

Endophytic Mycobiota of *Schinus molle* L.,
(Anacardiaceae) and *Peganum harmala* L.,
(Tetradiclidaceae) in Algeria

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Abstract

Schinus molle is an arborescent plant that is common in North Africa. It has insecticidal and pharmaceutical activities that are assigned to some active compounds. Harmel is a cosmopolitan species extensively distributed in the world and very common in Algeria especially in the Sahara. This plant is very rich in alkaloids of the β -carboline group of

which most important are harmine. It has pharmaceutical and agrochemical virtues. Endophytic fungi were isolated from aerial part (leaves and fruits) of Peruvian peppertree (*S. molle* L.) Anacardiaceae and for the first time of the Harmel (*Peganum harmala* L.) Tetradielidaceae. Twenty three mycotaxa were identified whose eleven isolated from *S. molle* and twelve isolated from the second plant. The vast majority are included in mitosporic group (Deuteromycota). The frequency of colonization or infection (FC%) at leaves of the two plants species is significant comparatively to the fruits.

Keywords: peruvian peppertree, harmel, mycoendophytes, fungi, identification, colonization frequency

1. Introduction

Endophytes are microbes that colonize living, internal tissues of plants without causing, any immediate overt negative effects (Stone *et al.* 2000). As almost all vascular plant species appear to be inhabited by endophytic bacteria or fungi, these represent important components of microbial diversity. The relationship between the host plant and its endophytic shows symbiotic characteristics as the endophytic occupant usually obtains nutrients and protection from the host plant and in return profoundly enhances the fitness of the host by producing certain functional metabolites (Bharathidasan and Panneerselvam 2015).

Schinus molle is an arborescent plant that is common in the tropical regions, more especially in South America and Africa, but spreads as well to the moderated zone of the northern hemisphere. *S. molle* has insecticidal and pharmaceutical activities (Orozco and Pentz 2005; Ferrero, Chopa *et al.* 2007). These virtues are essentially assigned to some active components; tannins, triterpenoids, flavonoids, saponins, alkaloids, sterols, terpens, resins and of the essential oils (Cavalher-Machado *et al.* 2008).

The Harmel; *Peganum harmala* is a cosmopolitan species extensively distributed in the world and very common in sandy lands, original of the arid, semi-arid zones and the steppes (Lamchouri *et al.* 2013). This plant is common in North Africa, the middle East, Pakistan, India, Iran, Turkey and has been introduced in America and Australia (Moshiri *et al.* 2013). In Algeria, this species is common in the high regions of the north, southern Sahara and the mountains of central Sahara.

It is a plant very rich in alkaloids, peganine, isopeganine, dipeganine and the quinazolic derivatives. This species has pharmaceutical and agrochemical virtues and is essentially assigned to the wealth of this plant in alkaloids of the β -carboline group of which most important are harmine, harmaline, harmol and harmalol.

It is this context justifies the present survey aiming to identify the mycobiota endophytes from these two plant species, and to see if these two plants, already well known for their medical and agrochemical virtues, can serve like source of endophytic fungi.

2. Materials and Methods

2.1 Sample Collection

We opted for the peruvian peppertree and the harmel as two plant species targeted at the rate of their importance in various domains particularly the agrochemical domain, as well as, their chemical compositions.

For the detection and the isolation of endophytic fungi, the aerial parts of the two plants; leaves and fruits were chosen. For the sampling of the foliar part, we chose the most aged and nature leaves because of the importance of limb surface as well as the chemical composition compared to the young leaves. On the other hand, the mature fruits are selected for the sampling. Leaves and fruits were collected from the surroundings of Batna city-Eastern Algeria-(N 35 54 18,56, E 006 15 05,52) in rainy period (January). The collected leaves and fruits were washed with steril water and carefully stored in polyphene bags and transported to the laboratory for the mycological examination.

2.2 Sterilization Surface

The objective of surface sterilization is to eliminate hyphes and spores of epiphytes organisms. We opted thus for the modified protocol of Tejesvi *et al.* (2006). Sterilized once, leaves of two plants are cut with sterilized blade. The resulting fragments vary in length of 2 mm to 4 mm. These fragments that will be seeded on the culture medium.

2.3 Culture of Fragments

The procedure of isolation of endophytic fungi opted for a sowing on the PDA medium (*Potato Dextrose Agar*) amended to the tetracycline (100µg/ml), a general antibiotic of action that prevent the bacterial contamination.

