Mycologia Iranica 10(2): 131_ 140, 2023 Original Article DOI: 10.22092/MI.2024.365215.1278

Phenotypic and molecular characterization of *Quambalaria cyanescens* from walnut kernels infested with codling moth (*Cydia pomonella*) in Iran

Z. Mahdizadeh
A. Narmani
M. Arzanlou✉
Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

Abstract: In a survey of fungal species associated with walnut kernel rot symptoms, a white fungal mycelial mass was observed in feces and larval debries of codling moths (*Cydia pomonella*) on walnut kernels in East and West Azerbaijan provinces in 2022. Infected samples were examined under a stereo microscope, white mycelial mass together with fungal spores were taken using a sterile needle, and pure cultures were established using the single spore method. Morphological characteristics were examined on potato dextrose agar (PDA) and malt extract agar (MEA) culture media in the dark at 21°C after one week of incubation. To confirm the identity of the isolated fungi, the ITS rDNA genomic region of representative isolates were amplified using a universal primer set (ITS1 and ITS4) via polymerase chain reaction, and PCR products were sequenced. Based on the combination of morphological features and sequencing data, the isolates were identified as *Quambalaria cyanescens*. Colonies grew slowly, reaching a diameter of 11-12 mm on PDA and MEA after one week, and produced a purple pigment in the medium. Conidiophores are undifferentiated from vegetative hyphae and conidiogenous cells are holoblastic with sympodial proliferation. The conidia are usually ovoid or pear-shaped, transparent and 2-8 × 1.5-2.5 µm. *Quambalaria cyanescens* is a rare basidiomycete species of the order *Microstromatales*, which also has a yeast phase. The pathogenic potential of the two isolates of *Q. cyanescens* was evaluated on larvae of *Ephestia kuehniella*; however, the survival rate of larvae treated with different concentrations of *Q. cyanescens* spores was the same as that of untreated control larvae, and it can be concluded that the *Q. cyanescens* isolates were not pathogenic to *E. kuehniella* larvae. To the best of our knowledge, this is the first report on the association of *Q. cyanescens* with feces and larval debries of codling moths on walnut kernels.

Submitted 7 March 2024, accepted for publication 8 May 2024
✉ Corresponding Author: E-mail: arzanlou@tabrizu.ac.ir

http://mij.areeo.ac.ir

Keywords: Eucalyptus, Quambalariaceae, Inesct damage, Mold contamination.

INTRODUCTION

Monotypic genus *Quambalaria*, J.A. Simpson resides in the family *Quambalariaceae* (order *Microstromatales*, class *Exobasidiomycetes*), along with *Quambalaria pitereka* (J. Walker & Bertus) J.A. Simpson as the type species (Simpson 2000). The genus was established to accommodate fungal species that cause leaf spot, shoot blight, and canker on *Eucalyptus* L'Hér. and its relative *Corymbia* K. D. Hill and L. A. S. Johnson (Simpson, 2000). *Quambalaria* species were previously treated as members of *Ramularia* Unger and *Sporothrix* Hektoen & C.F. Perkins, due to the similarity and overlap in the morphological characteristics of conidiogenous cells (Simpson 2000). Simpson (2000) revised three species: *Q. eucalypti* (M.J. Wingf., Crous & W.J. Swart) J.A. Simpson, *Q. pusilla* (U. Braun & Crous) J.A. Simpson and *Q. pitereka*. He also stated that *Quambalaria* spp. do not have affinity with *Ophiostoma*, as they are sensitive to cycloheximide and also proposed their taxonomic placement in basidiomycetes (Simpson 2000). The taxonomic affinity of *Quambalaria* spp. with basidiomycetes was later ascertained by de Beer et al. (2006) based on the phylogeny inferred using sequence data of the ITS rDNA and LSU regions, and the new family *Quambalariaceae* was erected in the order *Microstromatales*.

However, the taxonomic status of some *Quambalaria* species has proven highly problematic. For example, de Hoog and de Vries (1973) initially described *Q. cyanescens* as *Sporothrix cyanescens* based on isolates originating from human skin and air samples. Later on, Moore (1987) established the new genus *Cerinosterus* R.T. Moore 1987 to accommodate *Sporothrix* species with affinity to basidiomycetes and reclassified *S. cyanescens* as *C. cyanescens* (de Hoog) R. T. Moore. The name *C. cyanescens* was again subjected to taxonomic changes and was transferred to *Fugomyces* as *F. cyanescens* (de Hoog et de Vries) Sigler (Sigler and Verweij, 2003). Finally, de Beer et al. (2006) confirmed *Fugomyces* being congeneric with *Quambalaria* in

their phylogenetic study and proposed a new combination for *F. cyanescens* in *Quambalaria* as *Q. cyanescens* (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer.

Until the present eight *Quambalaria* species have been described (https://www.indexfungorum.org/ Names/Names.asp /accessed on 27 November. 2023) viz., *Q. coyrecup* Paap, Q. pitereka, *Q. eucalypti*, *Q. pusilla* (syn: *Q. simpsonii* Cheew. & Crous), *Q. fabacearum* J.D.P. Bezerra, Firmino,Souza-Motta & Crous, *Q. cyanescens*, *Q. rugosae* Crous and *Q. tasmaniae* Crous.

