Identification of *Armillaria* biological species in Iran

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Thirty-four isolates of *Armillaria* were collected from a variety of hosts in fruit orchard and forest regions in Iran. From each basidiocarp, monospore cultures were obtained. Haploid and diploid cultures were paired in all possible "haploid-haploid" and "diploid-haploid" combinations. Sexual compatibility was determined after 25-35 days based on differences in culture morphology of haploid colonies from white, with aerial mycelium (fluffy) to brownish, without aerial mycelium (crustose) which is characteristic of diploid cultures. Six compatibility groups named Iranian intersterility groups (IISG) were identified: IISG1 included one isolate, IISG2 seventeen isolates, IISG3 eight isolates, IISG4 one isolate, IISG5 two isolates and IISG6 five isolates. Haploid and diploid isolates from Iranian intersterility groups of *Armillaria* were paired with European and two Japanese haploid tester strains. Six intersterility groups were authenticated as *A. mellea, A. cepistipes, A. gallica, A. borealis, Armillaria* sp. (IISG5) and *Armillaria* sp. (IISG6). Two groups (IISG5 and IISG6) were, however, not compatible with any of tester strains representing different species.

Key words: Armillaria root rot, heterothallism, intersterility groups, pairing tests

Introduction

Fungi belonging to the genus *Armillaria* cause root disease of deciduous and coniferous trees and shrubs in forests, plantations, orchards and gardens throughout the world (Shaw and Kile, 1991). Armillaria root rot historically has been attributed to *A. mellea sensu lato*. However within the last 20-30 years, it has been shown that this taxon consists of several intersterility groups or biological species. In the early seventies, Hinitikka (1973) developed a technique that allowed the recognition of several biological species in *Armillaria*. On the basis of Hinitikka's technique, single spore isolates of *Armillaria* species are generally white and quite fluffy. When fusion of compatible mating types occurs, colonies become dark, brown and flattened,

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which is characteristic of diploid cultures. Using this method, Korhonen (1978) was able to distinguish biological species (BS) in Europe. Anderson and Ullrich (1979) applied the technique developed by Hinitikka and expanded by Korhonen and initially identified 10 biological species in North America (NABS) and then *A. mellea* (Vahl: Fr.) Kummer*, A. calvescens* Bérubé & Dessureault*, A. cepistipes* Velenovsky*, A. tabescens* (Scop.: Fr.) Emel.*, A. gallica* Marxmüller & Romagnesi*, A. gemina* Bérubé & Dessureault*, A. ostoyae (*Romagnesi) Herink*, A. sinapina* Bérubé & Dessureault and *A. nabsnona* Volk & Burdsall were identified (Anderson and Ullrich, 1979; Bérubé and Dessureault, 1988, 1989; Volk *et al*., 1996). In Australia Kile and Watling (1983) identified five species: *A. hinnulea* Kile & Watling*, A. luteobubalina* Watling & Kile*, A. novae-zelandiae (*Stevenson) Herink*, A. fumosa* Kile & Watling and *A. pallidula* Kile & Watling. In Japan 10 biological species of *Armillaria* were identified (Ota *et al.,* 1998).

In Iran, *Armillaria* is widely distributed throughout the country and is a well known cause of root rot (Saber, 1974; Shaw and Kile, 1991). Armillaria root disease was first reported on apple trees in 1956 (Saber, 1974). Since then, *Armillaria* has been reported in association with many cultivated and forest tree species (Ershad, 1995), but all of the isolates of *Armillaria* in Iran have been attributed to *A. mellea sensu lato*. Our objective in this study was to investigate the compatibility reactions among *Armillaria* isolates, and the identification of intersterility groups and biological species of *Armillaria* in Iran.