The fragments are seeded on Petri dishes containing the PDA. For each of these Petri dishes and for every plant species, we fix 10 fragments. To the total, we have 100 fragments by 10 Petri dishes for every organ/species (200 fragments to the total for each of the two plant species and 400 fragments to the general total). After the sowing, plates were incubated in a dust free cupbroad at the room temperature (25°C) for 5-7 days. Periodically, cultures are examined in order to observe the development of the emergent fungal colonies of the extremities of the fragments treated.

2.4 Observation

The colonies growing from leaves and fruits fragments on PDA plates with different morphology were observed. The fungal cultures were then transferred, subcultured and the pure cultures were maintained on PDA medium. A portion of the growing edge of the colony was picked up with the help of a pair of needles and mounted on a clean slide with lactophenol cotton blue strain. The slide was gently heated in a spirit lamp so as to facilitate the staining and remove air bubbles. If any, the excess stain was removed with the help of tissue paper and then the cover slip was sealed with transparent nail polish. The slide was observed under a compound microscope.

2.5 Frequency of Colonization (FC%)

The frequency of colonization or infection (FC%) is calculated while being based on the method of Fisher and Petrini (1987) In Tejesvi *et al.* (2006), given like follows:

$$FC \% = N_c / N_t \times 100$$

Where: **FC** = Frequency of colonization or infection.

N_c = The number of segments colonized by a species or a fungal group.

N_t = The total number of the segments.

2.6 Identification of Fungi

The isolates were grouped into sporulating and non sporulating forms. Sporulating structures were considered as diagnostic characters in the identification of fungi. Using standard taxonomic (Ellis 1971; Ellis 1976; Barnett and Hunter 1998), the isolates were identified and assigned to respective genera and species. In the absence of sporulation, the taxonomic identity of several non sporulating forms recovered is not known and they are recognized merely based on cultural characters such as colony morphology, growth rate and pigmentation (sterile mycelium).

3. Results

3.1 Frequency of Colonization or Infection (FC%)

The frequency of colonization or infection (FC%) for the selected fragments is represented in the table 1.

Table 1. Frequency of colonization (FC%) at *S. molle* and *P. harmala*

Species Organ	<i>S. molle</i>	<i>P. harmala</i>
Leaves	95%	98%
Fruits	88%	56%

3.2 Specific Composition of Fungal Isolates Detected at *S. Molle* and *P. Harmala*

We have twenty three (23) fungi isolates of which eleven (11) mycotaxons is identified from the *S. molle* and twelve (12) fungi are isolated from the second plant species (table 2 and figures 1, 2).

Table 2. Composition of mycoendophytes detected at *S. molle* and *P. harmala*

Plant species	Mycotaxon	Class
<i>S. molle</i>	Mitosporic fungi	Hyphomycota
	<i>Penicillium</i> sp.	
	<i>Penicillium italicum</i>	
	<i>Fusarium solani</i>	
	<i>Fusarium oxysporum</i>	
	<i>Alternaria</i> sp.	
	<i>Curvularia</i> sp.	
	<i>Trichoderma</i> sp.	
	<i>Torula</i> sp.	
	<i>Chaetomium</i> sp.2	Ascomycota
	<i>Mucor</i> sp.	Zygomycota
	Sterile shapes	-
Sterile mycelium (SM)		
<i>P. harmala</i>	Mitosporic fungi	Hyphomycota
	<i>Alternaria</i> sp.	
	<i>Fusarium</i> sp.	
	<i>Penicillium</i> sp.	
	<i>Epicoccum</i> sp.	
	<i>Gilmaniella</i> sp.	
	<i>Torula</i> sp.	
	<i>Ulocladium</i> sp.	
	<i>Botryoderma</i> sp.	
	<i>Chaetomium</i> sp.1	Ascomycota
	Ascomycete 1	
	Ascomycete 2	
	<i>Phomopsis</i> sp.	Coelomycota

According to results, the qualitative status of mycobiota endophytes detected on plant species, we reveal that the number of isolated mycoendophytes is relatively insignificant.

For the first plant species (*S. molle*), the group of mitosporic fungi and specifically the class of Hyphomycota contains the most dominant genus (*Penicillium*, *Fusarium*, *Alternaria*, *Curvularia*, *Trichoderma* and *Torula*) comes first, then the class of Ascomycota and Zygomycota with only one genus for each; *Chaetomium* and *Mucor* respectively.

