



## Fungal contamination associated with some dried fruits in Iran

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### Abstract

In order to identify fungal species associated with some dried fruits including; Common fig, Date palm, and White Mulberry, 35 fruit samples were collected from common markets in the south of Kerman province, Jiroft, Iran, during 2016. After surface disinfestation with 0.5% NaOCl, small fragments of each dried fruit were plated onto Potato dextrose agar (PDA) medium. Recovered fungal isolates were purified by single spore method. Morphological characteristics of each isolate were recorded. Results of identification recorded that about seven fungal species including; *Alternaria alternata*, *A. tenuissima*, *Arthrinium arundinis*, *Cladosporium cladosporioides*, *Clastospora gossypii* var. *polymorpha*, *Fusarium oxysporum* and *F. verticillioides* were obtained. To the best of our knowledge, this is the first report of isolating and identifying the fungi associated with dried fruits in Iran.

**Keywords:** Dried fruits, date, white mulberry, fungal species, morphology, Jiroft

### 1. Introduction

The wide range of temperatures and different climatic zones in Iran made the possibility to cultivate a diverse variety of crops. According to formal reports, more than 2000 plants were grown in this country. Agricultural products are very important in Iran especially for none-oil exports; in the meantime, dried fruits have the same important roles. Fungi are able to deteriorate food products and play important roles during their storage. So, it seems vital to conduct standard tests for checking fungal contamination of dried fruits and mycotoxins production. Many genera have been reported as contaminating fungi associated with dried fruits

worldwide such as; *Fusaria*, *Aspergilli*, and *Penicillium* spp. (Srivastava *et al.*, 2014; Tournas *et al.*, 2015). Therefore, the present investigation aimed to isolate and identify fungal species associated with some dried fruits; collected from different common markets in some regions of Iran.

### 2. Materials and methods

#### Sampling, Isolation and identification of fungi

Dried fruits tested in this study were collected from common markets in the south of Kerman province, Jiroft. Fungal isolation were carried out on

PDA plates from samples of dried fruits such as; Common fig (*Ficus carica*), Date palm (*Phoenix dactylifera*), and White Mulberry (*Morus alba*), according to Ho and Ko, (1997). Single spore colonies were transferred into PDA, synthetic nutrient poor agar (SNA), and 2% malt-extract agar (MEA) media; and then incubated at 25°C under continuous fluorescent light to promote their sporulation. Preparations were mounted in lactic acid; and then studied under a CH2 Olympus microscope (x1000 magnification), equipped with a Sony Digital Camera. Measurements of 10 conidiophores and 30 conidia were taken in 50% lactic acid in reference to Amirmijani *et al.*, (2014); (2015). Identification of each isolate was carried out using relevant literatures of Ellis, (1971), (1976); Gerlach and Nirenberg, (1982); Leslie and Summerell, (2006); Simmons, (2007); Bensch *et al.*, (2010), (2012).

### 3. Results and Discussion

49 fungal isolates were recovered on PDA plates including mainly eight fungal species that were identified as; *Alternaria alternata*, *A. tenuissima*, *Arthrinium arundinis*, *Cladosporium cladosporioides*, *Clastospora gossypii* var. *polymorpha*, *Fusarium oxysporum*, *F. prolioferatum* and *F. verticillioides*. All these fungi have been frequently reported from many substrates in Iran; but this is the first report of these fungi associated with dried fruits in this country. Moreover, *C. gossypii* var. *polymorpha* is a new record for Iranian Mycobiota. A brief description and illustrations of these 8 fungal species are presented.

**1- *Alternaria alternata*** (Fries) Keissler, Beit, Bot. Central bl. 29: 433 (1912).

After a week of incubation, colony on PDA reached 9-10 cm in diameter; had black, olivaceous to black green color with concentric rings, smooth to variously verrucose. Preliminary conidiophores arise singly from mycelia; whereas, the secondary conidiophores were in chains with a single cell or 2

(–4)–celled, up to 26 (–27) × (2.5–) 3-4 μm. Conidia were abundant, produced in multiple-branched chains. Initial conidia were 9–15 (–28) × 6–7 μm; with 3–4 transverse septa and up to 2–longitudinal septa. Secondary conidia 5–10 (–12) × 5– 9 (–10) μm, had 2–3 transverse septa and a few or no longitudinal septa. This is an opportunistic pathogen which has been recorded on around 400 host species; and causes several diseases such as; leaf spots, rots and blights on many plant parts (Fig. 1.)

