

Full Length Research Paper

The pattern of pathogen diversity and abundance in Lentil (*Lens culinaris*) fields in Constantine region, Algeria

Hiba Almi*, Laid Dehimat and Noredine Kacem Chaouche

Laboratory of Mycology, Biotechnology and Microbial Activity, SNV, University of Constantine -1 Constantine, 25000 Algeria.

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Lenses are a group of pulses having a socio-economic and nutritional significance. The study was conducted on two fields of *Lens culinaris* in Constantine region contaminated with molds, had the objective to put a relationship between pathogenic molds associated with these plants and their environment. This study revealed the presence of 20 genera in soils (*Absidia*, *Acremonium*, *Alternaria*, *Aspergillus*, *Bysochlamyces*, *Chaetomium*, *Cladosporium*, *Emericella*, *Eurotium*, *Fusarium*, *Mucor*, *Paecilomyces*, *Penicillium*, *Peronospora*, *Phytophthora*, *Pseudallescheria*, *Scopulariopsis*, *Scytalidium*, *Trichoderma* and *Ulocladium*) and 20 genera also in plants (*Absidia*, *Acremonium*, *Alternaria*, *Aspergillus*, *Botrytis*, *Chaetomium*, *Cladosporium*, *Cylindrosporium*, *Curvularia*, *Eurotium*, *Fusarium*, *Myrothecium*, *Onychocola*, *Phytophthora*, *Pseudallescheria*, *Penicillium*, *Peronospora*, *Rhizoctonia*, *Trichoderma* and *Ulocladium*). They contribute approximately 54% of the total micropopulation enumerated in studied samples. The development of these pathogenic strains is governed by environmental conditions namely the chemical elements in soil, pH, electrical conductivity, Nitrogen, Carbon and saturation. The results we have obtained shows that the chemical variations ground contribute to the right development fungi and their transfer to plants

Key words: *Lens culinaris*, soil, fungi.

INTRODUCTION

Lentil is a dicotyledonous legume plant and takes the fifth place in production of pulse crop in the world (Hymowitz, 1990). Lentil is still widely used today because of its high fiber, protein, vitamin and mineral content (Hnatowich, 2000) and considered as one of the best vegetable source of Iron especially important for adolescent and pregnant women. Furthermore, lentil is cultivated in

sandy loam soil and can be grown in nutrient deficient soil (Summerfield, 1981). Lentils are drought resistant and can be grown in water logged and saline soils (Muehlbauer et al., 2002).

In Constantine, lentil production has tripled in the last six years from 37,120 kg in 2006 to 710,074 kg in 2013. A survey of literature showed that many fungal species

*Corresponding author. E-mail: hibabio27@gmail.com

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have been reported for lentil seeds including species of *Pythium* sp., *Rhizoctonia* sp., *Sclerotium* sp., *Fusarium* sp., (Muehlbauer et al., 2002), *Alternaria* sp., *Aspergillus* sp., *Mucor* sp., *Chaetomium* sp., *Penicillium* sp., *Nigrospora* sp. (Hussain et al., 2007), *Uromyces fabae* and *Botrytis* sp. (Richardson, 1979). However, the presence of so much pathogenic fungi reduces the quality and quantity of crop which in turn lead to economic losses.

Soil is the most important resource of the nature (Sumithra et al., 2013), it is a thin layer of the earth's crust which serves as a natural medium for the growth of plants because the components of soils are mineral material, organic matter, water and air. Soil has important ecological functions in recycling resources needed for plant growth and microorganisms' development, the soil forms a large reservoir for many potential plant pathogens and especially plants with a decreased vitality (Katarzyna and Christel, 2011). An extremely simplified vegetation, such as a monoculture, selects a specific microbial community, including plant pathogenic microorganisms and sometimes also their parasites or antagonists (Bruggen et al., 2006).

This study aimed to reveal the complex interactions within the infected soil-lentil ecosystem and the aggressive behavior of molds.

MATERIALS AND METHODS

Studied area

The field experiments were conducted in 2011 and 2012 on two agricultural soils: AIN SEMARA and BARAOUIA situated in the North East of Algeria.

Sampling procedures in the study area:

Soil sampling

Soil samples taken at 5, 10 and 15 cm from the soil surface were collected from four locations in the vicinity of the two areas (AIN SEMARA and BARAOUIA), in sterile paper bags. The soils collected are ground and passed through 0.2 mm sieve and were used for the analysis.

