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Contribution to the mycobiota of Egypt: 25 new records and interesting fungal taxa from citrus and grapevine plantations

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Abstract: During a survey of mycobiota of citrus and grapevine plantations in Assiut area, Egypt, several interesting filamentous fungal isolates were recovered. These isolates were examined for their macroscopic, microscopic features and identified. In suspected isolates, molecular techniques targeting internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were employed to confirm their identification. Most of these species are recorded here for the first time in Egypt and some of them are rarely isolated. Reference strains of all species recorded and ITS gene sequences of some of them are deposited at Assiut University Mycological Center Culture Collection (AUMC) and National Center for Biotechnological Information (NCBI) respectively.

Key words: Citrus, grapevine, plantation, air, soil, phyllosphere, phylloplane, fruits, juices, ITS.

Introduction

During the course of a survey of mycobiota of citrus and grapevine plantations in Assiut Governorate, Egypt in 2008/2009, some interesting unfamiliar filamentous fungal isolates were recovered from the air, soil, phyllosphere, phylloplane, carposphere, carpoplane and fruit juices. The macro- and micro-morphological characteristics proved that these isolates are either recorded for the first time or they are rarely isolated in Egypt.

Various molecular methods have been used for phenotypic and genotypic studies of fungi (Rinyu *et al*. 2000, Varga *et al.* 2000). The internal transcribed spacer (ITS) region, located between the 18S and 28S rRNA genes, is an area of particular importance in discriminating between closely related species, because it has areas of high conservation and variability. ITS region has been used to identify fungal taxa (Henry *et al.* 2000). However, many authors (Varga *et al.* 2011, Visagie *et al.* 2014, Hubka *et al.* 2014, 2016, Chen *et al.* 2016, 2017) revealed that ITS has only limited discriminatory power in the genus *Aspergillus* in contrast to beta-tubulin, calmodulin and RPB2 loci.

Living cultures of these new records are deposited at the culture collection of the Assiut University Mycological Centre (AUMC), Assiut, Egypt. ITS genes of the sequenced strains are also deposited in the National Center for Biotechnological Information (NCBI) and the accession numbers could be found below the description of each species in section results and discussion.

Materials and Methods

Strains examined: The examined fungal strains were isolated from the air (by settle-plate method, Hoekstra *et al.* 2004), soil (by dilution-plate method, Johnson and Curl 1972), phyllosphere and carposphere (by washing-plate method), phylloplane and carpoplane (segment-plate method) and fruit juices (by direct-plating technique) of citrus and grapevine plantations. Plantations studied are found in Sahel-Saleem city at approximately 25 Km south-east of Assiut town, Egypt. The strains were isolated during 2008/2009 on dichloran yeast exract malt extract agar, DYM (Wickerham 1951 and modified by Moubasher *et al.* 2016), and dichloran rose Bengal chloramphenicol agar, DRBC (King *et al.* 1979) at 25°C, by Zeinab Soliman in a laboratory of Assiut University Mycological Centre (AUMC), Assiut, Egypt. Taxa registered in the GenBank are given accession numbers (http: //www.ncbi.nlm.nih.gov.).

Morphology: For macromorphological observations, the strains were grown in the dark at 25ºC for 7 days on the appropriate medium/media: poato sucrose agar (PSA, Booth 1971), Czapek yeast autolysate agar (CYA), yeast extract sucrose agar (YES), Blakeslee malt extract agar (MEA) and Oatmeal agar (OA) (for media formulations see Samson *et al.* 2004),. Colony diameters were obtained from one-point inoculation of at least three replicate agar plates (90 mm diam). Colors were identified according to Kornerup and Wanscher (1989).

A Sony Cybershot DSCW5 5.1MP Digital Camera with 3× Optical Zoom was used for plate photography. For micromorphological observations, microscopic mounts were made in lactophenol from colonies grown on the appropriate medium after 7-10

days old, and examined using a Carl Zeiss, Axiostar Plus microscope, Microimaging GmbH, Göttingen, Germany, magnification up to 1000×).

Growth of fungi and DNA extraction and sequencing

The tested fungal isolates were individually grown on CYA plates and incubated at 25°C for 7 days. A small amount of fungal mycelia was scraped and suspended in 100 µl of distilled water and boiled at 100° C for 15 minutes and samples were sent to SolGent Company (Daejeon, South Korea) to carry out the whole procedure from DNA extraction till the final step of DNA sequencing. Each sample was sequenced in the sense and antisense direction. Contigs were created from the sequence data using the CLCBio Main Workbench program. The sequence obtained from each isolate was analysed using BLAST from the National Center of Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov).

Results & Discussion

Taxa treated

***Absidia cylindrospora* Hagem, Skrifter Udgivne af Videnskabs-Selskabet i Christiania. Mathematisk-Naturvidenskabelig Klasse 7: 45 (1908)**
≡*Tieghemella cylindrospora* (Hagem) Naumov, Opredelitel Mukorovykh (Mucorales): 83 (1935)

Brief description: colonies grow rapidly at 25°C on PSA, whitish when young, turning greyish-brown with age; no growth at 37°C; sporangiophores in whorls (1-6); sporangiophores arising from stolons, never opposed to rhizoids sporangia pyriform or subpyriform, septum always present below sporangia, sporangia apophysate, multispored, sporangiospores cylindrical and smooth, no zygospores observed (heterothallic species) (Plate 1, for more details see Schjpper 1990, Moustafa 2006, Alastruey-Izquierdo *et al.* 2010, Hoffmann 2010, de Souza *et al.* 2017).

Ecology and distribution: from dung in Brazil (de Souza *et al.* 2017). This species was isolated in Assiut from the air (Moubasher *et al.* 2016), soil (Abdel-Sater *et al.* 2016) of citrus and grapevine and from citrus phyllosphere (Moubasher *et al.* 2018c).

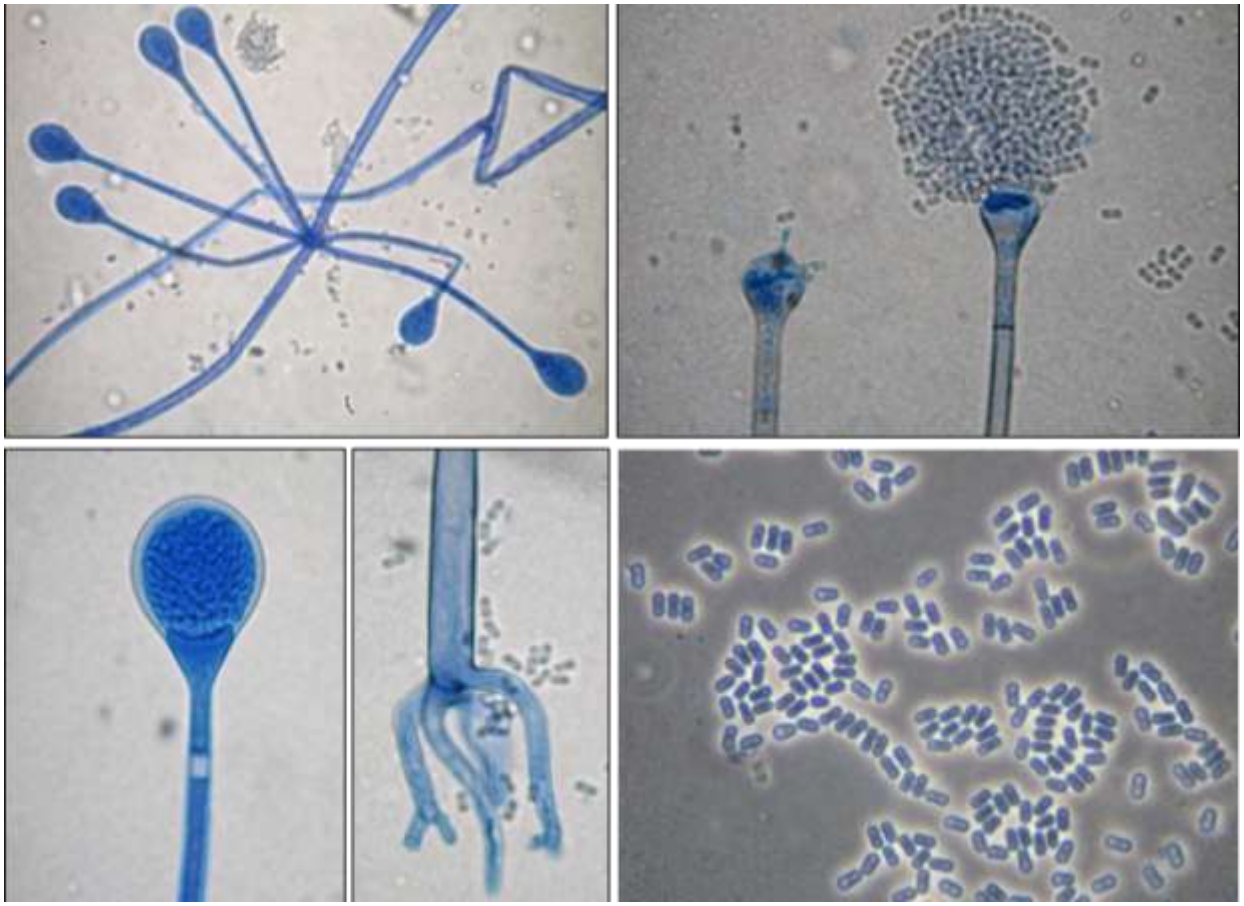

Plate 1: *Absidia cylindrospora* AUMC 6716: sporangiophores in whorls (upper left), sporangia releasing sporangiospores (upper right), sporangium-containing sporangiospores (lower left), rhizoids (lower middle), and cylindrical sporangiospores (lower right).

***Acremonium curvulum* W. Gams, *Cephalosporium*-artige Schimmelpilze 57 (1971)**

Brief description: colonies on PSA at 25ºC attaining 45 mm after 7 d, whitish; conidiophores usually simple, phialides erect arising from substrate mycelia or from aerial hyphae, 25-50 µm long; conidia hyaline, falciform, one-celled, 4-6 x 2-2.5 µm (Plate 2, for more details see de Hoog *et al.* 2000).

Ecology and distribution: originally reported from wheat field soil in Germany (refer to Giraldo *et al.* 2012), also it was reported from a case of endophthalmitis (Pflugfelder *et al.* 1988). In Egypt it was reported from grapevine soil on DRBC at 25°C (Abdel-Sater *et al*. 2016).

Plate 2: *Acremonium curvulum* AUMC 6734: colonies on PSA at 25°C (upper left), long phialides bearing curved, cylindrical conidia (upper right & lower photos).

***Acremonium furcatum* (Moreau & V. Moreau) ex W. Gams, Nova Hedwigia 18: 3 (1969)**
≡*Cephalosporium furcatum* Moreau & V. Moreau, Revue de Mycologie 6 (3-4): 65 (1941).

Brief description: colonies on PSA at 25ºC attaining 1.5-2 mm after 7 d, pale ochre-coloured; phialides often in whorls, often subapically proliferating without cross-walls, 10-25 µm, conidia short, ellipsoidal to short cylindrical, 3-4 x 1-2 µm (Plate 3, for more details see Domsch *et al.* 2007).

Ecology and distribution: cosmopolitan, common in cultivated, sandy and saline soils, and on decaying plant materials, from Belgium, British Isles, France, Germany, Hong Kong, India, Italy, Nepal, Netherlands, Nigeria, Portugal, Turkey (Domsch *et al.* 2007), from beach forest soil from eastern Bohemia, Czech Republic (Kubatova 1994). New bioactive compounds with antibiotic and antifungal activities were isolated from a marine strain of *A. furcatum* (Gallardo *et al*. 2006). In Egypt, it is reported from the air of citrus plantations on DYM (Zeinab Soliman 2012).

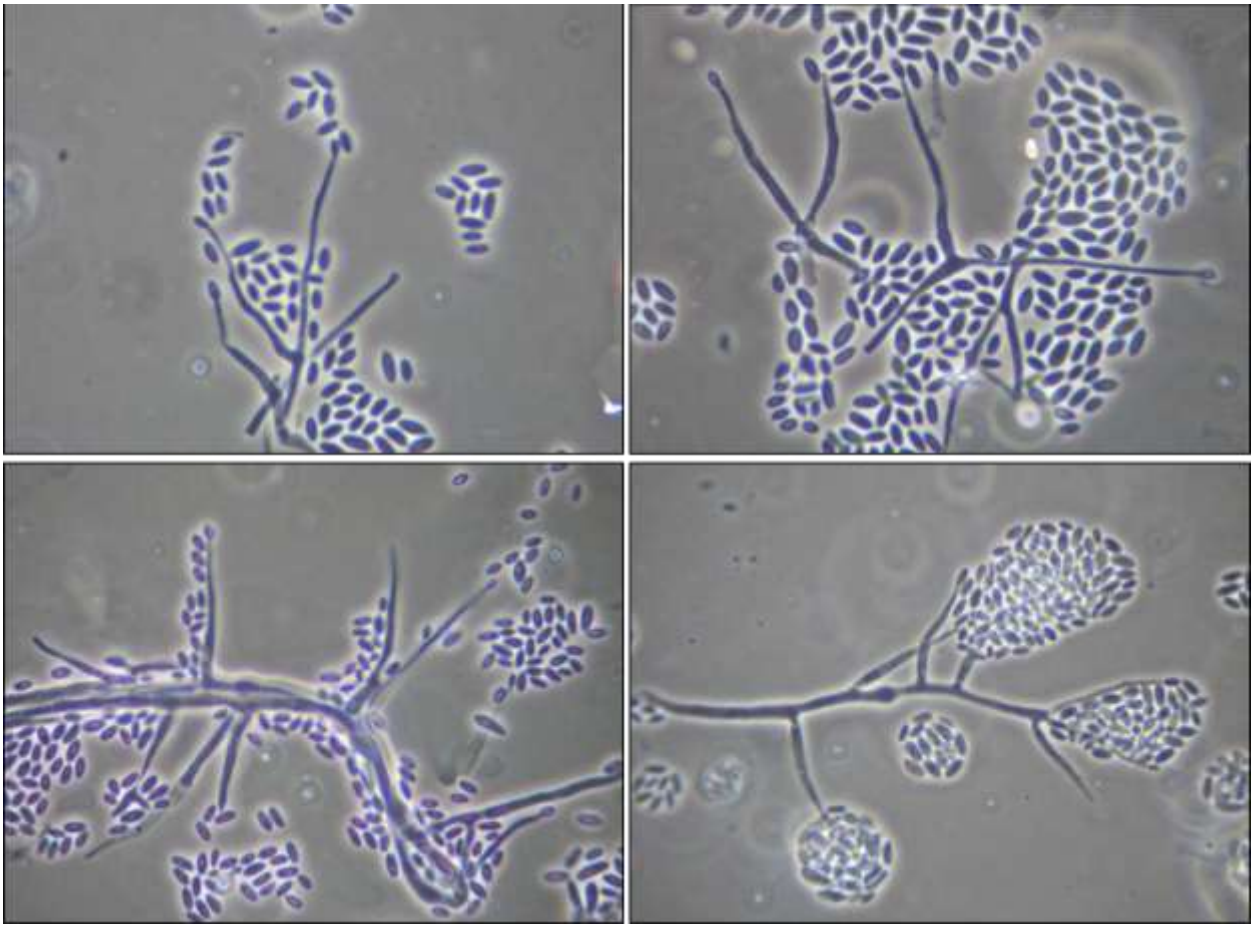

Plate 3: *Acremonium furcatum* AUMC 5779: phialides often in whorls & often proliferating and conidia in slimy masses, ellipsoidal to short cylindrical.

***Alternaria tuberculata* Meng Zhang & T.Y. Zhang, Mycosystema 25(1): 6 (2006)**

Brief description: conidia in long chains, muriform, tuberculate, with short peak and 6 transverse septa, germinating to short conidiophores bearing conidia in chains (Plate 4).

Ecology and distribution: ***A.*** *tuberculata* was described from *Helianthus annuus* in china (Zhang and Zhang 2006) and in Egypt we report it once from the air of grapevine plantation in Assiut area in February 2009 and this is the first record worldwide after its original description (Zeinab Soliman 2012).