**2-*Alternaria tenuissima*** (Nees & T. Nees: Fr.) Wiltshire, Trans, Brit, Mycol Soc. 18: 157 (1933).

Colony on PDA attained 10 cm in diameter after a week of incubation; was loosely cottony, olivaceous to black green. Conidia were abundant; produced in unbranched or less branched chains, ovoid, obclavate, (10–) 15– 30 (–37) × (4–) 6–8 (–9) μm, with 3–4 (–5) transverse septa; and up to 2–longitudinal septa. Conidia had pale olivaceous color; pale to brown, medium golden brown, smooth to slightly punctuate.

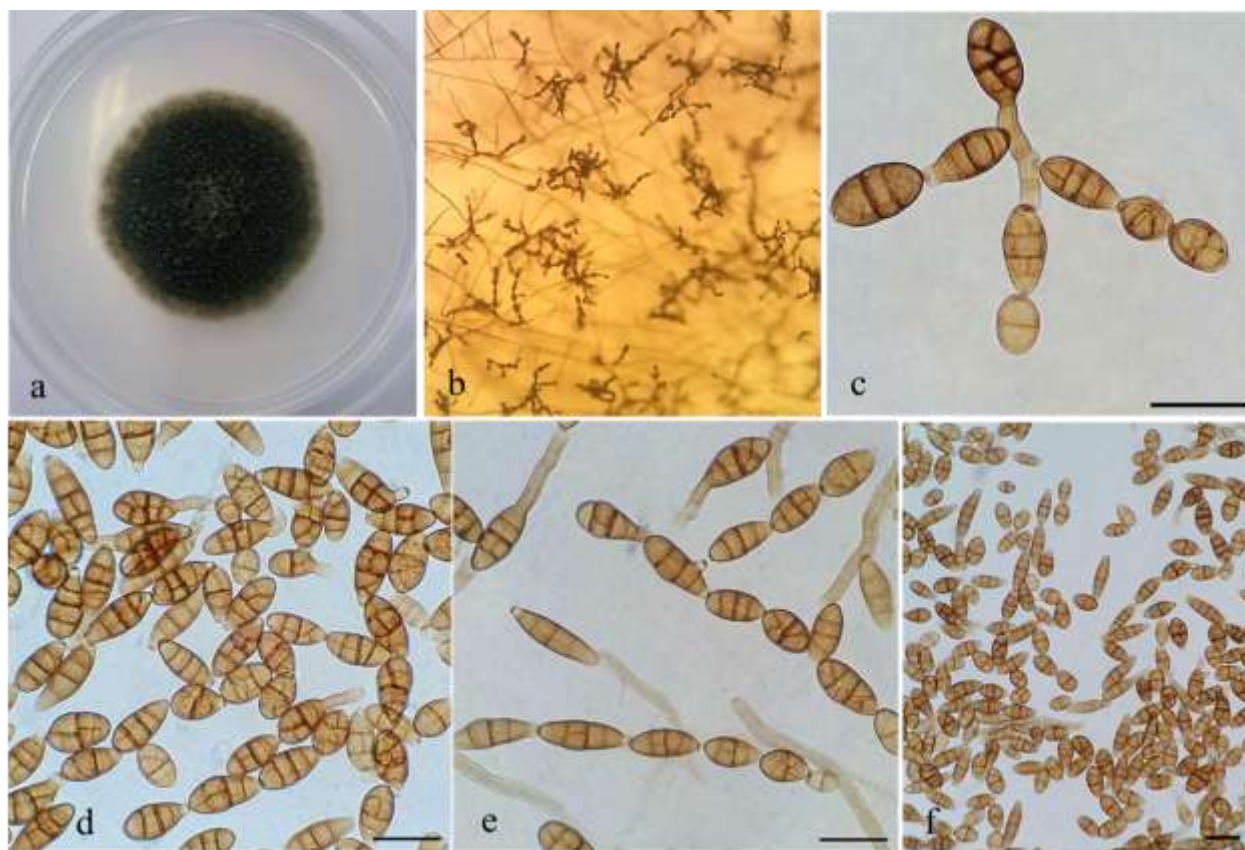
*A. tenuissima* is very similar to *A. alternata*; so it is difficult to identify and separate these species from each other by morphological data; however, there are some differences between them. One of the most important features for identifying these fungi was sporulation pattern. In *A. tenuissima*; conidia were produced in unbranched or less branched chains; whereas in *A. alternata* conidia were mostly produced in branched chains (Fig. 2.).

