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The *Pleurotus eryngii* species-complex in Kurdistan Region of Iran

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Abstract: *Pleurotus eryngii* species-complex are known to be well distributed in Kurdistan region of Iran. During a survey in 2003-2005 fourteen wild populations of the *Pleurotus eryngii* species-complex were collected from Sanandaj, Hane Gelan and Saral areas. Ecomorphological studies and pairing tests showed that all isolates were belong to *P. eryngii* and located into two groups associated with three Umbelliferous host plants including isolates associated with *Prangos* and *Pimpinella* with smaller spores and *Ferula haussknechtii* with larger spores. In this study we introduce *Ferula haussknechtii* as a new host species of *P. eryngii* in the world.

Key words: *Pleurotus eryngii*, Apiaceae, *Ferula haussknechtii*, Kurdistan, Iran

INTRODUCTION

The genus *Pleurotus* (Jacq.: Fr.) P. kumm. (Agaricales, Pleurotaceae) includes various lignotrophic species growing as saprotroph on deciduous and rarely on coniferous trees, or as weak parasites on the lower part of the stems and roots of some Apiaceae species (Hilber, 1982; Moser, 1983; Zervakis and Balis, 1996). Fungi of the genus *Pleurotus* are tetrapolar heterothallic; dikaryons produce edible reproductive structures (i.e., basidiomata) (Zerwakis *et al.*, 2001). Some *Pleurotus* species (e.g., *P. ostreatus*, *P. pulmonarius*, *P. eryngii*, *P. cornucopiae*, *P. dryinus* and *P. calyptratus*) are used in traditional medicine for at least 35 disorders or diseases (Chang, 1999; Lewinsohn *et al.*, 2002). *Pleurotus* species account for 15% of worldwide mushroom production (Chang, 1999). In addition, the use of *Pleurotus* fungi is linked to several agro-industrial activities of great economic importance, e.g., conversion of lignocellulosic residues to food and feed, biocontrol of plant diseases, degradation of noxious pollutants, production of enzymes and medicinal compounds (Zervakis *et al.*, 2001; Zervakis and Balis, 1996).

Pleurotus eryngii (DC.:Fr.) Qué. species-complex is especially distributed in the subtropical-temperate climate of the Mediterranean region (Italy, Spain, Greece, Cyprus, France, former Yugoslavia, Israel, Lebanon) and central Asia (Uzbekistan, Tadjikistan, Turkmenistan and Iran). It is also known from central Europe and North Africa (Lewinsohn *et al.*, 2002). *Pleurotus eryngii*

species-complex are known from the various hosts including *Eryngium campestre* L., *E. maritimum* L., *Ferula communis* L., *Ferula tingitana* L., *Cachrys ferulacea* (L.) Calestani, *Thapsia garganica* L., *T. villosa* var. *fuscus*, *Elaeoselinum asclepium* Bert., *Diplotaenia* sp., *Ferulago* sp., *Laserpitium siler* L., *L. latifolium* L., *Pimpinella* sp., *Opopanax chironium* Roch., *Prangos ferulacea* DC., *Peucedanum cervaria* (L.) Lapeyr, etc. The *Pleurotus eryngii* species-complex has been described by many authors (Hilber, 1982; Moser, 1983; Candusso and Basso, 1995). Despite the obvious economic importance, taxonomy within this group is still unclear and the relationship among different host-specific taxa are ambiguous since they present minut morphological differences and a partial intercompatibility which permits a limited outbreeding (Hilber, 1982; Zervakis and Balis, 1996). *Pleurotus eryngii* species-complex continues to be designated by one species (*Pleurotus eryngii*) which contain from five varieties to five independent species [*Pleurotus eryngii*, *P. ferulae* (Lanzi) Sacc., *P. nebrodensis* (Inzenga) Sacc., *P. fuscus* (Battarra) Bres., *P. caespitoso-terrester* (P. Henn.) Pil.]. Moser (1983) considered all types as one species *P. eryngii*, while Candusso and Basso (1995), stated that two species are valid, *Pleurotus eryngii* and *P. nebrodensis* for the mycobiota of Italy. Venturella (2000) illustrated the typification of *P. nebrodensis* and Venturella *et al.* (2002 and 2000) described two new varieties of *P. eryngii*, *P. eryngii* var. *elaeoselini* and *P. eryngii* var. *thapsiae*, for the mycobiota of Sicily,

respectively. Lewinsohn *et al.* (2002) described new var. of *P. eryngii*, *P. eryngii* var. *tingitanus*, for the mycobiota of the Israel.