Plant sampling

Lentil plants from Metropole variety (20 samples), were collected from different places in fields infected by many types of mold. These plants were collected in sterile paper bags.

Physical and chemical properties analysis

The physicochemical properties are executed in a laboratory of soil chemistry. The important parameters studied are: available Nitrogen, Organic Carbon, Electrical conductivity and pH value.

Available Nitrogen

The dosage of Nitrogen is effected using berthelot reaction (Krom,

1980; Searle, 1984, EN ISO 11732 (2013)) modified by Belahrache (Belahrache, 2013).

Organic Carbon

The Walkey and Black (1934) method described by Nelson and Sommers in 1982 is used for assaying the organic carbon in simple soils.

Electrical conductivity

The assay method involves wet ground with 100 g of distilled water slurry. The paste obtained was centrifuged at 300 revolutions / min for 30 min. In the end, the temperature of the float is noted in the resistance, is measured after adjusting the temperature to 25°C, using a conductivity meter (Anonymous, 1954).

pH

The method relies on the preparation of a solution (diluted suspension) of ground 10 g and 25 mL of distilled water, after magnetic stirring, the pH is measured at room temperature using a pH meter (Boudoudou et al., 2009).

Mycoflora isolation

From soil (Dilution plating method)

To isolate the fungi from the soil sample, 1 g was diluted in 9 mL of saline solution. 1 mL of the diluted sample was poured and spread on Petri plates containing sterilized PDA medium. The inoculated plates were incubated at a temperature of 27°C for 3 days (Warcup, 1950). To prevent bacterial growth, one milligram of chloramphenicol was added to the medium.

From plants

Infected plants collected from the websites surveyed (AIN SEMARA and ABARAOUIA), was cut into small pieces of about 0.5 cm. The pieces are disinfected with sodium hypochlorite (2%) for 3 min, then rinsed several times with sterile distilled water and ethanol (70%) and dried between sterile filter paper, then placed in Petri dishes (5 piece / Petri plates) containing a culture medium PDA, MEA5 or MS. Finally, the Petri plates were incubated at 27°C for 4 days (Belabid et al., 2000).

Mycoflora identification

Colony colour, odor, morphology and growth rate were noted besides hyphal structure, spore size, shapes and spores bearing structures. They were compared with the standard works of Packer and Thomas (1990), Rinaldi et al. (1998), Gams et al. (1998), Botton et al. (1999), and Snavi (1999).

Presentation of data

The number of genera is referred to as genera diversity. Population density is expressed in terms of colony forming unit (CFU) per gram of soil with dilution factors. In order to assert the dominance of the genus in site, percentage contribution was worked out as follows:

Table 1. Results of physico-chemical analysis of soils in Constantine region.

Parameter	BARAOUIA			AIN SEMARA		
	5	10	15	5	10	15
Depth (cm)	5	10	15	5	10	15
Organic matter (%)	1.720	1.978	1.686	1.892	1.858	1.686
Carbon (‰)	10.0	11.5	9.8	11.0	10.8	9.8
Nitrogen (‰)	0.76	0.89	0.70	0.95	0.82	0.80
C/N	13	13	14	12	13	12
pH	7.86	7.76	7.77	7.69	7.79	7.83
EC (milliohm/cm)	0.50	0.93	0.70	0.65	0.52	0.35

$$\% \text{ contribution} = \frac{\text{Number of colonies of the same genus in a sample}}{\text{Total number of all colonies of all the genera in a sample}}$$

RESULTS

Physical and chemical properties analysis

The results of the physical and chemical properties analysis of two soil samples (with different depth), have revealed that soils of AIN SEMARA and BARAOUIA are rich in organic matter (>1) and by consequence Carbon and Nitrogen. In addition, the organic matter is rich in carbon and poor in Nitrogen. Furthermore, results of pH analysis show that values are slightly alkaline (between 7.69 and 7.86). The measure of electrical conductivity (EC) and the salinity factor are indicated to lower than 1; which means that the soils were not salty (Table 1).

Mycoflora isolation

From soil

Altogether, six soil samples from two different stations representing the entire Constantine district were examined for fungal diversity. The study resulted in the presence of 44 species of fungi belonging to 14 genera from BARAOUIA and 42 species of fungi belonging to 16 genera from AIN SEMARA (Figure 1).

