

Studies on *Cytospora* canker disease of apple trees in Semiroom region of Iran

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Mehrabi, M., Mohammadi Golatapeh, E. and Fotouhifar, K.B. (2011) Studies on *Cytospora* canker disease of apple trees in Semiroom region of Iran. Journal of Agricultural Technology 7(4):967-982.

Identification of the fungal species associated with *Cytospora* cankers of apple trees in the Semiroom region of Isfahan Province, Iran, was established. One-hundred and fourteen isolates belonging to different species of this group of fungi were isolated and identified. Identification was based on morphological characteristics including; size of stromata, the color, shape and size of discs, the number of ostioles per disc, the presence or absence of a conceptacle, number and arrangement type of locules, size and shape of conidiophores, size of conidia, The teleomorphs including; the size of ascocarps, the number and size of perithecia, the size of asci and ascospores were all considered. Six species belonging to three genera were associated with cytospora canker disease of apple trees in Semiroom region, Iran which comprised of *Cytospora cincta*, *C. schulzeri*, *C. leucostoma*, *C. chrysosperma*, *Valsa malicola* and *Leucostoma cinctum*.

Key words: *Valsa*, *Leucostoma*, Semiroom region, disease.

Introduction

Apple trees (*Malus pumila* Mill.) are widely planted in the Semiroom region of Isfahan Province in Iran. Unfortunately, decline of mature apple trees in this region is generally caused by fungal disease, especially *Cytospora* canker. *Cytospora* canker is characterized by diffuse resinous branch cankers, with fruiting bodies of the causal fungi usually forming on infected parts. Cankers first occur on the lower branches, and then affect limbs progressively higher up the tree (Fig. 1). The damage caused by this disease has attracted attention from orchardists as well as plant pathologists. Species of the genus *Cytospora* Ehrenb., and their related teleomorphs, are among the major causal agents of annual and perennial canker diseases and sudden death of many hardwood and coniferous trees, rarely herbaceous plants and monocots (Adams

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et al., 2005). Members of *Cytospora* are associated with cankers and dieback on a wide range of trees and shrubs (Farr *et al.*, 1989; Speilman, 1985). *Cytospora* canker, caused by species of the genus *Cytospora* Ehrenb., is a worldwide problem and affects more than 70 species of woody shrubs and trees. The genus *Cytospora* was first described by Ehrenberg (1818). *Cytospora* refers to the anamorphic (asexual) stage of the causal fungi commonly found forming cankers. Numerous species of *Cytospora* are listed as causal agents of *Cytospora* cankers. However, species identification is difficult, because *Cytospora* fruiting and vegetative structures, as well as spore size, vary greatly even in the isolates of the same species (Spielman, 1985).

The species of *Cytospora* is the anamorphs of *Valsa* Fr., *Leucostoma* (Nitschke) Höhn., *Valsella* Fuckel and *Valseutypella* Höhn. (Adams *et al.*, 2005). Fruiting bodies consist of stromata that usually contain either labyrinthine chambers, having filamentous conidiophores and allantoid hyaline conidia. In moist conditions, the conidia exude from the fruiting bodies in gelatinous matrices, usually as yellow, orange, red or pallid droplets or tendrils (Adams *et al.*, 2006; Sutton, 1980). Kobayashi (1970) in Japan, Gvritishvili (1982) and Vasilyeva (1994) in the former Union of Soviet Socialist Republics (USSR), Spielman (1983, 1985) for North American species on hardwoods and Adams *et al.* (2005, 2006) in South Africa, described the numerous species of genus of *Cytospora*.

The first report on species of genus *Cytospora* in Iran was published by Fragozo (1918) to introduce *Cytospora silenes* Gonz. Frag. on *Silene boryi* Boiss. Thereafter, other researchers such as Petrak and Esfandiari (1941), Esfandiari (1946, 1948), Steyeart (1953), Ershad (1995), Ashkan (1979), Fotouhifar (2007) and Fotouhifar *et al.* (2007, 2008) introduced many other species of the genus *Cytospora* in Iran. These taxa have however, not been extensively investigated. Thus, awareness of existing species is essential for effective disease management. The main objective of the present study was to identify *Cytospora* species associated with canker diseases on apple trees in Semiroom region of Iran.

Materials and methods

Orchard canker survey and sampling

The apple orchards with dieback and canker symptoms were obesreveed during spring and autumn of year 2007 that cankered twigs showed fruiting bodies of *Cytospora* spp. and their related teleomorphs were collected. A total of 170 infected twigs were collected from apple orchards almost all regions of the Semiroom. The geographic origins of the collected isolates are listed in Table

1. Of these, 114 fungal specimens belonging to different anamorphic and teleomorphic species of this group of fungi were selected using morphological characteristics of sexual and asexual fruiting bodies (Fig. 1).



