**Neocosmospora caricae** sp. nov. and *N. metavorans*, two new stem and trunk canker pathogens on *Ficus carica* in Iran

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Received: 16 May 2022 / Revised: 9 August 2022 / Accepted: 12 August 2022
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**Abstract**

During 2018–2021, a survey was conducted in rainfed fig (*Ficus carica* L.) orchards throughout the Fars Province of Iran to investigate the occurrence of canker diseases, and to identify the causal organisms. Morphological and cultural characteristics, as well as multilocus phylogenetic analyses of the internal transcribed spacer (ITS) region of rDNA, RNA polymerase II second largest subunit (*RPB2*), and the translation elongation factor 1-alpha (*TEF1*), revealed that the recovered isolates from the infected fig trees clustered in clade 3 of *Neocosmospora* (*Nectriaceae*), including *N. metavorans*, and a new taxon described here as *N. caricae* sp. nov. *Neocosmospora caricae* is characterised by falcate, multiseptate, gently dorsoventrally curved macroconidia with poorly developed foot-shaped basal cells, ovoid, aseptate microconidia that cluster in false heads, and abundant terminal or intercalary chlamydospores. Pathogenicity tests indicated that isolates of both *Neocosmospora* species were pathogenic, causing stem canker and wood discolouration on fig saplings of “Sabz” and “Shah Anjeer” cultivars. The present study adds to existing knowledge on the aetiology of fig stem and trunk canker, and may provide essential information for developing effective integrated management strategies against canker diseases affecting fig orchards in Iran.

**Keywords** Fig · Multigene phylogenetic analysis · *Nectriaceae* · *Neocosmospora* spp. · 1 new taxon · Trunk canker

**Introduction**

The common fig (*Ficus carica* L.) is an ancient crop species belonging to the *Moraceae* family originating from the Mediterranean basin (Berg 2003). Iran is the fifth largest producer of figs after Turkey, Morocco, Greece, and Spain (FAOSTAT 2020) with 107,791 tons of fig production annually, and Fars Province is Iran’s leading dried fig producer, with 51,000 ha devoted to fig cultivation (Jafari et al. 2018). Despite the special significance of dried figs to Iran’s economy, some limiting factors such as fig canker disease decrease yield and export of this product.

In recent years, the most extensive collection of rainfed fig cultivars in Estahban, and other fig plantations in Fars Province have been at risk of a widespread decline caused by fig canker disease. The primary cause of fig canker disease in Iran is *Diaporthe cinerascens* Sacc. (syn. *Phomopsis cinerascens* (Sacc.) Traverso) (Banihashemi and Javadi 2009). However, different fungal plant pathogens are reported to attack fig and cause canker disease in other parts of the world, including species in the *Botryosphaeriaceae* such as *Neofusicoccum parvum* (Pennycook & Samuels) Crous et al. in Italy (Aiello et al. 2020), *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. in Turkey (Çeliker and Michailides 2012), and *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers in Australia (Elshafie and Ba-Omar 2002; Ray et al. 2010), a
species from Ceratocystidaceae, Ceratocystis ficiola
Kajitani & Masuya, in Japan (Kajitani and Masuya 2011),
and Stilbocrea banthashemiana Z. Bolboli, B. Tavakolian &
Mostowf., from Bionectriaceae, in Iran (Bolboli et al. 2022).

During a recent survey to identify fungal pathogens asso-
ciated with canker diseases of edible fig trees in southern Iran,
several Neocosmospora spp. isolates (formerly Fusarium
solani species complex = FSSC) were obtained from infected
tissues. Neocosmospora is one of the fusarioid genera that has
been segregated from the genus Fusarium sensu lato
(Lombard et al. 2015). Species of this genus affect an exten-
sive range of hosts, including humans, animals, and plants
(O’Donnell et al. 2008; Lombard et al. 2015). Several species
of Neocosmospora cause stem and trunk canker diseases of
trees. For example, N. perseae Sand.-Den. & Guar
caccia on avocado (Persea americana Miller.) in Italy (Guarnaccia et al.
2018), N. croci Guar
caccia et al. ( = N. martii (Appel &
Wollenw.) Sand.-Den. & Crous), N. macrospora Sand.-Den.
et al. and N. solani (Mart.) L. Lombard & Crous on English
walnut (Juglans regia L.) in Turkey (Sandoval-Denis et al.
2019; Polat et al. 2020), N. solani on pistachio (Pistacia vera
L.) trees in California (Crespo et al. 2019) and N. euwalliceae
(S. Freeman et al.) Sand.-Den. et al. on avocado in Israel and
California (Freeman et al. 2013). Still, to our knowledge, there
are not any reports of canker-causing species of
Neocosmospora on edible figs.

