A preliminary study on the genus Fusicladium s. l. in Iran

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Abstract: During 2011, twigs, leaves and fruits of symptomatic hosts containing Fusicladium species from Guilan, Mazandaran, Qazvin and Ardabil provinces were sampled and examined. Five species including F. carpophilum, F. fraxini, F. oleagineum, F. pomi and F. pyrorum were identified based on morphological data. Of these, F. carpophilum and F. fraxini are identified as new records for the mycobiota of Iran. Fraxinus sp., Prunus persica var. nectarina, Pyracantha sp., red apple cultivar of Malus pumila and Khoj (local variety of Pyrus communis in Guilan province) are identified as new hosts for the genus Fusicladium in Iran. Cultures of F. fraxini, F. pomi and F. pyrorum are deposited at fungal culture collection of Iranian Research Institute of Plant Protection. A brief description and illustration and a key to Fusicladium species reported from Iran are provided.

Key words: Fusicladium carpophilum, Fusicladium fraxini, scab, Spilocaea, Venturia

INTRODUCTION

Fusicladium Bonord. was established to accommodate Venturia Sacc. (Ascomycota, Pleosporales, Venturiaceae E. Müll. & Arx ex M. E. Barr) anamorphs with sympodial (denticulate) or percurrent (annellate) conidiogenous cells. Three genera were later distinguished based on the proliferation mode: Fusicladium with sympodial proliferation, Pollaccia E. Bald. & Cif. with monoblastic, determinate to percurrent conidiogenous cells (with few rather inconspicuous annellations) and Spilocaea Fr. with percurrent proliferation and numerous, conspicuous annellations.

Braun et al. (2002) suggested that the separation of these anamorphic genera is not tenable since the conidiogenesis and structure of the conidiogenous loci are uniform. Molecular examinations clearly showed that *Venturia* is a monophyletic clade that can not be separated into sub-clades relevant to these anamorphic genera (Beck et al. 2005). Therefore, it is proposed that these genera to be merged in one anamorphic genus. Hence, the name Fusicladium was conserved against other names (Braun 2005). In this new concept, Fusicladium s. 1. includes Fusicladium, Spilocaea, Pollaccia, Cycloconium and Pseudocladosporium (Braun et al. 2002, Braun 2005, Crous et al. 2007). They are phytopathologically relevant pathogens, causing leaf spot, necrosis, scab diseases as well as leaf and fruit deformations of at least 52 angiospermous plant genera. These fungi are host specific, mostly confined to a single host genus or allied host genera in a single family, e.g. Fusicladium pomi attacks members of Rosaceae (Schubert et al. 2003). A world monograph of the genus Fusicladium s. 1. Bonord. em. Schubert, Ritschel et U. Braun was published in 2003 and 57 taxa belonging to the anamorphic genus were described (Schubert et al. 2003). Some genera such as Cladosporium are taxonomically related Fusicladium. The structure of the conidiogenous loci and conidial hila proved to be the basic feature for a natural classification (David 1997), which could be confirmed by molecular examinations (Braun et al. 2003). True species of *Cladosporium* are characterized by having pigmented conidiophores with coronate conidiogenous loci, i.e. composed of a central convex dome surrounded by a raised periclinal rim, and pigmented conidia formed in acropetal chains (David 1997, Braun et al. 2003). Therefore, in a series of taxonomic studies of the genus Cladosporium s. 1. several new species and new combinations are proposed in the genus Fusicladium (Schubert 2005, Schubert & Braun 2005a, b, Schubert & Braun 2007, Braun et. al. 2008, Crous et al. 2007, 2010a, b). Although several species of the genus Fusicladium including F. pomi, F. pyrorum, F. oleagineum, F. eriobotryae, F. heterosporum, F. crataegi, F. dendritichum (= F. pomi), F. virescens (= F. pyrorum)and F. cerasiare so far reported from Iran (Hediaroude & Abbasi 2000, Mahdian 2006, Ershad 2009, Askari & Ghaderi 2010, Sanei & Razavi 2011), but most of them are not adequately described based on Iranian materials. This paper presents a primary finding on the genus Fusicladium species and their hosts in Iran.