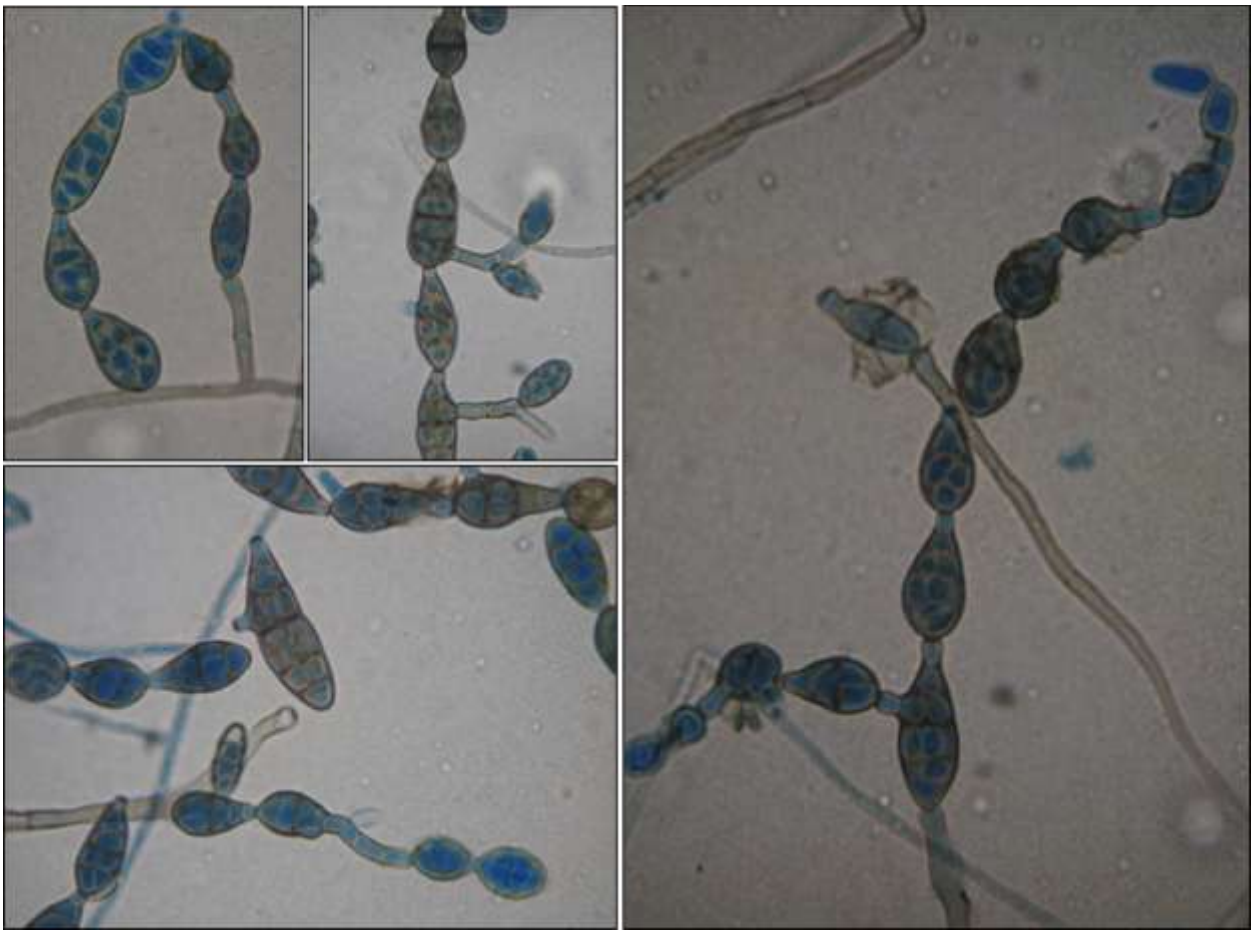

Plate 4: *Alternaria tuberculata* AUMC 5730: conidia in long chains, muriform, tuberculate, with short peak, germinating to short conidiophores bearing conidia.

***Arthrinium arundinis* (Corda) Dyko & B. Sutton, Mycotaxon 8 (1): 119 (1979)**
≡Sexual morph: *Apiospora montagnei* Sacchardo), Nuovo Giornale Botanico Italiano 7(4): 306 (1875)

Apiospora was treated as synonym on the basis that *Arthinium* is older (Crous and Groenewald 2013).

Brief description: conidiophores of *A. arundinis* reduced to conidiogenous cells; conidiogenous cells aggregated in clusters on hyphae, pale brown, smooth, ampulliform, 6-12 × 3-4 µm; conidia brown, smooth, globose in surface view, 5-7 µm, lenticular in side view, 3-4 µm diam, with pale equatorial slit (Plate 5).

Ecology and distribution: The genus *Arthrinium* is widespread and ecologically diverse. It commonly occurs as a saprobe on grasses, on leaves, stems and roots of a range of different plant substrates (Agut and Calvo 2004) and is extremely interesting for the pharmaceutical industry (Crous and Groenewald 2013). *A. arundinis* causes kernel blight of barley (Martínez-Cano *et al.* 1992). It is reported as an endophyte in plant tissue (Ramos *et al.* 2010), lichens (He and Zhang 2012), and marine algae (Suryanarayanan 2012). It was reported in Canada, Germany, India, Iran, the Netherlands, Switzerland, USA (Crous and Groenewald 2013) and China (Wang *et al.* 2018). In Egypt, it was reported once from citrus carposphere and carpoplane on DYM at 25°C (Moubasher *et al.* 2018a).

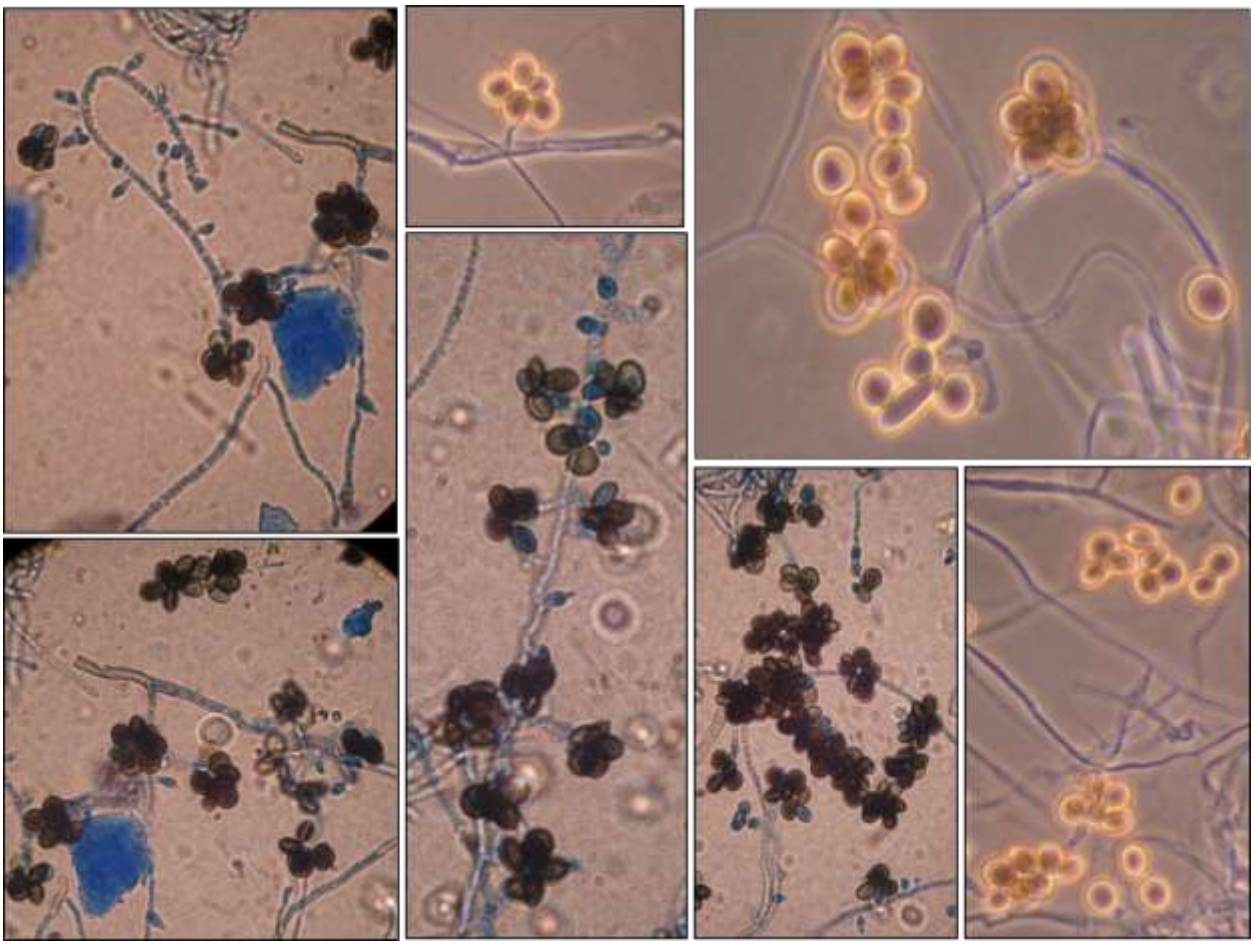

Plate 5: *Arthrinium arundinis* AUMC 5724: conidiogenous cells aggregated in clusters, smooth, ampulliform; conidia brown, smooth, globose in surface view and lenticular in side view with pale equatorial slit.

***Aspergillus stellatus* Curzi, Rendic. Accad. naz. Lincei 424-428 (1934)**
=*Emericella variecolor* Berk. & Broome, Introduction to Cryptogamic Botany: 340 (1857)
=*Aspergillus variecolor* Thom & Raper, Mycologia 31 (6): 663 (1939)
=*Aspergillus variecolor* var. *major* Bat. & H. Maia, Anais da Sociedade de Biologia de Pernambuco 15 (1): 235 (1957)
Aspergillus stellifer Samson & W. Gams, Advances in *Penicillium* and *Aspergillus* Systematics: 52 (1985)

Brief description: conidiophores smooth, yellowish brown, 320-610 × 4.5-6.5 µm; vesicles globose to subclavate, 13-18 µm wide, fertile over the upper half to two thirds; metulae and phialides hyaline, phialides flask-shaped; conidia echinulate, globose to subglobose, 2.5-3 µm; cleistothecia superficial, violet to reddish brown, globose to subglobose, 300–600 µm, surrounded by numerous Hülle cells; Hülle cells hyaline to pale yellowish brown, globose to ovoid, 10-25 µm; asci 8-spored, sub-globose to polygonal or stellate; ascospores reddish brown, in surface view stellate, 10-14 µm; spore bodies smooth, globose to subglobose, 3.5-4 × 3-4 µm; with two stellate equatorial crests; undissected part of crests, crests ornamented with longitudinal wide pleats (Plate 6, for more details refer to Chen *et al.* 2016). The stellate ascospores of *A. stellatus* resemble those of *A. stella-maris*, but *A. stellatus* differs from *A. stella-maris* by non-septate conidiophores (Moubasher *et al.* 2013).

Ecology and distribution: It was reported from soil in Algeria, India, Italy, Panama, Rhodesia, USA, dung in India, sewage in Pakistan and air, soil, salt marsh soils, wheat and corn grains in Egypt (refer o Moubasher 1993). Additionally, it was reported in Egypt from citrus and grapevine air, soil, phyllosphere and carposphere, citrus carpoplane and phylloplane in Assiut (Abdel-Sater *et al.* 2016, Moubasher *et al.* 2016, 2018a & c).

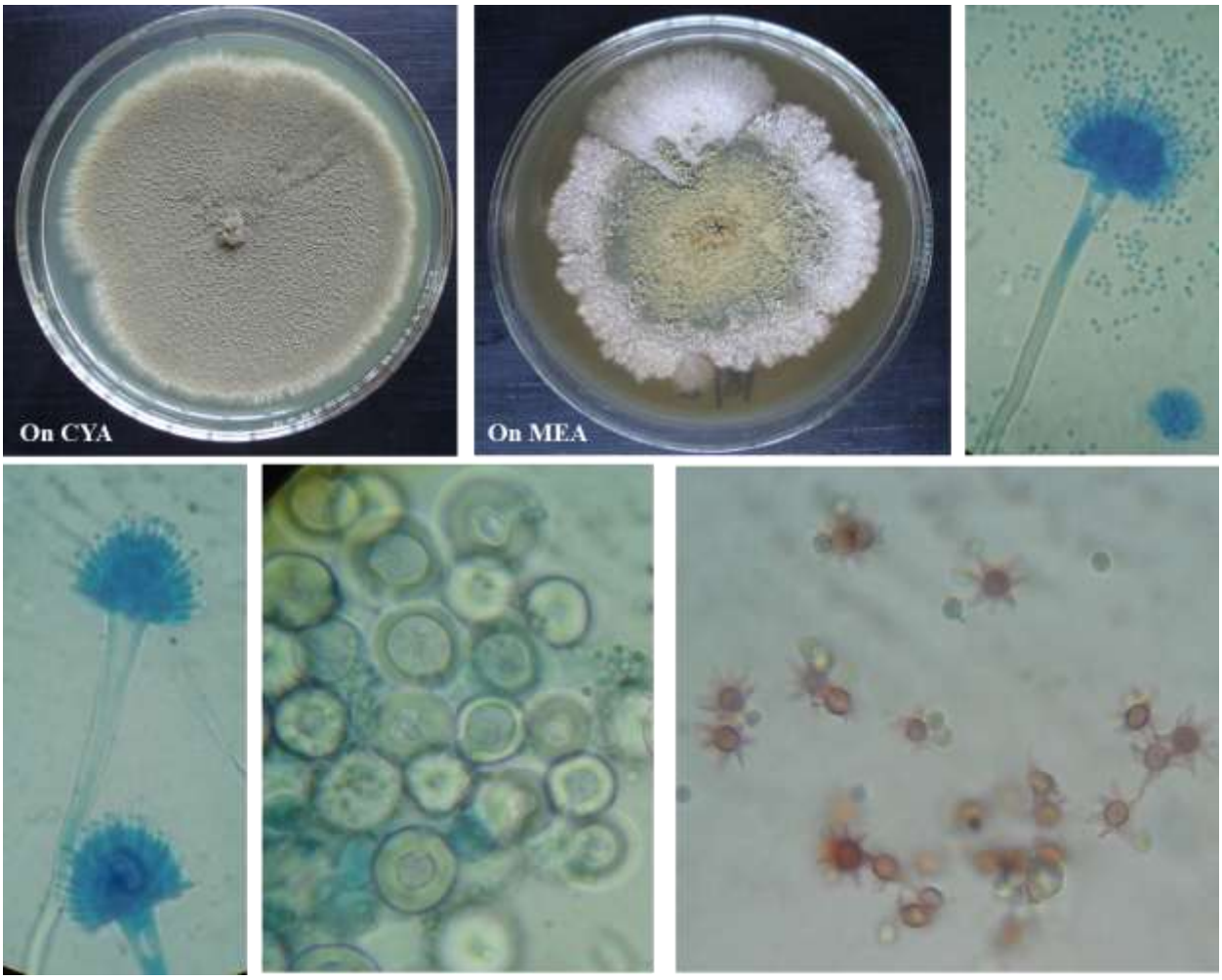

Plate 6: *Aspergillus stellatus* AUMC 5778: colonies on CYA and MEA, conidial heads, Hulle cells and stellate-shaped ascospores.

***Chuppia sarcinifera* Deighton, Mycological Papers 101: 32 (1965)**

Brief description: On PSA colonies golden yellow with orange pigmentation, conidia dark, golden brown, solitary, muriform, strongly constricted at the septa (sarciniform), irregular in shape, smooth to verruculose, up to 48 x 30 µm (Plate 7, Ellis 1971, Matsushima 1975, Seifert *et al.* 2011).

Ecology and Distriburion: reported on leaves of *Solanum* fom Venezuela (Ellis 1971), on the bark of *Eucalyptus falcata* from Faisalabad, Pakistan (Abbas *et al*. 2010), leaf litter of para rubber (*Hevea brasiliensis*) in Thailand (Seephueak *et al.* 2010) and from the phyllosphere of grapevine, air and soil of citrus plantation for the first time in Egypt (Zeinab Soliman 2012, Abdel-Sater *et al.* 2016, Moubasher *et al.* 2018c).

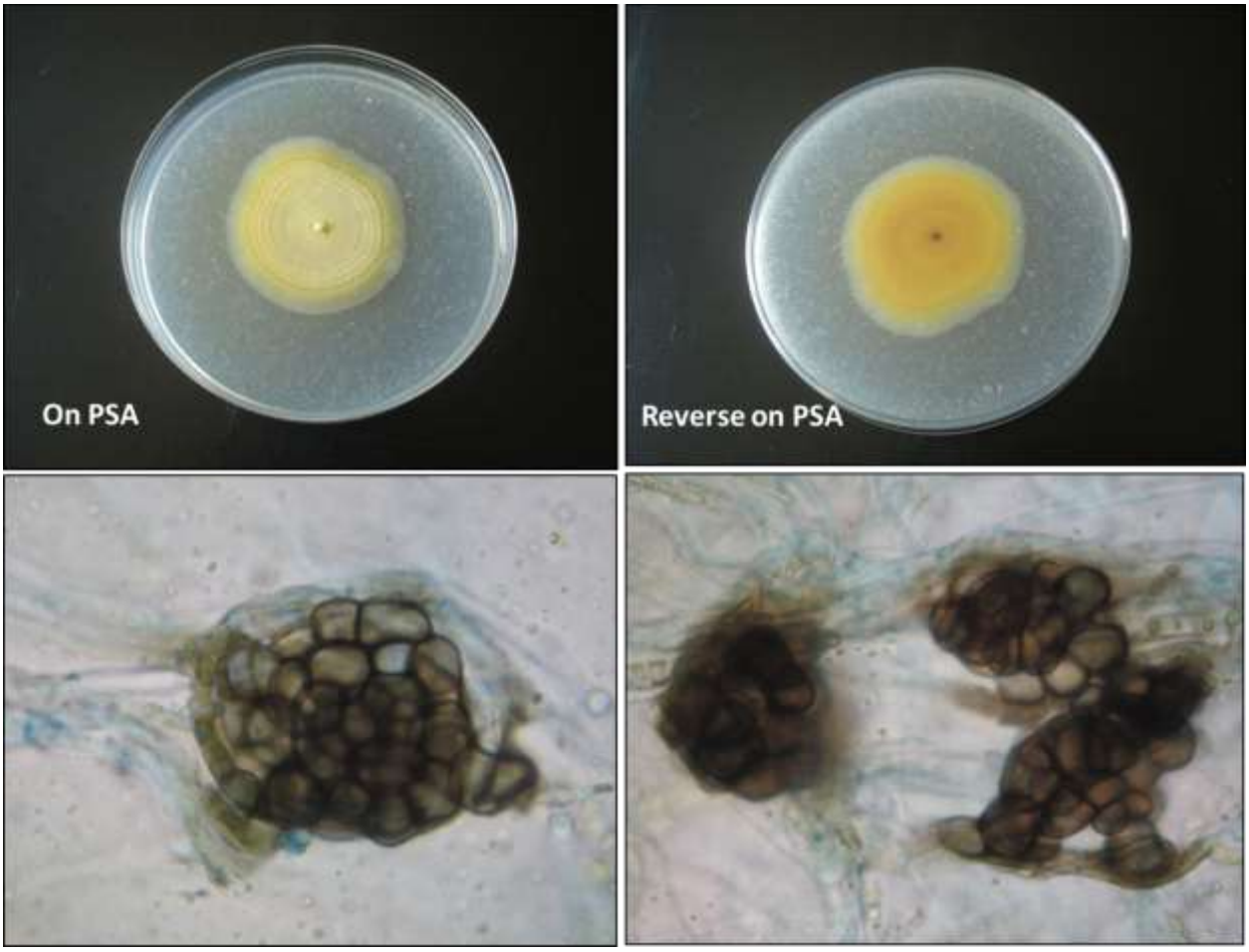

Plate 7: *Chuppia sarcinifera* AUMC 6217: colony obverse and reverse on PSA, and chlamydospores-like dictyospores.