Materials and methods

Thirty-four isolates from basidiocarps and rhizomorphs of *Armillaria* were collected during 1999 to 2001 from forests and orchards in Iran (Table 1). Monospore cultures were derived from each basidiocarp. Basidiospores of each collection were obtained from small squares of hymenial tissue suspended over sterile distilled water and then spread with a sterile glass rod on the surface of 2% malt extract agar and were incubated at 25ºC. Vegetative isolates were obtained by surface sterilizing small pieces of rhizomorphs with sodium hypochloride and cultured onto 2% malt extract agar amended with benomyl, streptomycin and penicillin (Worral, 1991). Stock cultures of all isolates were maintained on 2% malt extract agar at 6ºC in the dark, and working cultures on 2% malt extract agar at 23-25ºC. All isolates of *Armillaria* were deposited in culture collection of Plant Pests and Diseases Research Institute in Iran.

Mating experiments were conducted on 2% malt extract agar. Mycelial plugs (5 mm diam.) were derived from haploid and diploid cultures and

Table 1. Strains of Iranian *Armillaria* used in this study.

Isolate	Host	Isolate type	Ploidy	Location	IISG
EA1	*Ulmus minor*	Basidiocarp	N	East Azarbaijan	IISG 2
EA2	*Rosa* sp.	Basidiocarp	N	East Azarbaijan	IISG 2
EA3	*Populus nigra*	Basidiocarp	N	East Azarbaijan	IISG 2
EA4	*Amygdalus communis*	Rhizomorph	2N	East Azarbaijan	IISG 2
EA5	*A. communis*	Basidiocarp	N	East Azarbaijan	IISG 5
EA6	*Cerasus avium*	Rhizomorph	2N	East Azarbaijan	IISG 2
EA7	*Quercus macranthera*	Basidiocarp	N	East Azarbaijan	IISG 2
EA8	Stump	Rhizomorph	2N	East Azarbaijan	IISG 5
EA9	Stump	Basidiocarp	N	East Azarbaijan	IISG 2
TK1	*Platanus orientalis*	Basidiocarp	N	Tehran-Karaj	IISG 2
TK2	*Morus alba*	Rhizomorph	2N	Tehran-Karaj	IISG 3
TK3	*C. avium*	Basidiocarp	N	Tehran-Karaj	IISG 3
TK4	Stump	Basidiocarp	N	Tehran-Karaj	IISG 3
MB1	*Q. castaneifolia*	Basidiocarp	N	Mazandaran-Babol	IISG 3
MB2	*Q. castaneifolia*	Basidiocarp	N	Mazandaran-Babol	IISG 3
MB3	Stump	Basidiocarp	N	Mazandaran-Babol	IISG 6
MN1	Stump	Basidiocarp	N	Mazandaran-Noor	IISG 2
MN2	Stump	Rhizomorph	2N	Mazandaran-Noor	IISG 6
MN3	*P. orientalis*	Basidiocarp	N	Mazandaran-Noor	IISG 2
MS1	*Parrotia persica*	Basidiocarp	N	Mazandaran-Sisangan	IISG 2
MS2	*P. persica*	Basidiocarp	N	Mazandaran-Sisangan	IISG 2
MS3	*Q. castaneifolia*	Basidiocarp	N	Mazandaran-Sisangan	IISG 3
MS4	*Q. castaneifolia*	Basidiocarp	N	Mazandaran-Sisangan	IISG 4
MS5	*Q. castaneifolia*	Rhizomorph	2N	Mazandaran-Sisangan	IISG 6
MS6	*Q. castaneifolia*	Basidiocarp	N	Mazandaran-Sisangan	IISG 3
MS7	*Q. castaneifolia*	Basidiocarp	N	Mazandaran-Sisangan	IISG 3
MS8	*Alnus* sp.	Rhizomorph	2N	Mazandaran-Sisangan	IISG 6
MS9	*Acer* sp.	Basidiocarp	N	Mazandaran-Sisangan	IISG 2
MS10	*Acer* sp.	Basidiocarp	N	Mazandaran-Sisangan	IISG 2
MS11	*Fagus orientalis*	Rhizomorph	2N	Mazandaran-Sisangan	IISG 6
MS12	*F. orientalis*	Basidiocarp	N	Mazandaran-Sisangan	IISG 2
MS13	Stump	Basidiocarp	N	Mazandaran-Sisangan	IISG 2
MS14	Stump	Basidiocarp	N	Mazandaran-Sisangan	IISG 1
MS15	Unknown	Rhizomorph	2N	Mazandaran-Sisangan	IISG 2