MATERIALS AND METHODS

Plant parts (twigs, leaves, fruits) showing Fusicladium spp. symptoms were collected from Guilan, Mazandaran, Qazvin and Ardabil provinces of Iran. Moreover, all Fusicladium specimens preserved at fungal collection of the University of Guilan were reexamined. VANOX AHBS3 Olympus light microscope was used to examine fungal structures, and a BH2 Olympus light microscope equipped with a SONY DSCHX1 digital camera was used for photography. Structures were mounted in lactic acid, and 30 and 10 measurements (× 1000 magnification) determined for conidia and conidiophores respectively, with the extremes of spore measurements given in parentheses. The identification of fungal species was determined according to Schubert et al. (2003). Host plants were identified according to Mozaffarian (1998). All collected specimens were deposited in the fungal collection of the Department of Plant Protection, Faculty of Agriculture, University of Guilan, Iran.

RESULTS AND DISCUSSION

In our study, five species including *F. carpophilum*, *F. fraxini*, *F. pomi*, *F. pyrorum* and *F. oleagineum* were identified from newly collected plant materials in four provinces of Iran (Guilan, Qazvin, Ardabil and Mazandaran). *F. carpophilum* and *F. fraxini* are reported for the first time from Iran. Two species; *F.*

cerasi and F. crataegi, which have already been reported from Iran (Schubert et al. 2003, Khabiri 1952, Esfandiari 1948a, Scharif & Ershad 1966) were not found in this study. A brief description of identified taxa and a key to Fusicladium species reported from Iran are presented.

Fusicladium carpophilum (Thüm.) Oudem., Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk.: 388 (1900) Fig. 1A, 2A

On fruits and twigs, patches on the fruits superficial, circular to oval, small, greenish brown, conidiophores solitary, erect, somewhat flexuous or straight, unbranched, 29–105 \times 4–6.2 μm , septate, medium brown, smooth, sometimes swollen at the base, walls somewhat thickened. Conidiogenous cells integrated, terminal, 19–26 μm long, with 3–7 denticulate loci, 1–2 μm wide, unthickened, not darkened. Conidia catenate, cylindrical to fusiform, straight, 12.5–20(–22) \times 4–5 μm , aseptate, pale olivaceous, smooth, hila truncate, 1–1.5(–2) μm wide, unthickened, somewhat darkened.

Specimens examined: IRAN, Tajan Gokeh village, Astaneh Ashrafieh, Guilan Province, on fruits of *Prunus persica* var. *nectarine*, 12 Jun. 2012, M. Mousakhah, (1029).

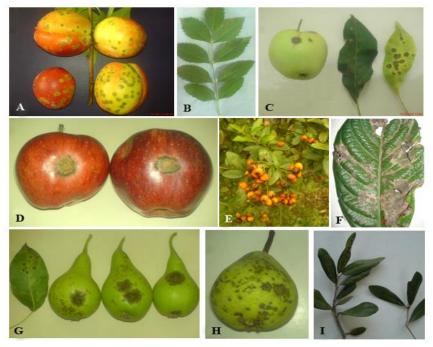


Fig. 1. Scab disease symptoms caused by *Fusicladium* s. l. species on leaves and fruits of few hosts in natural condition on: (A) *Prunus persica* var. *nectarine*, (B) *Fraxinus* sp., (C) and (D) *Malus pumila*, (E) *Pyracantha coccinea*, (F) *Eriobotrya japonica*, (G) *Pyrus communis*, (H) Khoj (local variety of *Pyrus communis* in Guilan Province, Iran), and (I) *Olea europaea*.



Fig. 2. Conidia and conidiophores of Fusicladium carpophilum (A) and F. fraxini (B). Scale bars = 10 µm.

Notes: According to Schubert et al. (2003) four species, *F. pomi*, *F. obducens*, *F. cerasi* and *F. carpophilum*, have been reported on *Prunus* species. In *F. obducens* and *F. cerasi* the conidia often are not catenate. The latter three species have sympodial conidiogenesis. *F. carpophilum* differs from *F. cerasi*, a previously reported species from Iran, by having catenate conidia, longer conidiophores (25–100 µm) than those of *F. cerasi* [(10–)20–40(–60) µm] and its broad host range (rarely occurring on *Prunus cerasus*) compared with *F. cerasi* with narrow host range, mostly occurring on *Prunus cerasus*. *Fusicladium carpophilum* is a new record for the mycobiota of Iran.