***Clonostachys chlorina* Schroers, Studies in Mycology 46: 174 (2001)**

Brief description: Colonies reaching 60 mm on PSA in 10 days at 25°C, aerial mycelium abundant, colony reverse pale to light yellow, conidiophores verticillate to penicillate with divergent branches, phialides in whorls 2-5, conidia forming round masses or short columns, smooth, one-celled, broadly ellipsoidal to ovoidal, Ascoma not observed (Plate 8).

Ecology and distribution: This species was first described from soil under *Theobroma cacao*, Brazil (Schroers 2001). To the best of our knowledge, the isolation of this species from soil of grapevine plantation on DRBC and DYM at 25°C in Assiut, Egypt (Abdel-Sater *et al.* 2016) is the first record after its original description.

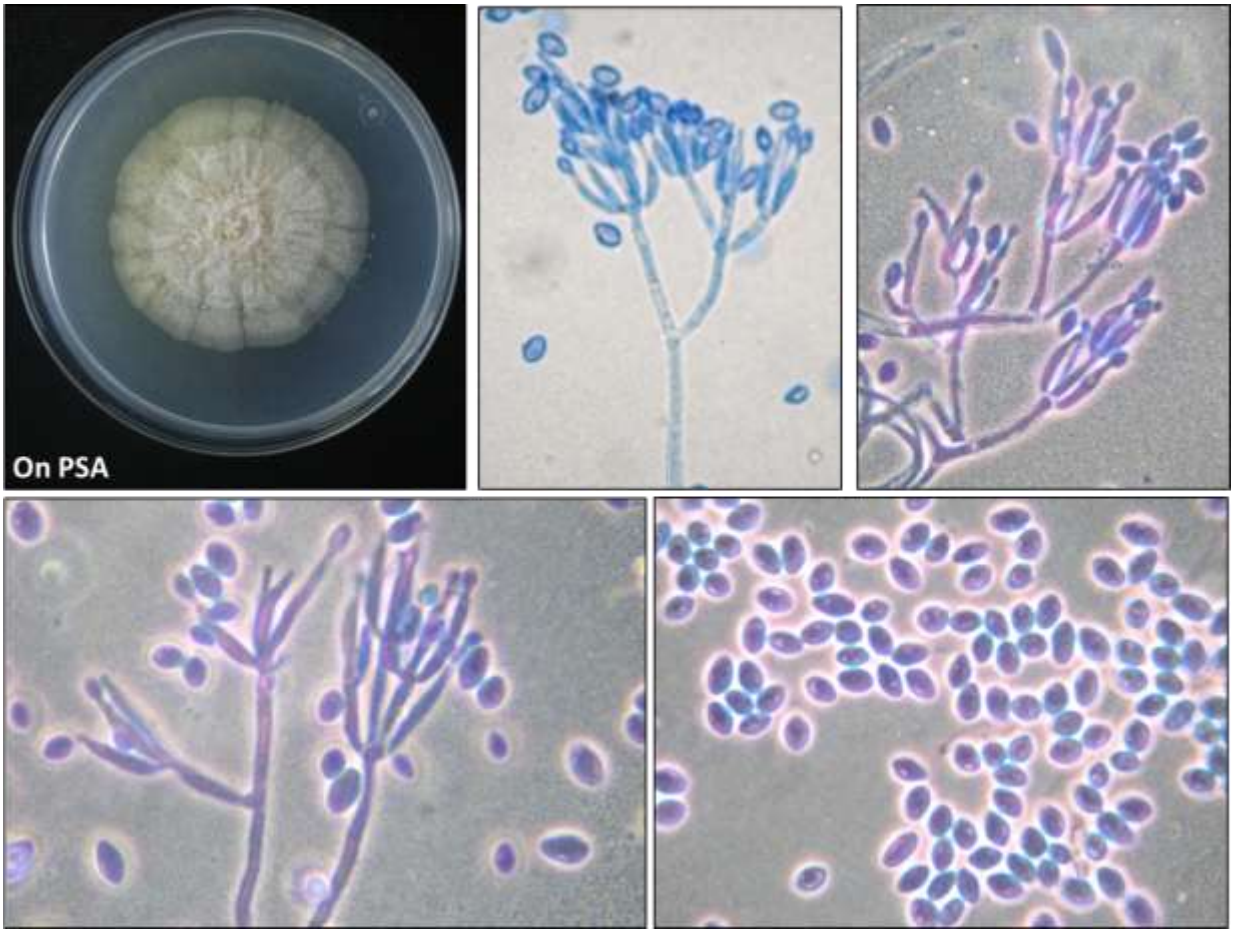

Plate 8: *Clonostachys chlorina* AUMC 6242: colonies on PSA in 10 days at 25°C (upper left), verticillate to penicillate conidiophores and ellipsoidal conidia (upper right & lower photos).

***Clonostachys rogersoniana* Schroers, Studies in Mycology 46: 109 (2001)**

Brief description: colonies attaining 40 mm diameter in 10 days at 25°C on PSA; reverse colourless, conidiophores of two types: verticillium-like with divergent phialides, phialides in whorls of 2-4 each producing a small hyaline drop of conidia, and penicillate-like conidiophores, solitary to gregarious, bi- to quaterverticillate, branches of the penicillus divergent with less divergent metulae and adpressed phialides of whorls of up to 6, conidia in long columns, hyaline, broadly ellipsoidal to oval, 5-9 x 3-4 µm, ascoma not observed (Plate 9).

Ecology and distribution: it was first described from soil under *Araucaria*, Brazil (Schroers 2001). In addition, he stated that it is mostly recorded from warmer or tropical countries, probably of cosmopolitan distribution (Australia, Brazil, Italy, Japan, Nrtherlands, Spain) and frequently isolated from soil, roots, bark of dead twigs and leaf litter. Pharmaceutically, it produced novel cytotoxic menthane-type monoterpenoid (Dong *et al.* 2017, 2018). In Egypt, it was isolated for the first time from the air of citrus plantation in Assiut (Moubasher *et al.* 2016).

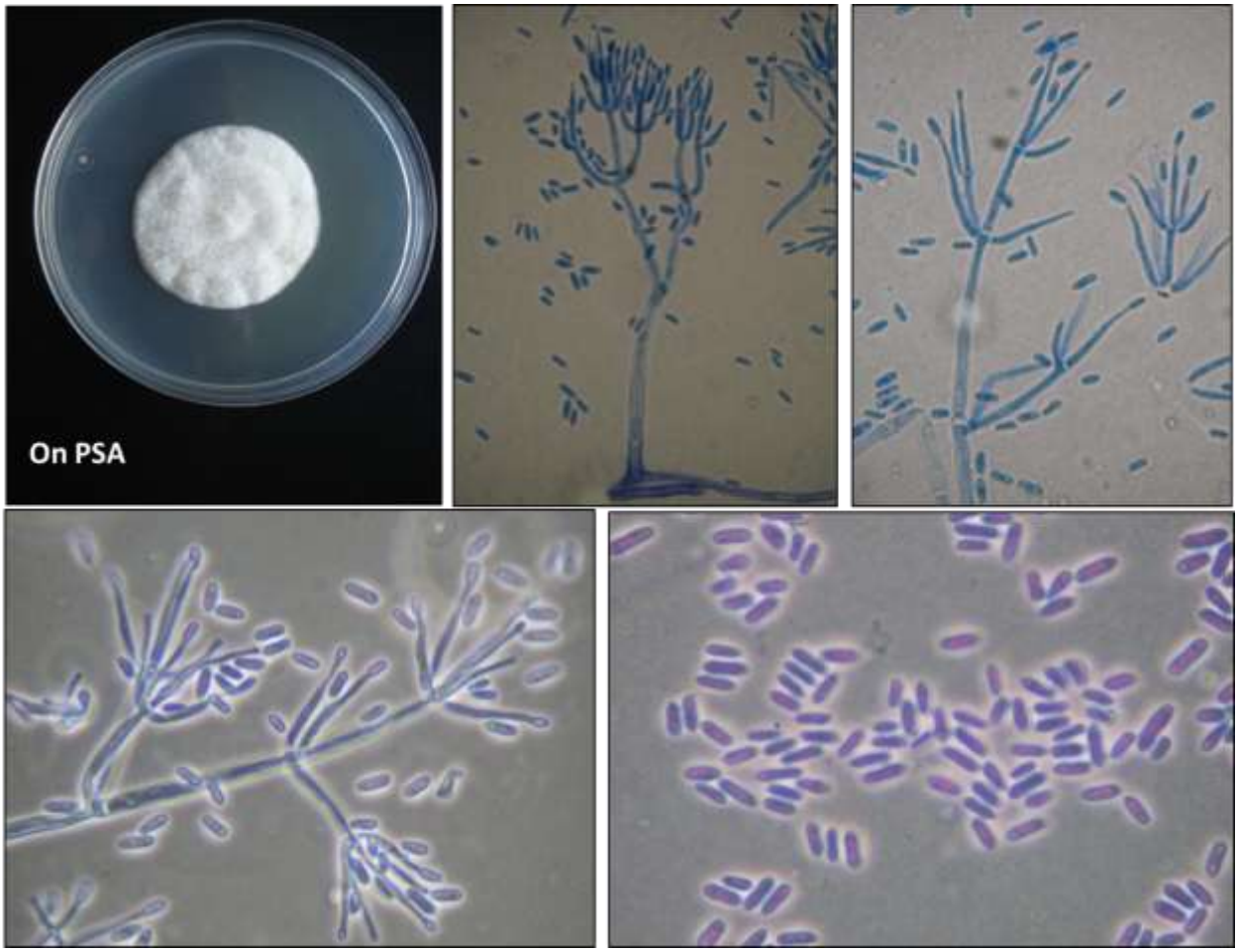

Plate 9: *Clonostachys rogersoniana* AUMC 6220: colonies on PSA (upper left), penicillate-like conidiophores (upper middle), verticillate-like conidiophores (upper right & lower left), and broadly ellipsoidal conidia (lower right).

***Curvularia inaequalis* (Shear) Boedijn, Bull. Jard. bot. Buitenz, 3 Sér. 13(1): 129 (1933)**
≡*Helminthosporium inaequale* Shear, Bull. Torrey bot. Club 34(6): 307 (1907)
=*Acrothecium arenarium* Moreau & V. Moreau, Revue de Mycologie 6 (3-4): 86 (1941)

Brief description: colonies on PDA at 25°C grey to black; conidiophores arising singly or in groups, septate, geniculate, smooth, brown; conidia straight or slightly curved, ellipsoidal or broadly fusiform, hilum scarcely protruding, 2-6 but predominantly 4-distoseptate, the central cell usually much longer and wider than the others, the 2 septa at each end close to each other, with one or both end cells much paler than the others, 25-40 x 10-15 µm (Plate 10, for more details see Ellis 1966, Sivanesan 1987).

Ecology and distribution: widely distributed in Australia, Canada, France, India, Japan, Malaysia, Turkey, UK, USA, and reported from grains of *Hordeum*, *Oryzae*, *Sorghum*, *Triticum*, *Pisum*, *Vaccinium*, roots of *Triticum* and sand dune soils (Ellis 1966), *C. inaequalis* and *C. spicifera* cause leaf blight of buffalograss in Nebraska (Amaradasa and Amundsen 2014) and leaf blight and fruit rot of strawberry in Iran (Ayoubi *et al.* 2017). The first report of human infection due to this fungus was a case of peritonitis in patient undergoing peritoneal dialysis (Pimentel *et al.* 2005), superficial mycosis of the breast (Tanabe *et al.* 2010), non-invasive fungal rhinosinusitis (Posteraro *et al.* 2010), and allergic rhinosinusitis (Cruz *et al.* 2013). Two antitumor constituents from *C. inaequalis* are reported (Pang *et al.* 2013). In Egypt, it was isolated once from the air of citrus plantation during February 2009 on DRBC at 25°C (Zeinab Soliman 2012).

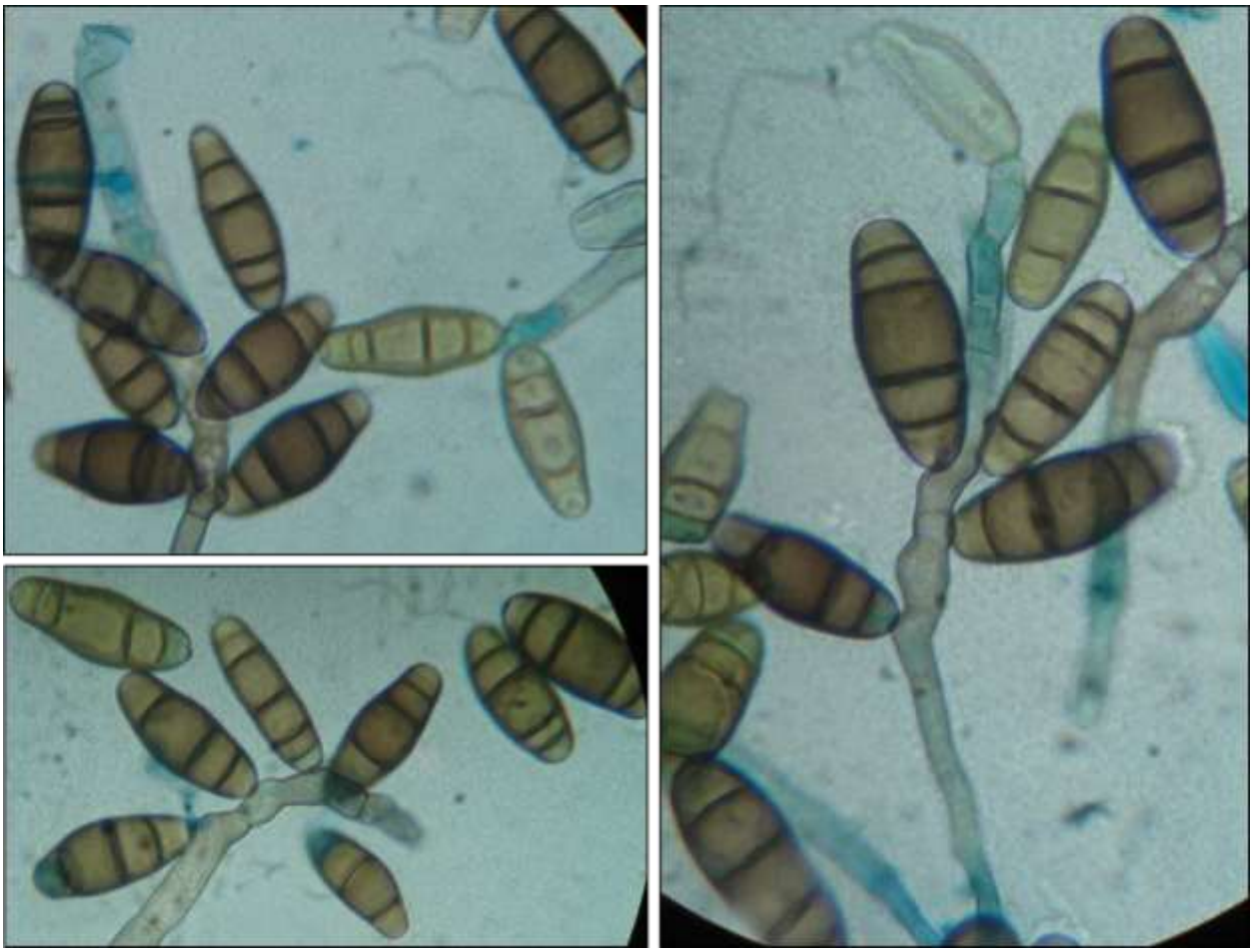

Plate 10: *Curvulaia inaequalis* AUMC 5713: conidiophores simple or branched, septate, geniculate, smooth, brown; conidia straight or slightly curved, predominantly 4-distoseptate.