Indeed, the soil sample of BARAOUIA is characterized by the presence of 68.21% Deutromycetes, 20.43% Ascomycetes, 6.82% Oomycetes and 2.27% of Phycomycetes and Zygomycetes. The soil samples of AIN SAMARA contain less Deutromycetes (50%), Ascomycetes (35.72%) and Zygomycetes (9.52%) but more Oomycetes (4.76%) (Figure 2).

The quantitative comparison of genera shows that high percentage belongs to the *Fusarium*, *Penicilium* and *Aspergillus* in two studied areas. By contrast, the other genera have variable percentages in both sites (Figure 3). The dominant genera from the field soils of BARAOUIA are *Fusarium* and *Penicillium* (9 species each), followed

by *Aspergillus* (8 species), *Phytophthora* (3 species), *Acremonium*, *Alternaria*, *Chaetomium*, *Cladosporium* and *Paecilomyces* (2 species each) and finally *Mucor*, *Peronospora*, *Scopulariopsis*, *Trichoderma* and *Ulocladium* (1 species each); whereas in AIN SEMARA soils, the dominant species were *Aspergillus* (9 species), *Penicillium* (5 species) and *Fusarium* and *Absidia* (4 species each) followed by *Eurotium* and *Scytalidium* (3 species each), *Alternaria*, *Paecilomyces*, *Phytophthora* and *Ulocladium* (2 species each), and finally *Acremonium*, *Bysochlamyces*, *Cladosporium*, *Emericella*, *Pseudallesheria* and *Trichoderma* (1 species each).

From plants

A total number of 110 species belonging to 18 genera of fungi: *Absidia*, *Acremonium*, *Alternaria*, *Aspergillus*, *Botrytis*, *Chaetomium*, *Cladosporium*, *Cylindrosporium*, *Curvularia*, *Eurotium*, *Fusarium*, *Myrothecium*, *Onychocola*, *Penicillium*, *Peronospora*, *Phytophthora*, *Pseudallesheria* and *Rhizoctonia* (Figure 4), were isolated from lentil plants (root, stem and leaf) in two areas of BARAOUIA and AIN SEMARA. These genera are classified in six divisions for BARAOUIA (Ascomycetes 66.24%, Basidiomycetes 1.78%, Deutromycetes 12.52%, Oomycetes 1.78%, Phycomycetes 12.5% and Zygomycetes 3.57%) and four divisions for AIN SEMARA (Ascomycetes 79.63%, Basidiomycetes 1.85%, Deutromycetes and Phycomycetes 9.25%).

The major genus in the area of BARAOUIA is *Fusarium* (9 species) and *Alternaria* and *Aspergillus* (8 species each). The other genera have a variable number of species: *Phytophthora* (7 species), *Penicillium* (4 species), *Cladosporium*, *Curvularia* and *Ulocladium* (3 species each), *Absidia*, *Acremonium* and *Chaetomium* (2 species each) and finally *Botrytis*, *Cylindrosporium*, *Myrothecium*, *Peronospora* and *Rhizoctonia* (1 species each). The *Alternaria* was the major genus with 11 species in the area of AIN SEMARA, followed by *Fusarium* (8 species), *Aspergillus* (6 species), *Curvularia*, *Penicillium* and *Phytophthora* (5 species each), *Ulocladium* (4 species),

