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Effect of the Number of Years of Soil Exploitation by Saffron Cultivation in Morocco on the Diversity of Endomycorrhizal Fungi

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The diversity of endomycorrhizal fungi in the rhizosphere of *Crocus sativus* has been studied at five sites in the Taliouine region (Tinfat), located in Taroudant Province (Morocco), according to the number of years of soil exploitation by Saffron cultivation. In all sites, the roots of *Crocus sativus* carry structures of endomycorrhizal fungi. Root mycorrhizal frequencies are very high in site 1 (93.33%); site 2 (96.67%); site 4 (90%) and in site 6 (93.33%). In these sites, the spore density is, respectively, 39, 58, 138, 99 spores / 100 g of soil. The frequency of root mycorrhization is lower at the site (76.66%) which also exhibited a spore density of 27 spores / 100 g of soil.

The identification of isolated spores made it possible to note the presence of 36 species belonging to 6 genera: *Glomus* (15 species), *Acaulospora* (10 species), *Scutellospora* (6 species), *Gigaspora* (2 species), *Pacispora* (2 species), *Entrophospora* (1 species). Species such as *Glomus clarum*, *G. etunicatum*, *G. aggregatum*, *G. intraradices*, *Acaulospora laevis*, *Scutellospora coralloidea*, were present in all studied sites.

The greatest richness of MA fungi was registers in the site at four successive years of exploitation by Saffron (24 species), with a Shannon diversity index H '= 2.82 which is the highest among all studied sites, followed by the site at six years of occupation by Saffron (21 species), with H '= 2.61, while the lowest number of species was recorded in sites of two, three and ten years of exploitation of sol by Saffron, with H '= 1.77, respectively; 2.12 and 2.44.

This decrease in endomycorrhizal species richness confirms that *Crocus sativus* residues are probably the cause. In fact, the prolonged occupation of plots with safrana has an allelopathic effect on mycoflora and on the yield of Saffron.

Keywords: Saffron, allelopathy, diversity, endomycorrhizal, Morocco.

In Morocco, saffron (*Crocus sativus* L.) has been cultivated for years in the Taliouine area (Taroudant Province) over an area of 565 ha, and more recently in the Taznakht area (Ouarzazate province) over an area of of 105 ha (Aboudrare et al., 2014). Although Morocco is a small producer (3 tons), it is ranked fourth producer of Safran after Iran, India and Greece, with only 1.5% of total world production (Dubois, 2010). Moroccan saffron is highly reputed nationally and internationally (Lage and Cantrell, 2009). It constitutes one of the main supports of the economy of the Taliouine-Taznakht region,

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characterized by difficult pedoclimatic conditions (Garcin and Carral, 2007), high rates of poverty and income inequality and a high level of rural-to-urban migration (Anonyme 1, 2004; Bouchelkha, 2009).

Among the objectives that have been highlighted by the strategy of the Green Morocco Plan in the Safran sector, 1. the increase in the area reserved for growing Saffron from 610 ha to 1,350 ha by 2020, 2. improvement of Safran's production to reach 9 tons by 2020, 3. increase of quantities exported to 6 tons per year and 4. strengthening of research and technology transfer programs (Anonyme 2, 2015).

The development of saffron's culture depends on the development of new production techniques adapted to the pedoclimatic conditions of the regions favorable to this crop. Several studies have shown that soil fertility and productivity are highly related to soil biological activity (Onguene, 2000). The development of plants depends on the interactions they have with the environment, particularly with soil microorganisms. This is the case of mycorrhizae which are symbiotic of plant roots (Smith and Read, 1997). The mutualistic association observed between vesicular and arbuscular mycorrhizas (MVA) and host plants are important in various natural and agricultural ecosystems (Sylvia and Williams, 1992). About 80% of the higher plants are associated with mycorrhizal arbuscular fungi (AMF) that provide the ecological stability of the environment (Harley and Smith, 1983; Strullu, 1991). Their importance in natural and semi-natural ecosystems is to improve the absorption of water and nutrients such as phosphorus, nitrogen and micronutrients, thereby improving plant growth and resistance to biotic and abiotic stresses (Goussous and Mohammad, 2009; Lone et al., 2015a, b).