Observing widespread decline and trunk cankers on fig
trees in several fig plantations in southern Iran (Fars Province),
we focused our studies on identifying the fig can-
ker’s causal agents during 2018–2021. The present study
identified two new stem and trunk canker pathogens of figs
belonging to the genus Neocosmospora, of which one repre-
sented a new species. Koch’s postulates were also confirmed
for both species.

Materials and methods

Sampling and fungal isolation

During 2018–2020, infected fig trees with decline and canker
symptoms were sampled from fig orchards in various parts of
Fars Province (Estahban, Firuzabad, Jahrom, Kazerun, and
Nayriz Counties). Transverse sections of infected branches
and trunks were prepared, and small pieces (5 × 5 mm) from
the margins between healthy and discoloured or decayed
wood tissues were cut, washed under running tap water, sur-
face disinfected for 1 min in a 70% ethanol, 1 min in a 2%
sodium hypochlorite solution and rinsed twice in sterile dis-
tilled water (Gonzalez-Dominguez et al. 2016). Surface
disinfected tissue samples were dried in sterile paper towels
under a laminar flow-hood, and subsequently plated on Petri
dishes containing potato dextrose agar (PDA; extract of 300-
g/L boiled potato, 20-g/L glucose monohydrate, 15-g/L aga-
rose, and distilled water) amended with tetracycline (1 mg/L).
Plates were incubated at 25 °C for 7 days. All isolates were
then transferred onto water agar (WA; 20-g/L agar, and dis-
tilled water) and single conidial isolates established once
sporulating.

Morphological characterisation

Isolates were transferred onto carnation leaf agar (CLA)
(Fisher et al. 1982), oatmeal agar (OA; extract of 30-g/L
boiled oatmeal, 15-g/L agar, distilled water), and PDA.
Morphological identification and characterisation for all
fusarioid isolates were performed based on Crous et al.
(2021). Average growth rates at 25 and 30 °C were obtained
from colony diameters on PDA (90 mm Petri dishes with
25 ml medium), after 7 days of incubation in the dark with
three replicates per isolate. Colony morphology and pigments
were recorded after 7 days of incubation at 25 °C in the dark
(Sandoval-Denis et al. 2019), using the colour chart of
McKnight and Rayner (1972).

DNA extraction, PCR amplification, and sequencing

Total fungal DNA was extracted using the method described
by Mirsoleimani and Mostowfizadeh-Ghalamfarsa (2013).
Mycelia were harvested from the isolates grown in potato
extract broth (extract of 300-g/L boiled potatoes in distilled
water) for 7–10 days, then freeze-dried, and DNA was extract-
ed with DNG-PLUS extraction kit (CinnaGen, Tehran, Iran).
DNA quality was examined with a MD-1000 Nanodrop spec-
trophotometer (NanoDrop Technologies, Delaware, USA).
The nc rDNA internal transcribed spacer (ITS) region
(ITS1–5.8S–ITS2) was amplified using the primer set ITS1
(5′-TCCTCCGTATTGATATGC-3′) and ITS4 (5′-
TCCTCGGTTATGATAGC-3′) following the protocol
of White et al. (1990). RNA polymerase II second largest
subunit (RPB2) was amplified using primers RPB2-5F2 (5′-
GGGGWGAYCAGAAGAAGGC-3′) and RPB2-7cR (5′-
GTAAGGARGACAAGAC-3′) (Sung et al. 2007) and
EF-2TR (5′-CCCCATRGGCTTGYRCCCAT-3′) (Liu
et al. 1999) and translation elongation factor 1-alpha (TEF1)
was amplified with primers EF-1H (5′-ATGG GTAAGGARGACAAGAC-3′) and EF-2T (5′-
GGARGTACCAGTACATG-3′) (O’Donnell 1998).
Temperature and time conditions for PCR amplification are
listed in Table 1. PCR amplifications were performed on a
Peltier Thermal Cycler (Techne, Germany). PCR products
were sequenced with the same primer pairs used for amplifi-
cation by a dye terminator cycle (Cardiogenetic Research
Center, Tehran, Iran). Sequenced data were deposited in
GenBank (www.ncbi.nlm.nih.gov/genbank). Accession
numbers are listed in Table 2.
Phylogenetic analysis

The isolates’ forward and reverse nucleotide sequences were edited, proofread, and assembled in BioEdit v. 7.0.9.0 (Hall 1999). Sequence alignment was conducted by Clustal X (Thompson et al. 1997) with subsequent manual adjustment. Partition homogeneity tests were conducted on the combined nuclear gene alignment by PAUP v. 4.0a136 (Swofford 2002) using 100 replicates and the heuristic general search option. Alignments derived in this study were deposited in Figshare (www.figshare.com; doi identifier https://doi.org/10.6084/m9.figshare.20455476.v1).

