JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2015. Vol. **9**(1), p. 97-107

Soil Fungi of Healthy and Infested Lupine (*Lupinus termis*) and its Role in Controlling of Lupine Root Rot *In vitro*

M.E. Ibrahim[1]*, A.M. Abdel-Azeem[2] and A.E.M. Abdelaziz[1,3]

[1]Department of Botany, Faculty of Science, University of Port Said, Port Said, Egypt.
[2]Department of Botany, Faculty of Science, University of Suez Canal, Ismailia, 41522, Egypt.
[3]Department of Biological sciences, University of Calgary, Alberta, Canada

(Received: 30 November 2014; accepted: 25 December 2014)

Soil mycobiota play an important function in agricultural economy of a nation. The current study was made to have the knowledge about soilborne fungi associated with lupine crop in healthy and infested soils. Fungal species and their population in the soil vary depending on many factors such as type of soil, vegetation, temperature, pH, organic matter, moisture content, aggregate size, predation, and agricultural management factors such as tillage, cover cropping, root exudates, fertilizer and crop rotation. All together these factors affected directly or indirectly soil mycobiota, especially the soilborne pathogenic ones. Thirty one fungal species belong to nineteen genera has been isolated from healthy and infested soils. The diversity as well as the count has been, to some extent, differed from healthy to infested soil. By comparison of the species lists of the fungal flora of healthy and infested soils it was evident that soil fungi behave differently toward soil status, while some species isolated only from healthy soil e.g. *Achaetomium*, *Chaetomium*, *Cladosporium, Epicoccum, Geotrichum, Gliocladium* and *Mucor*, some isolated from both healthy and infested soil e.g. *Alternaria, Aspergillus, Emericella, Fusarium Penicillium* and *Trichoderma,* still others isolated only from infested soil e.g. *Absidia, Acremonium* and *Paecilomyces.* Regarding the role of soil mycobiota for controlling lupine root rot caused by *Plectosporium tabacinum* in dual culture, the obtained data clearly show that some isolates were strong antagonist, others were moderate and some were weak.

Key words: soil mycobiota; lupine field; fungal diversity; healthy and infested soil.

Fungi are a diverse group of microorganisms that play a fundamental role in terrestrial ecosystems. They are generally involved in nutrient cycling, transportation of nutrients and minerals to plants, plant growth stimulation and antagonism against plant pathogens (Viebahn *et al.* 2005, Christensen 1989, Thorn 1997). Great number of plant associated fungi show a beneficial or neutral interaction with the host plant. Moreover, some fungi are used as biocontrol agents against phytopathogenic fungi; furthermore, they also include a large number of plant pathogens (Agrios 1997). It is evaluated that only 5% of all fungal species are known **(**Hawksworth & Rossman 1997**)** and that only 17% of the known species can be grown in culture (Bridge & Spooner 2001). These mean that the diversity of fungi is largely unrecognized. Besides, cultivating fungi from soil is not without partiality, because they can be dormant, occur as mycelium, as spores, or both (Bridge & Spooner 2001). Subsequently, in recent studies cultivation- independent molecular approaches have been used to assign the diversity of fungal communities through analysis of ribosomal (r) RNA genes (Ranjard *et al*. 2001, Schabereiter-Gurtner *et al*. 2001, Smit *et al*. 1999).

* To whom all correspondence should be addressed.
Tel: +201111215918; Fax: +20663657601;
E-mail: mohsenhbrahim@yahoo.com

Microbial variety in soil is one of the main components that appearing soil health (Garbeva *et al*. 2004), and is believed to be one of the main drivers in soil suppressiveness. Mycobiota and their population in the soil vary depending on many factors such as vegetation, type of soil, organic matter, temperature, pH, moisture content, aggregate size, agricultural management factors include tillage, fertilizer, cover cropping, root exudates, and crop rotation (Buyer *et al*. 2010). Performer of a range of fungal groups such as non-pathogenic *Fusarium* spp., *Penicillium*, *Trichoderma*, has been identified as antagonists of soil-borne plant pathogens (Garbeva *et al*. 2004). Moreover, arbuscular mycorrhizal (AM) fungi have been shown to inhabit or reduce plant root diseases; these interactions may lead to development of disease 'conducive soil', or prevent disease 'suppressive soil' (Gianinazzi *et al*. 2010, Whipps 2004).

Soil microbial composition is increasingly valued for their role in agro-ecosystem sustainability (Brussaard *et al*., 2007). Soil microorganisms render various roles in decomposition of organic matter, plant nutrient availability, and nutrient cycling, and are in turn influenced by plant species, crop management, and abiotic conditions (Zaady *et al*., 1996; Jones, 1998; Calderon *et al*., 2000). In addition, field boundaries can provide habitats for plant pathogens and/or beneficial microorganisms to control those pathogens. Nevertheless, only little is known about the status of soil microbial communities in boundary lands that is adjacent to agricultural fields (Masahiro *et al*., 2008).

It is a defy function to find appropriate methodologies for fully characterizing and assessment soil microbial communities and this is because only a small fraction of soil microorganisms is able to be cultured (Ward *et al*., 1990), methods of culture-dependent for microbial populations furnish limited information about soil microbial communities. However, recently some beneficial methods those are independent from culture survey have been developed for screening microbial communities. These methods include analyses of nucleic acids extracted from soil, denaturing gradient gel electrophoresis after PCR (Niemi *et al*., 2001; Smalla *et al*., 2001; Dierksen *et al*., 2002; Ibekwe *et al*., 2002), and community profiling based on fatty acid methyl esters (Cavigelli *et al*., 1995; Ibekwe and Kennedy, 1999; Dierksen *et al*., 2002; Larkin, 2003). In addition, carbon-based substrate utilization patterns have also been widely utilized for describing community-level physiological profiles using Biolog plates (Garland and Mills, 1991; Zak *et al*., 1994; Widmer *et al*., 2001; Larkin, 2003). There is no single method can describe an entire microbial community, a combination of the polyphasic data sets generally yields a better understanding of the community (Widmer *et al*., 2001).