Quambalaria species are mainly known as leaf and shoot pathogens of *Eucalyptus* and *Corymbia* species in Australia, South Africa, China, and other countries (Duong 2022). *Quambalaria coyrecup* and *Q. pitereka* affect *Corymbia* species, causing canker, leaf and shoot blight in Australia and China (Paap et al. 2006); while, the other *Quambalaria* species namely, *Q. eucalypti*, *Q. pusilla* and *Q. tasmaniae* are mainly restricted to *Eucalyptus* and cause leaf spot and shoot blight on this host; of those, *Q. eucalypti* has wider geographical distribution (Australia, South Africa, China, Brazil and Uruguay); while, *Q. pusilla* has been reported from Australia, China and Thailand and *Q. tasmaniae*, a recently described species, is only known to occur in Tasmania and Australia (Paap et al. 2008; Crous et al. 2019; Duong 2022). The pathogenic relevance of *Q. rugosa*, described in *Eucalyptus rugosa* in Australia, remains unknown (Crous et al. 2019). *Quambalaria fabacearum* is another species with endophytic nature, which has been described from *Mimosa tenuiflora* (Willd.) Poir. (*Fabaceae*) in Brazil (Bezerra et al. 2018). *Quambalaria cyanescens* is another species in this genus, which has been isolated from diverse range of substrates and ecological niches (de Hoog and de Vries, 1973; Narmani and Arzanlou 2019; Stupar et al. 2022).

In the present study, several isolates of *Quambalaria* were recovered from walnut kernels infested by codling moths in the East and West Azerbaijan Provinces. The aim of this study was to determine the identity of these isolates using a combination of morphological and molecular characteristics and to evaluate their pathogenicity in model insect larvae, *Ephestia kuehniella*, under laboratory conditions.

MATERIALS AND METHODS

Sample collection and fungal isolation

During a survey on fungal species associated with walnut kernel rot symptoms in 2022, a white fungal mycelial mass was observed on feces and larval debris of codling moth (*Cydia pomonella* L.) on walnut kernels. Therefore, walnut samples were collected from three orchards in the Firouragh district, Khoy County (West Azerbaijan province) and two orchards from Mamqan district Azarshahr County (East Azerbaijan Province). Nuts were unshelled and left to dry under indirect sunlight. Nuts were randomly picked and cracked using a manual nutcracker and subsequently checked for possible fungal contamination. Infected kernels were inspected under a stereo microscope and fungal mass were picked up using a sterile inoculation needle and transferred on to potato dextrose agar (PDA, Fluka, Hamburg, Germany) plates amended with 100 mg/L streptomycin sulphate and 100 mg/L ampicillin (Narmani and Arzanlou 2019). Pure cultures were established using single spore technique and were preserved at 4 °C in the Culture Collection of Tabriz University (CCTU) (Table 1).

Morphological studies

Morphological characteristics of the isolates were examined on PDA and MEA (Merck, darmstadt, Germany) Thus, 5 mm plugs were cut the margin of fresh fungal colonies using a cork borer and were centrally cultured on PDA and MEA in three repetitions. To monitor colony growth rate, PDA and MEA plates were incubated at 21 °C in the dark and cultural characteristics including growth rate, color and colony appearance were determined. Slide culture technique was used for microscopic studies. Microscopic characters of 30 fungal structures were evaluated and photographed using Olympus digital camera system BX41 (Olympus Corporation, Japan). Dimensions of fungal structures are given using the following format b–c, where the range 'b–c' represents at least 95% of the measured values.

Genomic DNA extraction and PCR amplification

Genomic DNA was extracted from fresh fungal mycelia grown on PDA according to Moller et al. (1992). For analysis by PCR and sequencing, the internal transcribed spacer (ITS-rDNA) region was amplified using the ITS1 and ITS4 primers (White et al. 1990). The amplification was performed by Bio RAD thermal cycler in a total volume of 25 µL. PCR mixture contained 12.5 µL of Taq DNA Pol (2x) Master Mix (Pishgam, Tehran), 0.3 µM of each forward and reverse primers and 50-60 ng of DNA template. The PCR condition were as follows: 94 °C for 5 min, followed by 36 cycles of denaturation at 94 °C for 45 sec, annealing at 55 °C for 45 sec, elongation at 72 °C for 45 sec and final extension at 72 °C for 5 min. The PCR products were visualized on 1.5% agarose gel in 1 × TAE buffer containing 0.1 µg/mg ethidium bromide by ultraviolet gel imaging. The purified amplicons were sequenced in both directions using the same primer set. The obtained sequence files were edited using DNA Dragon v. 1.6.0 (Hepperle 2017) and BioEdit v. 5.0.6 (Hall 1999) software. The consensus sequences were compared with sequences in the GenBank using the Basic Local Alignment. Search Tool (BLAST). ITS-rDNA sequences for the two isolates CCTU ZM1 and CCTU ZM2 were deposited in GenBank with the accession numbers PP757488 and PP757497, respectively.

Table 1. The list of reference isolates for fungal species used for phylogenetic analysis.