To reconstruct the phylogenetic trees, Bayesian inference analyses on individual and concatenated ITS, RPB2, and TEF1 loci were carried out with MrBayes v. 3.1 (Ronquist and Huelsenbeck 2003). Additional sequences included in this study were retrieved from GenBank and sequences of the ascomycete Geejayessia atrofusca (Schwein.) Schroers & Gräfenhan (NRRL 22316) served as the outgroup taxon in all analyses included (Supplementary Table 1) (Sandoval-Denis et al. 2019). The best nucleotide substitution model was determined by MrModelTest v. 2.3 (Nylander 2004). Two independent runs of Markov chain Monte Carlo (MCMC) using four chains were run over 1,000,000 generations. Trees were saved each 1000 generations, resulting in 10,001 trees. Burn-in was set at 25% generations. In order to conduct a phylogenetic comparison, maximum likelihood estimation was carried out using PHYLIP DNAML (Felsenstein 1993) with the same dataset. The robustness of the maximum likelihood trees was estimated by 1000 bootstraps. Phylogenetic trees were edited and displayed with TreeGraph (Stöver and Müller 2010).

Pathogenicity tests

Pathogenicity tests were conducted on detached woody shoots (fresh vegetative shoots, collected from 5–10-year-old fig trees and cut into 25–30 cm pieces (5–9 mm diam)) and mature 1-year-old fig saplings of Ficus carica cv. Shah Anjeer and cv. Sabz grown from cuttings in greenhouse conditions at 26 ± 3 °C. For both experiments, the outer bark at the inoculation site was cleaned and surface-sterilised with 70% ethanol, and a 6-mm wound was made using a sterilised cork-borer. A 6-mm diam mycelium plug taken from the margin of a 5-day-old PDA culture was inserted into the wound and covered with Parafilm (USA, Bemis Packaging) to prevent desiccation and contamination. Non-colonised PDA agar plugs served as the negative control (Roux et al. 2007). In the detached woody shoots experiment, the bases of inoculated shoots were inserted into Erlenmeyer’s flasks covered with Parafilm, with 500 ml of sterilised water, then kept under greenhouse conditions at 25 ± 2 °C. Inoculated detached shoots and saplings, as well as uninoculated controls, were returned to the laboratory 21 days after inoculation, their bark removed, and disease symptoms investigated. For re-isolation of fungal pathogens, five pieces (2 × 5 mm) from the margins of necrotic lesions were surface disinfected for 1 min in 70% ethanol, followed by 1 min in a 2% sodium hypochlorite solution, rinsed twice in sterile distilled water, and plated on PDA plates to recover and identify the inoculated fungi and complete Koch’s postulates.

Results

Field surveys and disease symptoms

Fig trees attacked by canker-causing fusarioid fungi displayed external and internal symptoms. External symptoms included leaf yellowing and defoliation, limb dieback, and three types of trunk cankers (Figs. 1 and 2). Type B cankers originated from the crown and developed upward (Fig. 2A), whereas type C was observed as well-developed sunken trunk lesions (Fig. 2E) and type D which consisted in cracked, discoloured, and dead areas on the main stem and branches (Fig. 1B). Internal symptoms included brown to dark brown discoloration of vascular tissues and different types of wood necrosis (Figs. 1 and 2). The occurrence of each symptom varied in an orchard from tree to tree, depending on cultivars.