On the other hand, *P. harmala* contains eight (8) genus in the class of Hyphomycota (*Alternaria*, *Fusarium*, *Penicillium*, *Epicoccum*, *Gilmaniella*, *Torula*, *Ulocladium* and *Botryoderma*). The class of Ascomycota is composed of three (3) genus with only one genus (*Phomopsis*) for the class of Coelomycota.

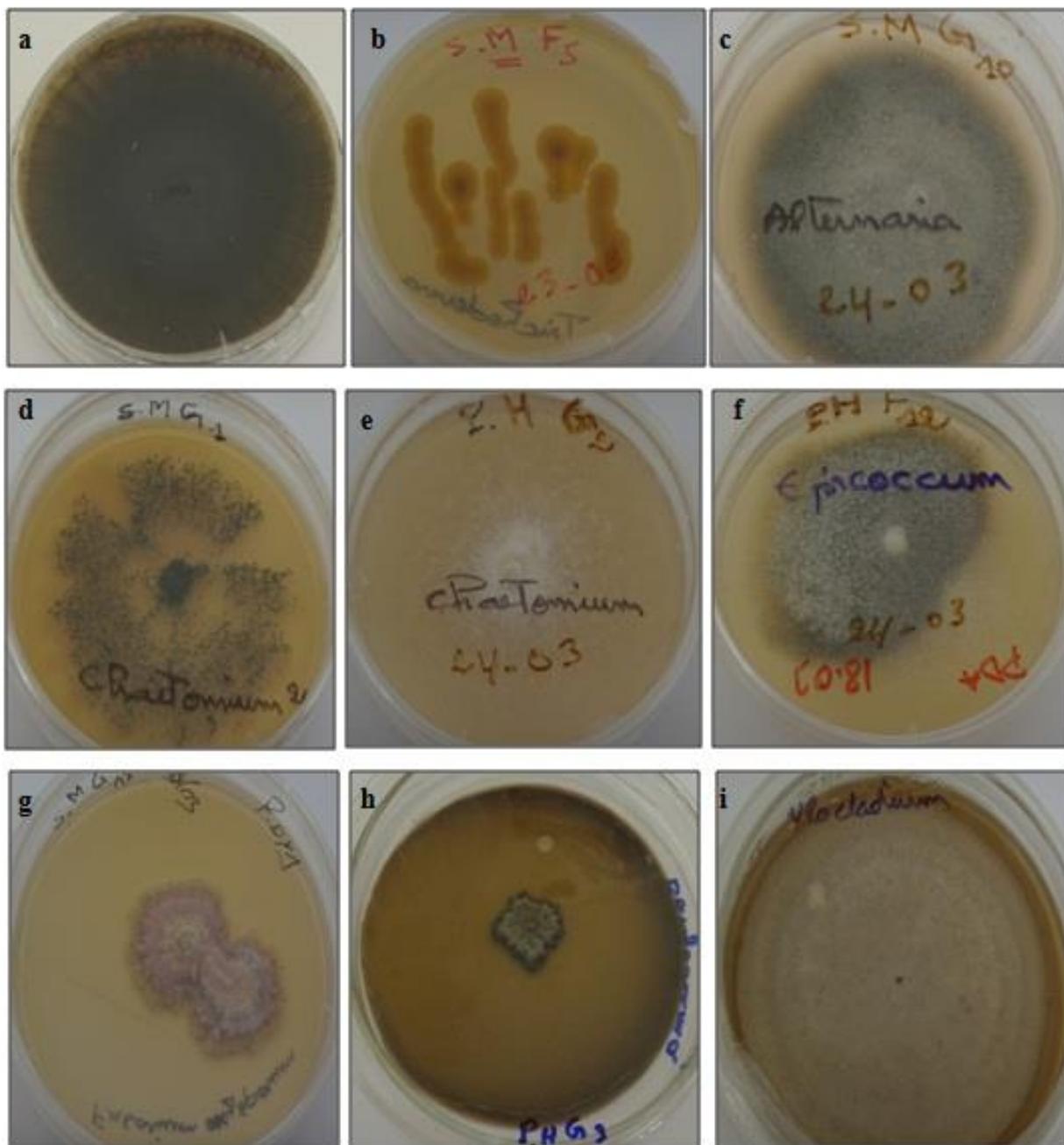


Figure 1. Examples of pure cultures of the endophytic fungi isolated from *S. molle* and *P. harmala*; (a): *Curvularia* sp., (b): *Trichoderma* sp., (c): *Alternaria* sp., (d), (e): *Chaetomium* sp., (f): *Epicoccum* sp., (g): *Fusarium oxysporum*, (h): *Botryoderma* sp., (i): *Ulocladium* sp.