***Dichotomopilus erectus* (Skolko & J.W. Groves) X.Wei Wang & Samson, Studies in Mycology 84: 217 (2016)**
≡ *Chaetomium erectum* Skolko & J. W. Groves, Canadian Journal of Research 26 (3): 277 (1948)

Brief description: Ascoma > 150 µm diameter, terminal hairs verrucose, branching starts from upper half part, branching angle acute to straight, apical branches erect, asci clavate, ascospores ovate to lemon shape (Plate 11, for more details see Wang *et al.* 2014a, 2017).

Ecology and distribution: this species was reported from *Petroselinum sativum* in USA, soil in Anqiu, Shandong Province, China (Wang *et al.* 2014a), and from citrus phylloplane in rare frequency for the first time in Egypt (Moubasher *et al.* 2018c).

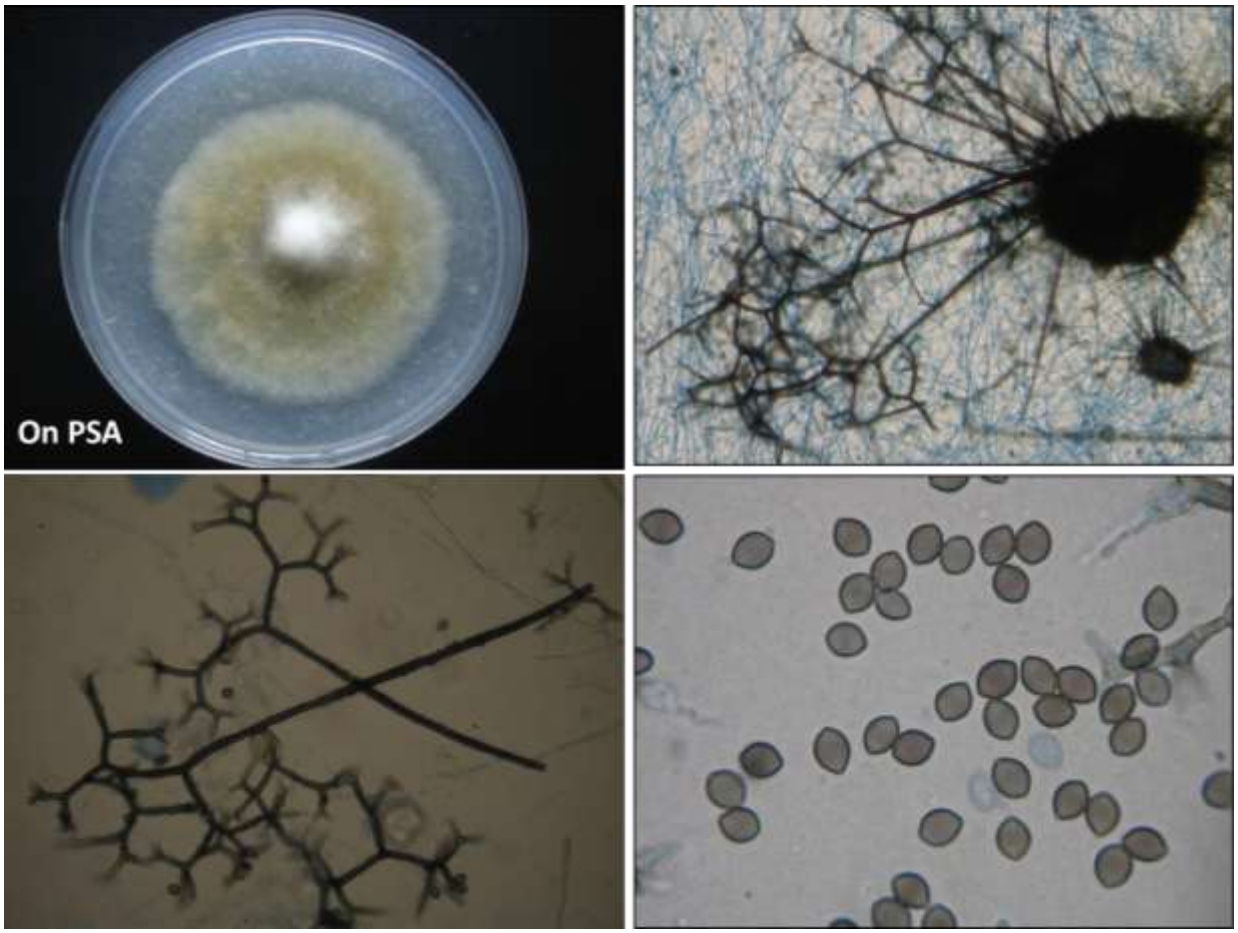

Plate 11: *Dichotomopilus erectus* AUMC 6296: colony on PSA (upper left), ascoma surrounding by hairs (upper right), dichotomous branching of hairs (lower left), and lemon-shaped ascospores (lower right).

Exserohilum pedicellatum (A.W. Henry) K.J. Leonard & Suggs, Mycologia 66(2): 291 (1974)
≡*Helminthosporium pedicellatum* A.W. Henry, Tech. Bull. Minn. agric. Exp. Stn 22: 42 (1924)
≡*Bipolaris pedicellata* (A.W. Henry) Shoemaker, Can. J. Bot. 37(5): 884 (1959)
≡*Trichometasphaeria pedicellata* R.R. Nelson, Mycologia 57 (4): 665 (1965)
≡*Drechslera pedicellata* (A.W. Henry) Subram. & B.L. Jain, Curr. Sci. 35: 354 (1966)
≡*Setosphaeria pedicellata* (R.R. Nelson) K.J. Leonard & Suggs, Mycologia 66: 295 (1974)

Brief description: colonies filling CYA plate within a week, obverse and reverse dark, conidiophores mostly single, straight to flexuous, conidia golden brown, straight, broadly fusoid, smooth, 4-6 distoseptate, 40-80 x 20-25 µm, with a distinguishing feature pedicel-like extension at the base, hilum protuberant. Ascoma not observed (the species is heterothallic) (Plate 12, for more details see Ellis 1971, Sivanesan 1987).

Ecology and distribution: reported from *Oryza sativa, Zea mays, Sorghum halepense, Paspalum dilatum, Setaria lutescens* and species of *Echinochloa* and *Triticum.* It also causes brown lesions on wheat roots and root rot of maize; found in India, Pakistan, South Africa, USA (refer to Sivanesan 1987). It was reported from barley roots in Poland (Lacicowa 1993), brown root rot of maize in Australia (Gilbert 2003), root rot of corn in Texas (Isakeit *et al.* 2007) and from a clinical specimen (McGinnis *et al.* 1986). In Egypt, it was reported from soil by AH Moubasher and from *Oryza sativa* by MA Hussein in 1969 (Sivanesan 1987) and from the air of grapevine plantation in rare frequency in February 2009 on DRBC at 25°C (Zeinab Soliman 2012).

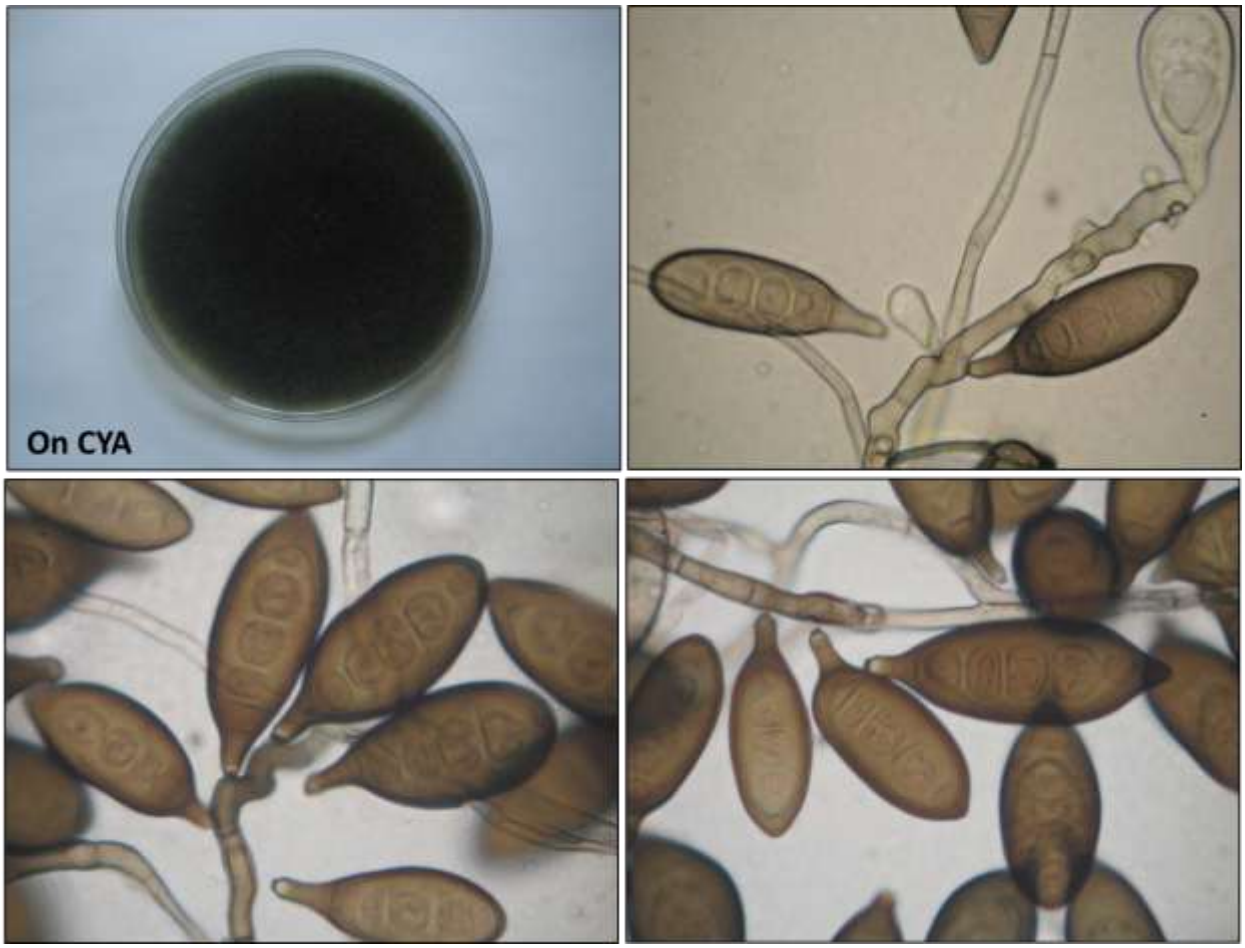

Plate 12: *Exserohilum pedicellatum* (*Setosphaeria pedicillata*) AUMC 5758: colonies filling CYA plate within a week, obverse dark (upper left), conidiophores mostly single, conidia brownish, 4-6 distoseptate, with a pedicel-like extension (upper right & lower photos).

***Fusarium circinatum* Nirenberg & O'Donnell, Mycologia 90: 442 (1998)**
≡*Gibberella circinata* Nirenberg & O'Donnell, Mycologia 90(3): 440 (1998)
≡*Gibberella circinata* Nirenberg & O'Donnell ex Britz, T.A. Cout., M.J. Wingf. & Marasas, Sydowia 54(1): 16 (2002)

Brief description: colonies on PDA after 7 d at 25°C grow rapidly, mycelium white, but may produce violet pigment; macroconidia sparse on carnation leaf pieces and may be difficult to find on other media; microconidia abundant, 0-septate, obovoid, oval or allantoid, produced singly or in aggregations of a few microconidia; conidiogenous cells polyphialidic and monophialidic; chlamydospores absent but some isolates produce swollen hyphae; coiled sterile hyphae on SNA are diagnostic for this species; sexual state not observed in our strain (this species is heterothallic) (Plate 13, for more details see Leslie and Summerell 2006).

Ecology and distribution: this fungus is reported as a pathogen on *Pinus* species and some other conifers; found in Brazil, Chile, Colombia, Japan, Mexico, South Africa, Spain, Uruguay and USA (Leslie and Summerell 2006, Pfenning *et al.* 2014), in roots of non-symptomatic mature pine trees Basque Country, NE Spain (Hernandez-Escribano *et al.* 2018), air (Schweigkofler *et al.* 2004), In Egypt, it was recorded from soil, air and juice of grapevine plantation at 25°C from Assiut area (Abdel-Sater *et al.* 2016, Moubasher *et al.* 2016, 2018a).

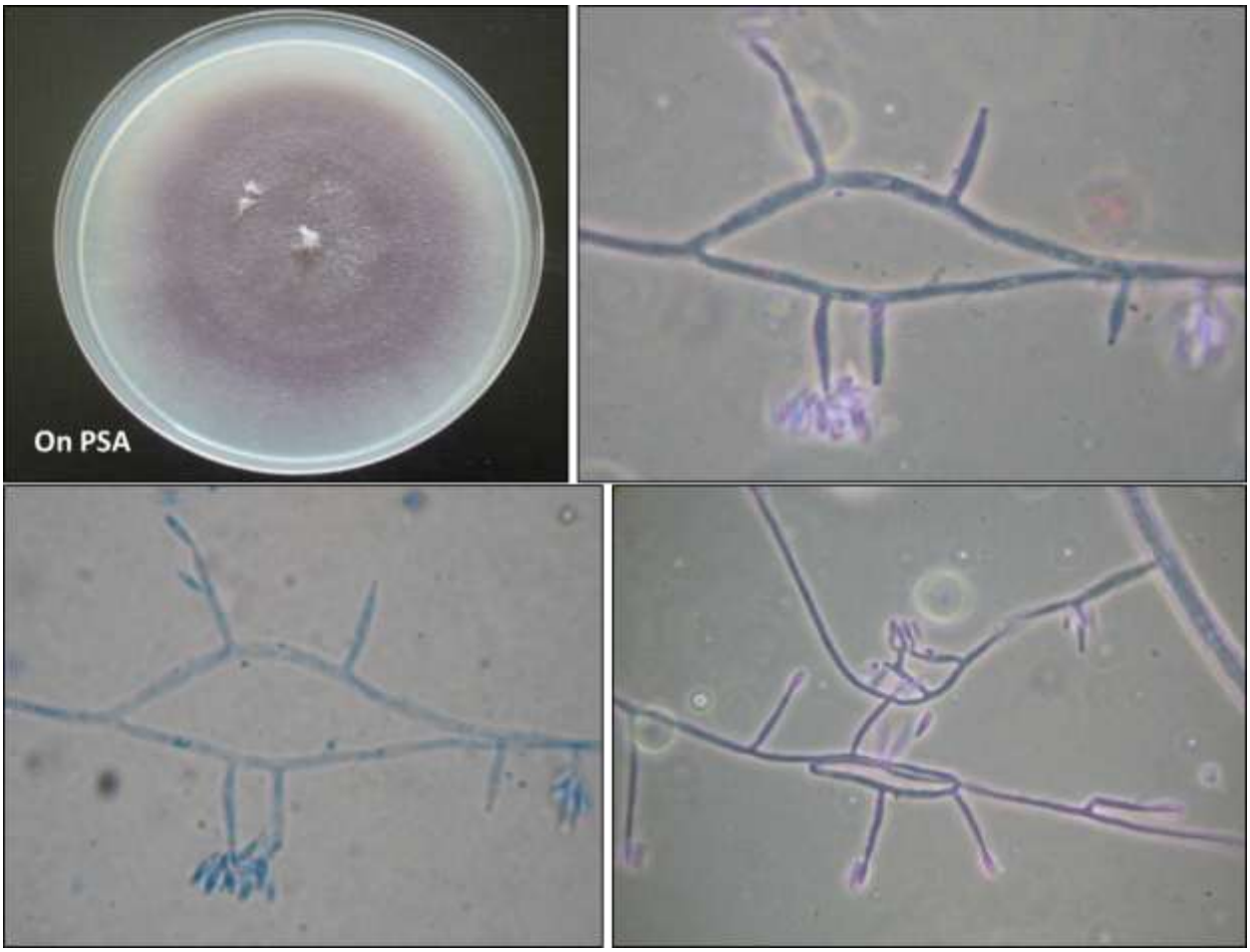

Plate 13: *Fusarium cicinatum* AUMC 6775: colonies on PSA at 25C after 7 days (upper left), monophialides and polyphialides bearing 0-septate, oval or allantoid conidia (upper right, lower left & right).

***Gibellulopsis nigrescens* (Pethybr.) Zare, W. Gams & Summerbell, Nova Hedwigia 85 (3-4): 477 (2007)**
≡*Verticillium nigrescens* Pethybr., Transactions of the British Mycological Society 6: 117 (1919)
≡*Verticillium amaranthi* Verona & Ceccar., Phytopathol. Z.: 379 (1935)
≡*Verticillium dahliae* f. *zonatum* J.F.H. Beyma, Antonie van Leeuwenhoek 6: 42 (1940)
≡*Verticillium serrae* (Maffei) J.F.H. Beyma, Antonie van Leeuwenhoek **6**: 40 (1940)

Brief description: colonies of *G. nigrescens* on PSA reaching 30 mm diam in 10 d at 25°C, whitish when young, finely floccose; becoming more or less dark grey with age, mainly in reverse, due to formation of chlamydospores; vegetative hyphae hyaline, smooth-walled; conidiophores short, arising from substratum or aerial hyphae, (sub-) erect, 50-100 µm long and 1.5-2.5 µm wide in the lower part, bearing 1-3 levels of phialides, with few phialides per whorl. Phialides aculeate, 25-50 µm long, tapering from near the base to the tip; conidia hyaline, smooth-walled, elongate-ellipsoidal, 4-7 × 1.0-2.5 µm; chlamydospores grey-brown, smooth-walled, single or in short chains, 4.5-7.5 × 2.5-5 µm (Plate 14, for more details refer to Zare *et al.* 2007).