The yield depends on the number of years of occupation of the plot by Saffron cultivation. In general, the yield decreases and over time the plot is no longer used for this crop. This decrease in yield is probably due to changes in the physicochemical and biochemical properties of the soil and changes in soil microorganism population structure that may occur after a period of Saffron cultivation on the same soil (Qarai and Beigi, 1995). Khozaei et al. (2015) observed that the continuous cultivation of Saffron on the same soil causes an undesirable change in the chemical and physical properties of the soil, the effect of these changes is very significant after six years of cultivation on the same soil, Jalali (1962) noted that after a period of cultivation, Saffron cannot be grown in the same soil. Azizi Zohan and Sepaskhah (2002) reported allelopathic effects and the accumulation of special salts in the root zone of saffron plants after a long period of cultivation on the same soil. The effect of these changes on the population structures of rhizosphere microorganisms in Saffron plants, the case of endomycorrhizal fungi, is not well known. This phenomenon is called allelopathy which is a problem that requires further research (Khozaei et al., 2015). Sharif and Moawad (2006) noted that the diversity of AM fungi species in agricultural systems is highly influenced by different types of inputs. The effect, for example, of the tissue extract of one plant species on the growth or reproduction of another species has been observed in many cases (Jadhav et al., 1997; Hoseini and Rizvi, 2003; Kobayashi, 2004).

In this work, the effect of the operating life of Saffron plants was studied on the diversity of endomycorrhizal fungi of Safran plants in the Taliouine (Tinfat) region of Morocco.

Materials and Methods

Prospecting and sampling

Soil samples were collected from six field sites in the Taliouine region (Tinfat), Province of Taroudant (Morocco). Each site is characterized by the number of years of land use by Saffron. The soil of the site 1 never carried saffron and the soils of sites 2, 3, 4, 5 and 6 were exploited, respectively, during 2, 3, 4, 5 and 6 years.

Soil samples were taken from the rhizosphere of *Crocus sativus* plants at a depth of 0–20 cm. Very fine roots, likely to be mycorrhized and easily observable under the microscope, were also taken with the soil.

Root coloring

The roots are cleaned of soil particles by thorough rinsing with tap water in a sieve. Then only the smallest fine roots are selected.

According to the lightening technique and Philips and Hayman (1970) coloring, the roots are cut into fragments of approximately 1 to 2 cm and placed in vials containing 10 ml of a potassium hydroxide solution (KOH) 10%. These flasks are then placed in a water bath at 90 °C for 15 min. The root fragments are then bleached by adding a few drops of H_2O_2 to the KOH solution. After 15 min, the fragments are rinsed with distilled water and then stained with a solution of Cresyl blue (0.05%) for 15 min.

Evaluation of mycorhization rate

Evaluation of the mycorrhizal parameters was performed by observing thirty root fragments of about 1 cm, randomly chosen to quantify the mycorrhizae (Kormanik and McGraw, 1982; Amir and Renard, 2003). These fragments are mounted parallel in groups of 10 to 15 in a drop of glycerine water between blade and coverslip (Kormanik and McGraw, 1982). Each fragment was thoroughly checked over its entire length, at magnifications $\times 100$ and $\times 400$.

The frequency and levels of arbuscules and vesicles of AMF within the root bark are measured by assigning a mycorrhizal index ranging from 0 to 5 (Derkowska et al., 2008):

0: absence; 1: traces; 2: less than 10%; 3: from 11 to 50%; 4: from 51 to 90%; 5: more than 91%

Frequency of mycorrhization (F%):

$$F\% = 100 \times (N - n_0) / N$$

– With, N: number of fragments observed and n_0: number of non-mycorrhizal fragments.

Intensity of mycorrhization (M%):

$$M\% = (95\,n5 + 70\,n4 + 30\,n3 + 5\,n2 + n1) / N$$

– With, n = number of affected fragments of the index 0, 1, 2, 3, 4 or 5

Teneur en arbuscules (A%):

$$A\% = (100\,mA3 + 50\,mA2 + 10\,mA1) / 100$$

Where mA3, mA2, mA1 are assigned, respectively, the notes A3, A2, A1, with, mA3 = (95 n5 A3 + 70 n4 A3 + 30 n3 A3 + 5 n2 A3 + n1 A3) / N. Similar is true for A1, A2.

In this formula, n5 A3 represents the number of fragments noted with A3; n4 A3 the number of fragments rated 4 with A3;

A0: no arbuscules; A1: few arbuscules 10%; A2: moderately abundant arbuscules 50%; A3: very abundant arbuscules: 100%.

Extraction of spores

The spores were extracted by the wet sieving method described by Gerdemann and Nicolson (Gerdemann and Nicholson, 1963; Nicolson and Johnston, 1979). In a 1-liter beaker, 100 g of each soil sample was immersed in 0.5 L of tap water and shaken with a spatula for 1 minute. After 10 to 30 seconds of settling, the supernatant is passed through four superposed sieves with decreasing meshes (500, 200, 80 and 50 μm). This operation was repeated twice. The contents retained by the 200, 80 and 50 micron sieves were divided into two tubes and centrifuged for 4 min at 9000 rpm. The supernatant is discarded and a viscosity gradient is created by adding 20 mL of a 40% sucrose solution into each centrifuge tube (Walker and Mize, 1982). The mixture is rapidly stirred and the tube returned to the centrifuge for 1 min at 9000 rpm. Unlike the first centrifugation operation, the supernatant is poured onto the 50 μm mesh sieve; the resulting substrate is rinsed with distilled water to eliminate sucrose, and then disinfected with an antibiotic solution (Streptomycin). The estimation of the number of spores in the soil was made by counting the spores contained in one mL of supernatant.