Table 1 PCR conditions for primers used in this study

| Gene          | Initial denaturation | Number of cycles | Desaturation | Annealing | Extension | Final extension |
|---------------|---------------------|------------------|--------------|-----------|-----------|-----------------|
| ITS<sup>1</sup> | 95 (120)<sup>4</sup>  | 30               | 95 (45)      | 64 (45)   | 72 (45)   | 72 (600)        |
| RPB2<sup>2</sup> | 95 (120)             | 35               | 95 (120)     | 66.5 (60) | 72 (45)   | 72 (600)        |
| TEF1<sup>3</sup>  | 95 (120)             | 35               | 95 (120)     | 64 (60)   | 72 (45)   | 72 (600)        |

<sup>1</sup> Internal transcribed spacers 1 and 2, and 5.8 S gene of nc rDNA
<sup>2</sup> RNA polymerase II second largest subunit
<sup>3</sup> Translation elongation factor 1-alpha
<sup>4</sup> Temperature, °C (time, s)
Table 2  List of *Neocosmospora* spp. isolates recovered from infected fig trees, their GenBank accession numbers, and their corresponding observed disease symptoms in fig orchards of Fars Province of Iran

| Species and isolates | Location | Cultivar | Date of sampling | Latitude | Longitude | GenBank accession number | External disease symptoms | Internal lesion types |
|----------------------|----------|----------|------------------|----------|-----------|--------------------------|--------------------------|----------------------|
|                      |          |          |                  |          |           |                          |                          |                      |
| *N. caricae*         |          |          |                  |          |           |                          |                          |                      |
| ES212-1              | Estahban | “Sabz”   | Dec. 2020        | 29°06’.852”N | 05°4’04’.487”E | OK539515 OK415856 OK422515 | + + + -               | + + -               |
| ES212-2              | Estahban | “Sabz”   | Dec. 2020        | 29°06’.852”N | 05°4’04’.487”E | OK539516 OK415857 OK422516 | + + + -               | + + -               |
| ES216                | Estahban | “Sabz”   | Dec. 2020        | 29°06’.793”N | 05°4’04’.473”E | OK539517 OK415858 OK422517 | + + + -               | - + -               |
| ES216-R              | Estahban | “Sabz”   | Dec. 2020        | 29°06’.793”N | 05°4’04’.473”E | OK539519 OK415860 OK422519 | + + + -               | - + -               |
| ES216-M              | Estahban | “Sabz”   | Dec. 2020        | 29°06’.793”N | 05°4’04’.473”E | OK539518 OK415859 OK422518 | + + + -               | - + -               |
| ES006                | Estahban | “Sabz”   | Nov. 2019        | 29°06’.792”N | 05°4’04’.481”E | OL6711097 -             | -                        | + + +               |
|                      |          |          |                  |          |           |                          |                          |                      |
| *N. metavorans*      |          |          |                  |          |           |                          |                          |                      |
| Esh191B              | Estahban | “Shah Anjeer” | Dec. 2020    | 29°06’.852”N | 05°4’04’.567”E | OK422512 OK415854 OK539520 | + + + +               | - + -               |
| NPDJ                 | Nayriz   | “Payves” | Dec. 2020        | 29°08’.777”N | 05°4’17’.480”E | OK422514 OK392021 OK539522 | + + + +               | + - -               |
| NPDJ-2               | Nayriz   | “Payves” | Dec. 2020        | 29°08’.838”N | 05°4’17’.480”E | OK422513 OK415853 OK539521 | + + + +               | + - -               |
| ES011                | Estahban | “Sabz”   | Nov. 2019        | 29°10’.657”N | 05°3’49’.596”E | OL671198 -             | -                        | + + -               |
| ES012                | Estahban | “Sabz”   | Nov. 2019        | 29°10’.656”N | 05°3’49’.598”E | OL671199 -             | -                        | + + -               |
| ES012-1              | Estahban | “Sabz”   | Nov. 2019        | 29°06’.807”N | 05°4’04’.476”E | OL671200 -             | -                        | + + -               |

External disease symptoms: CA canker, DF defoliation, DI dieback, Y yellowing

Internal shoot symptoms: CN crescent-shaped necrosis, IN irregular-shaped necrosis, WN wedge-shaped necrosis

*Ex-type*= CBS 148865
Fig. 1 Symptoms of canker disease caused by *Neocosmospora metavorans* observed on the main stem and branches of *Ficus carica* cv. Shah Anjeer (Estahban, Fars, Iran). A Yellowing of the leaves and dieback of branches. B Type D of fig canker disease: cracked, discoloured, and dead areas on the main stem and branches. C–D Wood decay of an infected tree in transverse and longitudinal view. E Transverse sections through a branch of an infected fig tree.