Most of the fungi found in the lupine and some rhizospheric crops, in Egypt and other countries all over the world, were registered in rhizosphere and non-rhizosphere soils (Cavalcanti & Maia 1994; Abdul Wahid *et al*. 1997; Maia & Gibertoni 2002; Mandeel 2002; Souza motta *et al*. 2003; Ananda and Sridhar 2004; Grishkan *et al*. 2006; Costa *et al*. 2006; Cavalcanti *et al*. 2006;). Conventional tactic to study microbial communities in soils are based on culturable protocols. These approaches are useful for isolation purposes, but are very limited in their field to comprehend microbial communities and variety (Kuske *et al*. 1997).

To determine practical routes of managing the soilborne diseases and microbial environments, it is required to understand effects of crop fields and their boundary areas on soil microbial communities. In this study, we aimed to characterize fungal communities of free soils of healthy and infested lupines roots under the influence of root rot caused by *Plectosporium tabacinum* in two consecutive years.

MATERIALS AND METHODS

Field experiment:

This experiment has been conducted in naturally infested soil at the Botanical Garden of Botany Department, Suez Canal University campus at Ismailia. The experiments were repeated in two consecutive years 2007 and 2008. Soils have been seeded by lupine "cultivar Giza 2" seeds at 3cm depth and 30 cm apart. After a growth period of 10 weeks under field conditions, diseased plants have been sorted out and free soils around healthy and infested plants were collected for subsequent isolation of mycobiota (Fig. 1).

Sampling:

Soil samples were collected from the upper soil layer (3 – 20cm deep) from healthy and infested soils. Thirty soil samples (500 gm. each) were collected from healthy and infested soils (15 samples each). Samples were transferred to the laboratory in tight sterilized polyethylene bags and kept at low temperature until plating.

Isolation and identification:

Fungi were isolated from subsurface layer (ca. 15 - 30 cm) by using dilution plate method (Johnson *et al.* 1960) in which six plates was used for isolation/sample. Czapek's agar supplemented with 0.5 % yeast extract (CYA) and potato dextrose agar (PDA), amended with rose bengal (1/15000) and chloramphenicol (50 ppm) was used for primary isolation. Plates were incubated at 28 °C for 10 days and developing fungi were counted. For maintaining cultures and proper identification, pure cultures of the isolated fungi were grown on standard media such as Vegetable Agar (V8), Oatmeal Agar (OA), Malt Extract Agar (MEA) Potato Dextrose Agar (PDA) and Potato Carrot Agar (PCA).

Taxonomic identification by morphology of fungal isolates was mainly based on the following identification keys: Raper & Thom (1949), Pitt (1980) for *Penicillium*; Raper & Fennell (1965) for *Aspergillus*; Ellis (1971 and 1976) for dematiaceous hyphomycetes; Booth (1971) for *Fusarium*; Arx (1981), Domsch *et al.* (1980) for miscellaneous fungi; Arx *et al*. (1986) for *Chaetomium*. The systematic arrangement follows the latest system of classification appearing in the 9th edition of Anisworth & Bisby's Dictionary of the fungi (Kirk *et al.* 2001).

Control of lupine root rot in Vitro:

Antagonistic potentiality *in Vitro*.

The competitive saprophytic potentiality of some isolated fungi has been tested against *Plectosporium tabacinum.* Discs of 5 mm diameter from actively growing colonies were used. The two candidates were inoculated (2 cm apart) on CYA & PDA plates. Plates were then incubated at 27 ^{O}C ± 2 for a period of 3 to 10 days depending on the growth rate of the two candidates.

RESULTS

During this study, a total number of 31 species belong to 19 genera, has been isolated from healthy and infested soils. Isolated fungi belong to four classes (Table 1) of which Ascomycota (anamorphic) comes first where represented by 22 species accounting for 70.96% of the total isolated taxa. It is followed by class Zygomycota which is represented by 5 species constituting 16.13% of the total fungi. While class Ascomycota (teleomorphic) exemplified by 3 species accounting for 9.69%. Mitosporic fungi came next represented by only one species (3.23%).

Species richness:

Species richness means the number of species belonging to each genus isolated throughout the present study. The genera recorded are given in Table 2. It is clearly evident, from the Table, that *Aspergillus* is the richest by showing a spectrum 6 species. *Fusarium* and *Penicillium* comes next by being represented by 3 species each. They are followed by *Acremonium*, *Cladosporium* and *Trichoderma* by showing 2 species each. The remainders are represented by only 1 species. *A. niger* is the most dominant among all Aspergilli, F. oxysporum among all Fusaria, while *P. chrysogenum* among all Penicillia.

Total fungal count:

Fungal counts were expressed as total number of colony forming units per gram dry soil (cfu/g). The data of Table 3 show nearly there is no difference in fungal counts between healthy and

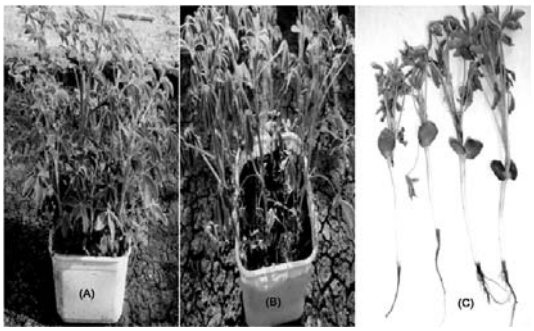
Table. 1. Number of isolated species in healthy and infested soil.