The characteristic structures (color, shape, size and number of separation membranes, etc.) of the spores are demonstrated by mounting between the slide and the plate of 0.1 mL of supernatant.

A preliminary identification of the spore genus was made based on the criteria proposed by Ferrer and Herrera (1981); Berch and Koske (1986); Schenck and Smith (1982); Hall (1987); Schenck and Perez (1987); Morton and Benny (1990); Walker and Mize (1982); Dalpé (1995); Mukerji and Kapoor (1986), and information available in different databases (Anonyme 3, 2016).

Specific richness and frequency of appearance of spores

The species richness represents the total number of species observed per collection site and the frequency of appearance of the species corresponds to the percentage of sites where each species is detected.

The Margalef index:

The Margalef index or Margalef biodiversity index is a measure used to estimate the biodiversity of a community based on the numerical distribution of individuals of different species according to the number of individuals in the sample (Margalef, 1958).

$$I_M = (S–1) / \text{Ln } N$$

Where:

S = number of species present;

N = total number of found individuals (belonging to all species);
Ln = natural logarithm of a number.

Shannon–Wiener index

The Shannon index is used to express diversity by taking into account the number of species and the abundance of individuals within each species. Thus, a community dominated by a species will have a lower coefficient than a community where all species co-dominate.

The index value ranges from 0 (one species, or one that largely dominates all others) to Ln S (when all species have the same abundance) (Shannon and Weaver, 1949).

$$H' = \sum_{i=1}^{S}\left[\left(\frac{ni}{N}\right)\ln\left(\frac{ni}{N}\right)\right]$$

Where:
S = total number of species;
ni = number of individual species in the sample;
N = total number of individuals of all species in the sample.

Results

In all the studied sites, the roots of Saffron were mycorrhizal. Different characteristic structures of arbuscular endomycorrhizae have been observed: arbuscules, intracellular and extracellular hyphae, spores and endophytes (Fig. 1).

Mean root mycorrhizal frequencies vary from one site to another (Table 1), the maximum value was 96.67% at the level of site 3, occupied for three years by Saffron, and the minimum value was 76.66% at the site operated for four years. The highest mean mycorrhizal intensity (45.9%) was noted in the roots of plants growing in a site operated for four years by Saffron and the lowest value (21.2%) in the site operated for ten years. The contents of the root on arbuscules were important at sites that carried the Saffron for four and six years, respectively, 44.9 – 45.3%.

The average number of spores noted in the rhizosphere of *Crocus sativus* plants is also a function of the studied sites. The lowest number is observed (27 spores / 100 g of soil) in the site exploited for 10 years (site 10) and the highest (138 spores / 100 g of soil) at the site which carried the saffron during 4 years.

Table 1

Mean frequencies and intensities of mycorhization and arbuscular content of the roots of saffron plants in the different studied sites

	Site 2	Site 3	Site 4	Site 6	Site 10
Frequency %	99.33	96.67	90	93.33	76.66
Mycorrhizal intensity %	31.96	30.33	45.9	32.3	21.2
Arbuscule %	25.6	28.2	44.9	45.3	21.2