were paired in all of the possible cases in "haploid-haploid" and "diploid-haploid" pairs. Isolates to be paired were placed on the agar surface 3-5 mm apart and were incubated at 23ºC in the dark. Morphological differences of the cultures were observed after 25-35 days. Compatibility was determined on the basis of differences in morphology of haploid colonies after compatible mating from white, with aerial mycelium (fluffy) to brownish without aerial mycelium (crustose) (Fig. 1). In incompatible mating, haploid colonies remained white

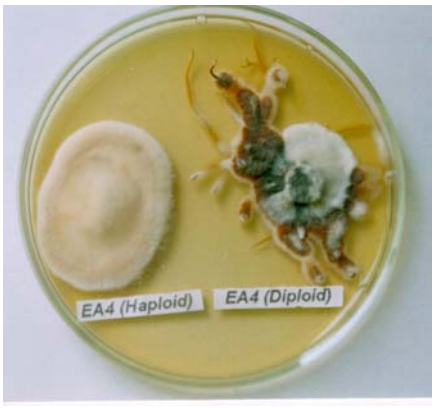

Fig. 1. Difference between haploid and diploid colonies of *Armillaria*.

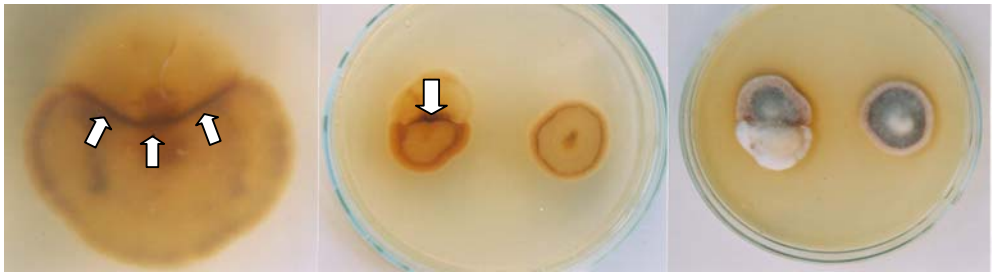

Fig. 2. Incompatible reaction and formation of black line (arrows) in contact zone of two incompatible colonies.

and fluffy and a black line was made in the contact zone of two colonies (Fig. 2).

After the determination of intersterility groups, strains from each group were paired with European and Japanese tester strains. Host and geographical source of test strains are indicated in Table 2. Two to four test strains were used from each biological species and Iranian isolates were paired with them in all possible combinations.

Results and discussion

From the results of pairing tests among the Iranian isolates (Table 3), these isolates were grouped into six intersterility groups named Iranian intersterility groups (IISG) (Table 4). After determination of intersterility groups, all strains from each group were paired with tester strains in all of the possible cases.