Fusicladium fraxini Aderh.,Nova Hedwigia 36: 74, 83 (1897) Fig. 1B, 2B

Leaf spots amphigenous, circular to irregular, about 5 mm wide, olivaceous-brown on the upper leaf surface, conidiophores mostly erect, straight to geniculate, flexuous, often unbranched, sometimes branched, (18–)26–45(–50) × 4–7 μ m, 0(–1) septate, pale olivaceous to medium brown, smooth, often conspicuously lobed at the base. Conidiogenous cells integrated, terminal or conidiophores usually reduced to conidiogenous cells, proliferation sympodial with 5–10(–14) loci, loci sub-denticulate, 1(–2) μ m wide, minutely thickened. Conidia solitary, fusiform to obclavate, straight, (12–)14–17(–18) × (4–)5–6 μ m, 1(–2)-septate, not or only slightly constricted at the septa, septa somewhat in the lower half, hila 1(–2) μ m wide, unthickened.

Specimens examined: IRAN, Faculty of Agricultural Science, University of Guilan, Rasht, on leaves of *Fraxinus* sp., 6 Jul. 2011, S. A. Hashemi, (1026), and IRAN, Ghalehroodkhan, Fuman, Guilan Province, on leaves of *Fraxinus* sp., 4 Aug. 2005, S. A. Khodaparast, (1025).

Notes: So far two species have been described on *Fraxinus*; *Fusicladium fraxini* and *F. nebulosum* that can be separated in conidiophore size, proliferation, colour, etc. (Schubert et al. 2003). *F. fraxini* is a new record for mycobiota of Iran. Conidiophores of Iranian

isolates of *F. fraxini* are conspicuously lobed at the base. This character was not mentioned in Schubert et al. (2003). Further studies on these isolates are in progress to evaluate this character value in terms of taxonomy.

Fusicladium pomi (Fr.) Lind, Danish fungi: 521 (1913) Fig. 1C-F, 3A-C

On living leaves and fruits, spots amphigenous, sub-circular to irregular, up to 10 mm wide, pale olivaceous-brown, becoming greyish black, on leaves sometimes surrounded by a yellowish halo, often around the main midrib, occasionally covering large leaf segments. On fruits forming small, circular to irregular spots, sub-cuticular, margin indefinite, olivaceous-brown.

Conidiophores mostly in loose to dense fascicles, erumpent through the cuticle, erect, straight to slightly flexuous, cylindrical to ampulliform, unbranched, 14– 22×7 – $10 \mu m$, aseptate (on yellow apple), 19– $31 \times 7 \mu m$, aseptate (on red apple), 10– 25×5 – $7 \mu m$, aseptate (on *Eriobotrya japonica*) and (14–)24– 58×5 – $6 \mu m$, (0–)1 septate (on *Pyracantha* sp.), walls thickened, often swollen at the base.

Conidiogenous cells integrated, terminal, with a single locus, proliferation percurrent, with conspicuous annellations, loci truncate, 4–5µm wide, unthickened, not darkened. Conidia solitary, shape variable, ovoid to obpyriform or obclavate, straight, (12-)16-25 $(-26) \times$ 7–9 μ m, 0(–1) septate (on yellow apple), (13–)17–24 $(-26) \times 7 \mu m$, 0(-1) septate (on red apple), (11-)13-18× 7–9 μm, aseptate (on *Eriobotrya japonica*) and $12-18 \times 7-10 \mu m$, 0(-1) septate (on *Pyracantha* sp.), walls somewhat thickened, narrowly pointed or broadly rounded at the apex, truncate at the base. Hilum (3-)4-5(-6) µm (on yellow apple), (3.5-)4-5(-6) μm (on red apple), 4–5(–6) μm (on Eriobotrya japonica) and 2.5-5 μm (on Pyracantha sp.) wide, truncate, unthickened to occasionally very slightly thickened, not darkened.