**3-*Arthrinium arundinis*** (Corda) Dyko & B. Sutton, Mycotaxon 8: 119 (1979).

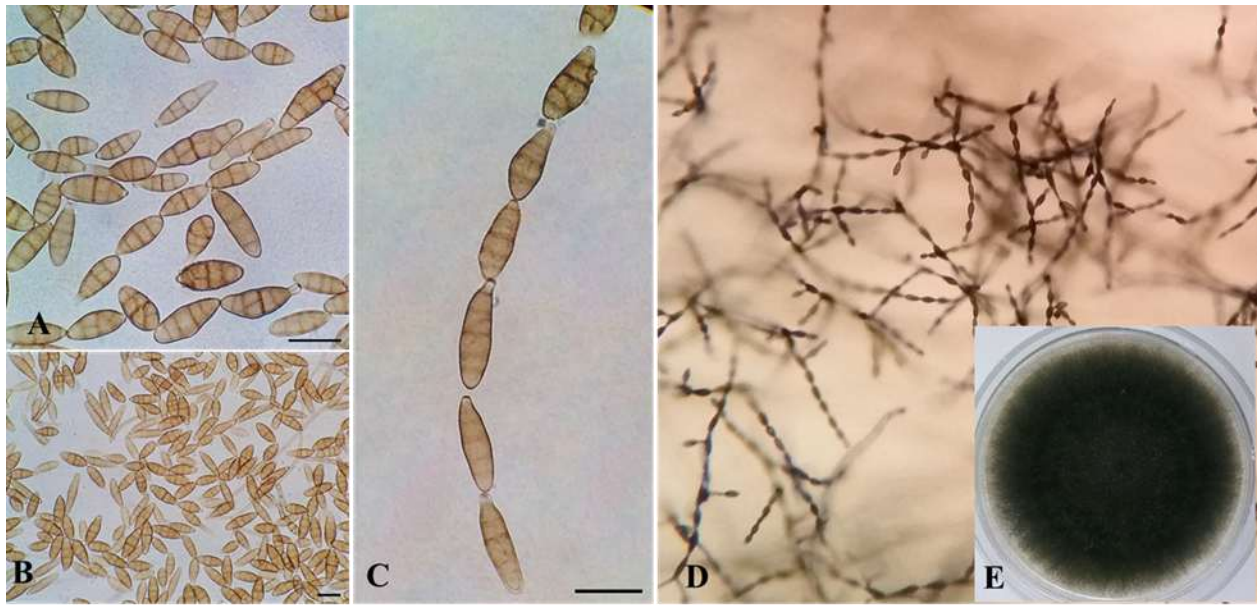
Colonies were flat; spreading, with moderate aerial mycelium. On PDA; MEA and SNA media, surface of colony was iron-grey with patches of dirty white. Mycelium was smooth; hyaline, branched, and septate, of up to 3 μm in diameter. Conidiophores were reduced to conidiogenous cells, which were aggregated in clusters on hyphae. They were pale brown, smooth, sometimes ampulli form, (4–) 6–11 × 3–4 (–4.5) μm, had apical neck of 3–4

$\mu\text{m}$  long; whereas, basal part was 4–6  $\mu\text{m}$  long. Conidia were pale brown to brown; smooth and globose in surface view of (4–) 5–7 (–8)  $\mu\text{m}$  diameter, but were lenticular in side view of (3–) 4–4.5  $\mu\text{m}$  diameter, with pale slit. This fungus was cosmopolitan; reported from tropical and temperate regions of Europe, Africa, Asia, North and South