Table 2. List of tester strains

Species	Isolate code	Code	Location	Host	Sourse
A. borealis	99 060\2	B1	Finland	*Picea abies*	Korhonen
A. borealis	99 065\5	B2	Finland	*P. abies*	Korhonen
A. borealis	99 068\4	B3	Finland	*P. abies*	Korhonen
A. borealis	MA 79.18.1	B4	Finland	*Betula* sp.	Guillaumin
A. cepistipes	98 008\2	C1	Finland	*Betula* sp.	Korhonen
A. cepistipes	99 086\2	C2	Finland	Ground	Korhonen
A. cepistipes	98 030\3	C3	Finland	Ground	Korhonen
A. cepistipes	MB 79.23.1	C4	Finland	*Alnus* sp.	Guillaumin
A. mellea	87 085\10	M1	Italy	*Opuntia* sp.	Korhonen
A. mellea	90 254\3	M2	Italy	Unknown	Korhonen
A. mellea	90 260\1	M3	Yugoslavia	*Malus* sp.	Korhonen
A. mellea	MD 79.30.1	M4	France	Unknown	Guillaumin
A. ostoyae	99 087\8	O1	Finland	*Pinus sylvestris*	Korhonen
A. ostoyae	99 088\3	O2	Finland	*P. sylvestris*	Korhonen
A. ostoyae	99 088\7	O3	Finland	*P. sylvestris*	Korhonen
A. ostoyae	MC 79.27.1	O4	Finland	*P. sylvestris*	Guillaumin
A. gallica	ME 70.1.2	G1	France	*Rubus fructicosus*	Guillaumin
A. gallica	ME 80.15.1	G2	France	*Corylus avellana*	Guillaumin
A. gallica	ME 81.24.4	G3	France	Ground	Guillaumin
A. sinapina	96-7-1	S1	Japan	*Larix kaempferi*	Yuko Ota
A. sinapina	96-7-2	S2	Japan	*L. kaempferi*	Yuko Ota
A. sinapina	96-7-3	S3	Japan	*L. kaempferi*	Yuko Ota
A. tabescens	MT 83.10.8	T1	France	*Quercus robur*	Guillaumin
A. tabescens	NT 1-8	T4	Japan	*Q. myrsinaefolia*	Yuko Ota

According to the results shown in Table 5, IISG1 was compatible with European *A. gallica,* IISG2 was compatible with European *A. mellea,* IISG3 was compatible with *A. cepistipes,* IISG4 was compatible with two European *A. borealis*. IISG5 and IISG6 were not compatible with any of the testers.

Of the collections identified, *A. mellea* was the most common, comprising 50% of the collections. *Armillaria mellea* was found on some important deciduous hosts. Isolates MS1 and MS2 were recovered from Iranian iron tree (*Parrotia persica*) and this is the first record of this host for *Armillaria.* Isolates EA4 and EA6 originated from two economically important hosts of Iranian orchards, *Amygdalus cummunis* L. and *Cerasus avium* (L.) Moench.. Isolates EA1, EA3, EA7, TK1 and MN3 were collected from *Ulmus minor* Miller*, Populus nigra* L.*, Quercus macranthera* Fisch. & Mey. and *Platanus orientalis* L..

Results of mating among a series of different monospore isolates from the same basidiocarp showed that Iranian isolates of *A. mellea* have clearly heterothallic life cycle as in Europe isolates (Guillaumin*,* 1989) but in contrast to Japanese isolates (Ota *et al*., 1998).

Table 3. Results of pairings among Iranian *Armillaria* isolates.

	EA1	EA2	EA3	EA4	EA5	EA6	EA7	EA8	EA9	TK1	TK2	TK3	TK4	MS1	MS2	MS3
EA1	+	+	+	+	-	/	+	-	+	+	-	-	-	+	+	-
EA2	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-
EA3	+	+	+	?	-	+	+	-	+	+	-	-	-	+	+	-
EA4	+	+	?	+	-	#	+	#	+	+	#	-	-	+	+	-
EA5	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
EA6	/	+	+	#	-	+	+	#	+	+	#	-	-	+	+	-
EA7	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-
EA8	-	-	-	#	+	#	-	+	-	-	#	-	-	-	-	-
EA9	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-
TK1	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-
TK2	-	-	-	#	-	#	-	#	-	-	+	+	+	-	-	+
TK3	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+
TK4	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+
MS1	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-
MS2	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-
MS3	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+
MS4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MS5	-	-	-	#	-	#	-	#	-	-	#	-	-	-	-	-
MS6	-	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-
MS7	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+
MS8	-	-	-	#	-	#	-	#	-	-	#	-	-	-	-	-
MS9	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-
MS10	+	+	+	+	-	+	+	?	+	+	-	-	-	+	+	-
MS11	-	-	-	#	-	-	-	#	-	-	#	-	-	-	-	-
MS12	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-
MS13	+	+	+	-	-	+	+	-	+	+	-	-	-	+	+	-
MS14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MS15	+	+	+	#	-	#	+	#	+	+	#	-	-	+	+	-
MB1	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+
MB2	-	-	-	?	-	-	-	-	-	-	+	+	+	-	-	+
MB3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MN1	+	+	+	+	-	+	+	?	+	+	-	-	-	+	+	-
MN2	-	-	-	#	-	#	-	#	-	-	#	-	-	-	-	-
MN3	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-