Species	GenBank Accession no. (ITS)	Isolation/ Herbarium no.	Host	Origin	Collector
Quambalaria cyanescens	DQ317622	CBS357.73 T	Skin of man	Netherlands	TF Visser
	DQ317623	CBS876.73	*Eucalyptus pauciflora*	New South Wales, Australia	MJ Wingfield
	KX377510	IRAN 2465C	*Punica granatum*	Iran	ME Vahedi-Darmiyan
	DQ823421	WAC 129555	*Corymbia calophylla*	Australia	T Paap
	DQ823419	WAC 12952	*C. calophylla*	Australia	T Paap
	DQ823422	WAC 12953	*Corymbia ficifolia*	Australia	T Paap
	HG799003	CBS 127353	*Betula pendula*	Russia	AB Antropova
	HG799002	CBS 127352	*B. pendula*	Russia	AB Antropova
	MN006031	CCTU 1684	*Vitis vinifera*	Iran	A Narmani
	MN013769	CCTU 1738	*V. vinifera*	Iran	A Narmani
	PP757488	**CCTU ZM1**	***Juglans regia*** ❋	**Iran**	**M Arzanlou**
	PP757497	**CCTU ZM2**	***J. regia*** ❋	**Iran**	**M Arzanlou**
Q. eucalypti	DQ317609	CBS118615	*Eucalyptus nitens*	Rooihoogte, South Africa	ZL Mthalane, J Roux
	DQ317610	CMW17253	*E. nitens*	Rooihoogte, South Africa	ZL Mthalane, J Roux
	DQ317611	CMW17254	*E. nitens*	Rooihoogte, South Africa	ZL Mthalane, J Roux
	DQ317612	CMW17255	*E. nitens*	Rooihoogte, South Africa	ZL Mthalane, J Roux
	DQ317613	CBS118616	*Eucalyptus grandis* clone	Kwambonambi, South Africa	J Roux
	DQ317614	CMW14329	*E. grandis* x *E. camaldulensis*	Kwambonambi, South Africa	J Roux
	DQ317625	CBS118844T CMW 1101	*E. grandis*	Kwambonambi, South Africa	MJ Wingfield
	DQ317626	CBS119680	*E. grandis*	Kwambonambi, South Africa	L Lombard
Q. pitereka					
	DQ823423	DAR 19773 T	*C. eximia*	New South Wales	Walker & Bertus
	DQ317627	CMW6707	*Corymbia maculata*	New South Wales, Australia	MJ Wingfield
	DQ317628	CBS118828, CMW 5318	*Corymbia citriodora* sub sp. *variegata*	Queensland, Australia	M Ivory
	DQ823428	WAC12956	*C. ficifolia*	Western Australia	T Paap
	DQ823427	WAC12958	*C. calophylla*	Western Australia	T Paap
Q. pusilla	GQ303291	CBS 124773	*Eucalyptus* sp.	Thailand	R Cheewangkoon
	GQ303290	CBS 124772 T	*E. tintinnans*	Australia	R Cheewangkoon
Q. fabacearum	NR160341	URM 7756	*Mimosa tenuiflora*	Brazil	J Bezerra
Q. rugosae	NR165610	CPC 20162 T	*Eucalyptus rugosa*	Australia	Crous
Q. tasmaniae	NR165611	CPC 25464 T	*Eucalyptus* sp.	Australia	Braun & Crous
	MN162016	CPC 25462	*Eucalyptus* sp.	Australia	Braun & Crous
Q. coyrecup	DQ823431	WAC12947 T	*C. calophylla*	Western Australia	T Paap
	DQ823433	WAC12948	*C. calophylla*	Western Australia	T Paap
	DQ823432	WAC12949	*C. calophylla*	Western Australia	T Paap
	DQ823429	WAC12950	*C. ficifolia*	Western Australia	T Paap
	DQ823430	WAC12951	*C. ficifolia*	Western Australia	T Paap
Microstroma album	DQ317624	RB2072	*Quercus robur*	Germany	R Bauer
M. juglandis	DQ317632	F3381	*Juglans regia*	Germany	M Göker
	DQ317633	RB2054	*J. regia*	Germany	R Bauer
	DQ317634	RB2024	*J. regia*	Germany	R Bauer
Rhodutorula bacarum	DQ317629	CBS6526 T	*Ribes nigrum*	UK	RWM Buhagiar
R. hinnulea	AB038130	CBS8079 T	*Banksia collina*	Australia	RG Shivas
R. phylloplana	DQ317630	CBS8073 T	B. collina	Australia	RG Shivas
Sympodiomycopsis paphiopedili	DQ317631	CBS7429 T	Nectar of Paphiopedilum primurinum	Japan	K Tokuoka
Volvocisporium triumfetticola	DQ317637	RB2070 T	Triumfetta rhomboidea	India	MS Patil
Tilletiopsis pallescens	DQ317635	F3370	fern leaf	Germany	JP Sampaio

❋ Walnut kernels infested by codling moth. Type strains are shown as (T).

Phylogenetic analysis

The dataset for ITS sequences from GenBank accession numbers and current study are listed in Table 1. The collected sequences, together with sequences obtained in this study were aligned by MEGA 6 (Molecular Evolutionary Genetics Analysis) (Kumar et al. 2016). The significant evolutionary models were achieved using MrModeltest v. 2.3 (Nylander 2004). To determine the identity of the studied isolates, Bayesian analyses were accomplished in PAUP v.4.0b10 and MrBayes v3.2.2 (Ronquist and Huelsenbeck, 2003). *Tilletiopsis pallescens* F3370 (accession no. DQ317635) was used as out group taxon. The generated phylogenetic tree was visualized using FigTree version 1.4.3 (Rambaut 2009).