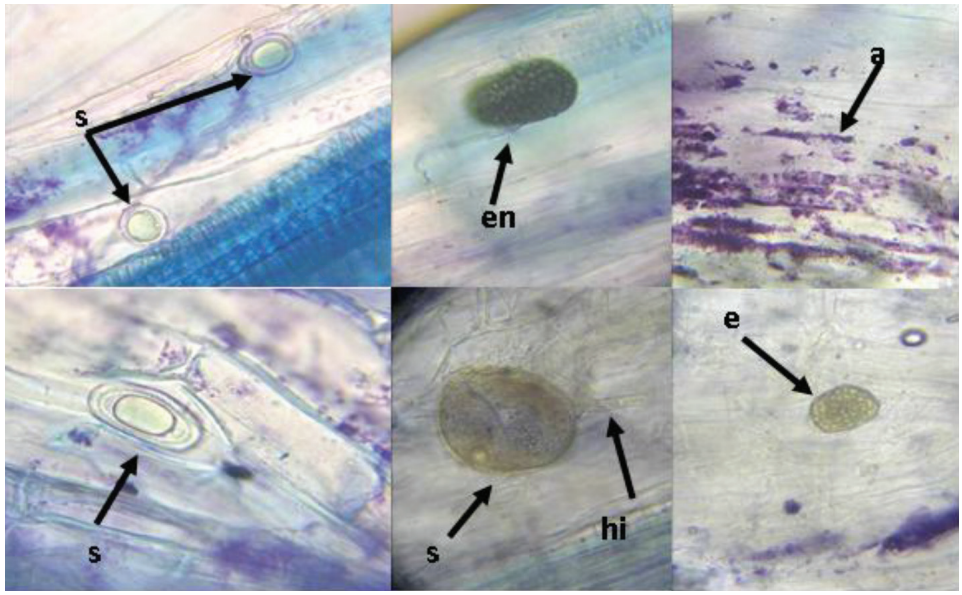

Fig. 1. *Crocus sativus* roots with arbuscular mycorrhizal structures: arbuscles (a); Intraductal hyphee (hi), spores (s); and endophytes (en). (G. ×400)

From these results, it appears that, in general, the optimum for all the mycorhization parameters is observed at the sites exploited during 3 (frequency of mycorrhization), 4 (intensity of mycorrhization and number of spores) or 6 (number of arbuscules) years.

MA fungi isolated from the saffron rhizosphere (*Crocus sativus*) of the studied sites were identified according to the morphological characteristics of the spores (Table 2 and Fig. 2).

Species richness (Table 4) varies from site to site. The highest number (24 species) is observed at the site occupied for 4 years by Saffron. The lowest number was recorded in sites operated during 2, 3 and 10 years of soil use by Saffron (9 and 10 species).

All the encountred species in the different studied sites belong to six genera: *Glomus* (15 species), *Acaulospora* (10 species), *Scutellospora* (6 species), *Gigaspora* (2 species), *Pacispora* (2 species), and *Entrophospora* (1 species).

In the site operated for two years by Saffron (Table 4), *Acaulospora* sp. 1 and *Glomus macrocarpum* are the most abundant species, with, respectively, a spore count of 16 and 6 spores / 100 g of soil. At the level of the exploited site during three years, the species which abound are *Acaulospora* sp. 1 (11 spore / 100 g of soil), *Glomus* sp. 1 and *Gigaspora decipiens,* with a number of spores count of 11 and 8 spore / 100 g soil. In the site occupied for four years by the Saffron, the most prominent species are *Entrophospora infrequens* (19 spores / 100 g soil), *Glomus aggregatum*, *Glomus etunicatum* (15 spores / 100 g soil), *Acaulospora laevis* and *Acaulospora* sp. 1*,* represented, respectively, by 13 and 10 spores / 100 g of soil). In the site cultivated for six years by Saffron, the most common species are *Glomus mosseae*, *G. aggregatum* and *Acaulospora denticulata*, with, respectively, 13, 9 and 8 spores / 100 g soil. In site 10, exploited during 10 years, the species encountered, with a low number of spores (4 spores / 100 g of the soil) are *Acaulospora acrobiculata*, *Glomus etunicatum*, *G. intraradices* and *Scutellospora coralloidea.*

In the five studied sites (Table 3), *Glomus macrocarpum, G. etunicatum, G. clarum* and *Acaulospora* sp. 1 are distributed in 4 different sites with a distribution percentage of 80%. *Acaulospora denticulata*, *A. laevis*, *Glomus aggregatum*, *G. intraradices*, *Glomus*. sp. 1, *Gigaspora decipiens*, *Scutellospora castanea* and are distributed in three different sites, with a distribution percentage of 60%. *Acaculospora mellea, A. morrowiae, Entrophospora infrequens, Glomus mosseae, G. heterosporum, Gigaspora* sp. 1, *Pacis-*

Table 2

Identification of mycorrhizal fungi isolated from the rhizosphere of *Crocus sativus* in the different study sites