Fig. 1. Symptoms of *Cytospora* canker on apple trees in the Semiram region. (A and B) Dieback of a 25-yr-old Golden Delicious cultivar of apple tree following the entire girdling of the trunk by a canker. (C) Diffuse resinous twig cankers on Golden Delicious cultivar (D & E) Conidiomata of a *Cytospora* species on Red Delicious cultivar.

Isolation of fungi

Single spore isolation of fungi from infected host tissues with fruiting bodies was carried out by removing a mass of conidia from the surface of the specimens and spreading the suspension on the surface of agar plates. Single germinating spores were picked off and placed on fresh plates. Alternatively, plant tissues were cut in almost 5 mm diameter discs and placed in 70% ethanol for 1 min and then surface sterilized by brief flaming. Masses of conidia from fruiting bodies were discharged by placing the treated tissues in a moist chamber for 1 hr at 25°C. Small amount of conidial mass from a single fruiting body was streaked on water agar medium using sterile needle and then the plates were incubated at 25°C for 24 hr. If perithecia were presented in infected tissues, the contents of a single perithecium were similarly transferred to an agar plate. Single-spore isolates were obtained by transferring 3-10 germinated conidia or ascospores to PDA plates separately (Fotouhifar, 2007). Colors of the surface and reverse parts of the pure fungal colonies were compared using a color chart of (Rayner, 1970).

Table 1. Fungal Species identified from canker symptoms of apple trees in different locations of the Samirom region.

Species	Isolate No.	Host	Location	Date
<i>Valsa malicola</i> (anamorph: <i>Cytospora schulzeri</i>)	82-68-78-86-76-95- 77-87-89-116-127- 97-146-83-101-75- 69-84-118-117-71- 111-100-103-88-93- 1-7-8-23-25-121- 114-140-151-155- 138-150-136-149- 132-152-160-125- 122-148-134-123- 135-161	<i>Malus pumila</i>	Semirom-Hanna- Sheibani-Barand-	March -September 2007
<i>Leucostoma cinctum</i> (anamorph; <i>Cytospora</i> <i>cincta</i>)	2-3-4-5-6-9-12-11- 15-67-28-17-18-31- 65-21-156-145-139- 157-159-106-153- 143-158-154-147- 133-124-137-131- 135-109-119-108- 107-120-105-130- 92-110-102-112-96- 94-128-104-99-98- 81-91	<i>Malus pumila</i>	Dorohan-Hanna- Sheibani-Barand- Kahangan- Mehrgerd- Komme-Bideh- semirom	March -September 2007
<i>Cytospora leucostoma</i>	135-126-144	<i>Malus pumila</i>	Semirom- Sheibani-Barand	September 2007
<i>Cytospora chrysosperma</i>	75-79-64	<i>Malus pumila</i>	Sheibani	March 2007

Morphological studies

Identification of species were based on morphological features of the sexual and asexual fruiting bodies produced on infected plant tissues. For this purpose, thin cross sections were prepared by hand from fruiting bodies. Morphological characteristics of the fruiting bodies including; size and arrangement of stromata, presence or absence of conceptacle in stromata, number and diameter of ostioles per disc, color, shape and size of discs, arrangement type of locules and perythecia, size and number of perithecia, size and shape of conidiophores and asci, size of conidia and ascospores were determined using a dissecting microscope. Microscopic measurements were made with a calibrated microscope. For each isolate, over 30 conidiomata were sectioned and diameters of 50 conidia were measured under an Olympus light microscope (SZH10). If the perithecia were presented, over 50 perithecia were sectioned and then over 100 ascospores were measured under an Olympus microscope. For other morphological features, at least 50 specimens were measured. Representative isolates and samples were deposited at the Iranian Research Institute of Plant Protection (IRAN, Tehran, Iran).

Results

Isolates were placed in three genera based on their morphological features including; *Cytospora*, *Valsa* and *Leucostoma*. In the genus *Cytospora*, the following species were identified; *Cytospora cincta*, *C. schulzeri*, *C. leucostoma* and *C. chrysosperma*. In the genus *Valsa* only *Valsa malicola* was identified and in the genus *Leucostoma* also a single species, *Leucostoma cinctum* was identified. Out of 107 isolates studied, 50 isolates belonged to *Valsa malicola* (anamorph: *Cytospora schulzeri*), 51 isolates belonged to *Leucostoma cinctum* (anamorph: *Cytospora cincta*), three isolates belonged to *C. leucostoma*, and finally, three isolates belonged to *C. chrysosperma*. The distinctive features of the identified species are shown as follows:

Valsa malicola Z. Urb., *Index of fungi* 2:357, 1956 (anamorph: *Cytospora schulzeri* Sacc. & P. Syd., *Syll. Fung.* 14(2): 918, 1899), (IRAN: 14246 F and IRAN: 14244 F) Figs. 2 & 3.

