greece (Ligoxigakis *et al.* 2013), root rot

disease of date palm (*P. dactylifera*) in Gatar (Nishad & Ahmed 2020), leaf spot on pygmy date palm (*P. roebelenii*) in China (Zhang & Song 2022), and is isolated from bracts of a palm tree (*Syagrus romanzoffiana*) in Brazil (da Silva Fonseca *et al*. 2020). There are no records on *N. phoenicum* on coconut palm (*Cocos nucifera*) in the world yet. The obtained fungus in this study from coconut fruits is the first report on this host and first report of the occurrence of *N. phoenicum* in Iran. The results derived from the present study confirm the pathogenicity of this species to coconut fruit. This will contribute to the knowledge of fungi infecting coconut. Further study is necessary to determine if this fungus has spread in coconut productions in southern regions of Iran.

Acknowledgments

This work was supported by Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman (Iran).

References

Carbone, I. & Kohn, L.M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3): 553–556. https://doi.org/10.1080/00275514.1999.12061051.

Crous, P.W., Slippers, B., Wingfield, M.J., Rheeder, J., Marasas, W.F., Philips, A.J., Alves, A., Burgess, T., Barber, P. & Groenewald, J.Z. 2006. Phylogenetic lineages in the Botryosphaeriaceae. Studies in Mycology 55(1): 235–253. https://doi.org/10.3114/sim0020.

da Silva Fonseca, M., Marchioro, M.L., Guimarães, D.K., Góes-Neto, A., Drechsler-Santos, E.R., Santos, V.A., Barbosa-Dekker, A.M., Dekker, R.F. & Cunha, M.A. 2020. *Neodeightonia phoenicum* CMIB-151: isolation, molecular identification, and production and characterization of an exopolysaccharide. Journal of Polymer Environment 28: 1954–1966. https://doi.org/10.1007/s10924-020-01744-5.

Dheepa, R., Goplakrishnan, C., Kamalakannan, A., Nakkeeran, S., Mahalingam, C. A., & Suresh, J. 2018. Coconut nut rot disease in India: Prevalence, characterization of pathogen and standardization of inoculation techniques. International Journal of Current Microbiology and Applied Sciences 7(2): 2046–2057. https://doi.org/10.20546/ijcmas.2018.702.245.

Dai, D.Q., Phookamsak, R., Wijayawardene, NN., Li, W.J., Bhat, D.J., Xu, J.C., Taylor, J.E., Hyde, K.D. & Chukeatirote, E. 2017. Bambusicolous fungi. Fungal Diversity 82: 1–105. https://doi.org/10.1007/s13225-016-0367-8.

FAO, 2021. FAOStat. Vol. 2014. Rome: Food and Agriculture Organization of the United Nations.

Goudarzi, A., Askari Seyahooei, M. & Bagheri, A. 2019. Coconut malformation: An emerging disease caused by *Fusarium proliferatum* in southern Iran. Journal of Phytopathology 167(11–12): 609–617. https://doi.org/10.1111/jph.12851.

Ligoxigakis, E.K., Markakis, E.A., Papaioannou, I.A. & Typas, M.A. 2013. First report of palm rot of *phoenix* spp. caused by *Neodeightonia phoenicum* in Greece. Plant Disease 97(2): 286. https://doi.org/10.1094/PDIS-08-12-0727-PDN.

Liu, J., Chomnunti, P., Cai, L., Phookamsak, R., Chukeatirote, E., Jones, E.B., Moslem, M. & Hyde, K.D. 2010. Phylogeny and morphology of *Neodeightonia palmicola* sp. nov. from palms. Sydowia 62(2): 261–276.

Nishad, R. & Ahmed, T.A. 2020. Survey and Identification of Date Palm Pathogens and Indigenous Biocontrol Agents. Plant Disease 104(9): 2498–2508. https://doi.org/10.1094/PDIS-12-19-2556-RE.

Phillips, A.J., Alves, A., Pennycook, S.R., Johnston, P.R., Ramaley, A., Akulov, A. & Crous, P.W. 2008. Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. Persoonia: Molecular Phylogeny Evolution of Fungi 21(1): 29–55. https://doi: 10.3767/003158508X340742 .

Punithalingam, E. 1969. Studies on Sphaeropsidales in culture. Mycological Papers 119: 1–24.

Swofford, D.L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland.

Taylor, J.E. & Hyde, K.D. (eds). 2003. Microfungi of tropical and temperate palms. Fungal Diversity Research Series 12, Fungal Diversity Press.

von Arx, J.A. & Müller, E. 1975. A re-evaluation of the bitunicate ascomycetes with keys to families and genera. Studies in Mycology 9. Centraalbureau voor Schimmelcultures.

White, T.J., Bruns, T., Lee, S.J. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds), PCR Protocols: A Guide to Methods and Applications. Academic Press Inc., New York, USA.

Xiong, Y., Manawasinghe, I.S., Liao, C., Hyde, K.D. & Dong, Z 2022. *Neodeightonia arengae* sp. nov., Botryosphaeriaceous taxa on *Arenga tremula* (Arecaceae) from Guangdong, China. Phytotaxa 530(2): 130–140. https://doi.org/10.11646/phytotaxa.530.2.1.

Zhang, W. & Song, X. 2022. Occurrence of Leaf Spot Caused by *Neodeightonia phoenicum* on Pygmy Date Palm (*Phoenix roebelenii*) in China. Plant Disease 106(8): 2269. https://doi.org/10.1094/PDIS-10-21-2190-PDN.