Fig. 2 Symptoms of canker disease caused by *Neocosmospora caricae* sp. nov. observed on the main stem of *Ficus carica* cv. Sabz (Estahban, Fars, Iran). A Type B of fig canker disease: a trunk canker originated from the crown and developed upward with holes in the wood produced by fig tree borer. B Wedge-shaped necrosis in transverse sections of infected fig trees. C–D irregular-shaped necrosis in longitudinal and transverse view. E Type C of fig canker disease: a well-developed sunken lesion on the trunk.
locations, and the orchards surveyed. From all sampled counties (Estahban, Firuzabad, Jahrom, Kazerun, and Nayriz), canker-causing fusarioid isolates were only isolated from the infected fig trees in Estahban and Nayriz. Thirteen fusarioid isolates were identified from diseased fig trees based on morphological and phylogenetic data. Trunk and branch cankers chiefly developed from pruning wounds, fig tree borer (Phryneta spinator Fabricius, Coleoptera: Cerambycidae) feeding sites, sunburn lesions, blighted shoots, and wounds that were caused by mechanical injuries (Table 2).

**Phylogenetic analyses**

Representative fusarioid isolates including, Esh191B, NPDRJ, NPDRJ-2 ES212-1, ES212-2, ES216-M, ES216, and ES216-R were subjected to multilocus sequence analyses. Polymerase chain reaction (PCR) amplification of the ITS, RPB2, and TEF1 regions generated 523–525, 863–870, and 686–688 bp fragments, respectively. BLASTn searches in GenBank showed that RPB2 sequences of some isolates (ESH191B, NPJ, and NPJ-2) had 99–100% identity with isolates previously described as *Neocosmospora metavorans* (Al-Hatmi et al.) Sand.-Den. & Crous (strain F201334 and KM520375 (Zhou et al. 2016)). The TEF1 sequences of these isolates also had 99–100% identity with isolates previously identified as *N. solani* (strain NRRL 22654 GenBank accession no. DQ247636 (Zhang et al. 2006)) and *N. metavorans* (strain NRRL44904, GenBank accession no. GU170621 (Migheli et al. 2010)). Furthermore, ITS sequences showed 99–100% identity with *N. solani* (strain CBS 143218 GenBank accession No. LR583743 (Sandoval-Denis et al. 2019)).

Results from maximum likelihood and Bayesian methods showed that *N. metavorans* isolates from fig canker (ESH191B, NPJ, and NPJ-2) were closely related to a *N. metavorans* isolate from *Malus sylvestris* L. (culture/specimen: CBS 233.36 = NRRL 22654 (Sandoval-Denis et al. 2019)), both of which were clustered strongly (1/100%) in a monophyletic subclade within *N. metavorans* (Fig. 3).

Several isolates with unique morphological features were recovered from trunks and branches of infected fig trees in plantations of southern Iran. BLASTn searches in GenBank showed that RPB2 sequences of these isolates had ca. 99% identity with isolates previously described as *N. parceramosa* Sand.-Den. & Crous (strains NRRL 31158, GenBank accession No. EU329559 (O’Donnell et al. 2008)). *N. liliodendri* Sand.-Den. & Crous (strains NRRL 22389, GenBank accession No. EU329506 (O’Donnell et al. 2008)) and *N. petroliphila* (Q.T. Chen & X.H. Fu) Sand.-Den. & Crous (strain JMR: NZ: 0086, GenBank accession No. MF467496 (Walther et al. 2017)). The TEF1 sequences of these isolates also had 98% identity with isolates previously identified as *Fusarium* sp. (strain NRRL 13414 GenBank accession No. MK818415 (Carrillo et al. 2020)) and *N. petroliphila* (strain NRRL 44904, GenBank accession No. KJ867424 (Ersal et al. 2015)). Furthermore, the partition homogeneity test between ITS, RPB2, and TEF1 loci resulted in a P value of ca 0.9 indicating statistical congruence, so the null hypothesis of congruence is accepted (P ≥ 0.05), which means these genes have co-evolved.

**Taxonomy**

The multigene genealogy using nuclear ribosomal and protein-coding loci (ITS, RPB2, and TEF1) showed that these isolates were significantly distinct from other known *Neocosmospora* species and clustered in a monophyletic clade with strong supporting values both in Bayesian and maximum likelihood trees. The new lineage is proposed here as a new species, *Neocosmospora caricae* sp. nov.