Soil Classes	Healthy soil No. of spp. isolated	Infested soil No. of spp. isolated	Total	%
Mitosporic fungi	1	-	1	3.23
Ascomycota (teleomorphic)	3	1	3	9.68
Ascomycota (anamorphic)*	18	13	22	70.96
Zygomycota	4	4	5	16.13
Total No. of species	26	18	31	100.00

infested soil soils. While healthy soils showed a mean colony count of 12778 cfu/g, infested soils revealed a mean colony count of 12360

Frequency of species:

It is based on the percentage number of cases of isolation (regardless of colony count). The data of Table 4 revealed that, in view of frequency values, recorded species could be temporarily divided into four ecological classes as follows:



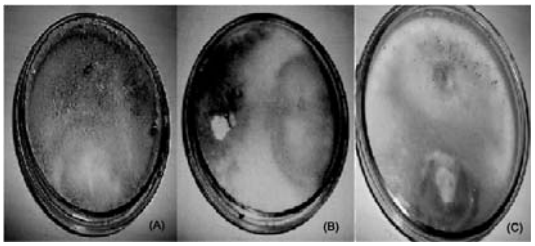
A: healthy plants (control)
B: infected plants (symptoms on shoot system)
C: infected roots (symptoms on root system)

Fig. 1. Symptoms of *Plectosporium tabacinum* root rot of lupine

High occurrence group (H), including species showing frequency values of 50 % or more out of 14 cases; Moderate occurrence (M), from 25 – 49 %; Low occurrence (L), species showing frequency values between 12 % and 24 %; Rare occurrence (R), less than 12 %.

Antagonistic potentiality of some isolated fungi:

A group of candidates consists of six taxa comprise eight isolates, which have been reported from healthy and infested soils, were in vitro tested for their biocontrol activity. To evaluate the antagonistic potentiality among these taxa, they



(A) And (B) *Trichoderma harzianum* mycoparasitism
(C) *Trichoderma pseudokoningii* clear zone

Fig. 2. Different types of antagonism

Table. 2. Genera and species richness of isolated fungi

No.	Genera	Healthy soil No. of species	Infested soil No. of species	Total No. of species
1	*Absidia*	-	1	1
2	*Achaetomium*	1	-	1
3	*Acremonium*	-	2	2
4	*Actinomucor*	1	1	1
5	*Alternaria*	1	1	1
6	*Aspergillus*	5	5	6
7	*Chaetomium*	1	-	1
8	*Cladosporium*	2	-	2
9	*Emericella*	1	1	1
10	*Epicoccum*	1	-	1
11	*Fusarium*	3	2	3
12	*Geotrichum*	1	-	1
13	*Gliocladium*	1	-	1
14	*Mucor*	1	-	1
15	*Mycocladus*	1	1	1
16	*Paecilomyces*	-	1	1
17	*Penicillium*	3	1	3
18	*Rhizopus*	1	1	1
19	*Trichoderma*	2	1	2
	Total	26	18	31

tested on agar plates, for their ability to antagonize the target organism (*Plectosporium tabacinum*). According to the growth pattern revealed by the two competitors (Fig. 2), taxa under test are classified into strong antagonists, moderate antagonists and weak antagonists.

DISCUSSION

Development of sustainable agricultural systems by manipulating soil microbial communities using soil and crop management practices is a basic tactic for improving crop

Table 3. Main of total count (MTC, colonies/ g dry soil), number of cases of isolation (NCI, out of 7 soil samples) and percentage frequency of fungal taxa recovered on Czapek's yeast extract agar at 28°C

Species	Healthy Soil			Infested Soil		
	MTC	NC1	% F	MTC	NC1	% F
Zygomycota						
Absidia glauca Hagem	0	0	0	95*	1	14
Actinomucor elegans (Eidam) C.R. Benj. & Hesselt.	309	1	14	214	1	14
Mucor circinelloid Tiegh.	833	2	28	95	1	14
Mycocladus corymbiferus (Cohn) J.H. Mirza	95	1	14	142	2	28
Rhizopus stolonifer var. *stolonifer* (Ehrenb.) Vuill.	547	4	57	880	7	100
Ascomycota (teleomorphic)						
Achaetomium sp	71	1	14	0	0	0
Chaetomium globosum Kunze	71	1	14	0	0	0
Emericella nidulans (Eidam) Vuill.	625	4	57	456	2	28
Ascomycota (anamorphic)*						
Acremonium terricola (Miller & Al.) W. Gams	142	1	14	79	2	28
Alternaria alternata (Fr.) Keissl.	214	2	28	95	1	14
Aspergillus flavus Link	571	4	57	785	5	71
A. niger var. niger Tiegh.	1428	7	100	1785	6	85
A. sydowii (Bain & Sart.) Thom & Church	380	4	57	309	2	28
A. terreus Thom	2595	5	71	2452	3	42
A. versicolor (Vuill.) Tirab.	261	3	42	404	3	42
A.wentii Wehmer	71	1	14	0	0	0
Cladosporium sp	166	2	28	0	0	0
C. herbarum (Pers.) Link	404	4	57	0	0	0
Clonostachys rosea f. *rosea* (Link) Schroers, Samuels, Seifert & W. Gams	261	1	14	0	0	0
Fusarium sp	333	3	42	0	0	0
F. oxysporum Schltdl.	1261	5	71	2833	5	71
F. solani (Mart.) Sacc.	1214	4	57	1428	5	71
Geotrichum candidum Link ex Pers	857	1	14	0	0	0
Gliocladium sp	261	1	14	0	0	0
Paecilomyces variotii Bainier	0	0	0	47	1	14
Penicillium brevicompactum Dierckx	23	3	42	0	0	0
P. chrysogenum var. chrysogenum Thom	309	5	71	0	0	0
P. citrinum Thom	95	1	14	71	2	28
Trichoderma harzianum Rifai.	238	3	42	0	0	0
T. pseudokoningii Rifai.	309	5	71	190	3	42
Mitosporic fungi						
Epicoccum nigrum Link	23	1	14	0	0	0
Total	12778	12360				