In Iran, for the first time, Petrak (1939) reported this fungus as *P. fuscus* (Batt.) Bres.. Also, Esfandiari (1948) reported this fungus as *P. eryngii* (Dc. ex. Fr.) Quél. *P. eryngii* was reported from different regions of Iran including Esfahan, Fars and Tehran Provinces (Saber, 1990). In Kurdistan region in the west of Iran, the *P. eryngii* species-complex has been widely distributed. Thus we studied the morphology, host association and mating experiments of this complex in order to identification of variation of *P. eryngii* species-complex.

MATERIALS AND METHODS

Biological material: Periodical field observations and collection of *Pleurotus eryngii* species-complex basidiomata growing on Umbellifers were carried out in Kurdistan region in 2003-2005. Fourteen population were studied in this survey (Table 1). These collections have been deposited in the fungus reference collection of the Ministry of Jihad-e-Agriculture (IRAN).

Microscopic features: Small pieces of fungal (basidiomata) tissues were taken from each sample and rehydrated in L4 or KOH 5%. The micromorphology, Length and width of 25 up to 50 basidiospores, cheilocystidia and basidioms of each isolates were measured with the aid of Olympus BH2 microscope.

Mating experiments: Basidiospore suspensions were prepared in sterile water (from a piece of lamellae or spore print). One microbiological loop from basidiospore suspension of each isolates was smeared on BSMA (benomyle sulphate streptomycin malt agar) surface on a petri dish. Two days after incubation, basidiospores were investigated under the microscope and individual germinated basidiospores were transferred into petri dishes with fresh MA (malt agar). In order to confirm of single spore transfer to the second MA, inoculated dishes were microscopically investigated for clamp connection absence and three of monosporous cultures were used for determination of mating types. Pairings were set up by placing mycelia plugs freshly cut from colonies with a cork borer (5 mm) about 15-20 mm apart on MEA 2% (Fig. 1). Inoculated dishes were incubated at 24°C in the dark. After 10 to 15 days when the colonies had grown over the zone of contact, the mycelium was examined for the occurrence of clamp connections. In order to investigate the sexual compatibility of isolates, two mating system (haploid-haploid and dikaryon-haploid) were used.

Table 1: Origin of the 14 populations of *Pleurotus eryngii* species-complex

Population no.	Host/Substrate	Location	No. of isolates
1	<i>Ferula haussknechtii</i>	Saral	1
2	<i>Prangos</i> sp.	Hane Gelan	9
3	<i>Prangos</i> sp.	Hane Gelan	12
4	<i>Pimpinella</i> sp.	Sanandaj	10
5	<i>Prangos</i> sp.	Sanandaj	8
6	<i>Pimpinella</i> sp.	Sanandaj	15
7	<i>Prangos</i> sp.	Sanandaj	9
8	<i>Prangos</i> sp.	Sanandaj	11
9	<i>Ferula haussknechtii</i>	Sanandaj	1
10	<i>Prangos</i> sp.	Sanandaj	8
11	<i>Prangos</i> sp.	Sanandaj	11
12	<i>Pimpinella</i> sp.	Sanandaj	9
13	<i>Pimpinella</i> sp.	Sanandaj	13
14	<i>Pimpinella</i> sp.	Sanandaj	15
Total			132



Fig. 1: Mating experiments of two *P. eryngii* haploid colonies on MEA

Dikaryon-haploid matings were conducted in cases that the haploid colonies were not accessible. In order to determination of the relationship of isolates to the *P. eryngii*, pairing tests were conducted with valid tester isolates of different biological species throughout of the world (details of the tester isolates are available for any request).

RESULTS

Geographical distribution and association between *Pleurotus eryngii* and Apiaceae plants in Kurdistan region: Present results showed that *P. eryngii* species-complex have a considerable distribution in Kurdistan altitudes higher than 2000 m in Sanandaj, Hane Gelan and Saral. Maximum distribution detected from 2100 to 2750 m. The most suitable growing season was from the later of March to the later of May. Host range was restricted to three genus including *Ferula*, *Pimpinella* and *Prangos* from Apiaceae.