	MS4	MS5	MS6	MS7	MS8	MS9	MS10	MS11	MS12	MS13	MS14	MS15	MB1	MB2	MB3	MN1	MN2	MN3
EA1	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
EA2	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
EA3	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
EA4	-	#	-	-	#	+	+	#	+	+	-	#	-	?	-	+	#	+
EA5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EA6	-	-	-	-	-	+	+	-	+	+	-	#	-	-	-	+	-	+
EA7	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
EA8	-	#	-	-	#	-	-	#	-	-	-	#	-	-	-	?	#	-
EA9	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
TK1	-	-	-	-	-	+	+	-	+	+	-	+	-	-	/	+	-	+
TK2	-	#	+	+	#	-	-	#	-	-	-	#	+	+	-	-	#	-
TK3	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-

Table 3. (continued).

	MS4	MS5	MS6	MS7	MS8	MS9	MS10	MS11	MS12	MS13	MS14	MS15	MB1	MB2	MB3	MN1	MN2	MN3
TK4	-	?	+	+	?	-	-	?	-	-	-	-	+	+	-	-	?	-
MS1	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
MS2	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
MS3	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
MS4	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MS5	-	+	-	-	#	-	-	#	-	-	-	#	-	-	+	-	#	-
MS6	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
MS7	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
MS8	-	#	-	-	+	-	-	#	-	-	-	#	-	-	+	-	#	-
MS9	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
MS10	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
MS11	-	#	-	-	#	-	-	+	-	-	-	#	-	-	+	-	#	-
MS12	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
MS13	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
MS14	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
MS15	-	#	-	-	#	+	+	#	+	+	-	+	-	-	-	+	#	+
MB1	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
MB2	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
MB3	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-
MN1	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+
MN2	/	#	-	-	#	-	-	#	-	-	-	#	-	-	+	-	+	-
MN3	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	+

+=Compatible mating; /=Pairing was not carried out; -=Incompatible mating; ?=Results abnormal and unknown; #=Pairing between two diploid isolates (was not carried out).

Table 4. Intersterility grouping of Iranian *Armillaria* isolates

Intersterility group	Isolates	Species
IISG1	MS14 EA1 EA2 EA3 EA4 EA6 EA7 EA9 TK1	*A. gallica*
IISG2	MS1 MS2 MS9 MS10 MS12 MS13 MS15 MN1 MN3	*A. mellea*
IISG3	TK2 TK3 TK4 MS3 MS6 MS7 MB2 MB1	*A. cepistipes*
IISG4	MS4	*A. borealis*
IISG5	EA5 EA8	*Armillaria* sp.
IISG6	MS5 MS8 MS11 MB3 MN2	*Armillaria* sp.

Table 5. Results of pairing among Iranian and test strains of *Armillaria*.

Group1	Group2																	
	MS14	EA1	EA2	EA3	EA4	EA6	EA7	EA9	TK1	MS1	MS2	MS9	MS10	MS12	MS13	MS15	MN2	MN3
B1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M1	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+
M2	-	+	+	+	+	+	+	+	+	+	/	+	+	+	?	+	+	+
M3	-	+	+	+	+	+	+	+	+	?	+	+	/	+	+	+	+	/
M4	-	+	+	+	+	+	/	+	+	+	+	+	+	+	+	+	+	+
O1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	Group3								Group4	Group5			Group6			
	TK2	TK3	TK4	MS3	MS6	MS7	MB1	MB2	MS4	EA5	EA8	MS5	MS8	MS11	MB3	MN2
B1	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
B2	-	-	-	-	-	-	-	-	?	-	-	-	-	-	-	-
B3	-	-	-	-	-	-	-	-	?	-	-	-	-	-	-	-
B4	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
C1	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
C2	+	+	+	?	+	+	+	+	-	-	-	-	-	-	-	-
C3	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
C4	/	+	+	+	+	+	+	/	-	-	-	-	-	-	-	-
M1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 5. (continued).