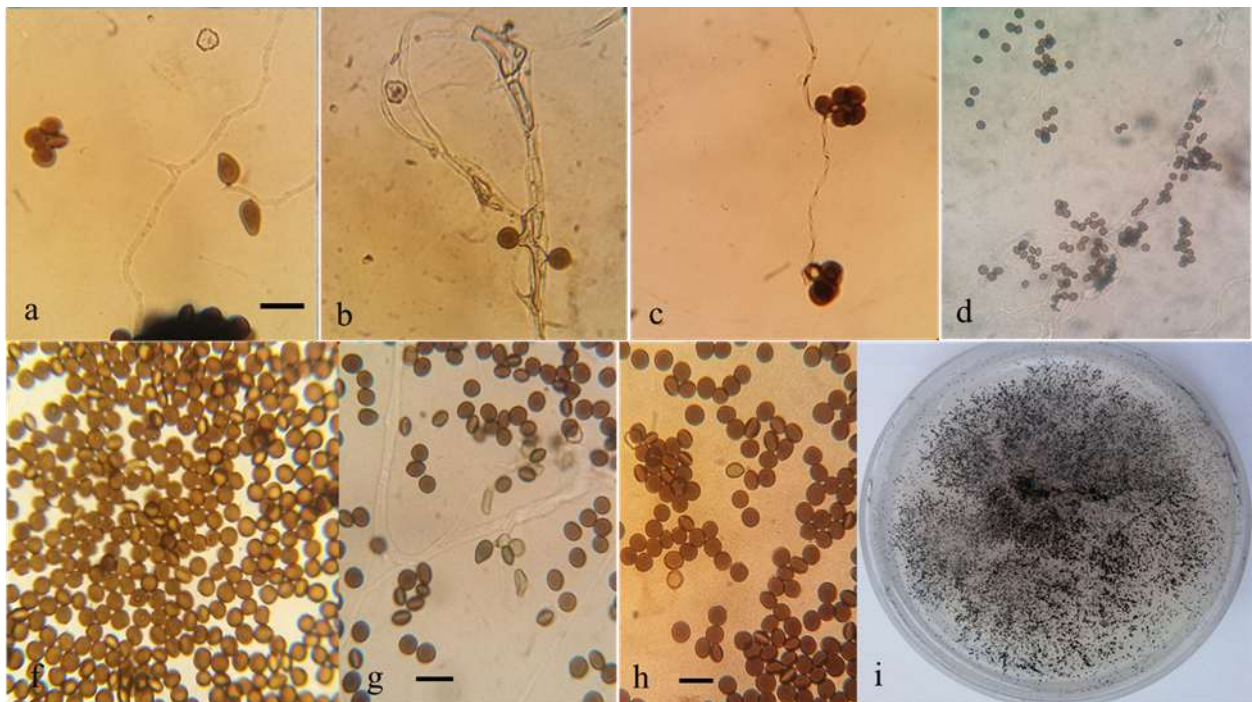
America. This species is very similar to *Arthrinium sacchari*; however, they may be differentiated by the diameter of their conidiophores. Conidiophore of *Arthrinium arundinis* was about 0.5  $\mu\text{m}$ , whereas in *Arthrinium sacchri* it was 1–1.5  $\mu\text{m}$  (Crous and Groenewald, 2013) (Fig. 3).



**Fig. 1.** *Alternaria alternata*. a: Colony growth on PDA, b: Sporulation pattern, c: Secondary conidiophore, d-f: Conidia. Bars: 10  $\mu\text{m}$ .



**Fig. 2.** *Alternaria tenuissima*. A, B and C: Conidia, D: Sporulation pattern, E: Colony growth on PDA. Bars: 10  $\mu$ m.



**Fig. 3.** *Arthrinium arundinis*. a-c: Conidiogenous cells giving rise to conidia, d-h: Conidia, i: Colony growth on PDA. Bars: 10  $\mu$ m.

**4-*Cladosporium cladosporioides*** (Fresen.) G.A. de Vries, Contr. Knowl. Genus *Cladosporium*: 57. 1952.

Conidia were pale brown; smooth, ovoid, lemon form to ellipsoid-ovoid,  $5-11 \times 2.5-3.5$  ( $-4$ )  $\mu\text{m}$ , aseptate, with 1–3 distal hila (Amirmijani *et al.*, 2014). This species was isolated from 23% of all dried fruits; and 36% of white mulberry samples. According to Bensch *et al.*, (2010), *C. cladosporioides* is one of the most common and wide-spread saprobic hyphomycetes. It is well-distinguished from other *Cladosporium* spp. by its long branched chain of conidia on the unbranched terminal part of the conidiophore; and their wider ramoconidia ( $3-5 \mu\text{m}$ ) (Fig. 4.).