Usually, telomorphic and anamorphic states are formed in the same stroma or may be discrete. Ascomata immersed in the bark. Scattered, erumpent, circular to ovoid, 0.7-2 mm in diam., euvalloid, discs usually obscured by tightly packed ostiolar necks, when apparent pale brown to beige, nearly flat, circular to ovoid, 0.35-1 mm in diam., 4-27 ostioles arranged circinately in a disc, brown to black, at unequal levels on the disc surface. Perithecia globose to subglobose, 350-630(500) μm in diam., 4-27 perithecia arranged at different depths in the entostroma, laterally inclined, surrounded by yellow to grey entostroma.

Asci free, subcylindrical to clavate, 44-70.4(62.7) \times 6.6-11(8.1) μm in diam., 8-spored. Ascospores biseriate, elongate, allantoid, hyaline, aseptate, 8.8-17.6(14.3) \times 2.2-3.3(2.8) μm in diam. Paraphyses are elongate, septate. Surface of the colony on PDA is light greenish yellow (sulphur, yellow, sulphureus) in color and moderate to strong yellow(amber, electrinus) to moderate orange yellow (ochreous, ochraceous) at reverse. Colony texture is felty, slightly raised and with no growth zones.

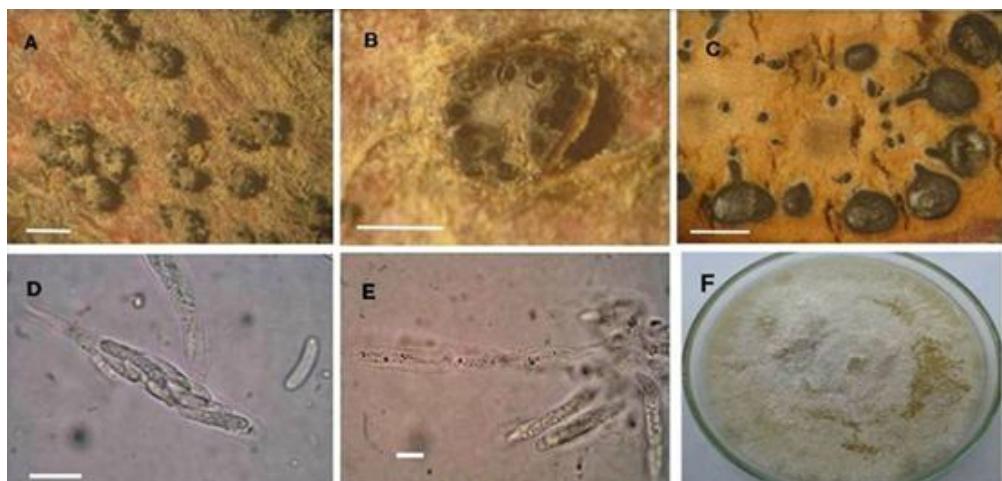


Fig. 2. Morphology of *Valsa malicola*, the telomorphic state of *Cytospora schulzeri*, isolated from *Malus* sp. in the Semirom region. (A) Ascostroma on bark, (B) Transverse section through the disc shows ostioles of the perithecia, (C) Transverse section through the bottom of ascoma showing globose perithecia surrounded by entostroma, (D) Ascus with ascoc pores, (E) Septate paraphyses and (F) Colony morphology of isolate 69 on PDA. [Scale bar: (A) 1mm, (B,C) 500 μ m and (D,E) 10 μ m].

Anamorph shows separately from teleomorphic stromata or in the same stromata. Conidiomata immersed in the bark, erumpent, rosette cytosporoid with regular radially arranged chambers, circular to ovoid, 0.7-2 mm in diam., disc light to medium grey, nearly flat, circular to ovoid, 0.28-0.5 mm in diam., with 2-11 ostioles per disc. Ostioles light to dark brown, circinately arranged, at the same level as the disc surface, about 35-42 μ m in diam. Locules multi-chambered, subdivided by entire invaginations into regular radially arranged chambers sharing common walls. Conidiophores hyaline, branched at the base and above the base, 11-28 μ m in diam. inclusive of phialides. Conidiogenous cells subcylindrical phialides, hyaline. Conidia hyaline, allantoid, aseptate, 4.5-8(6.3) \times 0.9-1.3(1.1) μ m in diam., color of mass of conidia is yellowish. Surface of the colony on PDA is light greenish yellow (sulphur, yellow, sulphureus) in color and moderate to strong yellow(amber, electrinus) to moderate orange yellow (ochreous, ochraceous) at reverse. Colony texture is felty, slightly raised and with no growth zones. In isolate 65, pycnidia were rarely produced on the agar and which exuded the cream to yellow cirri after 50 days.