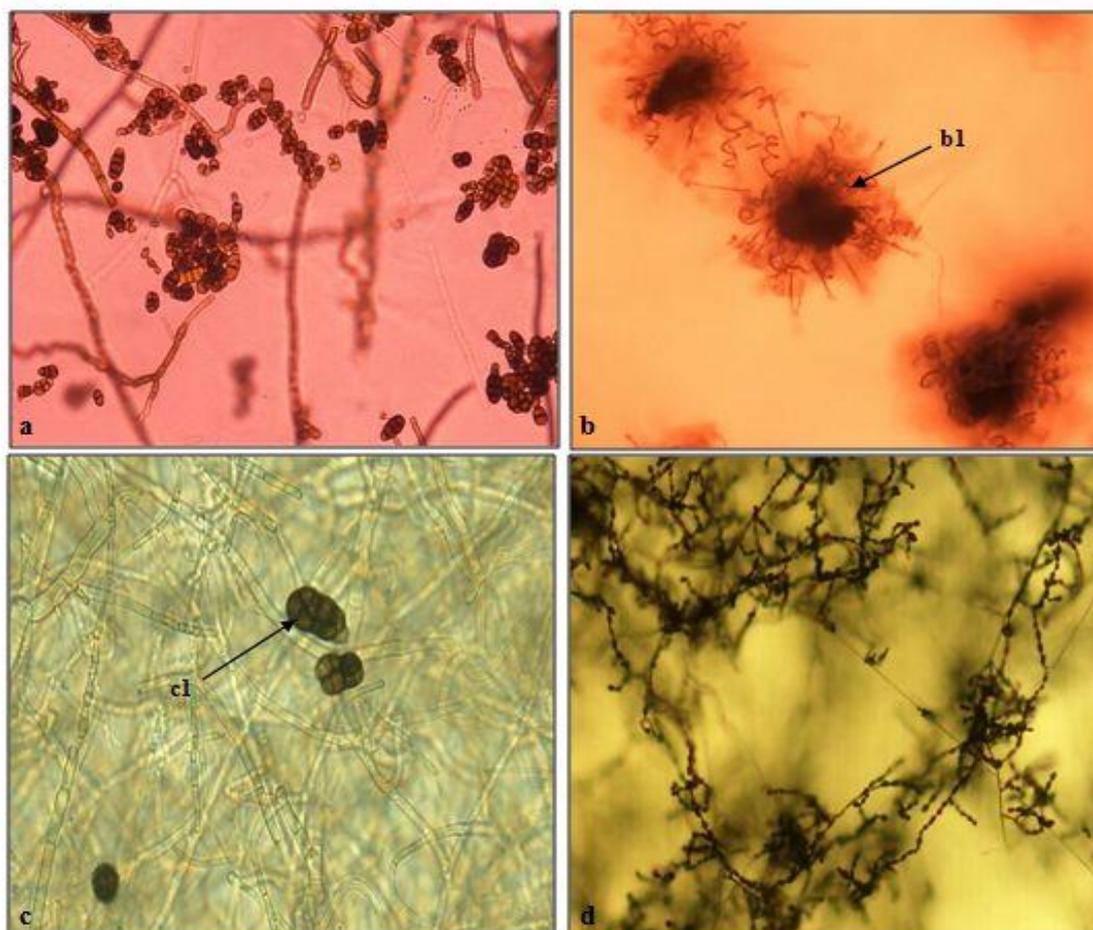


Figure 2. Microscopic illustrations of some mycotaxons; (a): *Curvularia* sp. (G×100), (b): *Chaetomium* sp. (b1: perithecium) (G×100), (c): *Ulocladium* sp. (c1: conidia) (G×100), (d): *Alternaria* sp. (G×40)

4. Discussion

Our work demonstrates the presence of endophytic fungi associated to leaves and fruits of the two studied plant species; peruvian peppertree (*S. molle*) and for the first time at the harmel (*P. harmala*). Endophytic fungi are one of the groups of microorganisms that are very diverse and unexplored. These make symbiotic associations and can produce beneficial substances for the host (Khan *et al.* 2007).