N_0	Name	Form	Color	Size of the spore µm	Wall size μm	Length of hyphae μm	Surface of the spore
1	*Scutellospora* sp. 1	Globular	yellow	119.88	6.66	–	granular
2	*Glomus macrocarpum*	Globular	light brown	116.55	9.32	–	smooth
3	*Glomus aggregatum*	Globular	light brown	113.22	10	–	smooth
4	*Glomus heterosporum*	Globular	dark brown	96.57	8.65	–	smooth
5	*Glomus* sp. 2	Irregular	dark yellow	125.4	2.33	–	smooth
6	*Pacisporaboliviana*	Oval	dark yellow	176.49	11.65	–	smooth
7	*Acaulospora denticulata*	Irregular	yellow	127.87	3.33	–	smooth
8	*Glomus etunicatum*	Globular	brown	139.86	11.65	–	smooth
9	*Acaulospora morrowiae*	Globular	brown	106.52	1.9	79.92	granular
10	*Glomus intraradices*	Irregular	brown	73.26	16.65	–	granular
11	*Glomus deserticola*	Globular	pale yellow	103.23	4.9	–	granular
12	*Acaulospora* sp. 1	Globular	pale yellow	83.25	1.67	23.31	granular
13	*Scutellospora fulgida*	Irregular	dark yellow	113.22	4.23	–	granular
14	*Gigaspora decipiens*	Irregular	dark brown	99.9	4.29	136.53	smooth
15	*Glomus microcarpum*	Irregular	light brown	129.87	9.99	–	granular
16	*Scutellospora* sp. 2	Globular	yellow	89.91	3.9	–	granular
17	*Gigaspora* sp. 1	Oval	green	93.24	2.9	–	granular
18	*Acaulospora foveata*	Jaune	oval	49.95	5.32	33.3	granular
19	*Acaulospora colossica*	Globular	yellow	96.57	8.32	–	granular
20	*Acaulospora gedanensis*	Globular	hyalin	123.21	13.2	–	smooth
21	*A. laevis*	Oval	brown	96.57	6.66	–	granular
22	*Glomus margarita*	Globular	yellow	139.86	8.35	–	granular
23	*Scutellospora castanea*	Globular	yellow	73.26	3.33	–	granular
24	*Glomus* sp. 1	Globular	brown	89.91	3.33	–	smooth
25	*Glomus mosseae*	Globular	hyalin	89.91	9.99	–	smooth
26	*Glomus clarum*	Globular	brown	106.56	13.32	116.55	granular
27	*Entrophospora infrequens*	Globular	dark brown	63.27	3.33	–	granular
28	*Scutellospora heterogama*	Globular	brown	113.22	8.9	–	smooth
29	*Acaulospora mellea*	Globular	yellow	76.59	5	53.28	smooth
30	*Acaulospora scrobiculata*	Irregular	yellow	103.23	7.9	–	granular
31	*Acaulospora* sp. 2	Globular	brown	119.88	10	–	granular
32	*Glomus corymbiform*	Irregular	hyalin	136.53	3.33	–	granular
33	*Glomus eburneum*	Irregular	yellow	56.58	3.66	–	granular
34	*Glomus fecundisporum*	Oval	dark yellow	76.59	3.33	–	granular
35	*Pacispora* sp.	Globular	yellow	63.27	4.9	–	smooth
36	*Scutellospora coralloidea*	Globular	dark brown	149.85	6.66	–	smooth