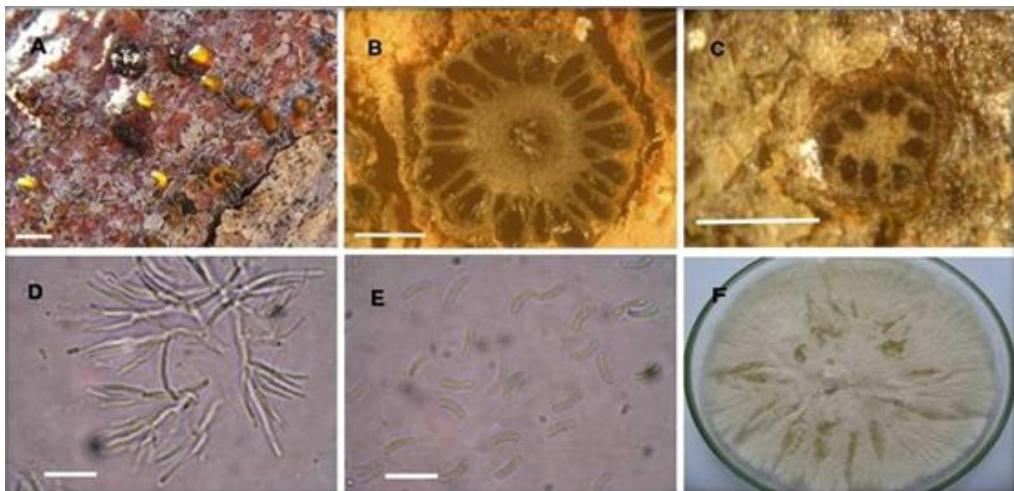


Fig. 3. Morphology of *Cytospora schulzeri*, the anamorphic state of *Valsa maliocla*, isolated from *Malus* sp. in the Semiroom region. (A) Conidiomata on the bark surface and yellowish tendrils of conidia exuded from conidiomata, (B) Transverse section through the bottom of the conidioma showing locules divided radially into about 23 chambers, (C) Transverse section through the disc showing brown tissue around and inside the circle of ostioles, (D) Conidiophores, (E) Conidia and (F) Colony morphology of isolate 146 on PDA. [Scale bar: (A) 1mm, (B,C) 500 µm and (D,E) 10 µm].

Leucostoma cinctum (Fr.) Höhn., Mitt. Bot. Inst. Tech. Hochsch. Wien 5:79, 1928 (anamorph *Cytospora cincta* Sacc., Syll. Fung. 3:254, 1884), (IRAN: 14247 F and IRAN: 14243 F) Figs. 4 & 5.

Telomorphic and anamorphic states are usually formed in the same stroma. Anamorph shows in the central part and teleomorph surrounding the anamorph, but sometimes discrete. Ascomata immersed in the bark, scattered, erumpent, circular to ovoid, 0.7-2.5 mm in diam., leucostomoid, in isolates 135 and 5, externally the black conceptacle on the bark surface are visible, discs usually obscured by tightly packed ostiolar necks, when apparent, pale brown to beige, nearly flat, circular to ovoid, 0.3-0.7 mm in diam., 9-26 ostioles of perithecia arranged circinately in the disc and ostiole of conidiomata in the centre, brown to black, at the same level as the disc surface. Perithecia globose to subglobose, 280-500(370) µm in diam., 9-26 perithecia arranged at different depths in the entostroma, laterally inclined, surrounded by yellow to grey entostroma. Ascii free, subcylindrical to clavate, 38-68(50.6) × 6-9(7) µm, 8-spored, ascospores allantoid, hyaline and aseptate. Paraphyses are elongated and septate.