Bioassay test on *Ephestia kuehniella*

In the present study, pathogenic potential of two isolates of *Q. cyanescens* were evaluated on larvae of *Ephestia kouhniela* (Zeller) (L.). For this purpose, *E. kouhniela* were reared in laboratory condition and the last instar larvae were used for bioassay test. Different concentrations of fungal spore suspensions (10^6, 10^7, 10^8 spores/ml) were applied for inoculation. For each spore concentration, 10 larvae of *E. kouhniela*, were soaked in spore suspensions for 10 seconds and then larvae were transferred on sterile filter paper to dry their excess moisture. After that, larvae were transferred to eight cm Petri dishes containing 1g of flour in the center. For the control, larvae were soaked in distilled water for 10 seconds. The experiment was carried out in three replicates for each treatment.

Treatments were incubated in dark at 25 °C. Inoculated larvae were inspected daily and the number of dead larvae in each treatment was counted up to 10 days and the percentages of mortality were calculated.

Bioassay test on *Ephestia kuehniella*

In the present study, pathogenic potential of two isolates of *Q. cyanescens* were evaluated on larvae of *Ephestia kouhniela* (Zeller) (L.). For this purpose, *E. kouhniela* were reared in laboratory condition and the last instar larvae were used for bioassay test. Different concentrations of fungal spore suspensions (10^6, 10^7, 10^8 spores/ml) were applied for inoculation. For each spore concentration, 10 larvae of *E. kouhniela*, were soaked in spore suspensions for 10 seconds and then larvae were transferred on sterile filter paper to dry their excess moisture. After that, larvae were transferred to eight cm Petri dishes containing 1g of flour in the center. For the control, larvae were soaked in distilled water for 10 seconds. The experiment was carried out in three replicates for each treatment.

Treatments were incubated in dark at 25 °C. Inoculated larvae were inspected daily and the number of dead larvae in each treatment was counted up to 10 days and the percentages of mortality were calculated.

RESULTS

Fungal isolates

Twenty fungal isolates were obtained from moldy walnut kernels infested with codling moth from the Firouragh region Khoy County (West Azerbaijan province) (17 isolates) and the Mamqan region Azarshahr County (East Azerbaijan province) (three isolates), Iran. All fungal isolates showed a similar growth pattern. Following a comprehensive morphological evaluation, two isolates (based on the locality of the isolates) were selected for further studies. Based on a combination of morphological characteristics and phylogenetic analysis, both isolates were identified as *Q. cyanescens*.

Quambalaria cyanescens (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer 2006

Colonies attained a diameter of 9 and 8 mm growth on PDA and MEA, respectively. The purple pigment was visible after seven days in the culture medium. Conidiophores undifferentiated from vegetative hyphae, terminal or arising as short lateral branches from vegetative hyphae, reduced to conidiogenous cells, up to 40 µm long, conidiogenous cells terminal or integrated, consisting cluster of small conidium bearing denticles, proliferating sympodially and repeatedly forming similar clusters. Conidiogenous loci sub-denticulate, inconspicuous, flattened. Conidia hyaline, smooth, aseptate, often guttulate; Primary conidia were variable in shape, ellipsoidal to fusiform, or obovoid, with basal scar and rounded apex, 3.7–6.9 × 1.4–2.6 µm; secondary conidia, formed on primary conidia, one to several, ellipsoidal or obovoid, 2–4.2 × 1.5–2.2 µm (Fig. 1).

DNA phylogeny

Megablast search analysis at NCBI's GenBank nucleotide database, showed 100% similarity with reference sequence of *Q. cyanescens* from GenBank (Table 1). A phylogeny inferred based on ITS-rDNA sequence data of representative isolates obtained in this study together with sequence data from GenBank. The final sequence alignment of the ITS-rDNA comprising 45 internal taxa had 692 characters and 314 unique site patterns. remaining 1074 (75%) generations were used to calculate the consensus Bayesian tree and posterior probabilities. Bayesian analyses were performed using the best-fitting substitution (GTR+G) model and resulted in 1432 generations. After discarding the first 25% of generations as burn-in, the Results indicated that the Iranian isolates used in this study (CCTU ZM1 and CCTU ZM2) clustered with *Q. cyanescens* isolates in same clade with highly supported value (Fig. 2).