Ecology and distribution: this species was originally described on potato tubers in England (Pethybridge 1919), then from soil in the Netherlands (Zare *et al.* 2007). It is typically a soil-borne fungus that colonizes only the roots, or at most the lower parts of the stem, of a wide variety of plants. An outbreak of wilt caused by *G. nigrescens* with symptoms of yellowing, defoliation and rapid death specific in potatoes was observed in Japan (Kitazawa and Sato 1984). Otherwise, it was found to be only slightly pathogenic to tomato, potato and eggplant (Isaac 1949), as well as peppermint and other crops (Melouk and Horner 1974). It attacked soybean flowers and pods without causing wilt (Phillips *et al.* 1983). It caused bright orange "tiger-stripe" discoloration of diseased leaves of some cultivars of cigar tobacco (Sheppard and Viswanathan 1974). *G. nigrescens* is more sensitive to benomyl than the pathogenic *Verticillium* species (Hall 1975, Wallbridge and Pinegar 1975). In Egypt, it was recorded in rare frequency from carposphere and phyllosphere of vine (Moubasher *et al.* 2018a &c).

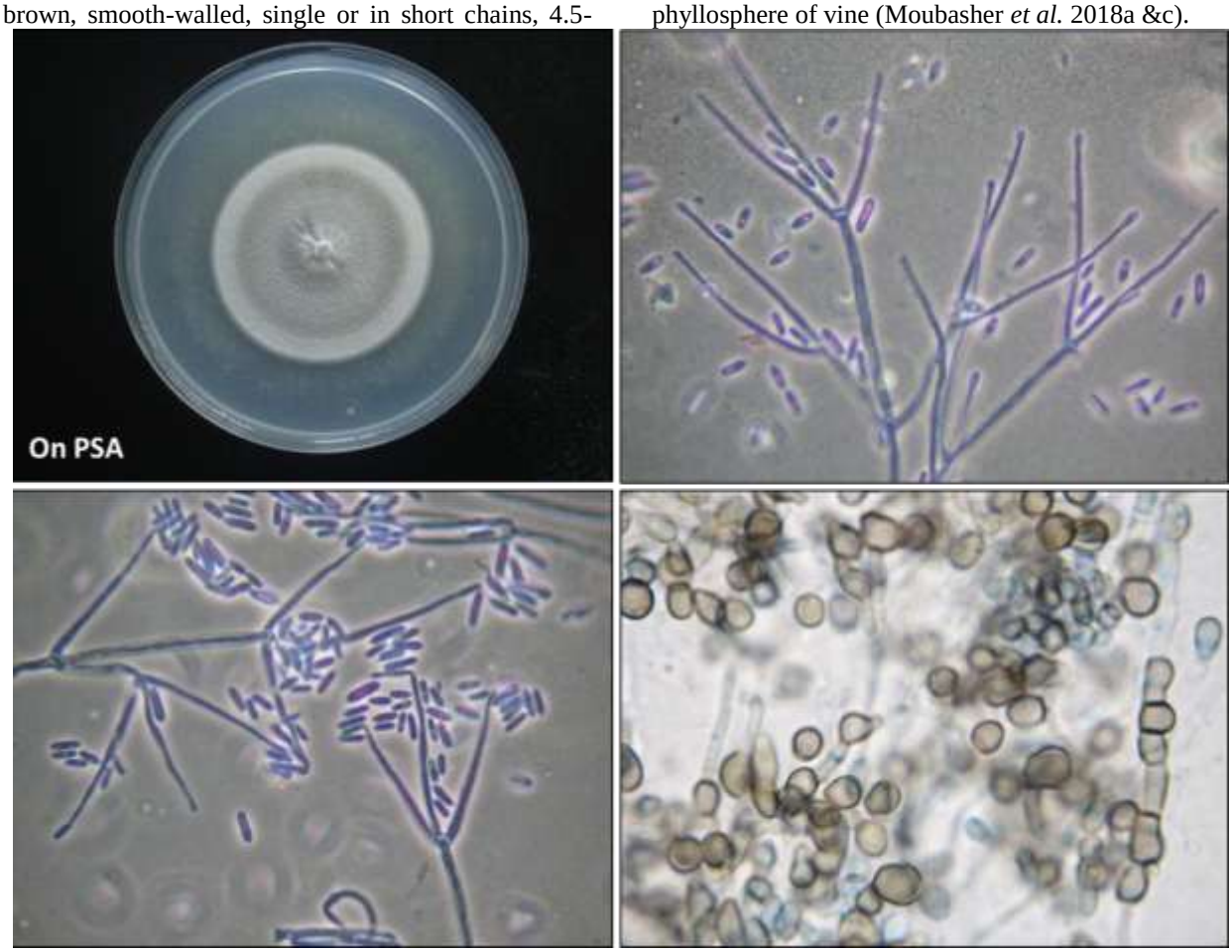

Plate 14*: Gibellulopsis nigrescens* AUMC 5737: colony on PSA (upper left), phialides in whorls and elongate conidia (upper right & lower left), and brownish chlamydospores in chains (lower right).

***Leptodontidium trabinellum* (P. Karst.) Baral, Platas & R. Galán, in Baral, Index Fungorum 271: 1 (2015)**
≡*Helotium trabinellum* P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 126 (1871)
≡*Rhinocladiella elatior* F. Mangenot, Revue Générale de Botanique 59: 44 (1952)
≡*Leptodontium elatius* (F. Mangenot) de Hoog, Studies in Mycology 15: 47 (1977)
≡*Leptodontidium elatius* var. *elatius* (F. Mangenot) de Hoog, Taxon 28: 348 (1979)

Brief description: colonies on CYA at 25°C attaining 20 mm in 10 d, colonies at first whitish, becoming grey to olivaceous black, reverse grey; conidiogenus cells occasionally acute, sometimes in groups or making small brushes, olivaceous brown, smooth, elongate, with thin septa but occasionally without basal septa, each with an acicular terminal conidiogenous cell; intercalary cells shorter than terminal ones, producing conidia singly on sympodial minute denticles, straight or flexuose rachis, hyaline, smooth, 3-5 x 1-1.5 µm (Plate 15, for more details see de Hoog 1977, 1979, Seifert *et al.* 2011)).

Ecology and distribution: reported in soil, litter and sometimes as mycorrhizas, from Africa, Europe, North America (de Hoog 1977), on *Fagus sylvatica*, dead wood, in Serbia (Savic and Karaman 2016), decaying wood of *Betula* from France (Hernandez-Restrepo *et al.* 2017), on dead wood in forest ecosystems (van der Wala *et al.* 2018). In the current study, it was recorded in rare frequency from citrus phyllosphere on DRBC at 25°C (Moubasher *et al.* 2018c).

Plate 15: *Leptodontidium trabinellum* AUMC 5709: colony on CYA at 25°C (upper left), conidiophores, conidioenous cells and conidia (upper right and lower photos).

***Neurospora tetraspora* D. Garcia, Stchigel & Guarro 2004**
≡*Gelasinospora tetrasperma* Dowding, Canadian Journal of Research 9 (3): 294 (1933)
≡*Gelasinospora calospora f. tetrasperma* (Dowding) C. Moreau & M. Moreau 1951

Strain examined: AUMC 6784 isolated from soil of grapevine plantation = Genbank accession No. JQ425383).

Brief description: colonies on PSA and CYA at 25ºC after 7 days: filling the agar plates (9 cm diam.), producing a rapid and dense growth of mycelium which is white at first becoming gray or slightly pinkish. Ascomata superficial or slightly immersed, black, pyriform, ostiolate, 450-600 x 270-450 µm. Asci cylindrical, 4-spored, but 3- and 5-spored asci were seen in the AUMC strain. Ascospores uniseriate, ellipsoidal or elongate, 22-33 x 12-20 µm, broadly rounded at the ends, dark brown, becoming black and opaque. Asexual stage (conidial state) not observed (Plates 16 & 17, for more details see Moubasher *et al.* 2018b).

Ecology and distribution: this fungus is reported in Canada, England, Finland, Norway, Russia, Spain, Sweden and USA, from dung and soil (Garcia *et al.* 2004). Also, it was one of the most frequent microfungi from rotting wood in Norhern Albert forests, Canada suggesting that these fungi are significant component of wood decaying fungal communities (Lumley *et al.* 2000). In Egypt, it was recovered for the first time once in June 2008 from a non-rhizosphere soil sample collected from a grapevine plantation in the village of El-Khawaled, Sahel-Saleem city, Assiut (Moubasher *et al.* 2018b). The presence of this fungus together with other fungal communities in grapevine soil (Abdel-Sater *et al.* 2016) may help in decaying the woody and non-woody debris of vine (Mouasher *et al.* 2018b).

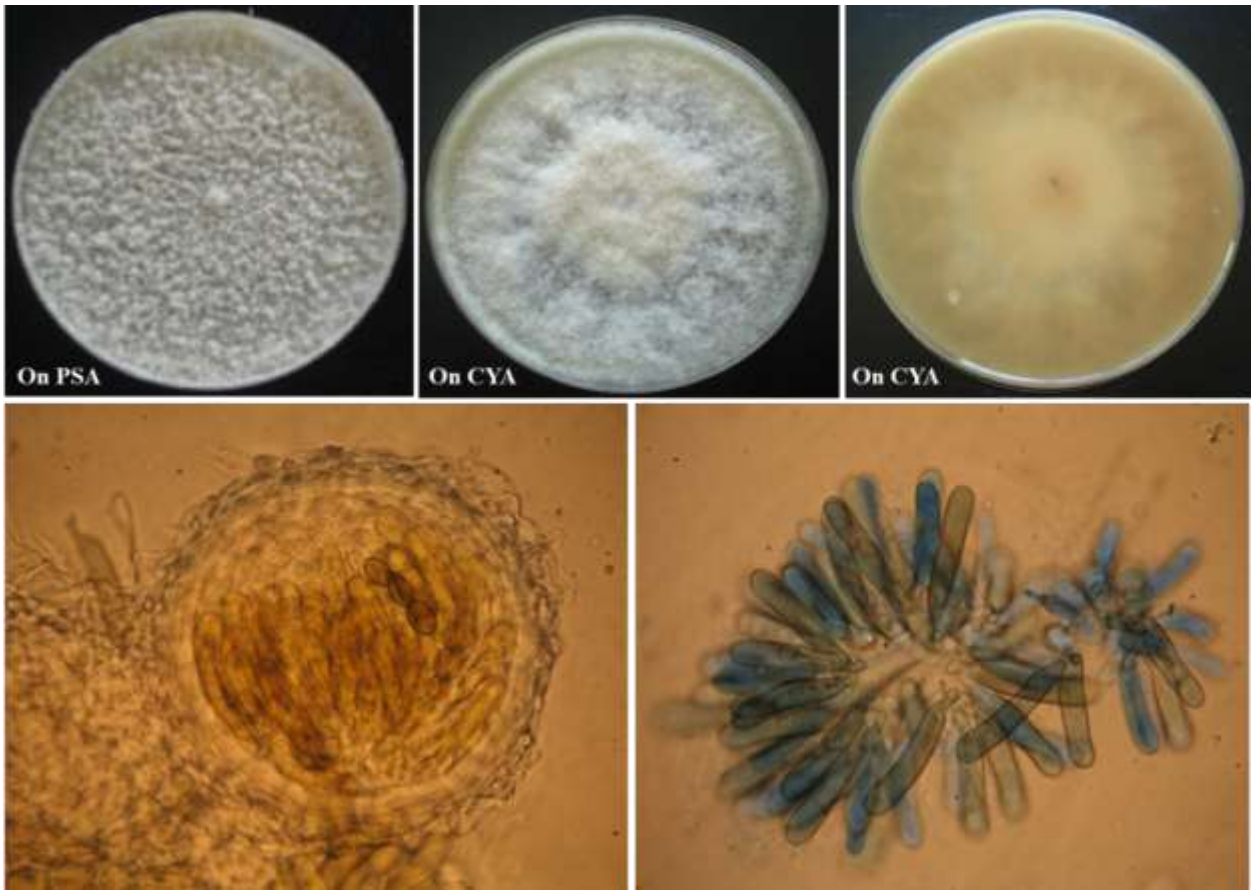

Plate 16: ***Neurospora tetraspora* (≡*Gelasinospora tetrasperma*) AUMC 6784**: 7-day-old colony on PSA (obverse, upper left) and on CYA (obverse upper middle, and reverse upper right), ascoma (lower left) and asci (lower right).

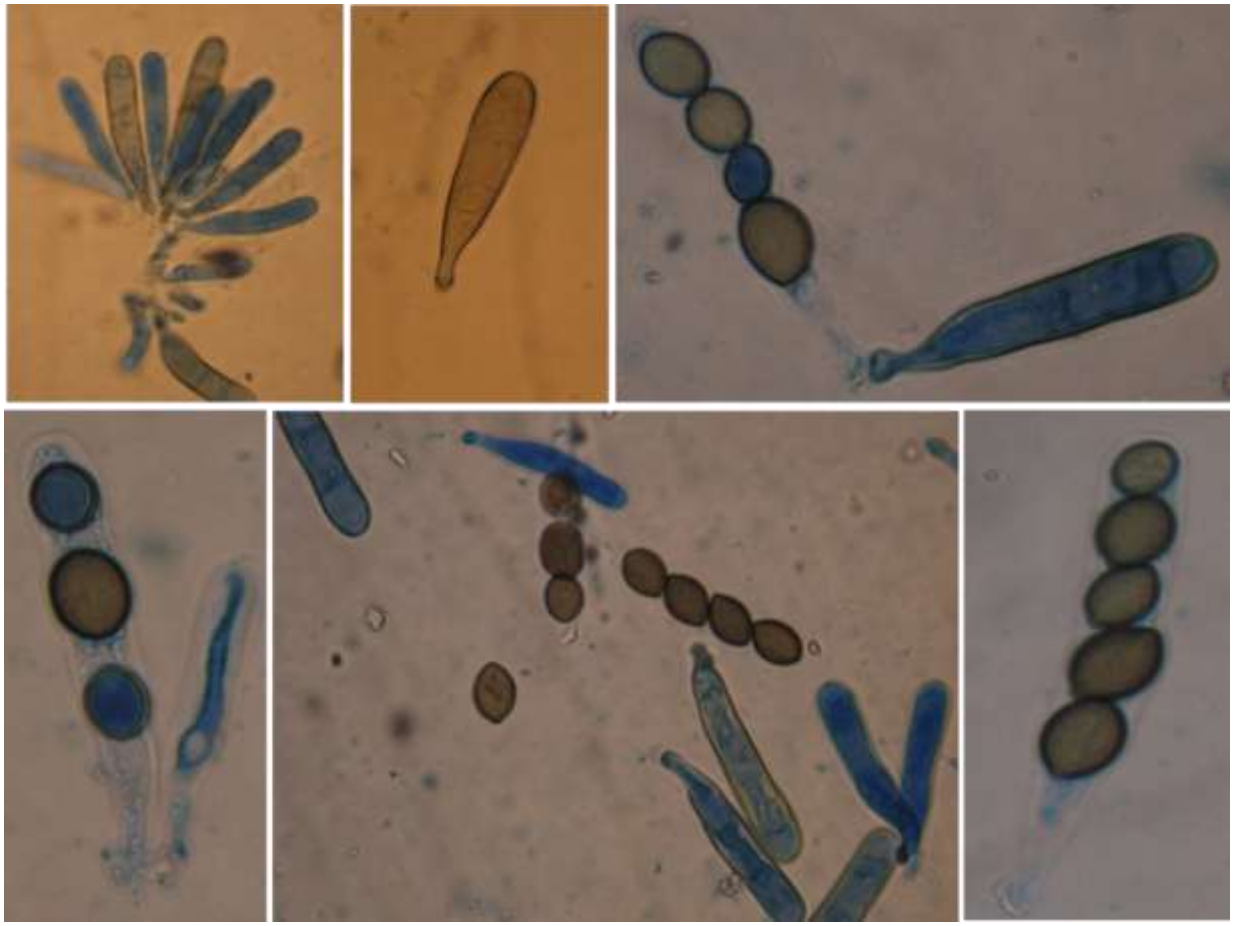

Plate 17: ***Neurospora tetraspora*** **(*Gelasinospora tetrasperma*) AUMC 6784**: cylindrical asci and 3-5 spored-asci.