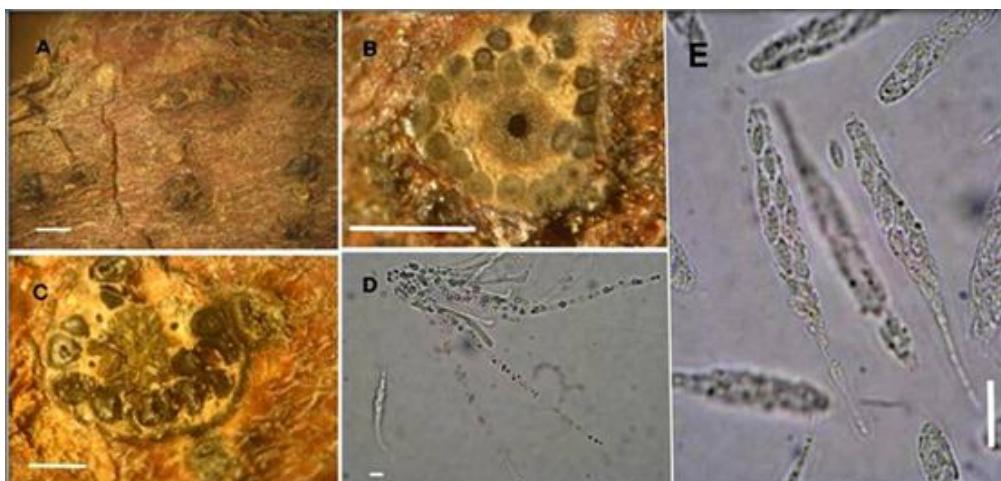


Fig. 4. Morphology of *Leucostoma cinctum*, the telomorphic state of *Cytospora cincta*, isolated from *Malus sp.* in the Semerrom region. (A) Ascomata on the bark, (B) Transverse section through the disc showing light brown tissue around the conidiomatal opening surrounded by the ostioles of the perithecia, (C) Transverse section through the ascoma showing chambers of central conidioma surrounded by perithecia and ascomata delimited by conceptacle, (D) Paraphyses and (E) Asci and immature ascospores. [Scale bar: (A) 1mm, (B, C) 500 µm and (D, E) 10 µm].

Anamorph shows separately from teleomorphic stromata or in the same stroma. Conidiomata immersed in the bark, erumpent, rosette leucocytosporoid, in some conidiomata conceptacle are not presented, conidiomata with regular radially arranged chambers, circular to ovoid, 0.7-2 mm in diam., disc light to medium grey, nearly flat, circular to ovoid, 0.28-0.56 mm in diam., with one ostiole per disc. Ostiole light to dark brown, circular, about 0.28-0.50 µm in diam. Locules multi-chambered, subdivided by entire invaginations into regular radially arranged chambers sharing common walls. Conidiophores hyaline, branched at their bases and above the base, 13-33 µm length, inclusive of phialides. Conidiogenous cells subcylindrical phialides, hyaline. Conidia hyaline, allantoid, aseptate, 4.5-6.7(6.1) × 0.9-1.2(1.1) µm, color of mass of conidia is reddish. Surface of the colony on PDA is pale greenish yellow (primrose, primulinus) to light greenish yellow (sulphur-yellow, sulphureus) in color and light yellow (pale luteous, luteolus) on the reverse. Colony texture is felty, slightly raised and with no growth zones. In most isolates, pycnidia were produced abundantly on the agar surface and exuded cream to yellow cirri after 50-60 days.



Fig. 5. Morphology of *Cytospora cincta*, the anamorphic state of *Leucostoma cinctum*, isolated from *Malus sp.* in the Semiroom region. (A) Conidiomata on the bark and reddish tendrils of conidia that exuded from conidiomata, (B) Transverse section through disc showing grey tissue around and inside the ostiole, (C) Transverse section through conidioma showing locule divided radially into about 21 chambers and the dark conceptacle delimiting the conidioma, (D) Conidiophores, (E) Conidia and (F) Colony morphology of isolate 65 on PDA. [Scale bar: (A) 1mm, (B) 500 µm and (C, D) 10 µm].

Cytospora chrysosperma (Pers.) Fr., *Sylv. Mycol. Berol.* 28: 1818, (IRAN: 14245 F) Fig. 6.

Teleomorph is not observed. Anamorph is separated from teleomorphic stromata. Conidiomata immersed in the bark, erumpent, scattered, usually rather flat, labyrinthine cytosporoid, with labyrinthine chambers, circular to ovoid, 1-2 mm in diam., disc grey to black, nearly flat, circular to ovoid, 0.14-0.49 mm in diam., with one ostiole per disc. Ostiole light to dark brown, circular, about 0.35-0.70 µm in diam. Locules multi-chambered, often irregular. With thick, dark wall. Conidiophores hyaline, divided into two types, first type branched and septate 15-30 µm length, inclusive of phialides, second type long, unbranched and aseptate or rarely septate. Conidiogenous cells subcylindrical phialides, hyaline, conidia hyaline, allantoid, aseptate, 3.6-5(4.05) × 0.9-1.1(1.03) µm, issuing in orange tendrils. Surface of the colony on PDA is moderate yellowish pink (rosy buff, roseo-bubalinus) in color and moderate to strong yellow(amber, electrinus) to moderate orange yellow (ochreous, ochraceous) at the bottom of the plate. Colony texture is felty, slightly raised and without growth zones. Conidiomata were produced abundantly on PDA medium.