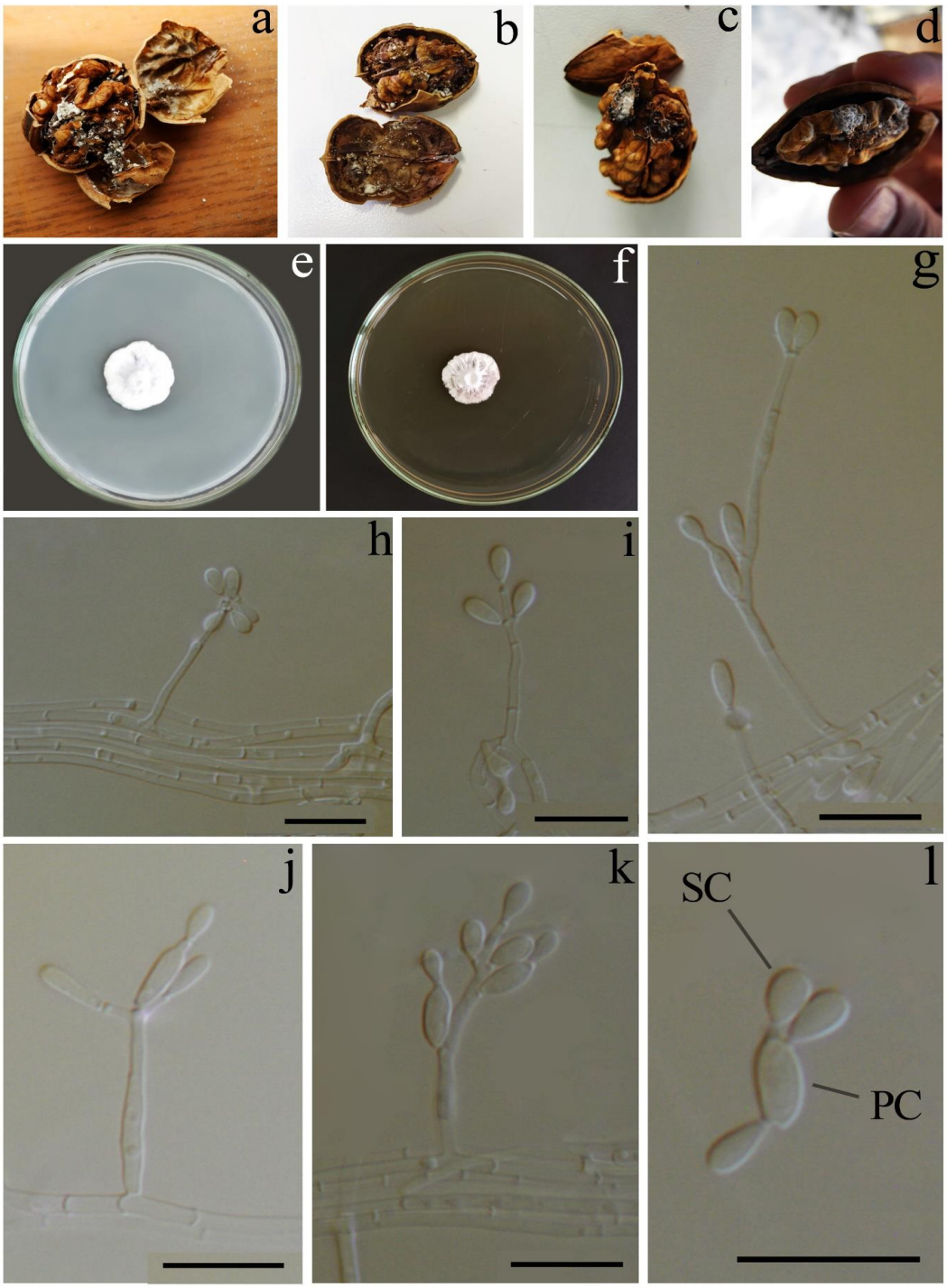

Fig 1: *Quambalaria cyanescens* a-b-c-d: Naturally infected walnut kernels; e: 7-day-old colony on MEA; f: 7-day-old colony on PDA; g-h-i-j-k: Conidiophores and conidia; l: primary (PC) and secondary conidia (SC). Scale bars: 10 µm.