***Penicillium digitatum* (Pers.) Sacc., Fung. Ital.: tab. 894 (1881)**
=*Aspergillus digitatus* Pers., Disp. meth. Fung.: 41 (1794)
=*Monilia digitata* (Pers.) Pers., Synopsis methodica fungorum: 693 (1801)
=*Mucor digitata* (Pers.) Mérat, Nouvelle flore des environs de Paris 1: 14 (1821)
=*Penicillium olivaceum* Wehmer, Beitr. Kennt. einh. Pilze: 73 (1895)
≡ *Penicillium digitatum* var. *californicum* Thom, The Penicillia: 245 (1930)
≡*Penicillium digitatum* var. *latum* S. Abe, J. gen. appl. Microbiol., Tokyo 2: 97 (1956)

Brief description: colonies on CYA at 25°C velutinous, 40 mm diam after 7 d, no growth at 37°C, conidiohores heavy, frequently biverticillate but terverticillate are found, metulae in verticils of 2-3, phialides in verticils of 3-5 ampuliform to cylindroidal, conidia ellipsoidal to cylindroidal, smooth walled, borne in disordered chains (for more details see Pitt 1979). It is distinct from other *Penicillium* species by the large size of metulae, phialides and conidia (Plate 18).

Ecology and distribution: rotting fruits of citrus species is the predominant habitat, universally distributed in soil and other substrates, cosmopolitan in Australia, Canada, Cyprus, India and UK (Pitt 1979). In this study, it was reported from citrus carposphere, carpoplane and juice (Moubasher *et al.* 2018a).

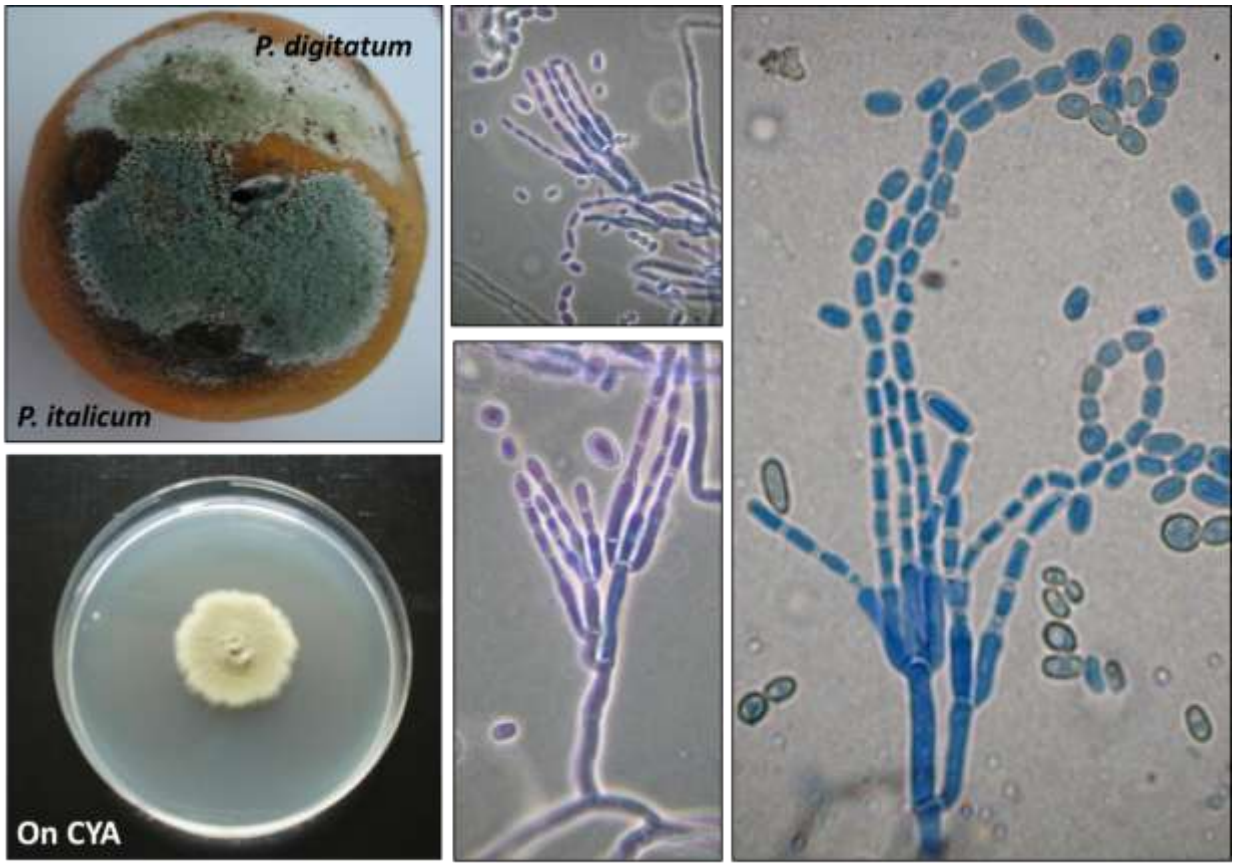

Plate 18: *Penicillium digitatum* AUMC 6250: *P. digitatum* deteriorating orange fruit (upper left), colony on CYA at 25°C (lower left), penicillus structure bearing cylindrical conidia (upper & lower middle and right photos).

***Penicillium olsonii* Bainier & Sartory, Annales Mycologici 10 (4): 398 (1912)**
=*Penicillium volgaense* Beliakova & Milko, Mikol. Fitopatol. 145-151 (1972)

Brief description: colonies velutinus, 35 mm diam on CYA at 25°C, reverse yellowish brown, no growth at 37°C; stipes very long up to 2 cm or more with closely appressed multiserriate penicilli, usually terverticillate but sometimes quaterverticillate, rami 2-3(-6) per stipe, metulae in verticills of 3-5 and phialides ampuliform 5-8 per metulae, conidia smooth 3-4 x 2.5-3 µm, borne in disordered chains. It is distinguished from other species by the large multiramulate tervericillate penicilli borne on wide stipes (Plate 19, for more details see Pitt 1979).

Ecology and distribution: *P. olsonii* has been isolated only from soil and water in Puertro Rico on a few occasion since its decription in 1912 (Pitt 1979). In Egypt, it is reported in high frequency from the air of citrus plantation (Moubasher *et al.* 2016) and in moderate occurrence from citrus soil on DRBC and DYM at 25°C (Abdel-Sater *et al*. 2016), in low and rare frequency from citrus carposphere (Moubaher *et al*. 2018a), moderate frequency from citrus phyllosphere while in rare frequency from citrus phylloplane (Moubasher *et al.* 2018c).

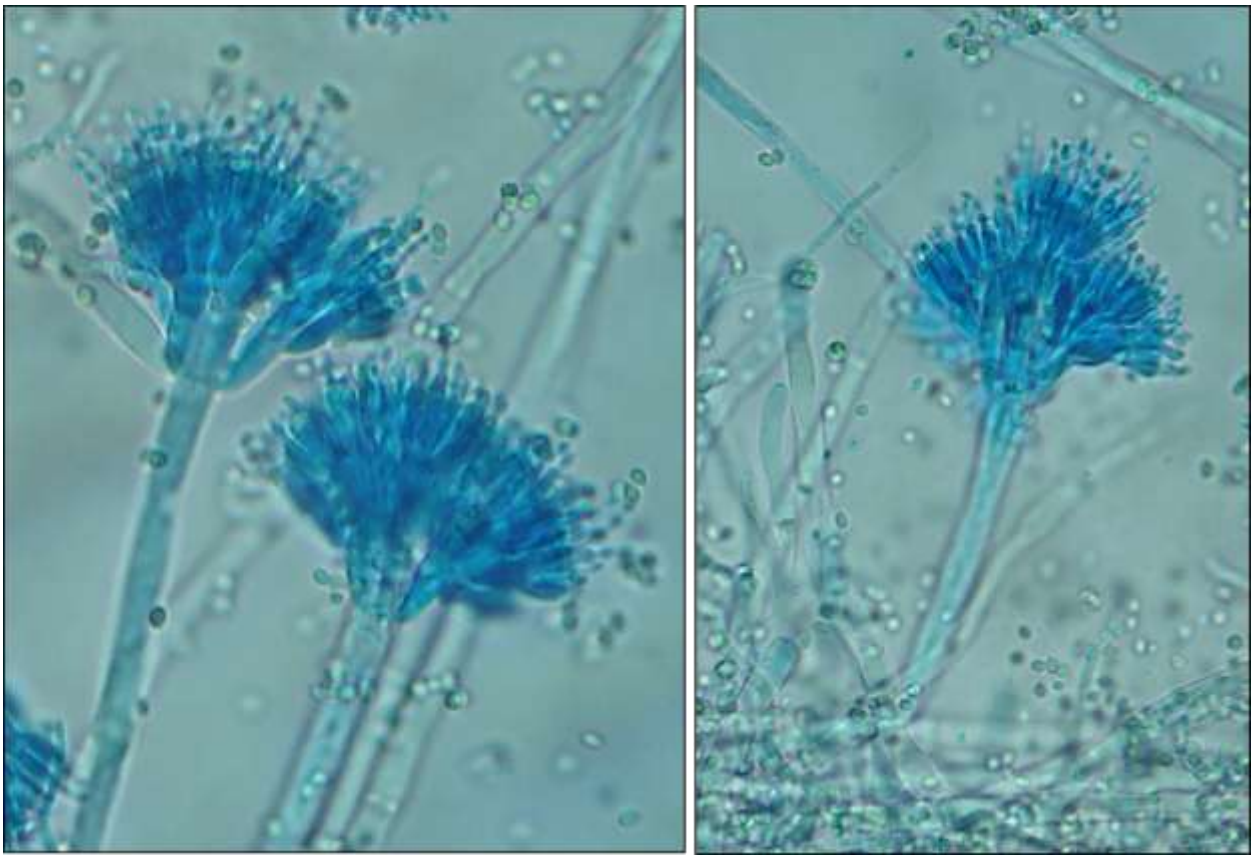

Plate 19: *Penicillium olsonii* AUMC 5708: very long appressed multiserriate penicilli beaing ovoidal to ellipsoidal conidia.

***Penicillium vinaceum* J.C. Gilman & E.V. Abbott, Iowa State College Journal of Science 1 (3): 299 (1927)**

Brief description: colonies attaining 18 mm diam after 7 d on CYA at 25°C; mycelium white at the margin, buff elsewhere; sporogenesis light, exudate vinaceous; reverse dark brown, soluble pigment widespread, yellow to brown; stipes short, 15-50 µm, smooth-walled, strictly monoverticillae, non-vesiculate, phialides in whorls of 2-8, ampuliform, 6-12 µm long, conidia globose to subglobose, smooth-walled, 2-3 µm diam, borne in short divergent chains (Plate 20).

Ecology and distribution: rare species and appears to be strictly a soil fungus, isolated in Egypt, Japan, USA, UK (Pitt 1979), Egypt, Qatar, Syria (refer to Moubasher 1993). Recently, it was recovered from soil of grapevine plantation in Assiut area (Abdel-Sater *et al*. 2016).

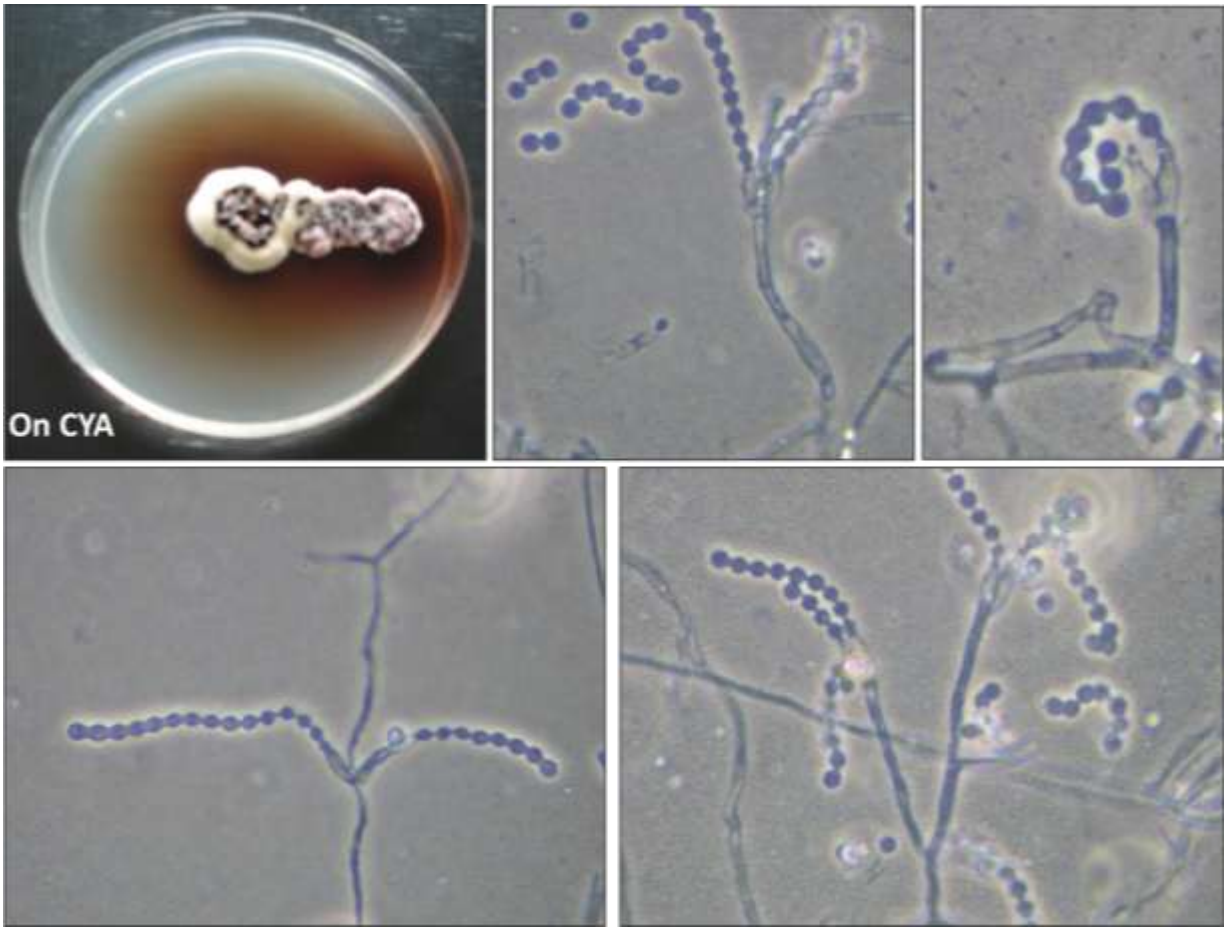

Plate 20: *Penicillium vinaceum* AUMC 6203: colonies on CYA with vinaceous exudate and soluble diffusing pigment (upper left), monoverticillae, non-vesiculate stipes bearing phialides in whorls and conidia globose to subglobose (upper right & lower left and right).

***Pestalotiopsis* sp.**

Brief description: colonies on PDA at 25°C filling the plate after 7 days, with dense whitish aerial mycelium on surface, reverse dark brown; conidiomata, with annellidic conidiogenous cells; conidia 5-celled with three concolourous median cells, hyaline terminal cells and 1-3 appendages arising from the apex of the apical cell (mostly 3), basal appendage single, tubular, unbranched, centric; sexual morph not observed (Plate 21, for more details see Steyaert 1949, Sutton 1980).

Ecology and distribution: The genus is widely distributed throughout tropical and temperate regions (Bate-Smith & Metcalfe 1957, Maharachchikumbura *et al.* 2014). Most species lack sexual morphs, and only 13 sexual morphs have been recorded in literature, which were previously treated as species of *Pestalosphaeria* (Maharachchikumbura *et al.* 2011). Species of *Pestalotiopsis* are common phytopathogens that cause a variety of diseases including canker lesions, shoot die back, leaf spots, tip blight, grey blight, scabby canker, severe chlorosis, fruit rots and various post-harvest diseases (Crous *et al.* 2011, Maharachchikumbura *et al.* 2012, 2013a,b, Zhang *et al.* 2012a, 2013), and have been isolated from dead leaves, bark and twigs (Ellis & Ellis 1997, Maharachchikumbura *et al.* 2013d), cause economic loss in apple, blueberry, coconut, ginger, grapevine, guava, hazelnut, mango, peach and tea due to disease (Sun & Cao 1990, Sangchote *et al.* 1998, Xu *et al.* 1999, Keith *et al.* 2006, Joshi *et al.* 2009, Keith & Zee 2010, Chen *et al.* 2011, Evidente *et al.* 2012, Ismail *et al.* 2013, Maharachchikumbura *et al.* 2013a,b,c, Ren *et al.* 2013), isolated as endophytes ((Watanabe *et al.* 2010, Maharachchikumbura *et al.* 2012, 2013a, b, c, Debbab *et al.* 2013, Zhang *et al.* 2012 a, b, 2013). *Pestalotiopsis* consists of around 300 names (Index Fungorum 2017). Due to their ability to switch nutritional-modes, many endophytic and plant pathogenic *Pestalotiopsis* species persist as saprobes (Hu *et al.* 2007, Maharachchikumbura *et al.* 2012), Several species have been recovered from soil, wood, paper, fabrics and wool (Guba 1961), and there are various reports that *Pestalotiopsis* species produce a diverse array of chemical compounds with medicinal, agricultural and industrial applications (Xu *et al.* 2010, 2014, Maharachchikumbura *et al.* 2011). Some species have been associated with human and animal infections (Sutton 1999, Monden *et al.* 2013) and others have also been isolated from extreme environments (Strobel *et al.* 1996, Tejesvi *et al.* 2007).