	Group3							Group4		Group5			Group6			
	TK2	TK3	TK4	MS3	MS6	MS7	MB1	MB2	MS4	EA5	EA8	MS5	MS8	MS11	MB3	MN2
G1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+=Compatible mating; /=Pairing was not carried out; -=Incompatible mating; ?=Results abnormal and unknown.

Armillaria cepistipes is a new report for the Iranian fungus flora and was the second most often collected species. It was collected from *Morus alba* L., *Quercus castaneifolia* Mey. and *C. avium*. Most of isolates collected from *Q. castaneifolia* were compatible with European *A. cepistipes* which is also known from North America and Japan (Anderson and Ullrich, 1979; Korhonen, 1978; Ota *et al*., 1998). Isolates TK2 and TK3 were collected from *Morus alba* and *C. avium*. Isolate MS14 in IISG1 which originated from a stump, was compatible with European *A. gallica*. *Armillaria gallica* has been found from North America (Anderson and Ulrich, 1978), Europe (Korhonen, 1978) and Japan (Ota *et al*., 1998). It is thought to be a weak pathogen of hardwoods and conifers (Guillaumin, 1989). The only isolate placed in IISG4, MS4, was clearly compatible with strains B1 and B4 but in cases of B2 and B3 many pairs were made, however that did not show any clear results.

Isolates of groups IISG 5 and IISG 6 were not compatible with any of the tester strains. Morphological observations did not help in identification of these isolates.

Data and field observations indicate that *A. mellea* is the most important species in forests and orchards in Iran as a primary pathogen of many hardwood tree species.

Results showed that at least six reproductively isolated *Armillaria* groups exist in Iran. Additional collections, mating tests and molecular techniques are needed to identify the isolates of IISG 5 and IISG6.

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References

Anderson, J.B. and Ullrich, R.C. (1979). Biological species of *Armillaria mellea* in North America. Mycologia 71: 202-414.

Bérubé, J.A. and Dessureault, M. (1988). Morphological characterisation of *Armillaria ostoyae* and *Armillaria sinapina* sp. nov. Canadian Journal of Botany 66: 2027-2034.

Bérubé, J.A. and Dessureault, M. (1989). Morphological studies of the *Armillaria mellea* complex: two new species, *A. gemina* and *A. calvescens.* Mycologia 81: 216-225.

Ershad, D. (1995). *Fungi of Iran.* 2nd edition. Agricultural Research, Education and Extension Organization. Iran.

Guillaumin, J.J. (1989). *Armillaria* species in the northern temperate hemisphere. In: *Proceedings of the 7th International Conference on Root and Butt Rot* (ed. D.J. Morrison). 9-16 August 1989, Vernon and Victoria.

Hinitikka, V. (1973). A note on the polarity of *Armillariella mellea*. Karstenia 13: 32-39.

Kile, G.A. and Watling, R. (1983). *Armillaria* species from South-eastern Australia. Transactions of the British Mycological Society 81: 129-140.

Korhonen, K. (1978). Interfertility and clonal size in the *Armillariella mellea* complex. Karstenia 18: 31-42.

Ota, Y., Matsushita, N., Nagasawa, E., Terashita, T., Fukuda, K. and Suzuki, K. (1998). Biological Species of *Armillaria* in Japan. Plant Disease 82: 537-548.

Saber, M. (1974). Armillaria root rot. Iranian Journal of Plant Pathology 9: 54-62.

Shaw, C.G. and Kile, A. (1991). *Armillaria Root Disease*. Agricultural Handbook No. 691, USDA Forest Service, Washington, USA.

Volk, T.J., Burdsall, H.H. and Banik, M.T. (1996). *Armillaria nabsnona*, a new species from Western North America. Mycologia 88: 484-491.

Worral, J. (1991). Media for selective isolation of hymenomycetes. Mycologia 83: 296-302.

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