**Neocosmospora caricae**  Z. Bolboli & Mostowf., sp. nov. MycoBank 844080 Fig. 4.

Etymology: Name reflects the host species, *Ficus carica*. Typification: IRAN, Fars Province, (29°06′.793″N – 054°04′.473″E) Estahban, on trunk of *Ficus carica*, Dec. 2020, Z. Bolboli (holotype CBS 148865, stored in a metabolically inactive state), Westerdijk Fungal Biodiversity Institute (CBS; Utrecht, The Netherlands).

Aerial conidiophores: highly abundant on aerial mycelium, straight rarely simple, often branched verticillately and sympodially, 64.2–80.1 × 2.1–3.2 μm (av. 71.4±6.8 × 2.6±0.4 μm) simple arial monopodial phialides, *Microconidia*: oval, obvoid to somewhat reniform, clustering in false heads at tip of monopodial phialides on slender, elongated arial phialides and aerial conidiophores, 0(–1)-septate, (5–)6–10.5 × (2.5–)3.5–5 μm (av. 8.2±2.1 × 4.1±0.6 μm), smooth- and thin-walled. *Sporodochia*: pale luteous to citrine, formed abundantly on the surface of carnation leaves after 14 d; *sporodochial conidiophores*: unbranched or branched multiple times, sporodochial phialides subcylindrical, subulate to doliiform, unbranched or branched multiple times, conidiophores: (ovoid to somewhat reniform, clustering in false heads at tip of monopodial phialides on slender, elongated arial phialides and aerial conidiophores, 0(–1)-septate, (5–)6–10.5 × (2.5–)3.5–5 μm (av. 8.2±2.1 × 4.1±0.6 μm), smooth- and thin-walled, with short apical collarette, pericilal thickening inconspicous or absent. *Sporodochial conidia*: fusoid, gently dorsivertically curved with somewhat

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Fig. 3 Phylogenetic relationships of *Neocosmospora* species from infected fig trees of Fars Province: relationships among 71 *Neocosmospora* species (92 isolates) based on Bayesian analysis of multigene genealogies of ITS (internal transcribed spacers 1 and 2) and *RPB2* (RNA polymerase II second largest subunit) and *TEF1* (translation elongation factor 1-alpha) sequences. Numbers on the nodes are Bayesian posterior probability values (BI-PP) followed by Maximum Likelihood bootstrap values (ML-BS) Full supported branches (ML-BS = 100/BP = 1). Ex-type isolates are indicated with T. *= ex-type of *N. caricae* sp. nov.
parallel walls or slightly widened above the mid line, basal cell with a poorly to well-developed foot shape, apical cell blunt and slightly curved, (3–)5–(6)-septate, hyaline, smooth-walled. Three-septate conidia: 28.1–40× 3.3–4.9 μm (av. 34.2±2.9× 4.2±0.3 μm); four-septate conidia: 34.2–51× 3.2–5.5 μm (av. 41.7±4.9× 4.2±0.6 μm); five-septate conidia: 33.6–45.3 × 4.3–5.6 μm (av. 39.9±2.6× 5.1±0.4 μm); six-septate conidia: 60.5–69.9 × 5.4–6.3 μm (av. 65.7±3.1 × 5.8 ±0.2 μm). *Chlamydospores*: abundant and rapidly formed on agar media (approx. 7 days), hyaline, globose to subglobose, 6.4–9 × 4.3–8.4 μm (av. 7.3±0.8 × 61±10 μm, n = 30), solitary or in chains, terminal, intercalary or borne on short lateral pegs, smooth- and thick-walled.

*Colonial characteristics*: Colonies on PDA growing in the dark with an average radial growth rate of 6.1–6.3 mm/days at 25 °C, reaching 64.3 mm diam in 7 days at 25 °C; white, pale luteous to luteous at centre, flat to slightly raised, cottony, with abundant aerial mycelium; colony margin filiform. Reverse pale straw to pale luteous. On OA incubated in the dark reaching 61.2 mm diam in 7 days at 25 °C; white to yellowish, flat, membranous with scant white aerial mycelia.

*Cardinal temperatures for growth*: Minimum 10 °C, maximum 37 °C, optimum 25 °C.

*Other specimens examined (paratypes)*: Iran, Fars Province: Estahban (29°06′.852″N–054°04′.487″E) from the trunk of *Ficus carica* cv. Sabz ES212-1, 23 Dec. 2020, Z. Bolboli, CBS 148933. Iran, Fars Province: Estahban (29°06′.852″N–054°04′.487″E) from the trunk of *Ficus carica* cv. Sabz ES212-2, 23 Dec. 2020, Z. Bolboli, CBS 148932. Iran, Fars Province: Estahban (29°06′.793″N–054°04′.473″E) from the trunk of *Ficus carica* cv. Sabz ES216, 23 Dec. 2020, Z. Bolboli. Iran, Fars Province: Estahban (29°06′.793″N–054°04′.473″E) from the trunk of *Ficus carica* cv. Sabz ES216-R, 23 Dec. 2020, Z. Bolboli, CBS 148930.