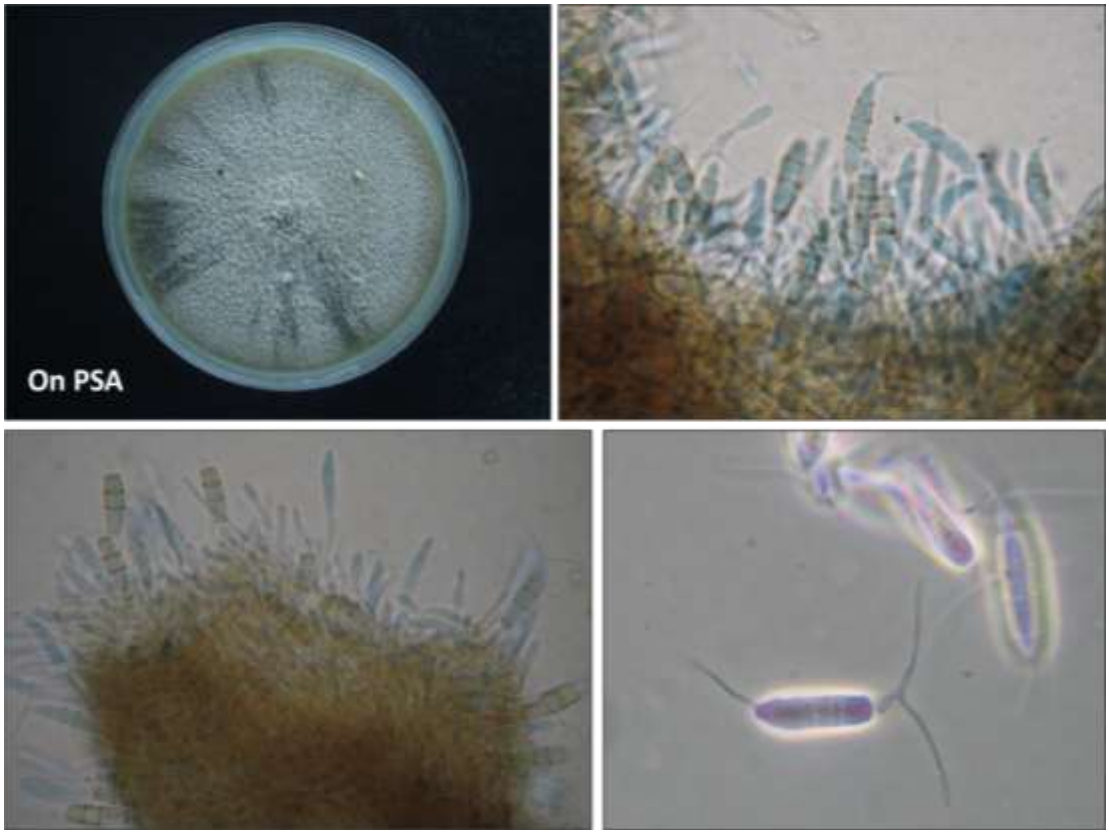

Plate 21: *Pestalotiopsis* sp. AUMC 5755: colony on PSA after 7 days at 25°C (upper left), conidiomata arising coidiogenous cells bearing 4-septate conidia (upper right and lower left) and conidia with 1-3 unbranched terminal appendages and one appendage at the base (lower right).

***Quambalaria cyanescens* (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer, Studies in Mycology 55: 295 (2006)**
≡*Sporothrix cyanescens* de Hoog & G.A. de Vries, Antonie van Leeuwenhoek 39 (3): 515 (1973)
≡*Cerinosterus cyanescens* (de Hoog & G.A. de Vries) R.T. Moore, Studies in Mycology 30: 216 (1987)
≡*Fugomyces cyanescens* (de Hoog & G.A. de Vries) Sigler, Manual of Clinical Microbiology: 1763 (2003)

Strains examined: AUMC 6293 isolated from citrus air =Genbank accession no. JQ425376 wih 100% similarity with the type strain & AUMC 6294 from citrus juice =Genbank accession no. JQ425382 with 99% similarity).

Brief description: colonies 10-15 mm diam after 10 d on CYA at 25°C, velvety or lanose, folded and raised in the centre, at first purely hyaline, after two weeks becoming purplish, finally bluish; reverse purple, diffusible; conidiogenous cells arising terminally or sometimes laterally from undifferentiated hyphae, apical part forming conidia by sympodial growth, denticles inconspicuous; conidiogenous cell often proliferates at or somewhat below the apex; conidia one-celled; hyaline, ellipsoidal, mostly 2.5-6.5 µm long, frequently longer, 2.5-3 µm wide, with a slightly apiculate base. Neither ascoma nor chlamydospores were observed. *S. cyanescens* differs from all species described in *Sporothrix* by the ability to produce a water-soluble pigment however, the pigment production is variable (Plates 22 & 23).

Ecology and distribution: *Q. cyanescens* (as *S. cyanescens*) has been isolated from human skin and blood and was involved in nosocomial infections in a patient with pneumonia (Jackson *et al*. 1990), pulmonary case in a transplant patient (Tambini *et al*. 1996), and fungemia in a neonate (Schmidt *et al*. 2000). In Egypt and for the first time, it was recorded in high frequency from citrus phyllosphere, moderate frequency from citrus phylloplane and grapevine juice, low frequency from grapevine phyllosphere, carposphere, and citrus juice, and rare frequency from the air of both plants, citrus soil, grapevine phylloplane and carpoplane (Zeinab Soliman 2012, Abdel-Sater *et al*. 2016, Moubasher *et al.* 2016, 2018a & b).

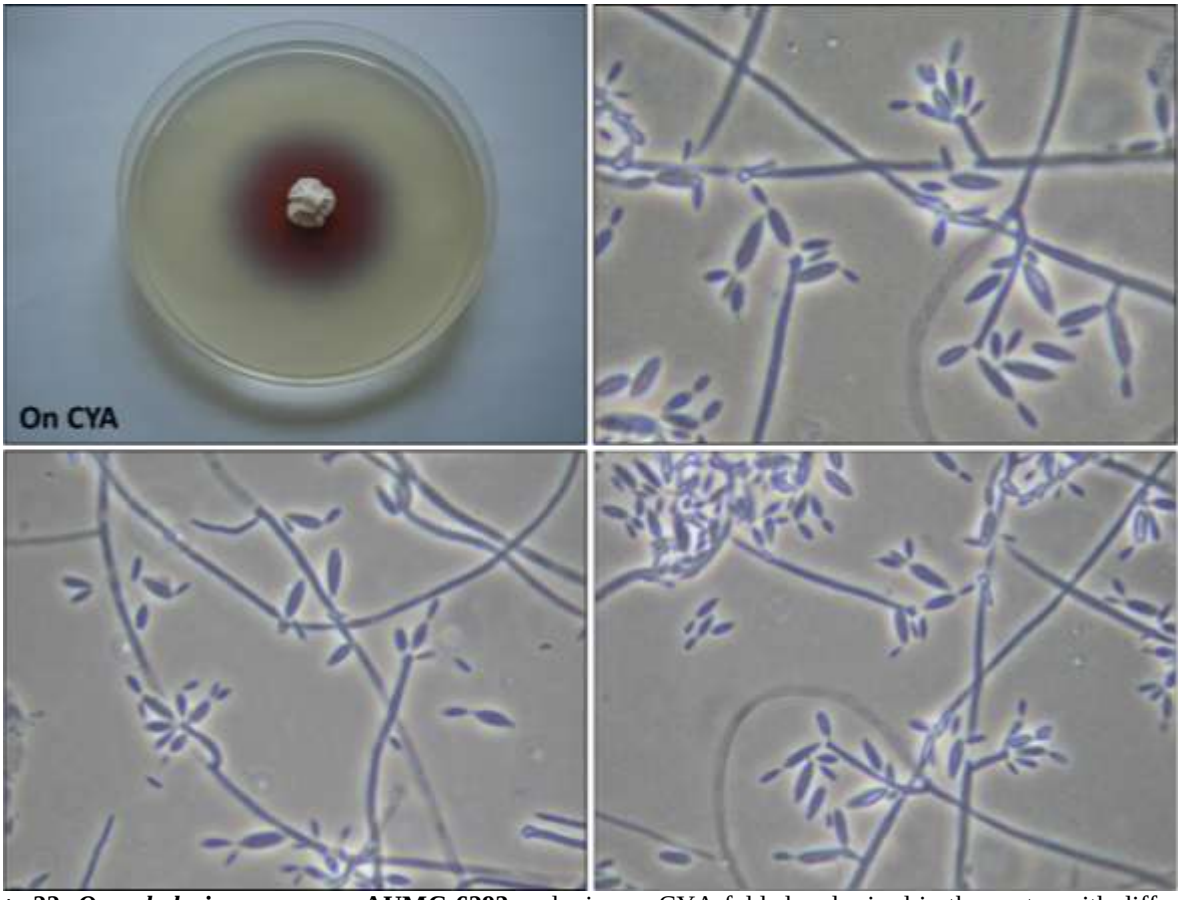

Plate 22: *Quambalaria cyanescens* AUMC 6293: colonies on CYA folded and raised in the centre with diffusing purplish pigment (upper left), coidiogenous cells forming one-celled conidia (upper right, lower left & right).

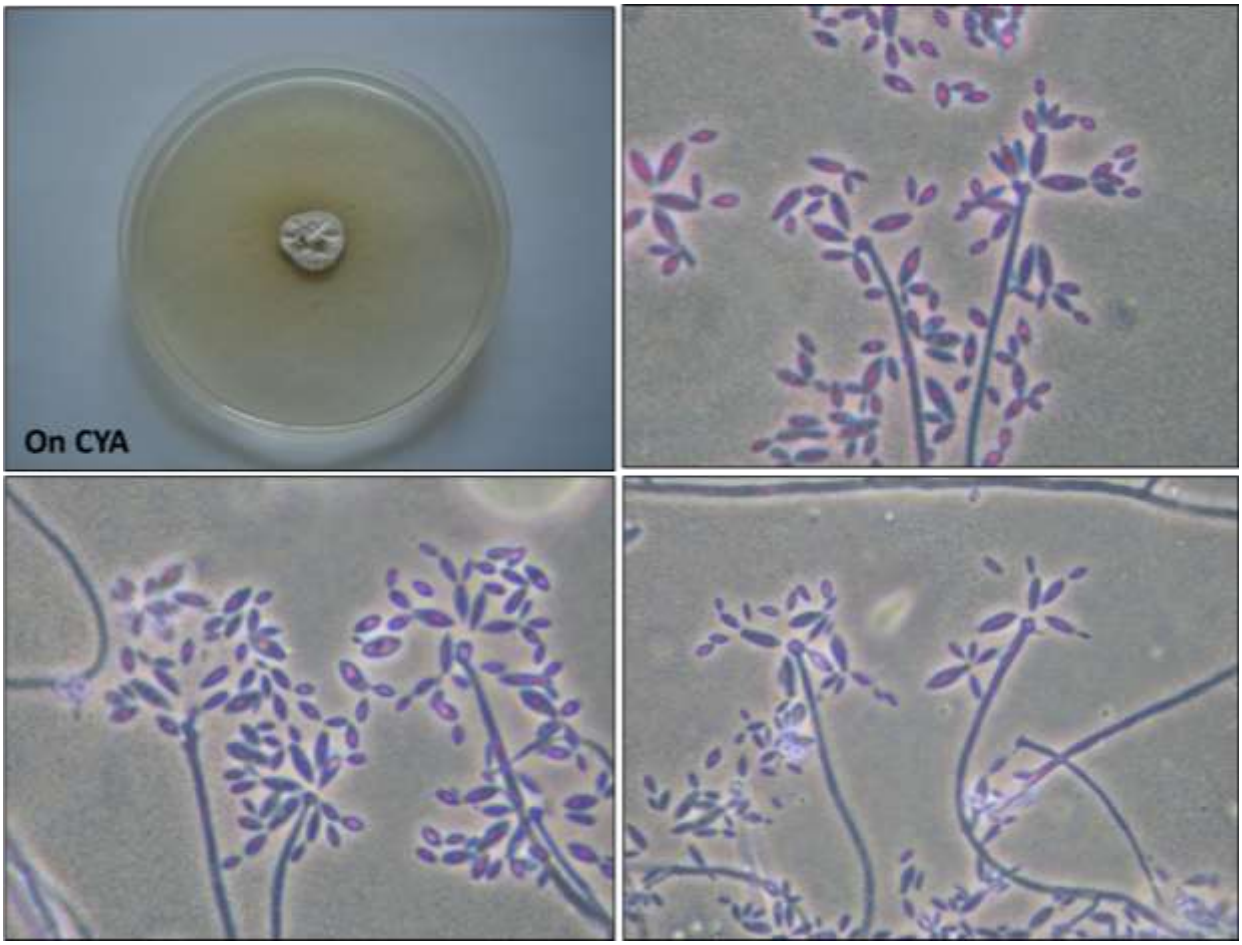

Plate 23: ***Quambalaria cyanescens*** **AUMC 6294:** colonies on CYA small, folded, and raised in the centre with faint pigment (upper left), coidiogenous cells forming one-celled conidia by sympodial growth (upper right, lower left & right).

***Ramichloridium apiculatum* (J.H. Mill., Giddens & A.A. Foster) de Hoog, Studies in Mycology 15: 69 (1977)**

≡*Chloridium apiculatum* J.H. Mill., Giddens & A.A. Foster, Mycologia 49 (6): 789 (1957)

≡*Veronaea apiculata* (J.H. Mill., Giddens & A.A. Foster) M.B. Ellis, More dematiaceous Hyphomycetes: 209 (1976)

≡*Rhinocladiella apiculata* (J.H. Mill., Giddens & A.A. Foster) Matsush., Matsushima Mycological Memoirs 1: 62 (1980)

=*Rhinocladiella indica* S.C. Agarwal, Lloydia 32: 388 (1969)

Brief description: colony on MEA olivaceous-green, olivaceous-black in reverse, oftenly with a spreading yellowish pigment; mycelium hyphae hyaline to subhyaline, smooth, thin-walled, conidiophores erect, arising at right angles from superficial hyphae, unbranched, 1–3-septate, thick-walled, dark brown, up to 75 µm long or more, conidiogenous cells terminal, brownish, thick-walled, smooth, 25–45 × 2–3.5 µm; proliferating sympodially, forming a straight rachis with distinct scars; scars crowded near the apex, slightly pigmented, conidia 3–6 × 2–4 µm, solitary, aseptate, pale brown, obovate, finely verrucose with conspicuous hilum (Plate 24).

Ecology and distribution: *R. apiculatum* was originally found in deteriorated materials, soil, and as a culture contaminant (de Hoog *et al.* 1977). It was reported as a causal agent of sooty blotch and flyspeck on apple and pear in China (Wang *et al.* 2014b). In Egypt, it was isolated once from grapevine soil on February 2009 on DYM at 25°C (Zeinab Soliman 2012).

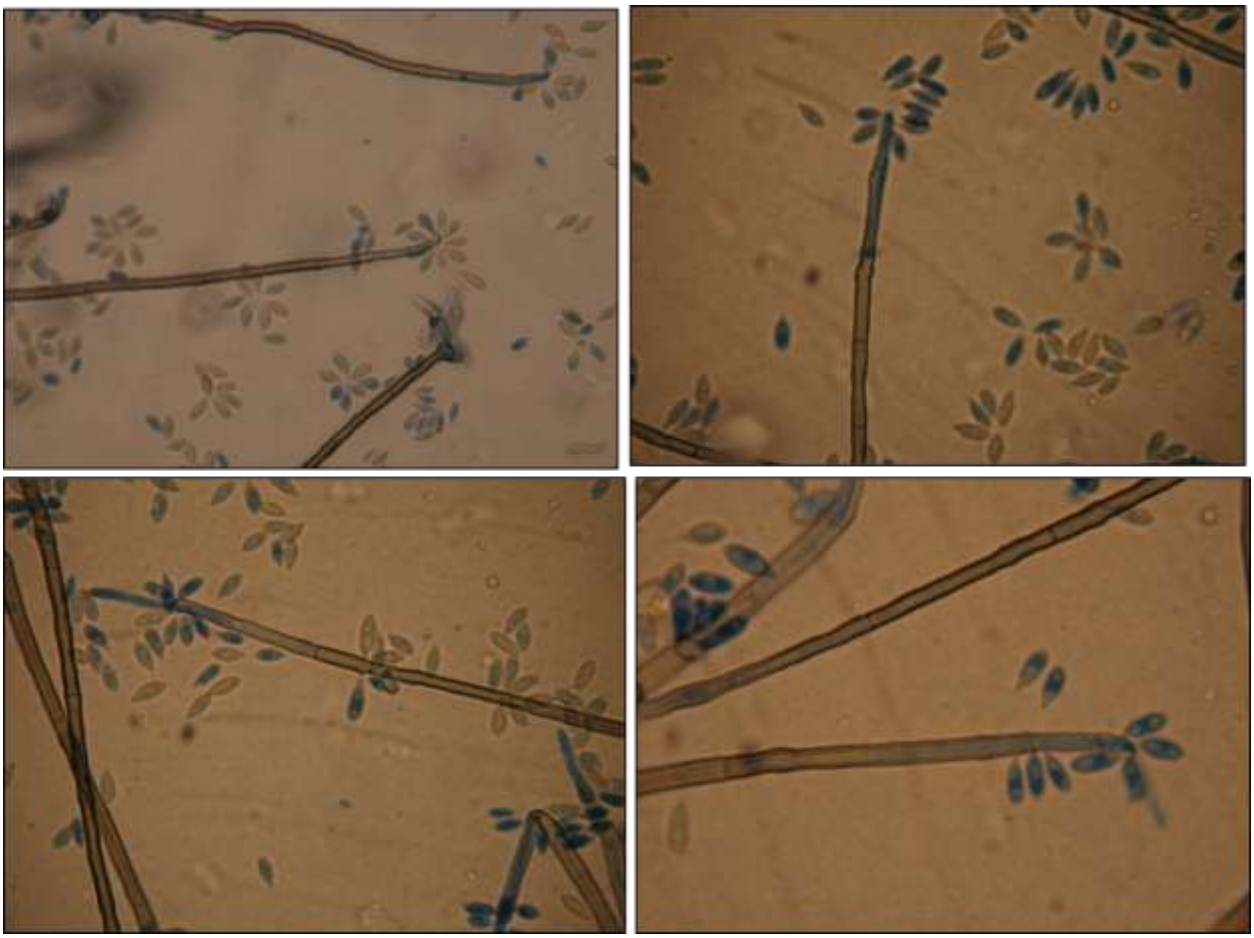

Plate 24: *Ramichloridium apiculatum* AUMC 5791: conidiophores erect, unbranched, 1–3-septate, dark brown; conidiogenous cells brownish, smooth, proliferating sympodially, forming a straight rachis with distinct scars; conidia solitary, aseptate, pale brown, with conspicuous hilum.