We noted that the colonization of leaves and fruits by mycoendophytes is significant on the quantitative statute, indeed, the ubiquity of endophytes is an aspect shared between various studied phytotaxons. This aspect can be bound to an anatomical, biochemical, and physiological predispositions of studied aerial parts, opposite to the infections and colonizations of endophytes. Thus, the ubiquity of endophytes in nature is always selective, this selective colonization can lead to the production of bioactives substances in the host plant (Huang *et al.* 2008).

The rate of infection of the aerial parts; leaves and fruits, generally evolves with the age of these last. Kumaresan and Suryanarayanan (2002) demonstrated that the rate of infection of *Rhizophora apiculata* (Rhizophoraceae) increases for the mature fruits and adult leaves. Goveas *et al.* (2011) demonstrated that the frequency of colonization of the medicinal plant *Coscinium fenestratum* (Menispermaceae) by endophytes increases with the age of leaves; 3,33% for the young leaves, 21,02% for the mature leaves and 53,33% for the adult leaves.

This colonization raised of the aged leaves is with time due to a superinfection of leaves by the inoculum carried with air (Suryanarayanan and Thennarasan 2004).

The same observations have been expressed for conifers, *Plumeria rubra*, *Trachycarpus fortunei*, *Azadirachta indica* (Suryanarayanan and Thennarasan 2004), the Gramineous (Bacon and White 1994), the Palmaceses (Rodrigues 1994), the Gymnospermeses and at some tropical plants (Kumaresan and Suryanarayanan 2002).

On the other hand, no information is available, concerning the mechanisms reacting the modes of the natural infection as well as the internalisation of fungi, within tissues of the two studied plant species; *S. molle* and *P. harmala*.

The number of isolated fungi is relatively insignificant with the predominance of the hyphomycetes fungi, in particular, *Fusarium*, *Torula* and *Alternaria*. This very low number can be explained by the presence of chemical factors illustrated by specific compounds within the host plant as the sesquiterpens, flavonoids, tannins as well as the resins (Goveas *et al.* 2011).

According to Clay (1993) the host plant can be at the origin of some secondary metabolites that cannot only inhibit the growth of endophytes on their tissues, but also to limit their propagation and even to restrict the number of associated fungi. This observation can be projected on our results to explain the fact that some endophytes, as *Fusarium* and *Chaetomium*, are especially frequent, notably on the fruits of *S. molle* and the leaves of the second plant; *P. harmala*.

The majority of endophytes detected are arranged within the mitosporic group (Deuteromycete). Practically, a multitude of conducted research on the subject, attest that the Deuteromycetes that represent the sexual formes, predominate regardless of the taxonomic nature of the host plant (Marquez *et al.* 2007; Huang *et al.* 2008; Costa *et al.* 2012; Singh 2013).

In contrast with our work, the survey of Rodrigues and Samuels (1999) range on a medicinal plant Anacardiaceae; *Spondias mombin*, proved that the dominance endophyte is for the Ascomycete; *Phomopsis* sp.

The mitosporic fungi specifically the class of the Hyphomycota has been considered as the most predominant group in the majority of the plants colonized by the mycoendophytes, it is justified by a lot of studies (Seena and Sridhar 2004; Khan *et al.* 2007; Mangunwardoyo *et al.* 2012; Jena and Tayung 2013; Han *et al.* 2013; Sharma and Kumar 2013; Shi *et al.* 2013; Suciati and Rahmansyah 2013; D'souza and Bhat 2013). The nature of dominance of the

Hyphomycetes can be assigned to their capacity to colonize their host quickly while producing abundant asexual spores (Jena and Tayung 2013).

This class predominates the species *Azadirachta indica* (Meliaceae) with a rate of colonization of 61%, followed by the Ascomycota (21%) and the sterile mycelium (18%). The genus *Fusarium*, *Trichoderma* and *Chaetomium* being the most frequently isolated (Tejesvi *et al.* 2006). The same results have been expressed for the two medicinal plants; *Solanum rubrum* and *Morinda pubescence* where the fungal composition for the first species is of 65% Hyphomycota, 10% Coelomycota and 10% sterile mycelium, while the rate of colonization is of 86% Hyphomycota, 8% Ascomycota, 4% Coelomycota and 16% sterile mycelium for the second medicinal plant (Jena and Tayung 2013).

The genus of *Trichoderma* and *Fusarium* are isolated in this study as endophytes. Whereas these two fungi are fundamentally pathogenic fungi, but sometimes, they are modified by mutation and become non-pathogenic endophytes (Nagaraja 2011). However, in the last years, *Trichoderma* sp. has also been returned like endophytic fungi (Chaverri *et al.* 2011).

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