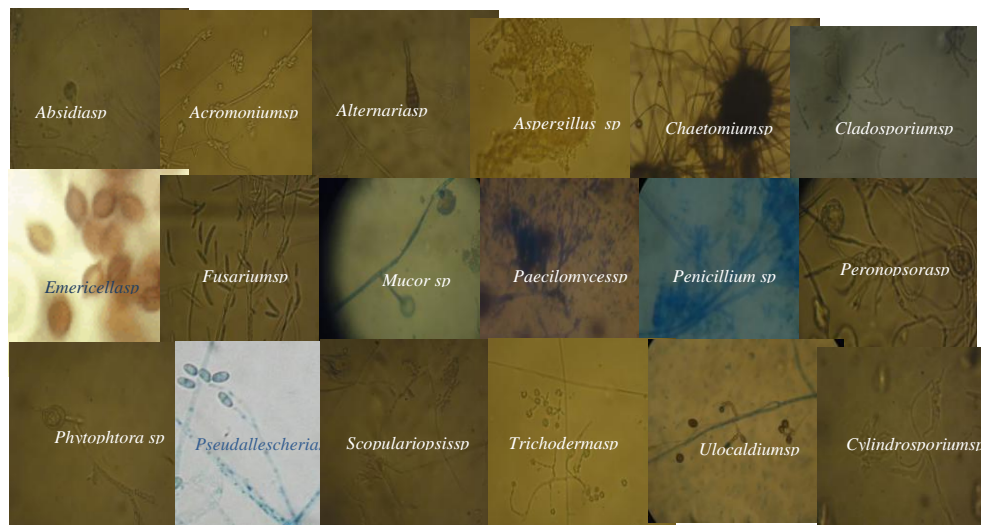


Figure 1. Examples of some species isolation from soils and plants.

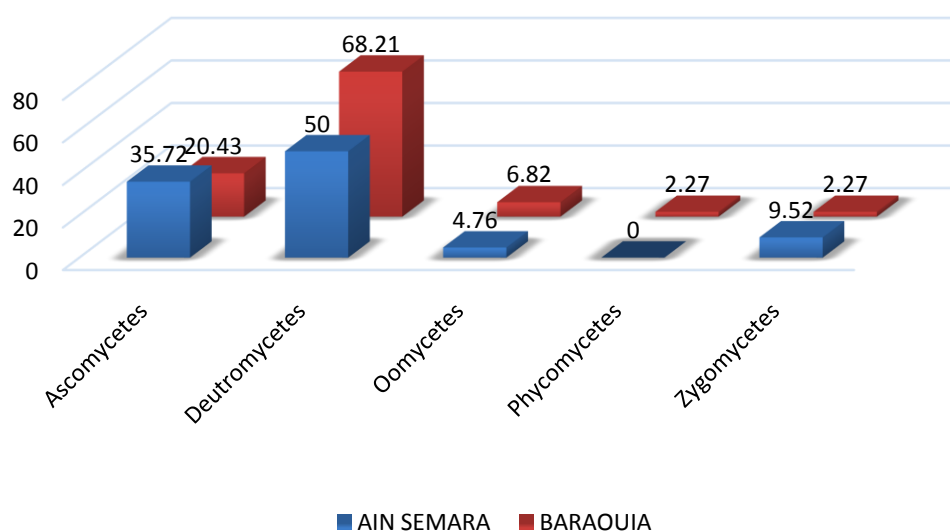


Figure 2. Percentage of different branch of fungi in the soil of BARAOUIA and the soil of AIN SEMARA.

Acremonium, *Onychocola* and *Trichoderma* (2 species) and finally *Rhizoctonia* (1 species) (Figure 5).

DISCUSSION

The samples of soil analysis were taken from the first horizon (0 to 20 cm) in which the essence of the biological activity is concentrated. This horizon is the most exposed one to the air and it contains mainly aerobic species.

The internal environmental conditions can assign the specific composition of the microbial communities and

their chemical potential (Alexander, 1997). Additionally, with an organic-matter equal to 1.79% in (BARAOUIA) and 1.81% in (AIN SEMARA), the studied soils are included in the interval of normal rates (1.5 to 2.5%) which are described in the standards of Duthil (1970).

The comparison of the pH values of the soil studied with the data standards by Madagascar (quoted by the interprocessor of the agronomist (1974)), shows that our soils are slightly alkaline. According to Baise and Jabiol (1995), the alkali soils have a pH between 7.3 and 8.5. These pH values should be perfect for fungi development.