***Sporormiella minima* (Auersw.) S.I. Ahmed & Cain, Pakist. J. scient. ind. Res. 12(3): 241 (1970)**
≡*Sporormia minima* Auersw., Hedwigia 7: 66 (1868)
≡*Sporormiopsis minima* (Auersw.) Breton & Faurel, Bulletin de la Société Mycologique de France 80 (2): 257 (1964)
≡*Preussia minima* (Auersw.) Arx, Proceedings van de Koninklijke Nederlandse Akademie van Wetenschappen Section C 76 (3): 294 (1973)

Brief description: colony color dark grey; ascomata abundant, dark brown to black, subglobose to pyriform, semi-translucent, 200 x 120 µm; asci cylindrical; asccospores obliquely bi- or triseriate, straight or curved, yellowish brown to dark brown, 30-35 x 4-6 µm, easily fragmenting at maturity into equal cells, each with a longitudinal germ slit (Plate 25, for more details see Moubasher 1993).

Ecology and distribution: coprophilous, reported on horse, cow, rabbit and fox dung (Ellis & Ellis 1988), however it was also rarely reported from soil in Egypt (Moubasher & Abdel-Hafez 1978) and from citrus carposphere (Moubasher *et al*. 2018a).

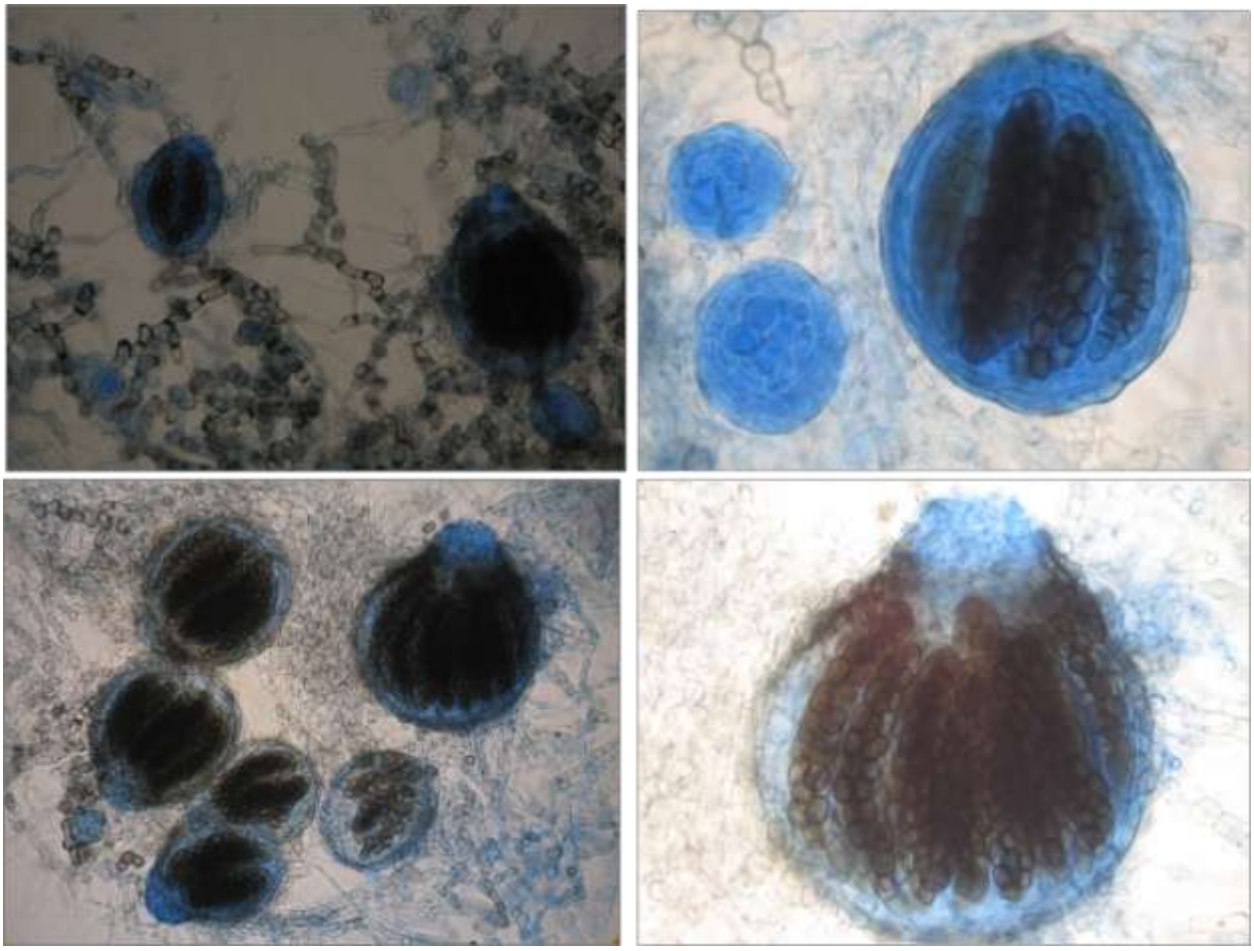

Plate 25: *Sporormiella minima* (*Preussia minima*) AUMC 5723: ascomata abundant, brown to black, semi-translucent; asci cylindrical; asccospores obliquely bi- or triseriate, easily fragmenting at maturity into equal cells.

***Stemphylium botryosum* Wallr., Flora Cryptogamica Germaniae 2: 300 (1833)**
≡*Stemphylium domesticum* (Sacc.) Mussat, in Saccardo, Syll. fung. (Abellini) 15: 402 (1901)
≡*Pleospora tarda* E.G. Simmons, Sydowia 38: 291 (1986)

Brief description: ascomata seen in slants after 9 months of preservation at 5°C, asci cylindrical, 8-spored, ascospores arranged as one row, brownish, ellipsoidal to cylindrical, muriform, transeverse septa up to 8 (Plate 26).

Ecology and distribution: leaf spot of spinach caused by *Stemphylium botryosum* Wallr. was reported in the United States (du Toit & Derie 2001, Everts *et al.* 2001, Koike *et al.* 2001, 2005), and as seed-borne fungus from spinach produced in the United States, Denmark, the Netherlands or New Zealand (Hernandez-Perez & du Toit 2006). In Egypt, it was reported for the first time from citrus air in Assiut (Moubasher *et al.* 2016).

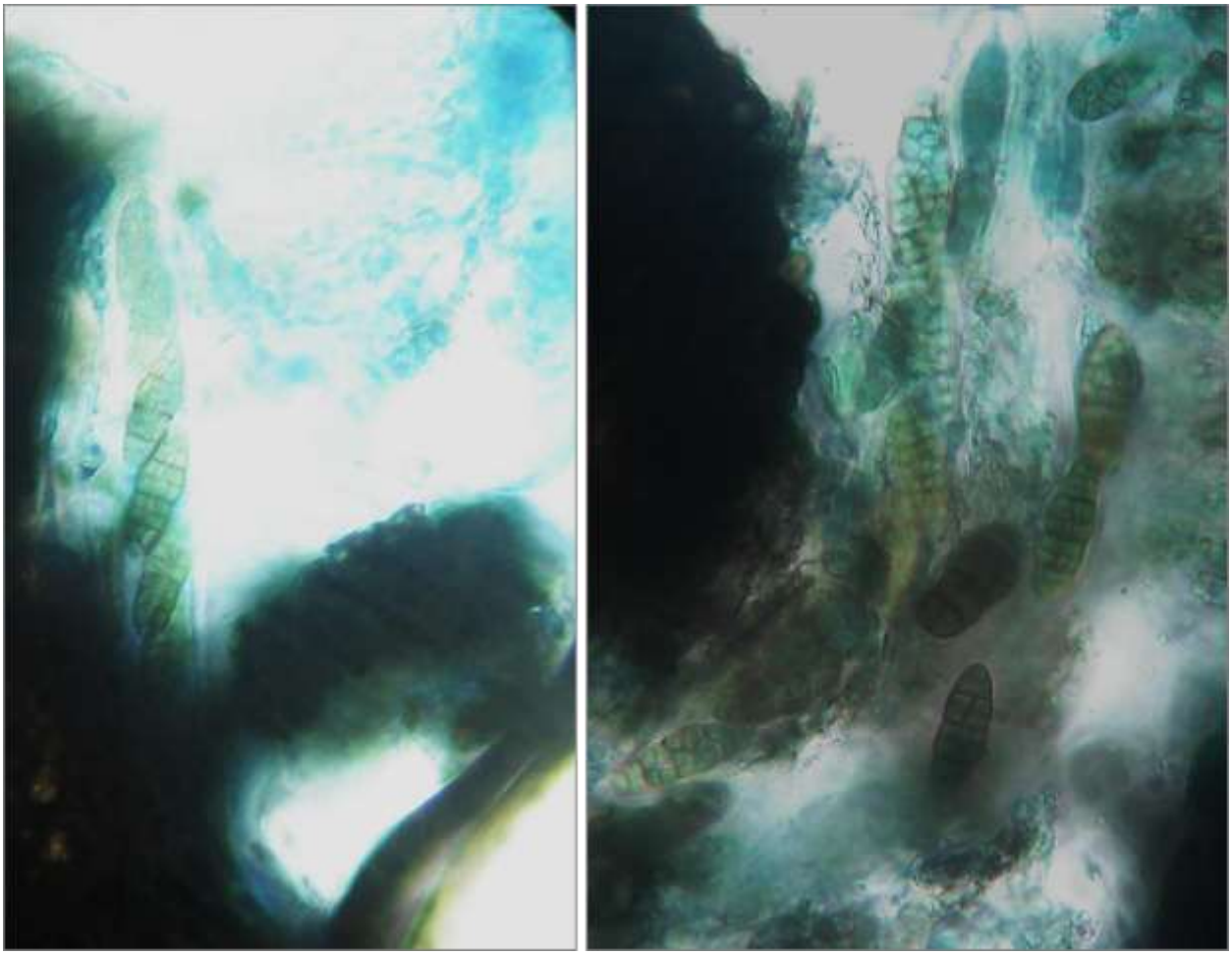

Plate 26: *Stemphylium botryosum* AUMC 5749: asci and ascospores (from slants after 9 months)

***Volutella consors* (Ellis & Everh.) Seifert, Gräfenhan & Schroers, Studies in Mycology 68: 109 (2011)**

≡*Dialonectria consors* Ellis & Everh., Journal of Mycology 4 (12): 122 (1888)
≡*Nectriella consors* (Ellis & Everh.) Sacc., Sylloge Fungorum 9: 941 (1891)
≡*Nectria consors* (Ellis & Everh.) Seaver, Mycologia 1 (2): 61 (1909)
≡*Cosmospora consors* (Ellis & Everh.) Rossman & Samuels, Studies in Mycology 42: 119 (1999)
≡*Volutellonectria consors* (Ellis & Everh.) J. Luo, X.M. Zhang & W.Y. Zhuang, Phytotaxa 44: 5 (2012)
≡*Nectria ignea* Höhn., Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Math.-naturw. Klasse Abt. I 118: 1475 (1909)
≡Nectria ignia Höhn. (1909)
≡*Volutella minima* Höhn., Fragm. Mykol. 1543 [83 repr.] (1909)

Brief description: sporodochial conidiomata short-stalked with straight, smooth-walled, unbranched, sterile, spine-like setae; phialidic conidiogenous cells arising from more or less penicillately branched conidiophores; phialoconidia simple, hyaline, slimy, rod-shaped, 3-5 x 1-1.5 µm (Plate 27).

Ecology and distribution: From New Zealand, North Carolina, Java, Colombia and Panama (Domsch *et al.* 2007), India, USA (refer to Gräfenhan *et al.* 2011). In Egypt, *V. consors* was reported once (as *Volutella* sp.) from soil of citrus plantation in Assiut (Zeinab Soliman 2012, Abdel-Sater *et al.* 2016), and to the best of our knowledge, this is the first record for this species in Egypt.

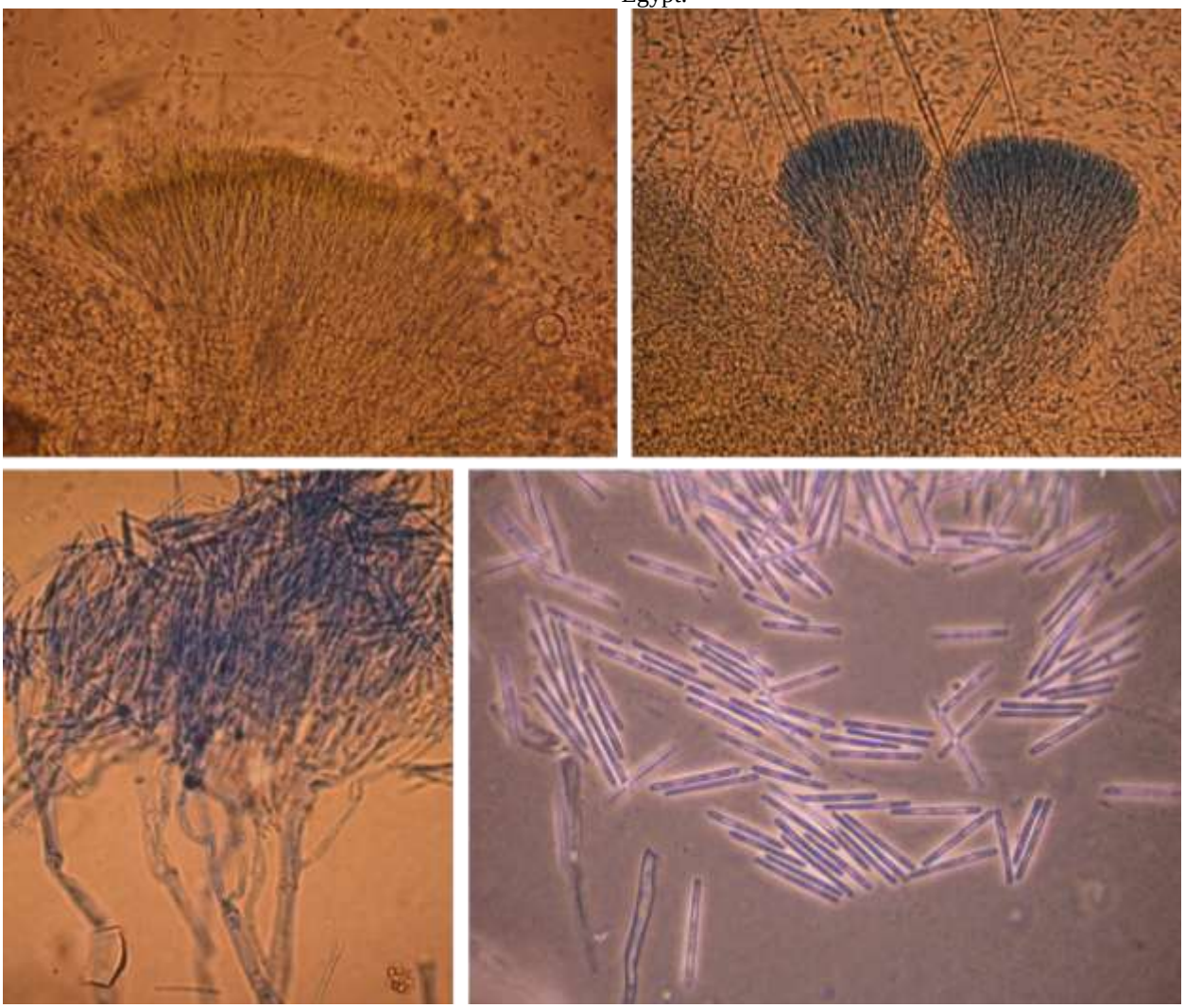

Plate 27: *Volutella consors* AUMC 5340: sporodochial conidiomata with unbranched, spine-like setae and rod-shaped conidia (upper left and right), conidiogenous cells arising from penicillately branched conidiophores (lower left) and conidiogenous cells (lower right).

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