Fig. 2: Basidiomata of *P. eryngii* (left) associated with *F. haussknechtii* (right)

Macroscopic features: *Basidiomata* sturdy and fleshy, single, seldom caespitose. *Pileus* 3-12 cm, convex then flat, finally centrally depressed seldom reflexed or funnel-shaped, cuticle not easily detachable, velvety and opaque, surface wrinkled, uniformly colored, white-ivory cream, Margin thin, acute, deeply involute, flat in the ripe basidiomata and the irregular, sometimes also lobate. *Lamellae* thick, white, arcuate, mixed by lamellulae, deeply decurrent. *Stipe* 4-7×1-2.5 cm, sturdy, filled and firm, irregularly cylindrical, attenuate at the base, sometimes radiating, central to eccentric, concolorous with the cap, then smooth and glabrous (Fig. 2).

Microscopic features: Spores measurements showed that isolates associated to *Ferula haussknechtii* was different from other isolates associated to *Prangos/Pimpinella* (Table 2). *Basidiospores:* cylindrical to ellipsoid, smooth hyaline, guttulate with pronounced apiculus. *Basidia* club-shaped, 4-strigmata, 6-12×25-50 μm . *Cheilocystidia* 6-12×35-60 μm , club-shaped. Basidiospores, basidia and trama hyaline in aqueous KOH and Melzer's reagent. Results of the comparing of size and shape of basidia, cystidia and pileal trama was not showed any variation between different isolates while spores size were different.

Cultural characteristics on PDA: Pigments absents, reverse colour unchanged, dikaryotic colonies mostly presenting a loose submerged and suppressed aerial mycelium, more or less zonate and radial, growth margin even and regular, colour white-ivory to cream; thin-walled hyphae, hyaline in aqueous KOH and Melzer's reagent, with abundant clamp-connections; occasional production

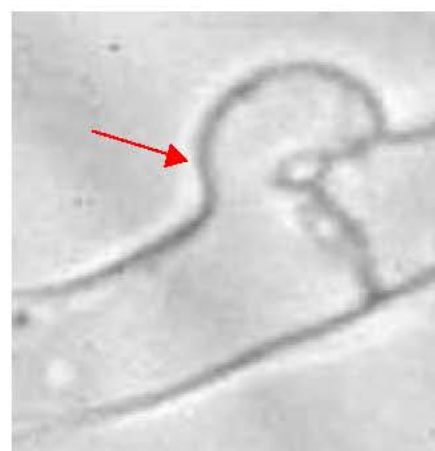


Fig. 3: Compatibility reaction and clamp connection formation (arrow) in mating experiments of this study

Table 2: Basidiospores measurements of all isolates located in three groups based on host plant or substrate

Spore width	Spore length	Host
6.5-7.5	11-13	<i>Ferula haussknechtii</i>
4.5-6	9.5-11	<i>Prangos</i> sp.
4-5	9-10	<i>Pimpinella</i> sp.

of micro droplets, singly on short secretory sterigmata on aerial hyphae (nematode trapping devices); optimal fungal growth 25-30°C (2-3 mm day⁻¹).

Mating experiments: Pairing tests results revealed compatibility (clamp connection formation) between all

isolates in present study (Fig. 3). In other word, no common incompatibility alleles existed between them and although all isolates are not belong to a common population, but they are still members of the same biological species and all isolates are belong to one species of *Pleurotus* and observed differences are in the lower taxonomic level (i.e., variety). Pairing tests with tester isolates showed that all isolates are belonging to *P. eryngii* species-complex (data are available for any request).

DISCUSSION

In Kurdistan region of Iran the *P. eryngii* species-complex is a spring mushroom (locally named as Kamma). Macroscopic, microscopic, cultural characteristics and mating tests showed that all isolates in this study are belong to *P. eryngii*. The host plants associated with these isolates were belong to three Umbelliferous genus including *Prangos*, *Pimpinella* and *Ferula*. Results of ecomorphological studies showed that all isolates, were located into two groups including, isolates associated with *Prangos* sp. and *Pimpinella* sp. with smaller spores and *Ferula haussknechtii* with larger spores. In Kurdistan region, specially Sanandaj, Hane Gelan and Saral area, Umbellifers show a high degree of diversity (Rechinger, 1987). In this study we introduce *F. haussknechtii* as a new host species for *P. eryngii* in the world.

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