The values of the electrical conductivity (EC) measures

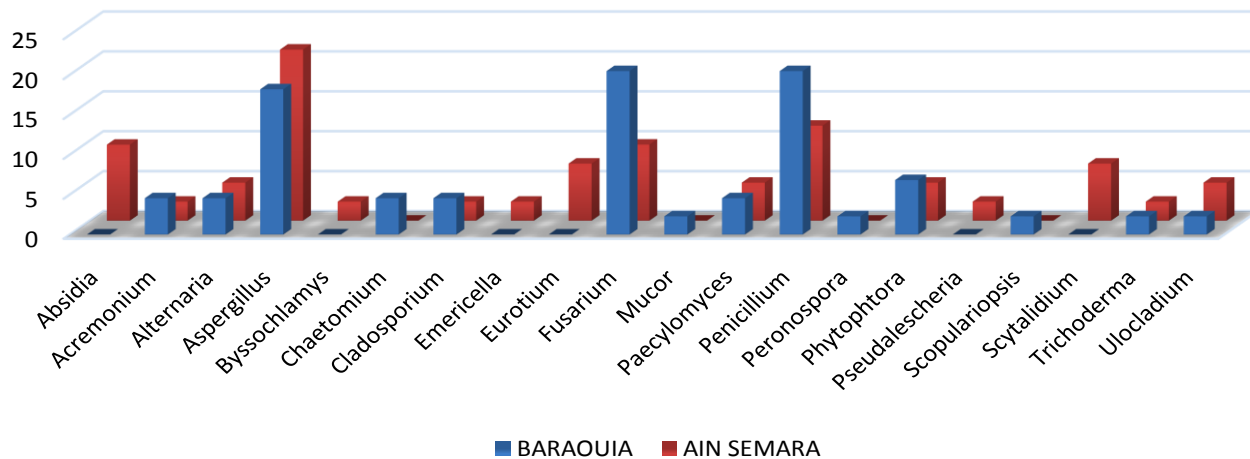


Figure 3. Percentage of contribution of different genera in the soil of BARAOUIA and the soil of AIN SEMARA.

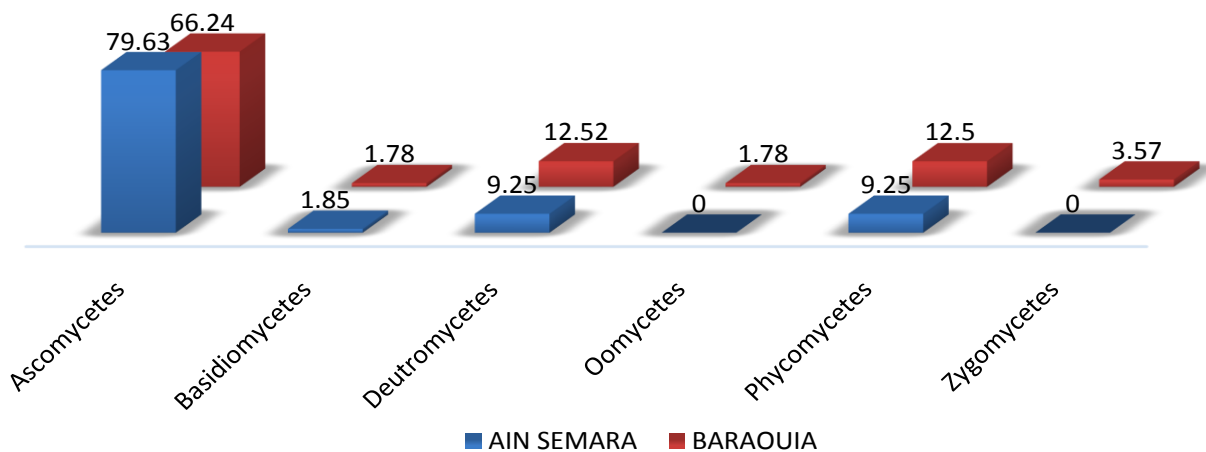


Figure 4. Percentage of branch divisions of fungi in the plants of BARAOUIA and the soil of AIN SEMARA.