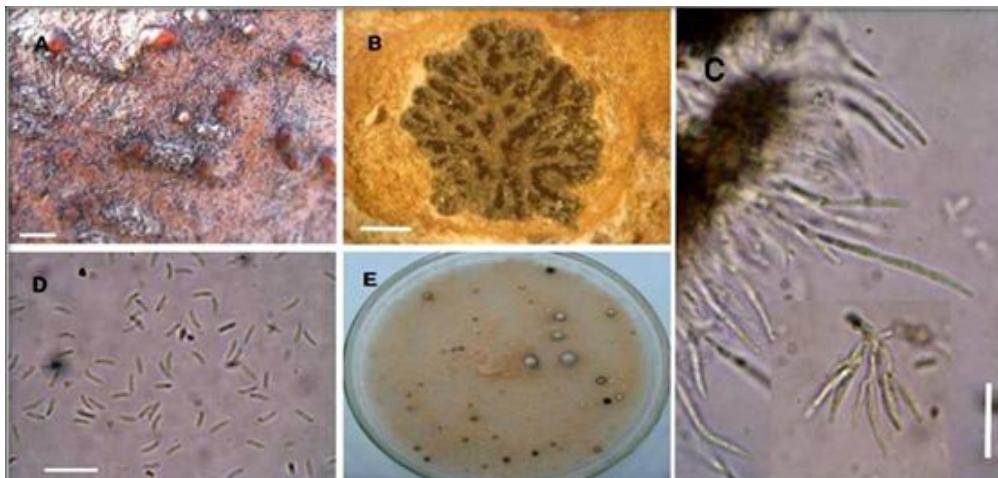


Fig. 6. Morphology of *Cytospora chrysosperma*, the anamorphic state of *Valsa sordida*, isolated from *Malus* sp. in the Semiroom region. (A) Conidiomata on the bark and orange tendrils of conidia that exuded from conidioma. (B) Transverse section through conidioma showing labyrinthine chambers. (C) Conidiophores, (D) Conidia and (E) Colony morphology of isolate 75 on PDA. [Scale bar. (A) 1mm, (B) 500 µm and (C, D) 10 µm].

Cytospora leucostoma (Pers.) Sacc., *Michelia* 2(7): 264, 1881. (IRAN: 14242 F) Fig. 7.

Teleomorph is not observed. Anamorph is separated from teleomorphic stromata. Conidiomata immersed in bark, erumpent, rosette leucocytosporoid with regular radially arranged chambers, circular to ovoid, 1-2 mm in diam., disc snow-white to grey, nearly flat, circular to ovoid, 0.28-0.5 mm in diam., with one ostiole per disc. Ostiole black, central, about 35-56 µm in diam. Locules multi-chambered, conidiophores hyaline, branched at base, 13-35 µm in diam. inclusive of phialides. Conidiogenous cells subcylindrical phialides, hyaline. Conidia hyaline, allantoid, aseptate, 3.5-5.5(4.5) × 0.9-1.2(1.1) µm, issuing in reddish tendrils. Surface of the colony on PDA is light olive gray to grayish olive (gray olivaceous, griseo-olivaceous) in color and dark grayish green (Olivaceous Black, olivaceo-niger) on the reverse. Colony texture is felty, slightly raised and with growth zones. Conidiomata are seldom produced on PDA medium.

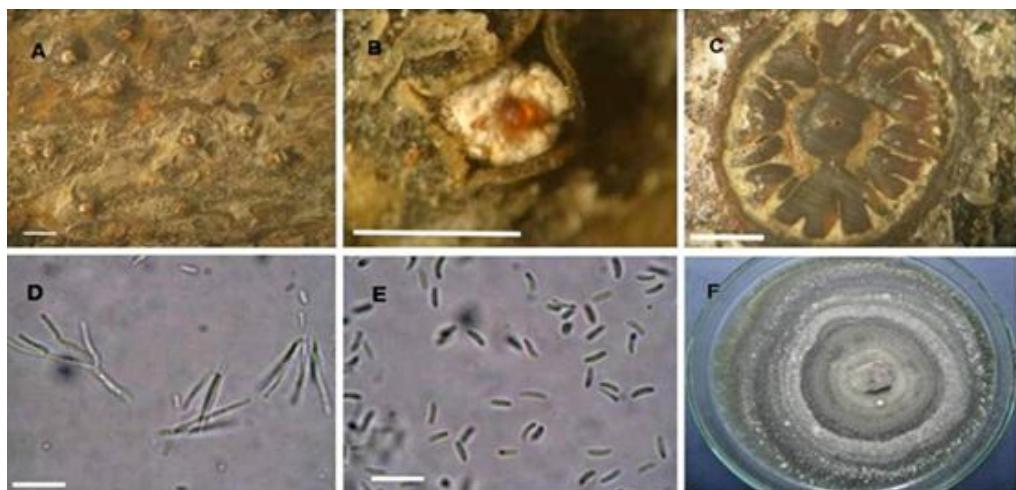


Fig. 7. Morphology of *Cytospora leucostoma*, the anamorphic state of *Valsa leucostoma*, from *Malus* sp. in the Semiroom region. (A) Conidiomata on the bark. (B) Surface view of the one conidioma with whitish disc having one ostiole. (C) Transverse section through conidioma showing radial locules in the dark and thick black conceptacle delimiting the conidioma. (D) Conidiophore, (E) Conidia and (F) Colony morphology of isolate 129 on PDA. [Scale bar: (A) 1mm, (B, C) 500 μ m and (D, E) 10 μ m].