**5-*Clastospora gossypii* var. *polymorpha***, (Jacz.) U. Braun & Crous, Persoonia 22: 139-161 (2009).

Colonies on PDA reached 6–8 cm in diameter after a week of incubation. Their surfaces were brown to olivaceous; reverse grey-black with regular margin, aerial mycelium was abundant, and sporulation was profuse. On SNA plates; mycelium was immersed and superficial, smooth, loosely branched,  $1-3$  ( $-4$ )  $\mu\text{m}$ , septate, without constriction at septa, sub hyaline to pale brown, and sometimes with Chlamydo-spore-like structure.

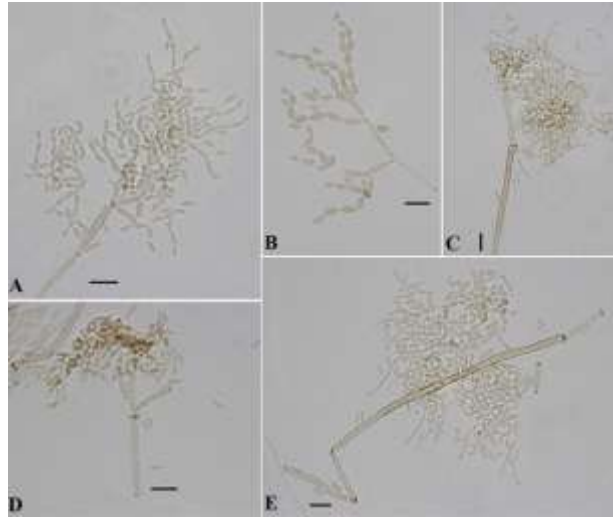
Conidiophores were arising terminally and laterally from hyphae; solitary, micronematous or semi-macronematous, unbranched,  $3-25 \times (2-)$   $2.5-4$  ( $-4.5$ )  $\mu\text{m}$ , and pale to medium olivaceous-brown. Conidiogenous cells were integrated; sympodial, mostly terminal, up to 16  $\mu\text{m}$  long, with up to 3 loci at the apex. Secondary ramoconidia were ovaloid; sub-cylindrical or cylindrical,  $9-26 \times 3-4.5$  ( $-5$ )  $\mu\text{m}$ , 3 ( $-4$ )-septa. Conidia were catenate; in simple or branched chains, sub-cylindrical or cylindrical, ovaloid to ovoid,  $5-18$  ( $-23$ )  $\times 2-3.5$  ( $-4$ )  $\mu\text{m}$ , 0–3 septa, smooth, pale brown to olivaceous-brown, produce alternarioid conidia with aging,  $18-40$  ( $-44$ )  $\times 6-12$  ( $-14$ )  $\mu\text{m}$ . However,

microcyclic conidiogenes were not observed (Fig. 5). *C. gossypii* has been previously reported from Iran as *Cladosporium malorum* (Ershad, 2009). Due to tetric nature of conidiation of this species; Braun *et al.*, (2003) identified it as *Alternaria malorum* with two varieties including; *A. malorum* var. *gossypii* and *A. malorum* var. *polymorpha*. The last variety was distinguished from other by producing alternarioid conidia; finally Simmons, (2007) placed it in the genus *Clastospora*. According to our knowledge, this is the first report of this variety in Mycobiota of Iran.

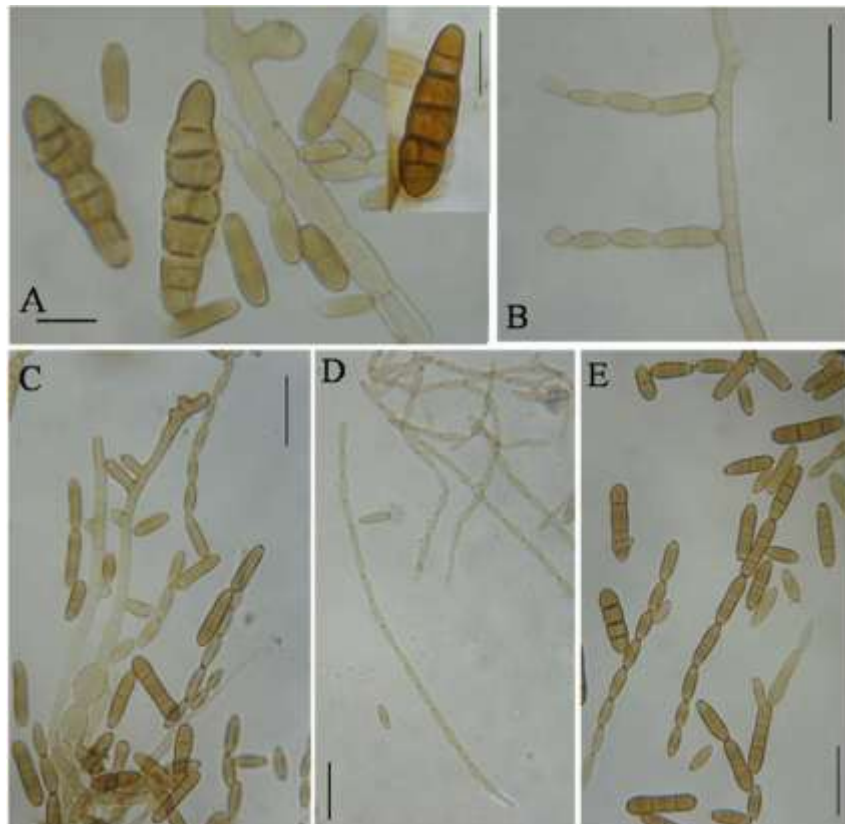
**6-*Fusarium oxysporum***, Schldl., Flora Berolinensis, Pars secunda: Cryptogamia: 106 (1824).