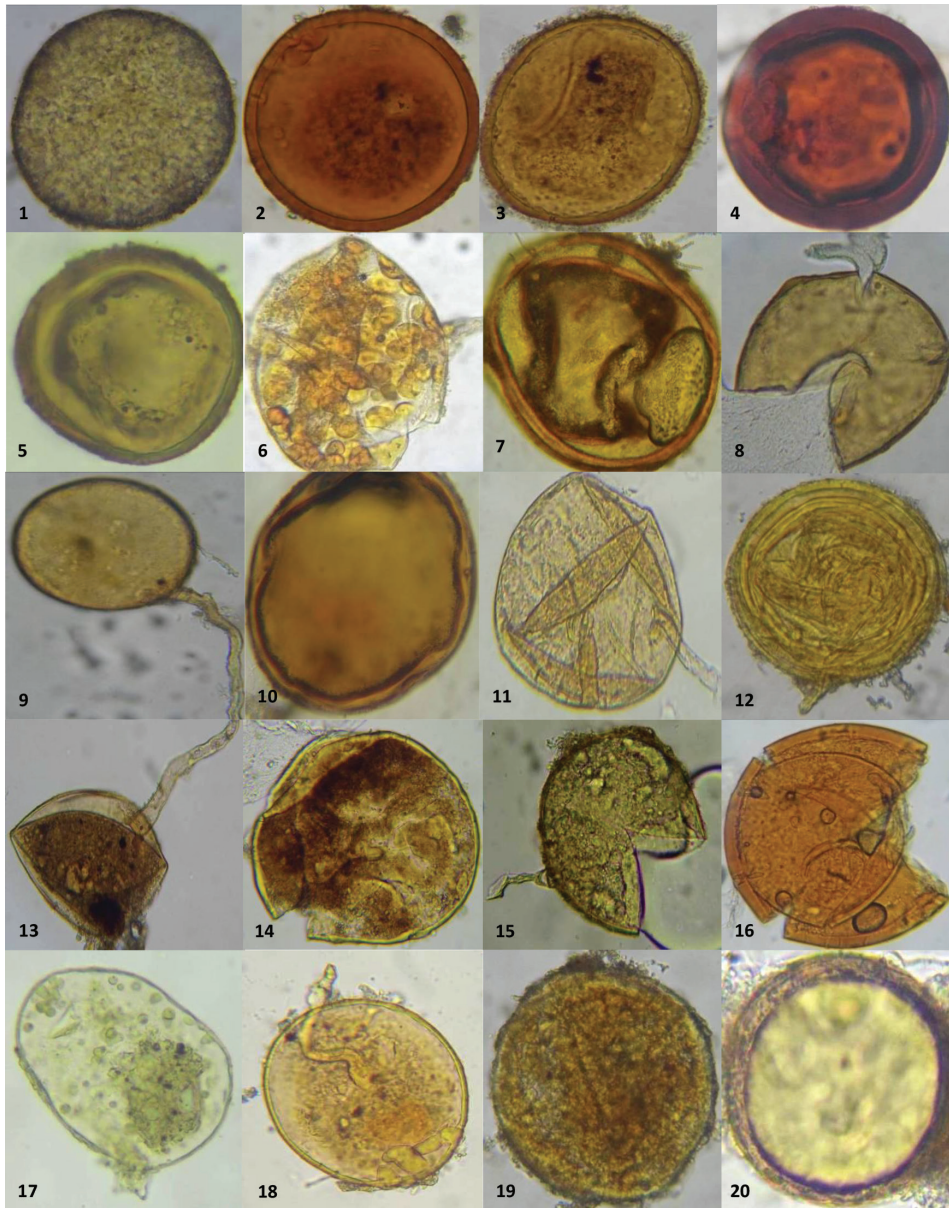

Fig. 2. Endomycorrhizal fungi isolated from the rhizosphere of *Crocus sativus*

pora boliviana, Pacispora sp. and *Scutellospora fulgida* are found in two different sites, with a distribution percentage of 40%. In contrast, *Acaulospora colossica*, *Acaulospora gedanensis, Acaulospora foveata, Acaulospora scrobiculata, Acaulospora* sp. 2, *Glomus corymbiform, G. adeserticola, G. eburneum, G. fecundisporum, G. margarita, G. microcarpum, Glomus* sp. 2, *Scutellospora heterogama, Scutellospora* sp. 1, *Scutellosporas* sp. 2 were observed in only one site, with a distribution percentage of 20%.

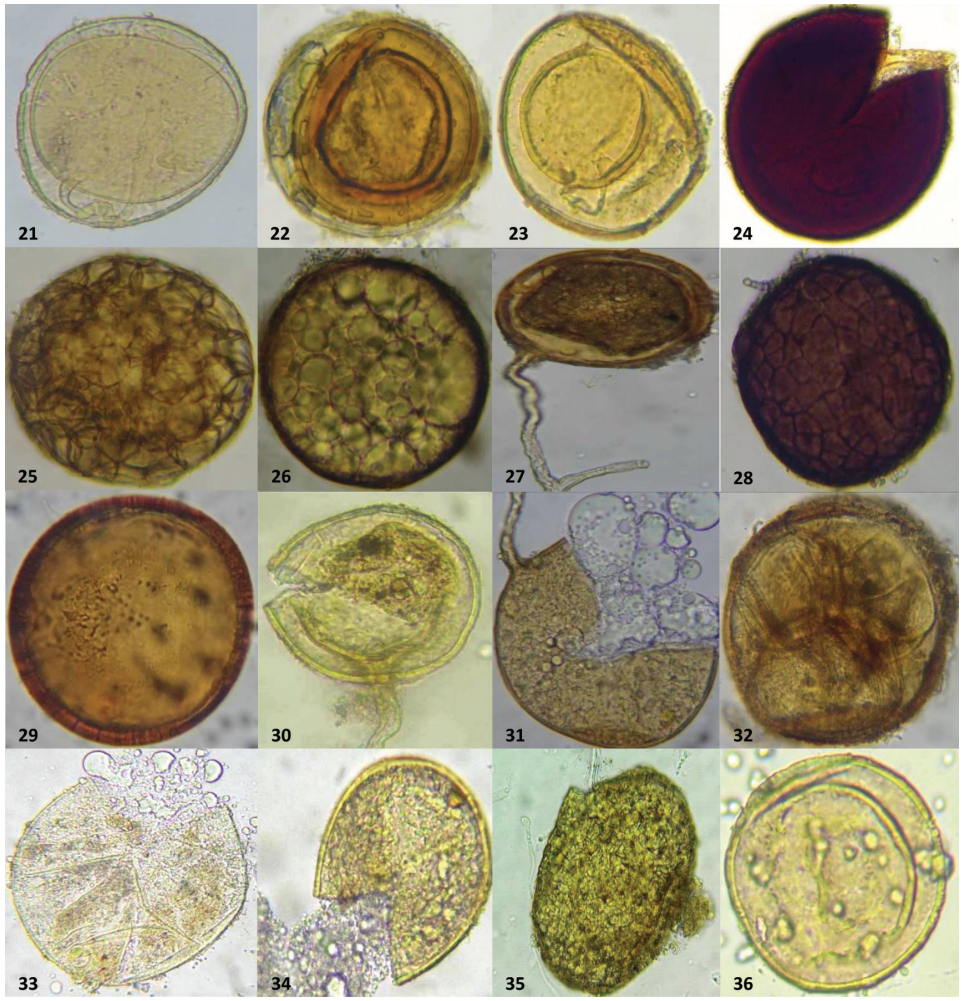

Fig. 2. (Cont.)