Discussion

In this study, five species belonging to three genera; *Cytospora*, *Valsa* and *Leucostoma*, including, *Cytospora cincta*, *C. schulzeri*, *C. leucostoma*, *C. chrysosperma*, *Valsa malicola* and *Leucostoma cinctum* were identified from Semiroom region of Iran. These species are described as fungi associated with perennial canker of apple trees in Semiroom region of Iran. Previously, *C. cincta* was identified on apple trees in this region but the other species are identified for the first time from Semiroom. *C. leucostoma* is reported for the first time on apple trees in Iran while the other species have been reported previously from other regions of Iran (Ashkan, 1979; Fotouhifar, 2007).

According to identification of the collected specimens from this region, *C. cincta* and *C. schulzeri* were the most dominant species on apple trees in Semiroom region. High morphological diversity was observed in the discs, ostioles, locules, size of conidia and conidiophores among the *C. chrysosperma*, *C. leucostoma*, *C. cincta* and *C. schulzeri* as well as within the isolates of the same species. These observations were agreed with results of Spielman (1983). Spielman (1983) believed that the characteristics of ostiole such as number and arrangement type in the disc, are extremely variable within species. In *Valsa malicola* and *Leucostoma cinctum* with high morphological diversity were identified in the disc, number of perithecia, size of asci and ascospores even

within the species. Authors believe that in the species identified in this study, the size of conidia, presence or absence of conceptacle and number of ostioles that are important in differentiating of anamorphic species. *Leucostoma cinctum* (anamorph: *Cytospora cincta*) previously described by Hayova and Minter (1998a), who has been observed on dead or dying, attached or fallen twigs of the family Rosaceae, mainly Prunoideae and rarely other subfamilies, including genera such as *Cotoneaster*, *Malus* and *Pyrus*. According to morphological characteristics, our specimens were shown a very similar to species that was described by Hayova and Minter (1998a). However, in our isolates the conidia were slightly smaller in size and ascospores were immature. This species was identified by Proffer and Jones (1989) in Michigan and by Ashkan (1994) in Iran as the causal agent of the canker disease on apple trees. It has also been described by Fotouhifar (2007) on different host plants in Iran (Table 2). Morphology of this species in all essential details agrees with the description of Fotouhifar (2007), but the diameter of perithecia in the isolates of our specimens was shown a smaller and the size of the ascii that longer than his description. *C. cincta* is also described by Fotouhifar (2007) on different host plants in Iran (Table 2). Morphological features of species were in agreement with description of Fotouhifar (2007), but the size of conidia of our isolates was smaller than that of his description. The characteristic features of this species are presence of one ostiole per disc, Locules multi-chambered, often regular with conceptacle surrounding chamberes and spore size of >5 μm that issue in reddish or blackish tendrils.

Valsa malicola (anamorph: *Cytospora schulzeri*) described by Hayova and Minter (1998b), This species has been observed on dead or dying twigs of *Malus* species, and other Maloideae. it can also be found on other members of the family Rosaceae. Morphology of this species in all essential details agrees with the description of Hayova and Minter (1998b) but morphology of teleomorphic isolates differ in ostiolar beaks. In our isolates ostiolar beaks are slightly longer and the number of perithecia in an ascoma varied from 4-27. Also, the size of ascii in the isolates of this species was longer and the size of the ascospores which showed smaller than their description. Morphological features of *Cytospora schulzeri* isolates were in agreement with description of Adams *et al.* (2006) on apple trees in South Africa. *Valsa malicola* is also described by Fotouhifar (2007) on different host plants in Iran (Table 2). Morphology of this species in all essential details agrees with the description of Fotouhifar (2007), but morphology of teleomorphic isolates (isolate 69) differ in ostiolar beaks. In our isolates the ostiolar beaks are almost longer and beaks of ostioles opened at a higher level from the disc surface. Also, the size of ascii in the isolates of this species was longer and the size of the ascospores were

almost smaller than those in his description (Table 2). Morphological features of the isolates of this species were in agreement with description of Fotouhifar (2007), but the size of conidia in our isolates was smaller than in his description. This species is distinguished from closely related ones by ostiole number of >1 per disc, Locules multi-chambered, often regular with absence of conceptacle in the periphery of chamberes and spores size of $>5 \mu\text{m}$ that issues in yellowish tendrils.