After a week of incubation on PDA, colonies attained more than 8 cm in diameter. Purple or violet colonies; aerial mycelia were abundant, becoming felt like or floccose. This mycelium produced microconidia cohering in false head, never forming chains. Microconidia were 1 or 2 celled; cylindrical to ellipsoid, oval, straight to slightly curved or reniform. Macroconidia at first were solitary scattered, and then become abundant on sporodochia. They were falcate; usually moderately curved, sub-cylindrical, with a pointed sometimes slightly hooked apical cell. They have mostly rather distinctly pedicellate basal cell; usually with 3 septa,  $15-33 \times 3.5-5 \mu\text{m}$ . Chlamydo-spores were abundant; terminal or intercalary, smooth or rough walled, globose to subglobose, single or in pairs.

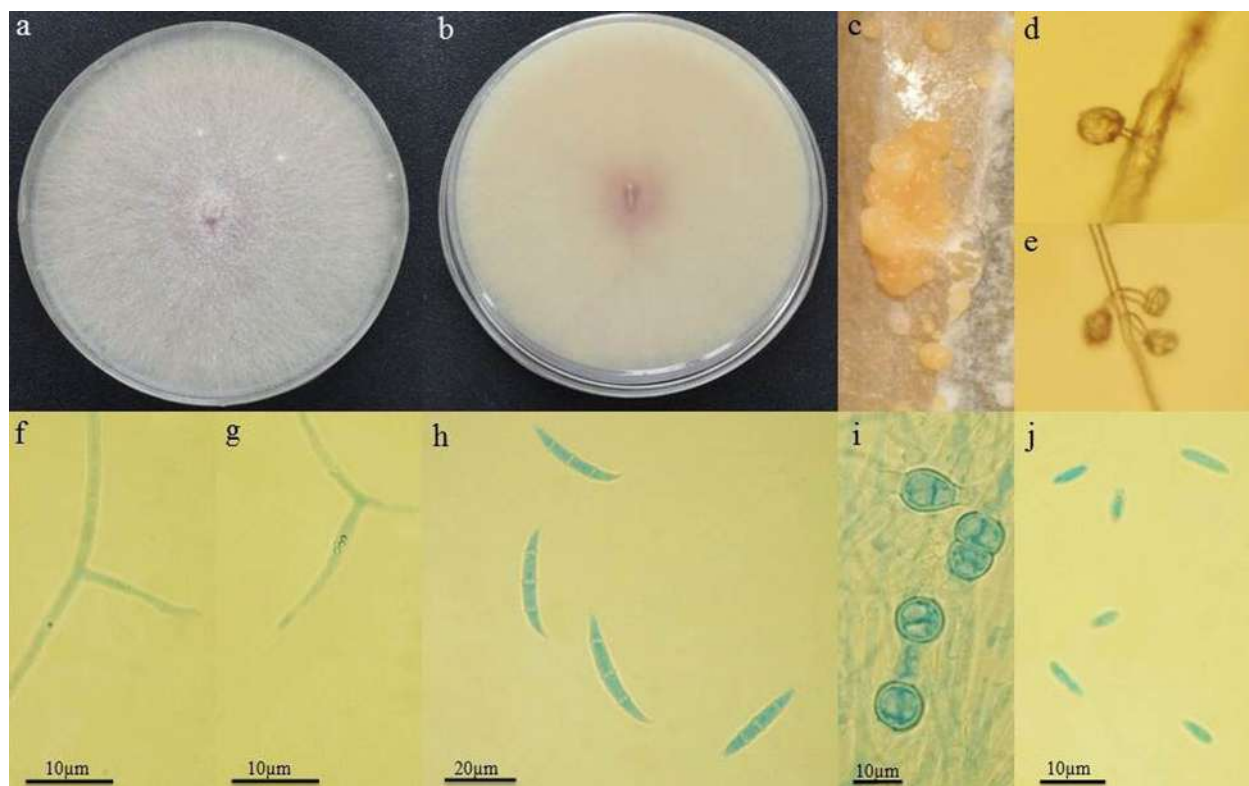
Conidiophores primarily formed were; short, single, lateral phialides on hyphae or loosely branched in the aerial mycelium. Later, they were formed in sporodochia that were densely irregular or verticillately branched. These conidiophores were monophialidic (Fig. 6). On PDA, *F. oxysporum* may be morphologically close to *F. proliferatum*, but it differs from this species by having false head and chlamydo-spores.