* Figure represent main of seven compost soil samples.
** According to the system of Kirk *et al* (2001).

production and management of plant diseases (Van Bruggen, 1995). Synthetic fertilizers and pesticides are not used in agriculture systems and instead, biological methods of pest and disease control are confirmed (National Research Council, 1989). It is recognized that a domain of specific soil microbes are playing an important role in the inhibition of soil-borne plant diseases as well as in plant growth progression (Kennedy & Smith 1995). In traditional farming regulation, controlling plant diseases and pests require the use of chemicals, which may cause environmental pollution, reduce soil microbial diversity and ultimately increase crop diseases (Sturz & Christie 2003, Garbeva 2005).

As a complex diversified habitat soil comprise a wide variety of organisms, comprehensive bacteria, actinomycetes, fungi, micro-algae, protozoan, nematodes and earthworms that play many functional roles in the ecosystem in which they exist. Their task as populations that interact with each other and their abiotic factors thereby participate in soil structure, soil fertility, plant nutrition, decomposition of organic matter, cycling of nutrients, suppression of soil-borne pathogens and removal of toxins (Kirk *et al*., 2004, Kozdroj and van Elsas 2000).

Populations of microorganisms in soil is the driving force of most terrestrial ecosystems,

Table 4. Frequency of occurrence of species isolated

No.	Species	No of cases of isolation (out of 14 samples)	Frequency %	Frequency class
1	*Aspergillus niger* var. *niger* Tiegh.	13	92.9	H
2	*Rhizopus stolonifer* var. *stolonifer* (Ehrenb.) Vuill.	11	78.5	H
3	*Fusarium oxysporum* Schltdl.	10	71.4	H
4	*A. flavus* Link	9	64.3	H
5	*F. solani* (Mart.) Sacc.	9	64.3	H
6	*A. terreus* Thom	8	57.1	H
7	*Trichoderma pseudokoningii* Rifai.	8	57.1	H
8	*Emericella nidulans* (Eidam) Vuill.	6	42.8	M
9	*A. sydowii* (Bain & Sart.) Thom & Church	6	42.8	M
10	*A. versicolor* (Vuill.) Tirab.	6	42.8	M
11	*Penicillium chrysogenum* var. *chrysogenum* Thom	5	35.7	M
12	*Cladosporium herbarum* (Pers.) Link	4	28.5	M
13	*Mucor circinelloid* Tiegh.	3	21.4	L
14	*Mycocladus corymbiferus* (Cohn) J.H. Mirza	3	21.4	L
15	*Acremonium terricola* (Miller & Al.) W. Gams	3	21.4	L
16	*Alternaria alternata* (Fr.) Keissl.	3	21.4	L
17	*F. sp*	3	21.4	L
18	*P. brevicompactum* Dierckx	3	21.4	L
19	*P. citrinum* Thom	3	21.4	L
20	*T. harzianum* Rifai.	3	21.4	L
21	*Actinomucor elegans* (Eidam) C.R. Benj. & Hesselt.	2	14.3	L
22	*Cladosporium sp*	2	14.3	L
23	*Absidia glauca* Hagem	1	7.14	R
24	*Achaetomium sp*	1	7.14	R
25	*A.wentii* Wehmer	1	7.14	
26	*Chaetomium globosum Kunze*	1	7.14	R
27	*Clonostachys rosea* f. *rosea* (Link) Schroers, Samuels, Seifert & W. Gams	1	7.14	R
28	*Geotrichum candidum* Link ex Pers	1	7.14	R
29	*Gliocladium sp*	1	7.14	R
30	*Paecilomyces variotii* Bainier	1	7.14	R
31	*Epicoccum nigrum* Link	1	7.14	R

because these populations that largely control the rates of transformation and mineralization of organic substrates. The fungi, as a group, are the organotrophs primarily responsible for the decomposition of organic residues. Expression as biomass (not numbers), fungi predominately dominate in soil microbiota. Fungi have a wide range of roles in soil, including their functions as saprotrophs, plant symbionts, as well as plant and animal pathogens. The majority of soil-borne fungi belong to the Deuteromycotina group. These fungi, which comprise more than half of the species of the entire soil fungi community, include *Penicillium, Aspergillus, Fusarium, Gliocladium, Scopulariopsis, Paecilomyces, Acremonium, Alternaria, Ulocladium, Drechslera, Cladosporium, Verticillium, Rhizoctonia* and many other genera.

The main intention of the present study has been to survey fungal biota of free soils of healthy and infested lupines roots under the influence of root rot caused by *Plectosporium tabacinum*.

To assure reasonable and fair characterization of the mycobiota of both healthy and infested lupine soils, two parameters have been adopted in order to avoid over or under estimation of fungal populations. These parameters are species density, based on total number of colony forming units (cfu) per gram dry soil; and species frequency, based on the number of cases of isolation of each species (regardless of its number of colonies on the isolation plates).