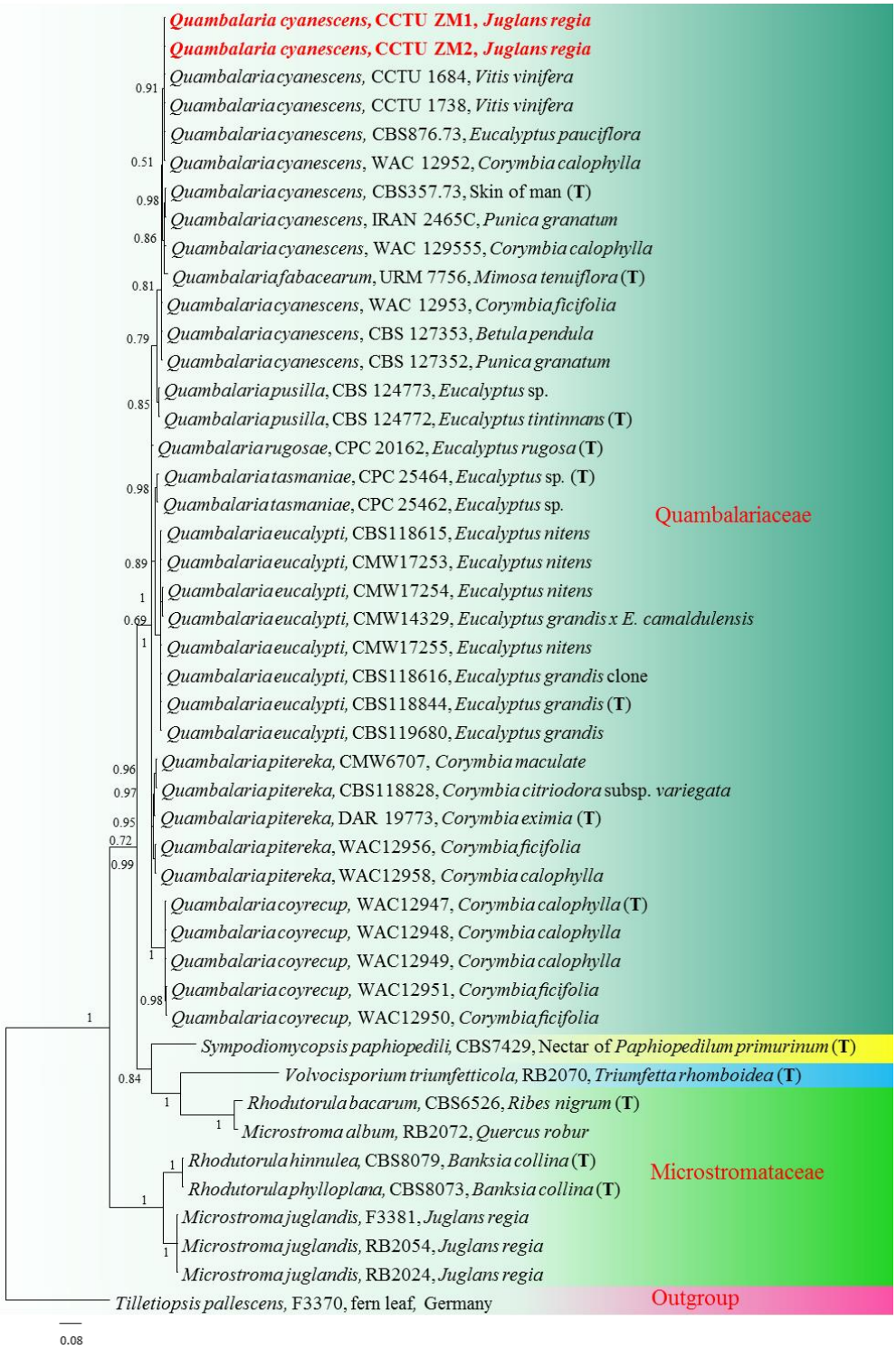

Fig. 2. Consensus phylogram obtained by a Bayesian analysis of the ITS-rDNA sequence alignment using MrBayes v. 3.2.6 of *Microstromatales*. The scale bar indicates 0.08 expected changes per site. The tree was rooted to *Tilletiopsis pallescens* (F3370, accession no. DQ317635).

According to a combination of morphological and phylogenetic data, our isolates were identified as *Q. cyanescens*.

Bioassay test

The isolates obtained in this study were evaluated for their insecticidal activity. However, in the case of isolates CCTU ZM1 and CCTU ZM2, significant mortality was not observed. The pathogenic potential of these isolates on codling remain to be tested.

DISCUSSION

Current study was initiated to characterize fungal isolates associated with moldy walnut kernels infested with codling moth larvae in Iran. In total, 20 fungal isolates were obtained from moldy walnut kernels. According to a combination of morphological and phylogenetic data, the isolates were identified as *Q. cyanescens*.

Quambalaria cyanescens has been reported from diverse range of substrates and ecological niches including human skin (de Hoog and de Vries, 1973); bark beetles on woody hosts in the Mediterranean, Hungary and Bulgaria (Kolařík et al. 2007); woody hosts (Vahedi-Darmiyan et al. 2017); larvae of olive moth (Preto et al. 2017) and Green Frogs' Skin (*Pelophylax esculentus* complex) (Stupar et al. 2022); and this species has been frequently isolated along with the other *Quambalaria* spp. from symptomatic (canker symptoms) and otherwise healthy *Corymbia* spp.; however, appears being no-pathogenic to *Eucalypts* and *Corymbia* (Paap et al. 2008). Very recently, *Q. cyanescens* has been reported as the causal agent of grapevine decline in Iran (Narmani and Arzanlou 2019). There are recent reports on the occurrence of *Q. cyanescens* as endophyte in woody hosts such as pomegranate, pistachio and almond (Vahedi-Darmiyan et al. 2017; Kari Dolatabad et al. 2019; Narmani and Arzanlou 2019).

There is report on the antagonistic property of *Q. cyanescens* against *Colletotrichum acutatum* J.H. Simmonds the causal agent of olive anthracnose disease (Oliveira et al. 2012). In addition it has shown that, *Q. cyanescens* produces a diverse range of bioactive metabolites such as napthoquinones quambalarine A and quambalarine B, with strong antifungal property against *Aspergillus funmigatus* and entomopathogenic fungus *Beauveria bassiana* (Stodůlková et al. 2015). Auambalarines are known as natural pigments with significant cytotoxic and antimicrobial properties (Prochazkova et al. 2020).

Quambalaria cyanescens is known as weak pathogen on humans, which was originally isolated from human skin (de Hoog and de Vries, 1973; Sigler et al. 1990). This species has been considered as a potential opportunistic human pathogen and has been reported from blood, skin and lung samples in immunodeficient patients (Kolarik et al. 2006; Stupar et al. 2022). This species has been recently isolated from skin of otherwise healthy green frogs (*Pelophylax esculentus* complex) in Serbia (Stupar et al. 2022). This species is known to assimilate complex benzene compounds (Middelhoven et al. 2000).