**Pathogenicity tests**

Pathogenicity of representative isolates Esh191B, ES212, and ES216 were evaluated in two experiments on detached twigs and 1-year-old saplings, respectively. All isolates used in both pathogenicity tests produced cankers, vascular tissue discoloration and yellowing on *Ficus carica* cv. Shah Anjeer and cv. Sabz saplings. The first visible symptom was the appearance of discoloration that began from the inoculation site and developed longitudinally on detached twigs and saplings. Based on pathogenicity tests, *N. metavorans* and *N. caricae* sp. nov. isolates produced canker disease symptoms on fig stems 10 and 21 days after inoculation, respectively (Fig. 5). Common symptoms included brown to dark brown discoloration of vascular tissues, wood necrosis, and branch dieback. Yellowing and defoliation of sapling were observed 5 months after inoculation. Symptoms were similar to those observed in infected fig trees in orchards. Inoculated isolates could be recovered from lesion margins. Control plants remained healthy.

**Discussion**

The primary cause of fig canker disease in Iran has been reported to be *Diaporthe cinerascens* (syn. *Phomopsis cinerascens*) (Banihashemi and Javadi 2009). Another causal agent of stem cankers and twig dieback of fig trees in southern Iran has been very recently reported to be *Stilbocrea banihashemiana* (Bolboli et al. 2022). Our results demonstrate that some *Neocosmospora* species (formerly *Fusarium solani* species complex = FSSC) cause fig trunk and branch canker in Estahban county, along with other parts of the Fars Province, which represent Iran’s largest fig producing region.

Although species of *Fusarium* have been associated with canker diseases on some horticultural and forestry trees such as sweet orange, *Citrus × sinensis* (L.) Osbeck, (*F. salinense* Sand.-Den., Guarnaccia & Polizzi), *Citrus* spp. (*F. citricola* Guarnaccia & Sand.-Den.), pines (*F. circinatum* Nirenberg & O’Donnell), and pistachio, *Pistacia vera* L., (*F. oxysporum* Smith & Swingle, and *F. proliferatum* (Matsush.) Nirenberg) (Pfenning et al. 2014, Sandoval-Denis et al. 2018, Crespo et al. 2019), there are no reports of *Fusarium* or *Neocosmospora* cankers from edible fig. However, some *Fusarium* species have been shown to be the causal agents of fig fruit diseases, e.g., *F. moniliforme* J. Sheld (now: *F. verticillioides* (Sacc.) Nirenberg) (Droby et al. 2011, Kosoglu et al. 2011, Crous et al. 2021; Guarnaccia et al. 2021), and *F. proliferatum* (Fawzi 2003). It seems that *F. proliferatum* isolates from many crops, including fig trees, are phylogenetically different from the original ex-type strain, and belong to a morphologically and phylogenetically diverse clade, *F. annulatum* Bugnic (Yilmaz et al. 2021).

Multi-locus phylogenetic analyses using three loci (ITS, RPB2, and TEF1), as well as morphological analysis, revealed that all fusarioid isolates in this study belong to clade 3 of the genus *Neocosmospora*, including *N. metavorans* and a new taxon, *N. caricae* sp. nov. Sandoval-Denis et al. (2019) provided a comprehensive phylogeny for *N. metavorans*, which included 19 isolates that originate from different substrates, namely humans, insects, and plants. These isolates are clustered in several subgroups in the clade. They are mostly known from human clinical samples, and only a single isolate
is associated with a plant, *M. sylvestris*. *Neocosmospora metavorans* isolates from fig canker were closely related to *N. metavorans* from *M. sylvestris*, which formed a subclade distinct from other isolates from humans and animals.