The distribution of the different genera of AM fungi in the studied sites is shown in Table 5. The *Glomus* and *Acaulospora* genera are present at six sites. The percentage of distribution of the genus *Glomus* varies from 8.33% to 30.56% and that of the *Acaulospora* genus, reaches 13.89% at the site which took six years of Saffron. The *Scutellospora* genus is present in four Saffron sites (site two, four, six and ten years), with distribution percentages ranging from 2.78% (two-year site) to 13.89% (six-year site). The *Entrospora*, *Gigaspora* and *Pacispora* genera are encountered in three different sites, with a distribution percentage not exceeding 5.56.

The diversity of AM fungi in the different studied sites varies from one site to another (Table 6). Shannon's diversity index is higher in the site operated for four years (H ' = 2.82), followed by the six-year site (H ' = 2.61). The site that has never been cultivated by Saffron recorded the lowest Shannon index (1.59).

Table 3

Frequency and percentage distribution of AM fungal species present in the soil of the different studied sites

Mycorrhizal species	Frequency of distribution	Percentage of distribution
Acaulospora colossica	1	20
Acaulospora gedanensis	1	20
Acaulospora denticulata	3	60
Acaulospora laevis	3	60
Acaulospora foveata	1	20
Acaulospora mellea	2	40
Acaulospora morrowiae	2	40
Acaulospora scrobiculata	1	20
Acaulospora sp. 1	4	80
Acaulospora sp. 2	1	20
Entrophospora infrequens	2	40
Glomus aggregatum	3	60
Glomus clarum	4	80
Glomus corymbiform	1	20
Glomus deserticola	1	20
Glomus eburneum	1	20
Glomus etunicatum	4	80
Glomus fecundisporum	1	20
Glomus intraradices	3	60
Glomus macrocarpum	4	80
Glomus margarita	1	20
Glomus microcarpum	1	20
Glomus mossea	2	40
Glomus heterosporum	2	40
Glomus sp. 1	3	60
Glomus sp. 2	1	20
Gigaspora decipiens	3	60
Gigaspora sp.	2	40
Pacispora boliviana	2	40
Pacispora sp.	2	40
Scutellospora castanea	3	60
Scutellospora coralloidea	3	60
Scutellospora fulgida	2	40
Scutellospora heterogama	1	20
Scutellospora sp. 1	1	20
Scutellospora sp. 2	1	20

The lowest species richness was recorded at the control site and at the operated sites for two, three and ten years, with Margalef (S) indices of 1.73, 2.18, 1.97 and 2.7. The greatest wealth was recorded at the sites exploited for four and six years, with S = 4.67 and 4.35).

Table 4

Number of spores of AM fungal types in all study sites (expressed as Nb spores/100 g of soil)

Endomycorrhizal species	Studied sites					
	Site 0	Site 2	Site 3	Site 4	Site 6	Site 10
A. colossica	1	5				
A. gedanensis					3	
A. denticulata			7	2	8	
A. laevis			5	13		3
A. foveata				3		
A. mellea				1	3	
A. morrowiae				1	3	
A. scrobiculata						4
Acaulospora sp. 1		16	11	10	2	
Acaulospora sp. 2	2			3		
E. infrequens	6		7	19		
G. aggregatum				15	9	2
G. clarum	3		6	1	4	3
G. corymbiform		2				
G. deserticola		1				
G. eburneum		1				
G. etunicatum			3	15	4	4
G. fecundisporum				4		
G. intraradices	5			6	5	4
G. macrocarpum	1	6	3	7	5	
G. margarita					3	
G. microcarpum					3	
G. mossea					13	3
G. heterosporum				5	2	
Glomus sp. 1			8		6	2
Glomus sp. 2					2	
G. decipiens		5	8	3		
Gigaspora sp. 2		2		5		
P. boliviana				1	3	
Pacispora sp.				2	3	
S. castanea		1		9		2
S. coralloidea				7	5	4
S. fulgida				3	7	
S. heterogama					6	
Scutellospora sp. 1				2		
Scutellospora sp. 2				1		
Total 36	18	43	58	138	99	27

Table 5

Frequency and percentage distribution of AM fungal genera in the studied sites (six sites)