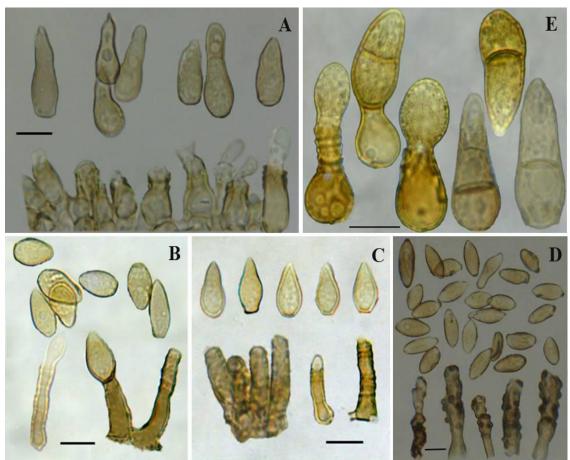


Fig. 3. Conidia and conidiophores of *Fusicladium pomi* on *Malus pumila* (**A**), on *Pyracantha* sp. (**B**) and on *Eriobotrya japonica* (**C**), *F. pyrorum* (**D**) and *F. oleagineum* (**E**). Scale bars = $10 \mu m$.

Specimens examined: On fruit of Malus pumila: IRAN, Khalkhal, Ardabil Province, 8 Jul. 2011, R. Ebrahimi, (1010); IRAN, Faculty of Agriculture, University of Guilan, Rasht, S. A. Khodaparast, (1011); IRAN, Eghbalieh, Qazvin, 17 Jul. 2011, S. A. Hashemi, (1013); IRAN, Eghbalieh, Takestan, Oazvin, 17 Jul. 2011, S. A. Hashemi, (1012); IRAN, fruit shop, Rasht, Guilan Province, 23 Jul. 2011, S. A. Hashemi, (1014); on fruit of red apple: IRAN, fruit shop, Rasht, Guilan Province, 14 Dec. 2011, S. A. Hashemi, (1030); on fruits of Pyracantha sp.: IRAN, Faculty of Agriculture, University of Guilan, Rasht, 17 Oct. 2011, S.A.Hashemi, (1027); IRAN, Faculty of Agriculture, University of Guilan, Rasht, 31 Oct. 2011, S. A. Hashemi, (1028); on leaves and fruits of Eriobotrya japonica: IRAN, Some-eh-sara, Guilan Province, 22 May 2007, S. A. Khodaparast, (1021); IRAN, Sarvelat, Guilan Province, 29 May 2009, S. A. Khodaparast, (1022); IRAN, Sost, Leisar, Hashtpar, Guilan Province, 25 Oct. 2011, S. A. Hashemi, (1023); IRAN, Ramsar, Mazandaran Province, 30 Dec. 2011, S.A. Elahinia, (1024).

Notes: Three *Fusicladium* species from *Malus*; *F. pomi*, *F. pyrorum* and *F. asperatum*, are different by having percurrent or sympodial conidiogenous cells, different conidial size and possessing solitary or catenate conidia. Schubert et al. (2003) reduced

Spilocaea amelanchieris, S. eriobotryae, S. photinicola and S. pyracanthae to synonyms of Fusicladium pomi. This species has previously been reported from Iran as F. pomi (Spilocaea pomi) on Malus pumila and eriobotryae (= Spilocaea pyracanthae) on Eriobotrya japonica (Ershad 2009, Askari & Ghaderi 2010) and here it is reported from yellow and red apple cultivars of Malus pumila, Eriobotrya japonica and Pyracantha sp. Some authors reported this species as F. eriobotryae on Eriobotrya japonica (Sánchez-Torres et al. 2009) and as F. pyracanthae on Pyracantha sp. (Raabe & Gardner 1972, Juhásová et al. 2004), but according to Schubert et al. (2003) all of them belong to F. pomi. Conidia from E. japonica and Pyracantha specimens are slightly, thought not significantly, shorter on average. Pyracantha sp. and red apple cultivar of Malus pumila are reported as new hosts for F. pomi in Iran. In this study, conidiophores on Pyracantha sp. are longer than those of the two other hosts. Conidia on Malus pumila are slightly longer than those of the two other hosts and hila from Pyracantha sp. are slightly shorter than others but, all of them fall in the range of F. pomi.

