First report of *Neurospora* on *Corylus avellana* in natural forest of Iran

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Teleomorph stage of the fungus *Neurospora udagawae* was formed abundantly on the culture media of potato dextrose agar, potato carrot agar and malt extract agar. Perithecia were visible on the culture medium and plant tissues (on the bark of tree) of hazelnut tree (*Corylus avellana* L.) from specimens of the Northwest forest of Iran. The fungus was identified for the first time in this area. The isolate was examined for its macroscopic and microscopic features and identified as nonconidiating species of *Neurospora*. Growth rate of the fungus hyphae was more than twice in 30 than 10 and 20°C. There was no growth at 5 and 40°C. This fungus is recorded here for the first time from hazelnut tree of Iran and so in the world.

**Key words:** *Neurospora udagawae*, morphology, homothallic.

**INTRODUCTION**

The *Sordariaceae* (*Ascomycota*) encompasses approximately 10 genera (Eriksson et al., 2003) which are coprophilous, soil borne or grow on plant debris. They are characterized by ampulliform or conical and usually ostiolate ascomata; asci are commonly cylindrical with an apical ring; and the ascospores are generally aseptate, with the surface smooth, pitted, reticulate or striate, sheathed or unsheathed, darkly pigmented and with one or several germ pores. The largest genera are *Gelasinospora* Dowding (1933) and *Neurospora* (Shear and Dodge, 1927). The main difference between them lies in the ornamentation of the ascospore wall.

The generic name *Neurospora* was introduced by Shear and Dodge (1927) for four species characterized by dark ascospores, with a grooved surface with longitudinal ribs. Different members of this genus use one of three different mating strategies: heterothallism, homothallism or pseudohomothallism (partial self-compatibility) (Perkins and Turner, 1988). However, some heterothallic species are difficult to separate morphologically and can only be differentiated by mating tests. The genus *Neurospora* includes five conidiating species: *Neurospora crassa*, *Neurospora intermedia*, *Neurospora sitophila*, *Neurospora discreta* (all heterothallic) and *Neurospora tetrasperma* (pseudohomothallic) studied in North America and as well as Europe (Jacobson et al., 2006). *N. crassa* is the best known and has been used extensively for genetic, biochemical and molecular studies.
Figure 1. The geographical location where fungi strain are collected (as red boll) ranging from the Longitude 28° 25'E and Latitude 48° 40', indicating the collection sites of Neurospora udagawae strain in Ardabil province, north west of Iran (from Google earth). Specimen Examined- Iran, East Azarbijan Prov., Ardabil city, Fandoghlo forest areas, on the healthy and fire burned twigs of Corylus avellana L. (Corylaceae), Longitude 28° 25'E, Latitude 48° 40', Co RAHNAMA K & Habibi R (NEUROSPORA IRAN2235C).

The five conidiating Neurospora species cannot be distinguished from one another on the basis of vegetative morphology or color. Morphology nonetheless continue to be useful for identifying N. tetrasperma because this pseudohomothallic species produces perithecia with asci containing four dikaryotic and binucleate spores, as opposed to the eight-spored asci found in all other Neurospora species (Turner et al., 2001).

The names Neurospora and Gelasinospora are synonymized and so far 49 species of Neurospora have been recognized in the genus by Garcia et al. (2004). The generic diagnosis was also expanded to include those with ascospores broadly fusiform, ellipsoidal or nearly spherical, 1-celled, hyaline to yellowish brown or olive-brown, becoming dark and opaque at maturity, ascosporic wall with longitudinal ribs or pitted, occasionally nearly smooth.

Anamorphs are known in only a relatively small number of species, belonging to Chrysosporia (Garcia et al., 2004). However, investigations on multiple-gene sequences and morphology concluded that although Gelasinospora and Neurospora are closely related, there is insufficient evidence to place currently accepted Gelasinospora and Neurospora species into the same genus (Cai et al., 2006). But, molecular studies in Lasiosphaeriaceae, Melanospora (Hypocreales) and Onygenales also suggested that generic distinction only on the basis of this character does not correlate with phylogenetic relationships inferred from DNA sequences (Cano et al., 2002; Miller and Huhndorf, 2002).