In the present study *Q. cyanescens* isolates were frequently isolated from walnut kernels with evident damage, feces and dead larval debris of codling moth in two different regions. This species has also been reported to occur on larvae of olive moth (Preto et al. 2017) and bark beetles on woody hosts in the Mediterranean, Hungary and Bulgaria (Kolařík et al. 2007). However, the pathologic relevance of *Q. cyanescens* isolates on the insect hosts remains unclear. In our study, the pathogenic potential of two isolates of *Q. cyanescens* were evaluated on larvae of *E. kouhniela*; however, the survival rate of larvae treated with different concentration of the *Q. cyanescens* spores, were the same as untreated control larvae and it can be concluded that the *Q. cyanescens* isolates were not pathogenic on *E. kouhniela* larvae. Bioassay tests on the larvae of codling moth or olive moth are required to further evaluate pathologic relevance of *Q. cyanescens* on the original hosts.

To the best of our knowledge, this is the first report on the association of *Q. cyanescens* with feces and larval debris of codling moth on walnut kernels. In this study, *Q. cyanescens* was only isolated in association with feces and debris of codling moth; it was absent in the other rotten and moldy walnut kernels. Different aspects of this species, including geographical distribution, host range (walnut and other woody hosts) and pathologic relevance on codling moth and other pest insects remain to be studied. In addition, it is known that insect damaged nuts display high levels of mold contamination (Campbell et al. 2003); hence, additional studies are required to explore biodiversity of fungal species associated with walnuts with specific reference to mycotoxigenic species.

REFERENCES

Baradaran Bagheri, M., Arzanlou, M. and Babai-ahari, A. 2015. Identification of the fungal agents associated with almond trunk diseases in East Azerbeijan province. Applied Research in Plant Protection 4: 27–41.

Bezerra, J.D.P., Machado, A.R., Firmino, A.L., Rosado, A.W.C., Souza, C.A.F.D., Souza-Motta, C.M.D., Freire, K.T.L.D.S., Paiva, L.M., Magalhães, O.M.C., Pereira, O.L. and Crous, P.W. 2018. Mycological diversity description I. Acta Botanica Brasilica 32: 656–666.

Crous, P.W., Wingfield, M.J., Cheewangkoon, R., Carnegie, A.J., Burgess, T.I., Summerell, B.A., Edwards, J., Taylor, P.W.J. and Groenewald, J.Z. 2019. Foliar pathogens of eucalypts. Studies in Mycology 94: 125–298.

Campbell, B.C., Molyneux, R.J. and Schatzki, T.F. 2003. Current research on reducing pre-and post-harvest aflatoxin contamination of US

almond, pistachio, and walnut. Journal of Toxicology. Toxin Reviews 22: 225–66.

Duong, H,T,, Mazanec, R., McComb, J.A., Burgess, T. and Hardy, G.E. 2022. Quambalaria shoot blight resistance in marri (Corymbia calophylla): genetic parameters and correlations between growth rate and blight resistance. Tree Genetics & Genomes. 18: 8.

de Beer, Z.W., Begerow, D., Bauer, R., Pegg, G.S., Crous, P.W. and Wingfield, M.J. 2006. Phylogeny of the Quambalariaceae fam. nov., including important Eucalyptus pathogens in South Africa and Australia. Studies in Mycology 55: 289–298.

de Hoog, G.S. and de Vries, G.A. 1973. Two new species of *Sporothrix* and their relation to *Blastobotrys nivea*. Antonie van Leeuwenhoek 39: 515–520.

FAO (Food and Agriculture Organization of the United Nations). 2023. FAO Statistical Databases.

Kari Dolatabad, H., Asadi Rahmani, H. and Rejali, F. 2019. Identification and evaluation of growth promoting and biocontrol properties of isolated endophytic fungi from the leaves and fruits of *Pistacia vera*. Journal of Soil Biology 7: 53–71.

Kolarik, M., Slavikova, E. and Pazoutova, S. 2006. The taxonomic and ecological characterisation of the clinically important heterobasiodiomycete *Fugomyces cyanescens* and its association with bark beetles. Czech Mycology 58:81.

Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874.

Middelhoven, W.J., Guého, E. and De Hoog, G.S. 2000. Phylogenetic position and physiology of *Cerinosterus cyanescens*. Antonie van Leeuwenhoek 77: 313–320.

Narmani, A., and Arzanlou, M. 2019. *Quambalaria cyanescens*, a new fungal trunk pathogen associated with grapevine decline in Iran. Crop Protection 124: 104875.

Oliveira, I,, Pereira, J.A., Lino-Neto, T., Bento, A. and Baptista, P. 2012. Fungal diversity associated to the olive moth, *Prays oleae* Bernard: a survey for potential entomopathogenic fungi. Microbial Ecology 63: 964–974.

Paap, T., Burgess, T.I., McComb, J.A., Shearer, B.L., and Hardy, G.E.S.J. 2008. *Quambalaria* species, including *Q. coyrecup* sp. nov., implicated in canker and shoot blight diseases causing decline of Corymbia species in the southwest of Western Australia. Mycological Research 112: 57–69.

Preto, G., Martins, F., Pereira, J.A., and Baptista, P. 2017. Fungal community in olive fruits of cultivars with different susceptibilities to anthracnose and selection of isolates to be used as biocontrol agents. Biological Control 110: 1–9.

Prochazkova, E., Kucherak, O., Stodůlková, E., Tošner, Z., Cisarova, I., et al. 2020. NMR Structure elucidation of Naphthoquinones from *Quambalaria cyanescens*. Journal of Natural Products 84: 46–55.