Isolates of *N. metavorans* were also recovered from the intestines and mouth parts of *Phryneta spinator* larvae. This longhorn beetle from *Cerambycidae* is a wood borer that attacks fig trees in Iran. The larvae tunnels were also
observed on the canker sites of fig trunks. These observations agreed with previous reports of symbiotic relationships between canker-causing Neocosmospora species and fruit and nut tree borers. For example, *N. euwallacea* and *N. ambrosia* (Gadd & Loos) L. Lombard & Crous, associated with symbiotic *Euwallacea* beetles in avocado (Freeman et al. 2013), and *N. metavorans* isolates from the guts of the wood-boring cerambycid beetles, *Anoplophora glabripennis* Motschusky (Herr et al. 2016). Hence, fig tree borer larvae can be considered as potential vectors or transmitters of canker-causing Neocosmospora species in fig. More experiments, however, should be conducted to confirm this hypothesis.

Several *N. caricae* sp. nov. isolates were recovered from trunks and branches of infected fig trees in plantations of southern Iran. Morphological and multigene phylogenetic studies using ribosomal and protein-coding loci (ITS, RPB2, and TEF1) showed that these isolates were significantly distinct from other known Neocosmospora species. The differences were more evident in the TEF1 phylogeny than in the other genes. Neocosmospora caricae sp. nov. appeared as a sister taxon to *N. petroliphila*, one of the most prevalent species associated with human infections (Sandoval-Denis et al. 2019). Morphologically, the apical cells of sporodochial conidia in *N. caricae* sp. nov. were short, and the basal cells poorly developed foot-shaped, vs longer and more curved apical cells of sporodochial conidia in *N. petroliphila*. Furthermore, sporodochial conidia in *N. caricae* sp. nov. were shorter than those of *N. petroliphila* and *N. metavorans* (Short et al. 2013, Sandoval-Denis et al. 2018). The morphological differences, as well as the phylogenetic analyses, supported describing these isolates as a new species.

Four different types of canker were observed in the infected fig orchards; we named them as types A–D (Bolboli et al. 2022). Only the previously reported *Diaporthecina cinerascens* (syn. *Phomopsis cinerascens*) (Banijashemi and Javadi 2009) was recovered from the type A cankers: trunk lesions with zonation. Our observations, combined with these results, revealed that the fig canker-causing Neocosmospora isolates can induce types B, C, and D cankers. Type B cankers that originate from the crown were more widespread than type C, with well-developed sunken lesions on the trunks, and type D, cracked, discoloured, and dead areas on the main stem and branches. However, *N. caricae* sp. nov. may cause type B, or C in the orchards, whereas type C and D can result from *N. metavorans* infections of the fig trees. Types C and D cankers were also caused by the recently described *S. banihashemiana* (Bolboli et al. 2022). Two types of discoloration were also observed in the transverse sections of the infected fig trees. Neocosmospora caricae sp. nov. isolates caused irregular-shaped and wedge-shaped necrosis, whereas *N. metavorans* necrosis was crescent-shaped and wedge-shaped in the transverse sections of infected trees.

Since Neocosmospora species could have a non-pathogenic endophytic or pathogenic lifestyle (Sandoval-Denis et al. 2019), our pathogenicity results demonstrate that both *N. metavorans* and *N. caricae* sp. nov. were pathogenic and responsible for fig stem and trunk canker. Based on our observations, these newly reported pathogens may represent a severe threat to fig plantations.

In conclusion, this study identified two new pathogenic fungal species from the Nectriaceae, *N. metavorans* and *N. caricae* sp. nov., associated with trunk and branch canker diseases of fig orchards in Iran. These species were pathogenic to the “Sabz” cultivar, the most widely planted fig cultivar in Iran. The current results add to the previous knowledge on the aetiology of fig stem and trunk canker and may provide essential information for developing effective integrated management strategies against canker diseases affecting fig orchards in Iran. Future research on disease integrated management of fig canker diseases should focus on fast and accurate detection of the inoculum sources in fig nurseries and orchards as well as the evaluation of susceptibility of various Iranian fig cultivars to these pathogens.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11557-022-01834-9.

**Acknowledgements** The authors would also like to thank the National Union of Agricultural Cooperatives of Iran Orchards Owners, especially the chairman of the union, Mr. Kamal Yadollahi, for support.

**Author contribution** All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Zeinab Bolboli and Reza Mostowfizadeh-Ghalamfarsa. The first draft of the manuscript was written by Zeinab Bolboli and Reza Mostowfizadeh-Ghalamfarsa, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Funding** This research was supported by grant number 97010489 from the Iran National Science Foundation (INSF). Data availability

The datasets generated during and analysed during the current study are in supplementary table or available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Informed consent was obtained from all individual participants included in the study.
Competing interests  The authors declare no competing interests.

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