Today about 3500 fungus have been extensively identified and reported in Iran, and most reports of new records are limited to check lists with rare descriptions. Thus, fungi of Iran have received more attention in the past few decades. The recent indexed of fungi by Ershad (2009) and, the most reports on the fungal species from different substrates indicated recently, one report of N. tetrasperma from fire burned twigs of plane trees (Platanus orientalis) in the north east of Iran (Aghapur et al., 2010). In order to look at distribution of fungus in various natural conditions, this study determines to find possibility of new host for Neurospora in the distribution areas from North West of Iran.

MATERIALS AND METHODS

The plant material for this investigation was obtained from the north west of Iran, Ardabil province (Longitude 28° 25'E & Latitude 48° 40') in the autumn of 2013 (Figure 1). At the time of sampling of
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Figure 2. Macroscopic characteristics of *Neurospora udagawae* on *Corylus avellana* branch. A-B, Artificial perithecia formation of burned twigs on the bark of *Corylus avellana* (scale bar: A,B=2 mm); C, Formation of perithecia (small black dot) on WA 2% after 12 days; D-F, 12 days old cultures of *Neurospora udagawae* on PDA at 30±1°C; PCA at 20±1°C; on MEA at 20±1°C.

hazelnut trees (*Corylus avellana* L.), twigs with no sign of the fungus on the surface were collected. In order to produce sexual within Petri dishes and incubated at 25°C (Figure 2A and B). Perithecia formed after 14 days on the bark surface in the laboratory. The specimens with fruiting bodies were observed by using an Olympus light microscope (model Olyvia Bio Report). Pieces of plant tissue were placed on potato dextrose agar (PDA) culture medium after disinfestations then sub cultured on water agar (2%) medium and pure fungal cultures were obtained by transferring hyphal tips. The fungal isolates were grown on PDA culture medium then incubated at 25±1°C under dark condition. The specimens with fruiting bodies were observed by using an Olympus light microscope (model Olyvia Bio Report). Pieces of plant tissue were placed on potato dextrose agar (PDA) culture medium after disinfestations then sub cultured on water agar (2%) medium and pure fungal cultures were obtained by transferring hyphal tips. The fungal isolates were grown on PDA culture medium then incubated at 25±1°C under dark condition. The macro and microscopic morphological characteristics of the fungi were studied on potato dextrose agar (PDA), malt extract agar (MEA, Oxoid) and potato carrot-agar (PCA; potato 20 g, carrot 20 g, agar 20 g/l) at various temperatures of 5±1, 10±1, 20±1, 30±1 and 40±1°C (Garcia et al., 2004; Nygren et al., 2011), as well as growth rates of the fungal isolates, were measured. The morphology of the ascospores, mainly the ornamentation pattern, number and placement of germ pores, and the presence or notch of ostiole in the ascomata are the most important features for species distinction. The fungal isolate were identified (based on morphological species concept) by comparison with the descriptions of Perkins and Turner (1988), Turner et al. (2001) and Garcia et al. (2004).

RESULTS AND DISCUSSION

The growth rate of fungus was measured on various media and different temperatures under continuous dark conditions for 24 h (Table 1). There was no growth at 5 and 40°C temperatures after 24 h incubation.

**Colony characteristics**

Colonies on PDA and PCA filled the plates (9 cm) within 24 h at 30±1°C. Ascomata were produced abundantly after 12 days, but on water agar 2% with very little numbers as compared to those on PCA which was more regular and many ascomata at 20±1°C. Ascomata produced pale brown to dark brown color on PDA, with scanty aerial hyphae especially at the periphery of the plate and dark reverse (Figure 2D). Colonies on MEA filled the plate within 48 h, with white color mycelia (Figure 2F). The margins of fungal colonies on PDA and PCA medium were smooth or partly irregular but on MEA medium were branchy. Colony color in PCA and MEA medium were white (Figure 2E and F), but subsequently, the fungus metabolites turned culture to pale orange just on PDA medium at 30±1°C (Figure 2D). The colony expanded quickly and the expanding hyphae were broad, septate, thick-walled, hyaline and branched.
Table 1. Effect of temperature and media culture on mycelial growth of *N. udagawae* after 24 h incubation.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Mycelial growth in culture medium (mm/h)</th>
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<tr>
<td></td>
<td>PDA</td>
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<tr>
<td>5±1</td>
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<td>10±1</td>
<td>23.0 ± 0.39</td>
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<td>20±1</td>
<td>15.0 ± 0.08</td>
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<tr>
<td>30±1</td>
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<td>40±1</td>
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Potato dextrose agar (PDA), potato carrot-agar (PCA), malt extract agar (MEA).