Fusicladium pyrorum (Lib.) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 357 '1869' (1870) Fig. 1G-H, 3D

On fruits and leaves, leaf spots amphigenous, diffuse, sub-circular to somewhat irregular, olivaceous to dark brown, surrounded by a paler brown halo. Conidiophores solitary or in loose fascicles, erect, straight to flexuous, unbranched, $17-32 \times 4-5 \mu m$, and aseptate (on Pyrus communis), $(12-)20-37(-41) \times 5-$ 8(-10) µm, (0-)1(-2) septate (on Khoj, a local varietyof Pyrus communis in Guilan Province), olivaceous, thick-walled. Conidiogenous integrated, terminal, 17-32 µm long (on Pyrus communis) and 9-25(-37) µm long (on Khoj), with numerous conidiogenous loci (2-4 loci on Pyrusc ommunisand 2-5(-10) loci on Khoj), proliferation sympodial, loci denticulate, 2(-3)µm wide (on Pyrus communis) and (1–)2–3(–4) µm wide (on Khoj), walls unthickened. Conidia solitary, fusiform to pyriform, ellipsoid to obovoid, straight, $14-26 \times 6-7.5(-8) \mu m$, 0(-1) septate (on Pyrus communis), $(11-)13-23 \times 8 10(-11) \mu m$, 0(-1) septate (on Khoj), olivaceous, hila (1.5-)2(-4) µm wide (on Pyrus communis) and 2-3(-4) µm wide (on Khoj), unthickened or slightly thickened.

Specimens examined: On Pyrus communis:IRAN, Qazvin, S. A. Khodaparast, (1015); IRAN, Qazvin, 26 May 2011, S. A. Khodaparast, (1016); on Khoj fruits: IRAN, Lahijan, Guilan Province, 5 Jul. 2011, S. A. Hashemi, (1017); IRAN, Lahijan, Guilan Province, 21 Aug. 2011, S. A. Hashemi, (1018).

Notes: Fusicladium pyrorum occurs on some host species in the Rosaceae (Aronia, Chaenomeles, Eriobotrya, Malus and Pyrus) and except of F. pyrorum other three species of Fusicladium occur on Pyrus (F. ahmadii, F. pomi and F. nashicola). These species differ in possessing percurrent or sympodial conidiogenous cells and specific host of Pyrus species (Schubert et al. 2003). In this study, F. pyrorum on Khoj has broader conidiophores and conidia compared with specimens on Pyrus communis, but all of them fall in the range of F. pyrorum. The Khoj variety of Pyrus communis is reported as a new host of F. pyrorum in Iran.

Fusicladium oleagineum (Castagne) Ritschel & U. Braun, Schlechtendalia 9: 70 (2003) Fig. 1I, 3E

On living leaves, leaf spots 5–10 mm wide, dark to greyish brown. Mycelium mostly superficial. Conidiophores solitary, sub-globoseor ampulliform, 17–33(–37) \times 7–13 μm , erect, straight, unbranched, mostly aseptate, usually thick-walled, conidiophores reduced to conidiogenous cells, with a single conidiogenous loci, proliferation percurrent, with 4–6(–7) conspicuous annellations, loci 5.5–6 μm wide, unthickened, not darkened. Conidia solitary, obclavate, straight or occasionally slightly curved, (16–)19–27(–29) \times 8–11(–12) μm , 1(–2) septate, medium to dark olivaceous-brown, thick-walled, apex rounded, hila (5–)6(–7.5) μm wide, unthickened, not darkened.

Specimens examined: IRAN, Rice Research Institute, Rasht, Guilan Province, on leaves of Olea

europaea, 25 Feb. 2011, N. Zareian, (1019); IRAN, Rice Research Institute, Rasht, Guilan Province, on leaves of *Olea europaea*, 12 Dec. 2011, S. A. Hashemi, (1020).

Notes: This species occurs only on *Olea* (Schubert et al. 2003) and is a common species in olive growing regions of Iran. Olive scab and its causal agent (*F. oleagineum*) have been studied previously in different locations of Iran such as Mazandaran, Golestan and Guilan Provinces, Iran (Mahdian 2006, Ershad 2009, Sanei & Razavi 2011).