Sigler, L., Harris, J. and Dixon, D. 1990. Microbiology and potential virulence of *Sporothrix cyanescens*, a fungus rarely isolated from blood and skin. Journal of Clinical Microbiology 29: 1009–1115.

Stodůlková, E., Císařová, I., Kolařík, M., Chudíčková, M., Novák, P., et al. 2015. Biologically active metabolites produced by the basidiomycete *Quambalaria cyanescens*. PLoS One 10: p.e0118913.

Simpson, J.A. 2000. *Quambalaria*, a new genus of eucalypt pathogens. Australasian Mycologist 19: 57–62.

Stupar, M., Savković, Ž., Breka, K., Stamenković, S., Krizmanić, I., et al. 2023. A variety of fungal species on the green frogs' skin (*Pelophylax esculentus* complex) in South Banat. Microbial Ecology 86: 859–871.

Vahedi-Darmiyan, M.E., Jahani, M., Mirzaee, M.R. and Asgari, B. 2017. A noteworthy record of endophytic *Quambalaria cyanescens* from *Punica granatum* in Iran. Czech Mycology 69: 113–123.

White, T.J., Bruns, T., Lee, S.J.W.T. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols. A Guide to Methods and Applications, 18.1: 315–322.

شناسایی ریخت‌شناختی و مولکولی گونه *Quambalaria cyanescens* در میوه‌های گردو آلوده به کرم سیب

زهرا مهدیزاده، مهدی ارزنلو✉ و ابوالفضل نرمانی

گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه تبریز، تبریز، ایران.

چکیده: طی بررسی گونه‌های قارچی مرتبط با علایم پوسیدگی مغز میوه‌های خشک گردو در استان‌های آذربایجان غربی و شرق در سال ۱۴۰۱، یک توده میسیلیوم قارچی سفیدرنگ روی مغز میوه‌های گردو با نشانه های خسارت کرم سیب (فضولات و بقایای لارو) مشاهده گردید. نمونه‌های آلوده زیر استریو میکروسکوپ بررسی شد و با یک سوزن سترون مقداری از میسیلیوم همراه با توده سفیدرنگ اسپوری برداشته شد و به روش تک اسپور کردن خالص‌سازی گردید. ویژگی‌های ریخت‌شناختی جدایه‌های قارچی روی محیط‌های کشت عصاره سیب‌زمینی-دکستروز-آگار (PDA) و عصاره مالت آگار (MEA) در دمای ۲۱ درجه سانتی گراد و شرایط تاریکی بررسی گردید. به منظور تایید شناسایی قارچ‌های جداسازی شده، ناحیه ژنومی ITS-rDNA با استفاده از آغازگرهای عمومی این ناحیه (ITS1 و ITS4) طی واکنش زنجیره‌ای پلی‌مراز تکثیر و محصول واکنش توالی‌یابی گردید. با مطالعه صفات ریخت‌شناختی و ترکیب آن با داده‌های مربوط به توالی یابی، جدایه‌های بدست آمده شده در این تحقیق *Quambalaria cyanescens* شناسایی شدند. توالی ناحیه ITS جدایه‌های مورد مطالعه با توالی موجود برای جدایه تیپ گونه *Q. cyanescens* در بانک ژن، ۱۰۰ درصد شباهت نشان داد. قطر پرگنه این گونه بعد از یک هفته روی محیط کشت PDA و MEA به ترتیب ۱۱ و ۱۲ میلی‌متر ارزیابی گردید و مشخص شد که این گونه تولید رنگدانه بنفش در محیط کشت می‌کند. کنیدیفورها غیر متمایز از هیف‌های رویشی بوده و کنیدیوم‌زایی به شیوه هولوبلاستیک می‌باشد. کنیدیوم‌ها غالبا تخم مرغی یا گلابی شکل، شفاف و در اندازه های ۲/۵-۱/۵× ۸-۲ میکرومتر می‌باشند. *Q. cyanescens* از گونه‌های نادر بازیدیومیستی از راسته *Microstromatales* می‌باشد که دارای مرحله مخمری نیز می‌باشد. توانایی بیماری‌زایی دو جدایه از این گونه روی لارو شپ پره مدیترانه‌ای (*Ephestia kuehniella*) در شرایط آزمایشگاهی ارزیابی گردید. با این وجود نرخ بقا و زنده‌مانی لاروهای تیمار شده با غلظت‌های مختلف سوسپانسیون اسپور قارچ *Q. cyanescens* با تیمار شاهد تفاوتی نشان ندادند. بنابراین می‌توان نتیجه‌گیری کرد که این گونه روی لارو شب پره مدیترانه‌ای توانایی بیماری‌زایی ندارد. این مطالعه اولین گزارش از همراهی *Q. cyanescens* با فضولات و بقایای لاوری کرم سیب می‌باشد.

کلمات کلیدی: اکالیپتوس، پوسیدگی مغز گردو، خسارت حشرات، Quambalariaceae

مکاتبه کننده: مهدی ارزنلو Email: arzanlou@tabrizu.ac.ir

تاریخ دریافت: ۱۴۰۲/۱۲/۱۷ تاریخ پذیرش: ۱۴۰۳/۲/۱۹