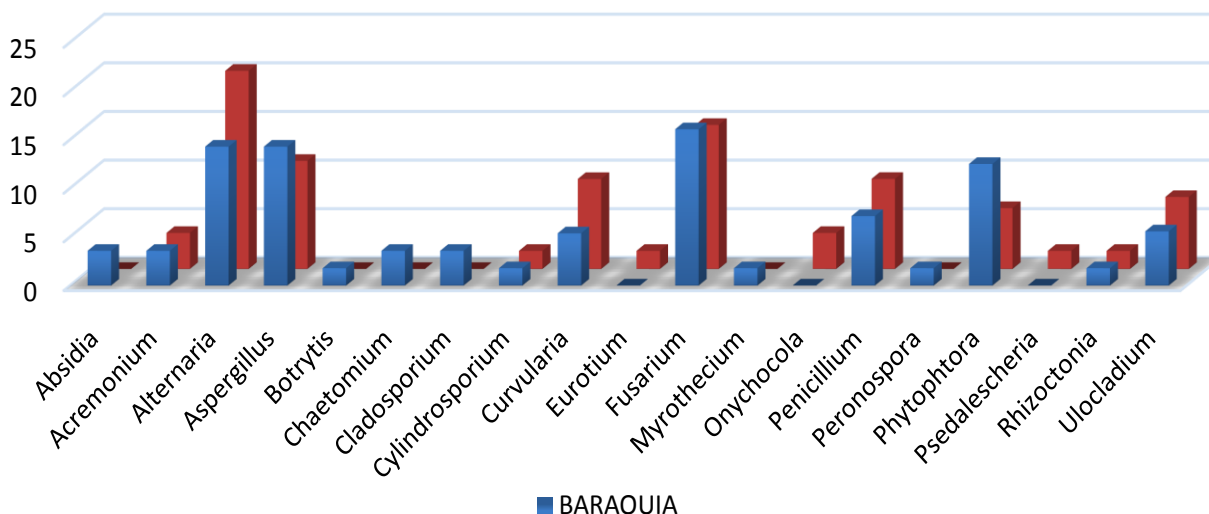


Figure 5. Percentage of contribution of different genera in the plants of BARAOUIA and the plants of AIN SEMARA.

of the soil which are taken from BARAOUIA and AIN SEMARA show that the soils are not saline ($EC < 1$) and this is according to the norms given by DURAND (quoted by the interprocessor of the agronomist, 1974).

Furthermore, the C/N report that was calculated after the determinations of Carbon and Nitrogen, has given the results < 15 , which means that the organic material has been normally mineralized. Thus, it provided mineral nitrogen.

The analysis of the results obtained after the isolation of the fungi of the two prospected sites shows that 84.09% of the strains isolated from the BARAOUIA site (genera: *Acremonium*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Peronospora*, *phytophthora*, *Penicillium*, and *Ulocladium*) and 90.73% of the AIN SEMARA site (genera: *Acremonium*, *Alternaria*, *Aspergillus*, *Eurotium* suggests, *Penicillium*, *Phytophthora*, *Fusarium*, *Pseudallescheria*, *Ulocladium* and *Trichoderma*) are isolated from the different parts of infected plants and also from the sampled soils. These results showed that the strains have been transmitted from the soil through the sap which can take for granted the soil as a middle of storage of fungal spores. These results are in relationship with those of Estelle Levetin and also of Abigail Jenkis (2005).

Furthermore, the remains of the soil isolates (15.91% of BARAOUIA and 9.27% of AIN SEMARA) are plants only. It means that these strains are transmitted through the seed or the air (Estelle Levetin).

Actually, the study of the different isolates shows that only 55.34% of the strains of the BARAOUIA site (genera: *Alternaria*, *Botrytis*, *Cladosporium*, *Cylindrosporium*, *Fusarium*, *Peronospora*, *Phytophthora* and *Rhizoctonia*) and 46.3% of the strains of the AIN SEMARA site (genera: *Alternaria*, *Cylindrosporium*, *Phytophthora* and *Fusarium*) are of pathogenic strains associated with *Lens culinaris* plants. These results are in coincidence to those of Richardson (1979), Ahmed et al. (1993), Muhlabauer et al. (2002) and Hussain et al. (2007).

The great diversity of isolates obtained after identifications (soil and plants) is mainly due to the environmental conditions favouring the development of molds: rate of rainfall between 19 and 68 mm, 60 to 71 humidity and temperature between 19 and 26°C.

The majority of the identified isolates belong to large families of the Ascomycetes and Deuteromycetes. These strains are indigenous, usually isolated from the ground (Alvarez-Rodriguez et al., 2002).

The obtained varieties after isolation from the ground are: *Absidia*, *Acremonium*, *Alternaria*, *Aspergillus*, *Bysochlamyces*, *Cladosporium*, *Eurotium* suggests, *Emericella*, *Fusarium*, *Mucor* species, *Peronospora*, *Paecylomyces*, *Penicillium*, *phytophthora*, *Pseudallescheria*, *Trichoderma*, *Ulocladium*, *Chaetomium* and *Scopulariopsis* species are often dating in the soils, this is related with the results obtained by Prince et al. (2011).

Conflicts of Interest

The authors declare no conflicts of interest.

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