Furthermore, three species of *Fusicladium* such as *F. crataegi* on *Crataegus melanocarpa* (from Gorgan, Iran), *F. cerasi* on *Prunus domestica* (from Babolsar & Borujerd, Iran) and *F. heterosporum* (and related teleomorph *Venturia adusta*) on *Epilobium hirsutum* (from Shahrestanak, Iran) have been reported from Iran (Ershad 2009, Hedjaroude & Abbasi 2000), but were not found in this study. *Fusicladium heterosporum* is already transferred to the genus *Passalora* (Schubert et al. 2003).

Key to Fusicladium species reported from Iran:

1a. Conidiogenous cells sympodial
b. Conidiogenous cells percurrent 6
2a. Conidia catenate, conidiophores 29–105 μm long,
conidia $12.5-20(-22) \times 4-5 \mu m$, on fruits of <i>Prunus</i>
persica var. nectarine F. carpophilum
b. Conidia not catenate or rarely in chains; if so
conidia 0–1(–3)-septate
3a. Conidia 1(–2), sometimes 3-septate
b. Conidia 0–1-septate5
4a. Conidiophores with distinct lobed foot cell, conidia
1(-2) septate, more or less thickened and darkened
at septa, $14-18 \times 5$ µm, conidiophores 26-45 µm
long, on Fraxinus sp F. fraxini
b. Conidiophores without distinct lobed foot cell,
conidia 0–2 septate, 10–25 \times 4–6 μ m,
conidiophores 20-54 µm long, on Crataegus spp.
F. crataegi
5a Conidia $0(-1)$ -septate, $13-26 \times 6-10 \mu m$,
conidiophores 9-32 µm long, on Pyrus communis
and Khoj (local cultivar of P. communisin Guilan
Province) F. pyrurum
b. Conidia solitary, rarely in chains, $0-1(-3)$ -septate,
$11-25 \times 4-7 \mu m$, conidiophores (10–)20–40(–60) μm
long, with narrow host range, mostly on Prunus
cerasus F. cerasi
6a. Conidia usually without septa, $12-25 \times 7-10 \mu m$,
conidiophores 10–58 µm long, on <i>Malus</i> sp.,
Pyracantha sp. and Eriobotrya japonicaF. pomi

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...... F. oleagineum

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مطالعه مقدماتی جنس .Fusicladium s. l در ایران

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چکیده: جنس های Fusicladium در برابر Spilocaea Fusicladium و Spilocaea Pollaccia و Pseudocladosporium و Pseudocladosporium در برابر Fusicladium در برابر Spilocaea Pollaccia Cycloconium در استان- Fusicladium در استان- Fusicladium در استان- Fusicladium در استان- Fusicladium در استان- های گیلان، مازندران، قزوین و اردبیل در سالهای ۲۳۹۰-۱۳۹۰ نمونه برداری شد و نمونه ها مورد بررسی های آزمایشگاهی قرار گرفتند. بر اساس این مطالعه، گونههای Fr fraxini و سالهای ۴. و جرای نخستین بار در ایران از روی زبان گنجشک (Sp. گرفتند. بر اساس این مطالعه، گونههای (Prunus persica var. nectarine) گزارش می شوند. همچنین زبان گنجشک (Prunus persica var. nectarine) و شبرنگ (Prunus persica var. nectarine) بشیرخشتی (Prunus persica var. nectarina) به تر آزمیش می شوند. نمونههای جدید گونه های آزمیش آزمیش آزمیش آزمیش و شبرنگ (Prunus persica var. nectarina) به آزمیش می شوند. نمونههای کشت شده سه گونه نامبرده نخست در مجموعه قارچهای زنده موسسه تحقیقات گیاه پزشکی کشور موجود است. بر این اساس، تاکنون هفت گونه از جنس آزمیش شده است که پنج گونه نخست در این پژوهش نیز جداسازی شده اند. در این معرفی شده در این پژوهش توصیف و کلید شناسایی برای گونه های معرفی شده در این پژوهش توصیف و کلید شناسایی برای گونه های Fusicladium در ایران ارائه شده است.

واژه های کلیدی: Spilocaea Fusicladium carpophilum Fusicladium fraxini لکه سیاه، Priscaea Spilocaea الکه سیاه،