In view of species richness i.e. number of species revealed by each genus, the genera *Aspergillus, Fusarium* and *Penicillium* were the richest by exhibit a broad spectrum of 6, 3, 3 species respectively. They were followed by *Acremonium, Cladosporium* and *Trichoderma* by representing by 2 species each. The same finding was reported by Latiffah et. al., (2011), Coutinho et. Al., (2010) Grishkan *et al.* (2006), Noveriza and Quimio (2004) Azaz (2002), Mandeel (2002), Maia & Gibertoni (2002), Lee *et al*. (2000) and Ibrahim (1994), Abdul Wahid O. A. (1990), Ismail and Abdulla (1977).

As for total count i.e. total number of cfu/g dry soil in healthy and infested soils, data shows that counts in both soils (healthy and infested) are of the same order although healthy soils apparently tend to hold higher counts than infested ones. While healthy soils showed a main total count of 12778 cfu/g, infested soils revealed a main of 12360 cfu/g.

According to the frequency value, fungi isolated were diversified into four ecological groups: High, Moderate, Low and rare frequency classes. High frequency group, involved species showing frequency of about 50 % or more, specified to groups: *Aspergillus niger* var. *niger, Rhizopus stolonifer* var. *stolonifer, Fusarium oxysporum,* and *Trichoderma pseudokoningii*. Moderate frequency group, encompass species rendering frequency of 25 – 49 %. Assigned to this group species of common isolated aspergilli and penicilli, such as *Emericella nidulans, A. sydowii, A. versicolor,* and *Penicillium chrysogenum* var. *chrysogenum*. Low frequency group, consisting of species having frequency from 12 – 24 %. Among these species: *Mucor circinelloid, Mycocladus corymbiferus, Acremonium terricola, P. brevicompactum,* and *T. harzianum*. Rare frequency group, comprised taxa showing frequency less than 12%. *Absidia glauca, Achaetomium sp, A.wentii, Chaetomium globosum,* and *Clonostachys rosea* f. *rosea*.

The data, of frequency and total count, in this research are in consistent with the result obtained in Egypt and elsewhere allover the world, by Ismail and Abdulla (1977) in Araq; Abdul Wahid (1990), Ibrahim (1994), Abdul Wahid *et al*., (1995), Abdul Wahid *et al*., (1998), in Egypt; Azaz (2002) in Turkey; Garbeva *et al*., (2004) in Netherland; Noveriza and Quimio (2004) in Philippine Grishkan et. al. (2006) in Palestine; Majchrzak *et al*., (2010) in Poland; Coutinho et. Al., (2010) in Brazil; Wahegaonkar *et al*., (2011) in India; Latiffah et. al., (2011) in Malaysia; Xu et. Al., (2012) in Denmark; Florina (2013) in Romania.

Selection of a proper candidate is by far the most challenging issue in the biocontrol through the application of antagonistic microorganisms. Generally the activities of antagonistic candidates *in vitro* do not guarantee their success *in vivo*. Broadbent *et al*. (1971) tested more than 3500 isolates from 60 soils; only 40% inhibited one to nine pathogens on agar, and out of these 40% only 3% were active in soil. The relationship between antagonistic potentiality *in vitro* and *in vivo* is seldom, if never, straightforward (Fokkema 1978, Andrews 1985). This has been

referred to several interacting factors such as: a-presence of wide range of populations with different relations, b- the nutritional status, c- as well as many factors related to physical and chemical properties. However, the *in vitro* test looks attractive as its shows clear and visible results (inhibition, lysis or coiling of the pathogen). It has also another advantage i.e. relatively easy and quick to perform with large number of isolates.

Four isolates of *Trichoderma pseudokoningii* and *T. harzianum* two each and four isolates of *Aspergillus niger, Fusarium oxysporum* (non pathogenic), *Penicillium chrysogenum* and *Cladosporium* sp, were evaluated for their effectiveness as antagonists against *Plectosporium tabacinum*, the causative agent of root rot of lupine using the paired culture mechanism. Data of screening tests revealed that the antagonistic potentiality differ markedly from species to another. While some strains proved to be strong antagonist represented by *Trichoderma harzianum* and non pathogenic *Fusarium oxysporum*; some consider as moderate antagonist comprised by *Aspergillus niger* and *Penicillium chrysogenum;* still other regard as weak antagonist represented by *Cladosporium* sp.

This divergence among isolates in their ability to control the growth of pathogen has been apprised by several investigators (Tjamos and Fravel 1995; Duffy *et al*., 1996; Haran *et al*., 1996; Naik *et al*., 2000; Upmanyu *et al*., 2002; Singh *et al*., 2008) indicating the necessity for selection of the best isolates for applying as biocontrol agents against speciûc pathogens and under speciûc agro-climatic conditions (Suriachandraselvan *et al*., 2004). Difference in the inhibitory ability between isolates has been often been attributed to factors such as antibiosis, myco-parasitism, competition for space and nutrients and over growth (Naik *et al*., 2009; Manjunatha and Naik, 2011, Singh *et al*., 2013).

CONCLUSION

Thirty one fungal species belong to nineteen genera have been isolated from healthy and infested soils of lupine (*Lupinus termis*). The Ascomycota (anamorphic) species among the isolated fungi were the most dominant, represented about 70.96% of the total isolated taxa. The two Isolates, *Trichoderma harzianum* and the non pathogenic *Fusarium oxysporum* proved to be strong antagonist against *Plectosporium tabacinum*, the causative agent of root rot of lupine in vitro. This study flourish the diversity knowledge of fungi associated with the healthy and infested soil of the lupine plant and reveals that *Trichoderma harzianum* is a strong biocontrol agent against the root rot disease of lupine.