Figure 3. Sexual stage of *Neurospora udagawae* on *Corylus avellana* branch. A, Globose or subglobose perithecia (scale bar: A=100 μm); B, Formation perithecia with lengthy neck; C, Ostiolate ascomata (scale bar: C=100 μm); D, Asci 8 spored (scale bar: D=20 μm); E, Uniseriate ascospores (scale bar: E=20 μm); F-G, Detail of the ascus (scale bar: F-G=40 μm); H, One celled ascospore and global ornate pits (scale bar: H=100 μm); I, Abnormal hyaline sheath (arrows) (scale bar: I=100 μm); J-K, Developing perithecia from coiled ascogonia (scale bar: J-K=100 μm); L, Irregularly and remotely septate (scale bar: L=100 μm).
Sexual stage

The sexual stage of the fungus was formed abundantly on the WA 2% (Figure 2C) and PCA especially at 20±1°C (Figure 2E) and the perithecia were visible on the culture medium as very small black dots. Teleomorph stage was confirmed as homothallic, perithecia developing from coiled ascogonia (Figures 3J and K), were superficial to somewhat immersed covered with white hyphal-like hairs, scattered (Figure 2C) to aggregated (Figure 2E), hairs hyaline, flexuous, irregularly and remotely septate (Figure 3L), rarely branching with slightly thickened walls, globose or subglobose (Figure 3A) with one lengthy neck with 40-84 (63) μm in length (Figure 3B). Ascomata were ostiolate (Figure 3C), dark brown to black, smooth or downy with loose hyphae and 226-240 (235) μm in diameter. Asci were uniloculate, hyaline, cylindrical, thin-walled having ring-like thickening at the tip, short stalked, often 8-spored (Figure 3D, F and G), 103–109 (105) × 13–18 (16) μm in diameter. Ascospores were uniseriate (Figure 3E) or somewhat overlapping, initially hyaline, becoming yellowish brown to black with maturity, one celled ellipsoidal or elongate, ascospore wall surface with global ornate pits (Figure 3H), 0.7-1.6 μm in diameter and abnormal hyaline sheath (arrows) (Figure 3I). Ascospores were 19–26 (23) × 12–15 (14) μm in diameter and had circular and apical germ pores at each end. Based on morphological features of teleomorphic stages of the fungus on host plant and culture media, it was identified as *Neurospora udagawae* Krug & Khan. In this study conidia were not produced.

In general, *Neurospora* species are common primary colonizers of trees and shrubs killed by forest fires in cold and dry temperate regions (Jacobson, 2003). In this study, based on morphological characters of ascospores, sexual stage and the effect of temperature, *N. udagawae* is reported as new for the mycoflora of Iran. Maximum mycelia growth was observed at 30°C but there was no growth at 5 and 40°C (Table 1). According to the description of Garcia et al. (2004), the fungus *N. udagawae* has been reported in Pakistan from soil (Khan and Krug, 1989), and there were no reports of *N. udagawae* from somewhere else. Our results represent the first occurrence of *N. udagawae* species from Iran (Ershad, 2009) and it seems a new host plant, hazelnut tree (*C. avellana* L.) in the world (www.Mycobank.org/IMI).

There are no obvious morphological differences between the reproductive structures of our isolates when compared with those described by Garcia et al. (2004), except their dimensions, which could be attributed to different hosts and the environmental conditions. Previous studies identified *N. tetrasperma* from fire burned twigs of plane tree by producing four ascospores from northeast of Iran, but in this study, *N. udagawae* from north west of Iran produced eight ascospores recognized as homothallic species (Figure 3H and I). The *Neurospora* species can often be found on the surface following forest or grass fires or soil, as our isolates were obtained from healthy twigs and fire burned twigs in artificial condition *in vitro*. Homothallic species is reported from Hungary previously (Krug and Khan, 1991). Sexual structures were not frequently observed in the nature because of difficulty in condition for black perithecia formation on the hazelnut trees and *in vitro* conditions. We hope to continue our studies on phylogenetic and biological species diversity among reported *N. udagawae* strains in Iran in addition, with some other collected strains of the fungus from other plants in the future.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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