Valsa sordida (anamorph: *Cytospora chrysosperma*) also described by Hayova and Minter (1998c). This species has been found on *Populus* spp., *Salix* spp. and rarely on other woody angiosperms. In our specimens the teleomorphic state of the fungus was not observed. But, morphological characteristics of anamorphic state are in agreement with descriptions of Hayova and Minter (1998c) and Adams *et al.* (2006). *Cytospora chrysosperma* has also been described by Fotouhifar (2007) on different host plants in Iran (Table 2). Morphological features of this species isolates were in agreement with those of Fotouhifar (2007), but the size of conidia in our isolates was smaller. This species is separated from closely related or similar species by the presence of one ostiole per disc, Locules multi-chambered, often irregular, absence of conceptacle and spores size of $<5 \mu\text{m}$ that ooze out in orange tendrils.

Leucostoma persoonii (anamorph: *Cytospora leucostoma*) has been found on dead or dying, attached or fallen twigs mainly on members of the family Rosaceae such as, *Malus*, *Persica*, *Prunus* and *Alnus* spp. Teleomorph state of the fungus was not observed among the collected specimens. But, morphological characteristics of anamorphic state are in complete agreement with description of species as given by Hayova and Minter (1998d). *Cytospora leucostoma* has also been described by Fotouhifar (2007) on different host plants in Iran (Table 2). Morphological features of this species isolates were in agreement with description of Fotouhifar (2007), but the size of conidia in the isolates of this species was smaller and the size of conidiophores almost longer than in his description. Also, shape of locules in the isolates of this species was variable. In some isolates, such as 129, locules were fewer and larger and in other isolates, such as isolate 144, locules were smaller and numerous. This species is separated from closely related or similar species by the presence of one ostiole per snow-white disc and with Locules multi-chambered, often regular with presence of conceptacle around chamberes and spores size of $<5 \mu\text{m}$ that exude in the form of reddish to blackish tendrils.

Table 2. Fungal Species and their host plants in Iran.

Species	Host
<i>Valsa malicola</i> (anamorph: <i>Cytospora schulzeri</i>)	<i>Malus pumila</i> Mill., <i>Prunus domestica</i> L., <i>Cydonia oblonga</i> Mill., <i>Cerasus vulgaris</i> Mill., <i>Colutea</i> sp., <i>Thuja orientalis</i> L. and <i>Crataegus</i> <i>pseudoheterophylla</i> Pojark.
<i>Leucostoma cinctum</i> (anamorph: <i>Cytospora cincta</i>)	<i>Malus pumila</i> Mill., <i>Cydonia oblonga</i> Mill., <i>Armeniaca vulgaris</i> Lam., <i>Armeniaca vulgaris</i> L., <i>Amygdalus communis</i> L., <i>Cerasus avium</i> (L.) Moench., <i>Crataegus azarolus</i> L., <i>Juglans</i> <i>regia</i> L., <i>Vitis vinifera</i> L. and <i>Prunus</i> <i>domestica</i> L.
<i>Cytospora leucostoma</i>	<i>Persica vulgaris</i> Mill., <i>Armeniaca vulgaris</i> Lam., <i>Rosa canina</i> L. and <i>Vitis vinifera</i> L.
<i>Cytospora chrysosperma</i>	<i>populus</i> spp., <i>salix</i> spp., <i>Platanus orientalis</i> L., <i>Fraxinus excelsior</i> L., <i>Olea sativa</i> L., <i>Julans regia</i> L., <i>Thuja orientalis</i> L., <i>Armeniaca vulgaris</i> Lam., <i>Malus pumila</i> Mill., <i>Ligustrum latifolium</i> Hook. F., <i>Morus alba</i> L., <i>Robinia pseudoacacia</i> L. <i>Prunus domestica</i> L., <i>Amygdalus communis</i> L., <i>Crataegus azarolus</i> L., <i>Juglans regia</i> L., <i>Vitis vinifera</i> L. and <i>Prunus domestica</i> L.

According to the results, in areas such as Sheibani, Kahangan and Bideh, *Cytospora* species were affected most of the apple trees. This group of fungi had not been observed in the other areas such as Noghol and Dangezlo. Low temperature was reported to injure the buds and dormant twigs and frost injury of such tissues predisposes apple trees to *Cytospora* canker (Helton, 1961; Tekauz and Patrick, 1974 and Reich and van der Kamp, 1993). The greatest morphological diversity of species belonged to Sheibani area and the highest number of isolates of *C. schulzeri* and *C. cincta* were collected from these areas. Correct and valid identification of *Cytospora* species would be very important selected in apple species, hybrids, or clones for orchard development in these areas. More studies on pathogenicity and physiology of this fungal group would be needed to reduce the damage caused by these diseases in this region of Iran.

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(Received 19 January 2011; accepted 30 May 2011)