**Fig. 4.** *Cladoporium cladosporioides*, A: Conidia, B: Secondary ramoconidia, c: Denticulate loci at the apex of conidiophore, d: Ramoconidia, E: Conidiophore with terminal conidia. Bars: 10  $\mu$ m.



**Fig. 5.** *Clastospora gossypii* var. *polymorpha*, A: Alternarioid conidia, B: Micronematous conidiophores, C: Chlamydospore-like structures and semi-macronematous conidiophores, D-E: Conidia, Bars: 10  $\mu$ m.



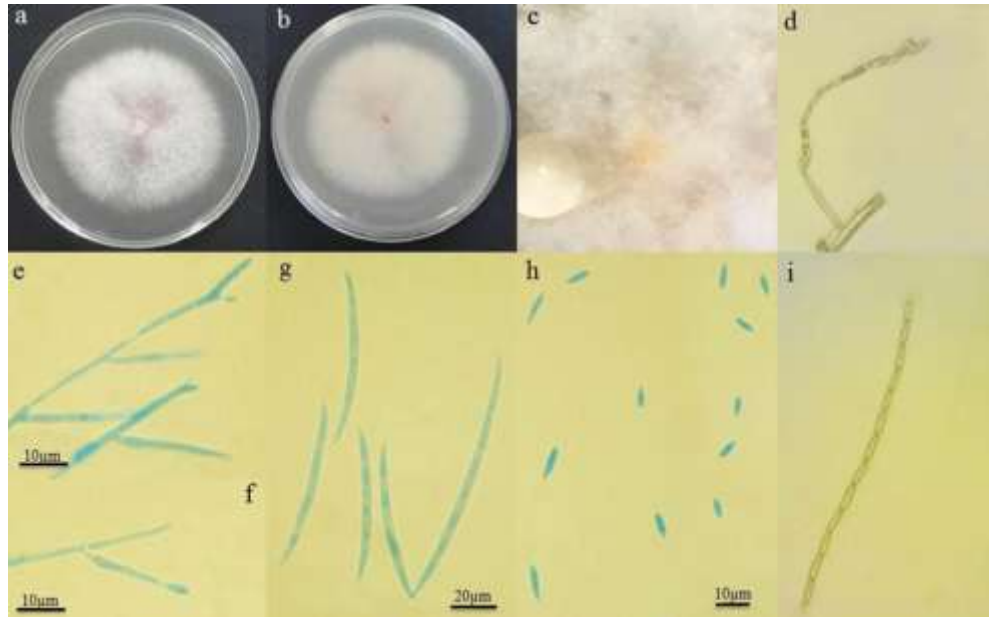
**Fig. 6.** *Fusarium oxysporum*. a-b: Colony on PDA, c: Sporodochium, d-e: false head, f-g: Monophialids, h: Macroconidia, i: Chlamydospore, j: Microconidia

**7-*Fusarium proliferatum*** (Matsush.) Nirenberg, *Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft* 169: 38 (1976).