REFERENCES

1. Abdul Wahid O. A. Fungal flora of cultivated soils and their role in the biological control of tomato *Fusarium* wilt in Ismailia governorate. Ph. D. thesis, Faculty of Science, Suez Canal University, Ismailia, 1990; Egypt. 115 pp.
2. Abdul Wahid O. A., Moustafa A. F., Ibrahim M. E. Biological control of tomato *Fusarium* wilt by using single and mixed inocula. *J. Union Arab. Biol.,* Cairo., 1995; **2**(B) Botany: 65-75.
3. Abdul Wahid, O. A, A. F. Moustafa, and M. E, Ibrahim. Soil mycoflora in tomato fields. *Mycoscience*, 1997; **38**(2): 237-241.
4. Abdul Wahid O. A., Ibrahim M. E. Omar M. A. Occurrence of soil suppressiveness to *Fusarium* wilt disease of broad bean in Ismailia governorate. *J. Phytopathology*, 1998; **146**: 431 – 435.
5. Ananda, K. & Sridhar, K.R. Diversity of filamentous fungi on decomposing leaf and woody litter of mangrove forests in the southwest coast of India. *Current Science of India*. 2004; **87**(10): 1431-1437.
6. Andrews J. H. Strategies for selecting antagonist microorganism from the phylloplane. In biological control of the phylloplane (ed. C. E. Windles & S. E. Lindow), pp 33- 44. Philadelphia, U. S. A.: The American Phyopathological Society, 1985.
7. Agrios GN; Plant Pathology. Academic Press, San Diego, CA, USA. Bridge P, Spooner B 2001 - Soil fungi: diversity and detection. *Plant Soil* 1997; **232**, 147–154.
8. Arx JA von, Guarro J, Figueras MJ. The ascomycete genus *Chaetomium. Nova Hedwigia Beihefte*. 1986; **84**: 1-162.
9. Arx JA von, The genera of fungi sporulating in pure culture. 3rd Ed. J. Cramer, Vaduz. 424 pp.
10. Azaz AD. 2002. Isolation and Identification of Soilborne Fungi in Fields Irrigated by GAP in Harran Plain Using Two Isolation Methods. *Turk J Bot.* 1981; **27** (2003) 83-92.
11. Bianchi A, Bianchi M. Microbial diversity and ecosystem maintenance: an overview In Allsopp,

R.R., Colwell, R.R., Hawksworth D.L. (Eds.), Microbial diversity and Ecosystem function. CAB International, Wallingford, UK, 1995; pp185-198.

12. Read DJ. Plants on the web. *Nature*. 1998; **396**: 22-23
13. Booth, C.: The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, 1971; London.
14. Bridge, P., Spooner, B., Soil fungi: diversity and detection. *Plant Soil*. 2001; **232**, 147– 154.
15. Broadbend P. Baker K. F., Waterworth Y. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. *Australian Journal of Biological Science*. 1971; **4**: 925-944.
16. Brussaard L, de Ruiter PC, Brown GG. Soil biodiversity for agricultural sustainability. *Agriculture Ecosystems and Environment*. 2007 ; **121**, 233–244.
17. Buyer JS, Teasdale JR, Roberts DP, Zasada IA, Maul JE. Factors affecting soil microbial community structure in tomato cropping systems. *Soil Biology & Biochemistry*. 2010; **42**, 831- 841.
18. Caldero´ n FJ, Jackson LE, Scow KM, Rolston DE. Microbial responses to simulated tillage and in cultivated and uncultivated soils. *Soil Biology & Biochemistry*, 2000; **32**, 1547–1559.
19. Cavalcanti, M.A. & Maia, L.C. Cellulolytic fungi isolated from allluvial soil in semi-arid area of the northeast of Brazil. *Revista de Microbiologia* 1994; **25**: 251–254.
20. Cavalcanti, M.A.Q.; Oliveira, L.G.; Fernandes, M.J. & Lima, D.M. Fungos filamentosos isolados do solo em municípios na região Xingó, Brasil. *Acta Botanica Brasilica*. 2006; **20**(4): 831-837.
21. Cavigelli MA, Robertson GP, Klug MK. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. *Plant and Soil* 1995; **170**, 99–113.
22. Christensen M 1989 - A view of fungal ecology. Mycologia 81, 1–19.
23. Costa, I.P.M.W.; Cavalcanti, M.A.Q.; Fernandes, M.J.S. & Lima, D.M.M. 2006. Hyphomycetes from soil of an area affected by copper mining activities in the state of Bahia, Brazil. Brazilian Journal of Microbiology 37: 267-275.
24. Coutinho F.P., Cavalcanti, M.A.Q.Yano-Melo A.M. 2010. Filamentous fungi isolated from the rhizosphere of melon plants (*Cucumis melo* L. cv. Gold Mine) cultivated in soil with organic amendments. Acta bot. bras. 24(1): 292-298.
25. Dierksen KP, Whittaker GW, Banowetz GM, Azevedo MD, Kennedy AC, Steiner JJ, Griffith SM 2002 -High resolution characterization of soil biological communities by nucleic acid and fatty acid analyses. Soil Biology & Biochemistry 34, 1853–1860.
26. Domsch KH, Gams W, Anderson TH. 1980. Compendium of soil fungi. London, England: Academic Press. 865 p.
27. Duffy B. K., Simon A., Weller D. M. 1996. Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. Phytopathology 86: 188-1194.
28. Ellis, M.B. 1971. Dematiaceous Hyphomycetes. The Commonwealth Mycological Institute, England. pp. 1-608.
29. Ellis, M.B. 1976. More Dematiaceous Hyphomycetes. The Commonwealth Mycological Institute, England. pp. 1-507.
30. Florina U. (2013).New versions Romanian pots of local peat bio composites quantitative and qualitative analysis of fungal micro flora. Journal of horticulture, forestry and biotechnology volume 17(1), 148- 154.
31. Fokkema N. J. 1987. Fungal antagonism in the phyllosphere. Annals of Applied Biology. 89: 115-119.
32. Garbeva P 2005 - The Significance of Microbial Diversity in Agricultural Soil for Disease Suppressiveness
33. Garbeva P, van Veen, JA, van Elsas JD, 2004 - Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annual Review of Phytopathology 42, 24-270.
34. Garland JL, Mills AL 1991- Classification and characterization ofheterotrophic microbial communities on the basis of patterns ofcommunity-level sole-carbon-source utilization. Applied and Environmental Microbiology 57, 2351–2359.
35. Gianinazzi S, Gollotte A, Binet MN, van Tuinen D, Redecker D, Wipf D 2010 - Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20, 519-530.
36. Grishkan, I.; Zaady, E. & Nevo, E. 2006. Soil crust microfungi along a southward rainfall gradient in desert ecosystems. Journal of Arid Environments 53(3): 409-417.
37. Haran S., Schickler H., Chet I. 1996. Molecular mechanisms of lytic enzymes involved in the biocontrol activity of Trichoderma harzianum, Microbiology 142(9), 2321-2331.
38. Hawksworth DL, Rossman, AY 1997 - Where are all the undescribed fungi? Phytopathology 87, 888–891.
39. Ibekwe AM, Kennedy AC 1999 - Fatty acid

methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils. Plant and Soil 206, 151–161.
40. Ibekwe AM, Kennedy AC, Frohne PS, Papiernik SK, Yang C- H, Crowley DE 2002. Microbial diversity along a transect of agronomic zones. FEMS Microbiology Ecology 39, 183–191.
41. Ibrahim M.E. (1994). Fungi as biocontrol agent of tomato fusarial-wilt. B. Sc. thesis, Faculty, of Science, Suez Canal University, Ismailia, Egypt 80 pp.
42. Ismail ALS, Abdullah SK (1977). Studies on the soil fungi of Iraq. Proc Indian Acad Sci 3: 151-154.
43. Jones DL 1998 - Organic acids in the rhizosphere—a critical review. Plant and Soil 205, 25–44.
44. Kennedy AC, Smith KL 1995 - Soil microbial diversity and sustainability of agricultural soil. Plant Soil 170: 75-86.
45. Kirk JL, Beaudette LA, Hart M, Moutoglis P, Klironomos JN, Lee H, Trevors JT 2004 - Methods of studying soil microbial diversity. Journal of Microbiological Methods 58 (2004) 169 – 188.
46. Kirk PM, Cannon PF, David JC, Stalpers JA (eds) (2001). Ainsworth & Bisby's dictionary of the fungi. 9th edition. CABI Publishing, Wallingford.
47. Kuske CR, Burns SM, Busch JD 1997 - Divers uncultivated bacterial groups from soil of the arid Southwestern United States that are present in many geographic regions. Appl Environm Microbiol. 63:3614-3621.
48. Larkin RP 2003. Characterization of soil microbial communities underdifferent potato cropping systems by microbial population dynamics, substrate utilization, and fatty acid profiles.SoilBiology&Biochemistry35, 1451–1466.
49. Latiffah Z., Yee T. L., Zakaria M. Salleh B. (2011). Diversity of microfungi in sandy beach soil of Teluk Aling, Pulau Pinang. Tropical Life Sciences Research, 22(1), 71–80.
50. Maia, L.C. & Gibertoni, T.B. 2002. Fungos registrados no semi-árido nordestino. Pp. 163-176. In: E.V.S.B. Sampaio; A.M. Giulietti; J. Virginio & C.F.L. Guamarra-Rojas (eds.). Vegetação & Flora da Caatinga. Recife, Centro Nordestino de Informações sobre Plantas.
51. Majchrzak B., Kurowski T. P., Wachowska U., JazwiDska E. 2010. Changes in soil microbial communities as a result of growing brassicaceae crops. Acta Agrobotanica Vol. 63 (1): 161–169.
52. Mandeel Q.A. 2002. Microfungal community associated with rhizosphere soil of *Zygophyllum qatarense* in arid habitats of Bahrain. Journal of Arid Environments 50(4): 665-681.
53. Manjunatha, S.V., Naik, M.K., 2011. Mechanism of antagonism of *Trichoderma* isolates on *Macrophomina phaseolina* causing dry root rot in chickpea. J. Plant Dis. Sci. 12, 131-133.
54. Masahiro S, Kazunori S, Hidemi Y, Noriaki M, Shun-Iichiro M 2008 - Changes in microbial communities in an apple orchard and its adjacent bush soil in response to season, land-use, and violet root rot infestation Soil Biology & Biochemistry 40 (2008) 1460–1473.
55. Naik, M.K., Singh, S.J., Sinha, P., 2000. Mechanism of bio-control of wilt of chilli caused by *Fusarium oxysporum f. sp. capsici*. In: Proc. Golden Jubilee Int. Conf., on Integrated Plant Disease Management for Sustainable Agriculture. Indian Phytopathological Society, New Delhi, p. 665.
56. Naik, M.K., Madhukar, H.M., Devika Rani, G.S., 2009. Evaluation of biocontrol efficacy of *Trichoderma* isolates and methods of its applications against wilt of chilli caused by *Fusarium solani*. J. Biol. Control 23, 31-36.
57. National Research Council 1989 - Alternative Agriculture. National Academy Press, Washington, D.C.
58. Nascimento, J.P. & Laranjeira, D. 2003. Identification and characterization of filamentous fungi isolated from the sunflower (*Helianthus annus* L.) rhizosphere according to their capacity to hydrolyse inulin. Brazilian Journal of Microbiology 34(3): 273-280.
59. Niemi RM, Heiskanen I, Wallenius K, Lindstron K 2001 - Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. Journal of Microbiological Methods 45, 155–165.
60. Noveriza R., Quimio T. H. (2004). Soil mycoflora of black pepper rhizosphere in the Philippines and their in vitro antagonism against *Phytophthora capsici* L. Indonesian Journal of Agriculture Science 5(1): 1 – 10.
61. Pitt JI. 1980, '1979'. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. London,Academic Press. 634 p.
62. Ranjard L, Poly F, Lata J-C, Mougel C, Thioulouse J, Nazaret S 2001 - Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. Appl. Environ. Microbial. 67, 4479–4487.
63. Raper KB & Fennell DI (1965). The Genus *Aspergillus*. Baltimore: Williams & Wilkins