	Site 0	Site 2	Site 3	Site 4	Site 6	Site 10
Acaulospora	5.55	5.56	8.33	19.44	13.89	5.56
Entrospora	2.78	0	2.78	2.78	0	0
Glomus	8.33	11.11	13.89	19.44	30.56	16.67
Gigaspora	0	5.56	2.78	5.56		0
Pacispora	0	0	0	5.56	5.56	5.56
Scutellospora	0	2.78	0	13.89	8.33	8.33

Table 6

Diversity of endomycorrhizal species in the studied sites

Ecological zones	Number of species (S)	Total number (N)	Margalef diversity index	Shannon diversity index
Site 0	6	18	1 .73	1.59
Site 2	9	39	2.18	1.77
Site 3	9	58	1.97	2.12
Site 4	24	138	4.67	2.82
Site 6	21	99	4.35	2.61
Site 10	10	27	2.73	2.44

Discussion

The analysis of the rhizosphere soils of Saffron has shown the existence of a diverse and extensive community of mycorrhizal fungi. Indeed, in this study, up to 36 arbuscular fungal species belonging to six genera were isolated and identified. The highest AM fungal richness was recorded in site 4, occupied for four years by Saffron (24 species), followed by site 6 (21 species), six years of occupation by Saffron, and the lowest number of species was recorded in the sites exploited for two, three and ten years, 9 and 10 species, respectively.

The *Glomus* genus exists in six studied sites with a proportion of distribution that varies between 8.33% in site 0 (control) and 30.56% in site 6. Various authors have associated the predominance of *Glomus* with its ability to produce, in a short time, more spores than other genera, case of *Gigaspora* and *Scutellospora* (Bever et al., 1996), and adapt to drought and soil salinity (Haas and Menge, 1990; Blaszkowski et al., 2002).

The diversity of AM fungi varies from one site to another, the site cultivated for four years by Saffron has recorded a value of Shannon's highest diversity index (H ' = 2.82). Mohankumar and Mahadevan (1987) noted that environmental factors such as soil pH, temperature, moisture, organic matter, and soil physical and chemical properties play an important role in the distribution of AM fungi. Other authors have noted that the diversity of AM fungal species decreases natural ecosystems to input-intensive farming systems (Hoseini and Rizvi, 2003), which is why plants can interact directly with other plants or with microorganisms by transmitting, receiving and responding to chemicals and other signals in their environment (Inderjit and Callaway, 2003). Saffron, medicinal plants, is among the plants that are known as strong allelopathic plants (Fuji et al., 1991). Some

studies have reported the presence of flavonoid components in the saffron petal extract, and high concentrations of aqueous extracts of these petals reduced the growth of the length of radicles and cotton coleoptiles (*Gossypium hirsutum* L.) (Eskandari et al., 2007).

The significant decrease in spore counts and changes in AM fungi populations during the years of soil use by Saffron are probably due to the accumulation of allelopathic substances in the soil. This allelopathic effect of the plant on MA fungi has been reported by Pellissier and Trosset (1989). These authors noted that the presence of *Molinia caerulea* has a negative effect on endomycorrhizal populations. For its part, Rice (1984) reported that allo-chemical substances released into the environment have a relationship with their concentration and age.

Javaid et al. (1995) reported that the roots of allelopathic grasses are less colonized by endomycorrhizae than non-allelopathic Gramineae. Afzal et al. (2000) noted that aqueous extracts of leaves of *Imperata cylindrica*, allelopathic grass, reduce root colonization of *Vigna radiata* (L.) Wlczek and *Phaseolus vulgaris* L. plants by endomycorrhizae. Rashed-Mohassel et al. (2006a) reported that aqueous extract of corms and saffron leaves inhibit seed germination and root growth in *Rapistrum rugosum* and *Rapistrum rugosum in vitro*. Rashed-Mohassel et al. (2006b) reported that extracts of Saffron and corms have an inhibitory effect on redroot pigweed (*Amaranthus retroflexus* L.).

Many countries use controlled mycorrhization of plants, a biotechnological technique used in nurseries to obtain more robust plants that are also resistant to attack by pathogenic organisms and to water stress after transplantation (Branzanti et al., 1992; Rai, 2001; Guissou, 2001; Estrada-Luna et al., 2003).

Conclusion

This study showed the existence of AM fungi in the rhizosphere of *Crocus sativus* in the Taliouine region (Tinfat) and showed a significant decrease in the number of spores and changes in AM fungi populations during the years of land use by Saffron due to the accumulation in the soil of substances of allelopathic nature.

The soil of this region is rich in endomycorrhizae provides favorable conditions for the growth and development of *Crocus sativus* and increased tolerance to abiotic stress conditions (allelopathic substance, drought, salinity of water or soil) and microbiotic pathogenic organisms. The soil of *Crocus sativus* has a reserve of mycorrhizal fungi, can be isolated and used in the improvement and production of Saffron.

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