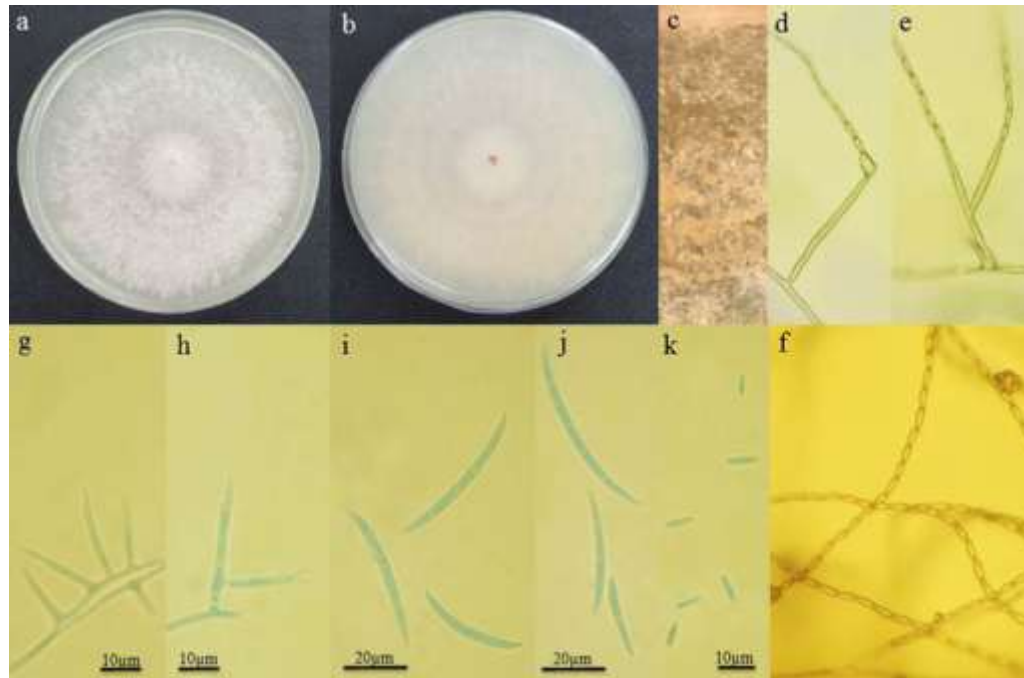
Colonies on PDA reached more than 7 cm in diameter after one week of incubation; white to buffish in color, with abundant aerial mycelia. Sclerotial bodies were not observed. Sporulation started within 2 days on the aerial mycelium; as microconidia attached to each other in short or long chains. Microconidia were clavate; oval, subglobose to globose, pyriform, and usually 1-celled,  $5-7 (-9) \times 2.5-5 (-7) \mu\text{m}$ . Macroconidia were falcate but rather straight, with 5-6 septa,  $25-59 \times 3-4.1 \mu\text{m}$ . Chlamydospores however, were not recorded. Sporodochia was white to yellowish; and appeared after two weeks. Sometimes it was monophialidic, but often polyphialidic with more than two conidiogenous loci,  $18-60 \times 3-3.3 \mu\text{m}$  (Fig. 7).

**8-*Fusarium verticillioides*** (Sacc.) Nirenberg, *Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft* 169: 26 (1976).

On PDA; colonies reached 5–6 cm in diameter after a week; white to grey in color, sometimes becoming purple with aging. Aerial mycelia were abundant. Sclerotial bodies were not produced. Sporulation started within 2 days on the aerial mycelium as microconidia attached to each other in long chains. These microconidia were clavate; oval,  $5-14 (-15) \times 2-2.5 \mu\text{m}$ , aseptate. On the other hand, macroconidia were falcate; with up to 4–5 septa,  $32-56 (-58) \times (2.5-) 3-4 \mu\text{m}$ . Chlamydospores were not observed. Sporodochia were rare; yellowish and monophialidic,  $(14-) -16-18 (-19) \times 2-2.5 (-3) \mu\text{m}$ . *F. verticillioides* is morphologically close to *F. proliferatum*, but the last species differs by having short chains of conidia on polyphialidic conidiophores.



**Fig. 7.** *Fusarium proliferatum*. a-b: Colony on PDA, c: Sporodochium, d and i: Chain of conidia, e-f: Mono and polyphialids, g: Macroconidia, h: Microconidia



**Fig. 8.** *Fusarium verticillioides*. a-b: Colony on PDA, c: Sporodochium, d-e: Chain of conidia, g-h: Monopialids, i-j: Macroconidia, k-f: Microconidia.



## Conflict of interests

The authors declare no conflict of interests.

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