Company. 686 p.
64. Raper, K. B. & Thom, C., 1949 - A manual of the Penicillia. Baltimore : Williams& Wilkins Company . 875 p.
65. Schabereiter-Gurtner C, Pinar G, Lubitz W, Rolleke S 2001 - Analysis of fungal communities on historical church window glass by denaturing gradient gel electrophoresis and phylogenetic 18S rDNA sequence analysis. J. Microbiol. Meth.47, 345–354.
66. Singh, V., Ranaware A. M., Nimbkar N. 2008. Bioefficacy of antagonists against root-rot fungus (*Macrophomina phaseolina*) of safflower. In Knights, S. E. and Potter, T. D. (Eds.) (2008). Safflower: Unexploited potential and world adaptability. Proceedings of the Seventh International Safflower Conference, Wagga Wagga, New South Wales, Australia.
67. Singh V., Naik, M.K., Khan F.R., Goswami R..S. 2013 Evaluation of bio-control agents for management of dry root rot of chickpea caused by *Macrophomina phaseolina* (Short communication). Crop Protection 45 (2013) 147-150.
68. Smit E, Leeflang P, Glandorf B, Dirk van Elsas J, Wernars K 1999 - Analysis of fungal diversity in the wheat rhizosphere by sequencing of cloned PCR-amplified genes encoding18S rRNA and temperature gradient gel electrophoresis. Appl. Environ. Microbial. 65, 2614–2621.
69. Smalla K, Wieland G, Buchner A. Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg, G 2001- Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Applied and Environmental Microbiology 67, 4742–4751.
70. Souza-Motta, C.M.; Cavalcanti, M.A.Q.; Fernandes, M.J.S.; Lima, D.M.M.; Sturz AV, Christie BR 2003- Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. Soil Tillage Research 72: 107-123.
71. Suriachandraselvan, M., Salarajan, F., Aiyanathan, K.E.A., Seetharaman, K., 2004. Inhibition of sunflower charcoal rot pathogen, *Macrophomina phaseolina* by fungal antagonists. J. Mycol. Plant Pathol. 34, 364-366.
72. Thorn G 1997 - The fungi in soil In: Modern Soil Microbiology (van Elsas, J.D., Treves, D.S. and Wellington, E.M.H., Eds.), pp. 63–127. Marcel Dekker, Inc., New York.
73. Tjamos E. C., Fravel D. R. 1995. Detrimental effects of sublethal heating and *Talaromyces flavus* on microsclerotia of *Verticillium dahliae*. Phytopathology 85: 388-392.
74. Upmanyu, S., Gupta, S.K., Shyam, K.R., 2002. Innovative approaches for the management of root rot and web blight (R. solani) of French bean. J. Mycol. Plant Pathol. 32, 317-331.
75. Van Bruggen AHC 1995- Plant disease in high-input compared to reduced - input and organic farming systems. Plant Dis. 79: 976-983
76. Viebahn M, Christiaan V, Karel W, Leendert Cvan Loon, Eric S , Bakker PAHM 2005 - Assessment of differences in ascomycete communities in the rhizosphere of field-grown wheat and potato. FEMS Microbiology Ecology 53 (2005) 245–253.
77. Wahegaonkar N., Salunkhe S.M., Palsingankar P.L., Shinde S.Y. 2011. Diversity of fungi from soils of Aurangabad, M.S., India. Annals of Biological Research, 2011, 2 (2): 198-205.
78. Ward DM, Weller R, Bateson MM 1990 - 16s rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature 345, 63–65.
79. Whipps JM 2004 - Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Canadian Journal of Botany-Revue Canadienne de Botanique 82, 1198 -1227.
80. Widmer F, FlieXbach A, Laczko´ E, Schulze-Aurich J, Zeyer J 2001- Assessing soil biological characteristics: a comparison of bulk soilcommunity DNA-, PLFA-, and Biolog-analyses. Soil Biology &Biochemistry 33, 1029–1036.
81. Xu L., Ravnskov S., Larsen J., Nilsson R. H., Nicolaisen M. 2012. Soil fungal community structure along a soil health gradient in pea fields examined using deep amplicon sequencing. Soil Biology & Biochemistry 46 (2012) 26 - 32
82. Zak JC, Willig MR, Moorhead DL, Wildman HG 1994 - Functional diversity of microbial communities: a quantitative approach. Soil Biology & Biochemistry 26, 1101–1108.
83. Zaady E, Groffman PM, Shachak M 1996 - Litter as a regulator of N and C dynamics in macrophytic patches in Negev desert soils. Soil Biology & Biochemistry 28, 39–46.