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Entomopathogenic fungi in soils from Alicante province

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Abstract

We have used *Galleria mellonella* (Linnaeus, 1758) larvae as a bait for detecting insect pathogens in soils from Alicante (SE Spain). Soil from 61 sites was collected including agricultural fields, forests and a mediterranean shrub (*Nerium oleander* L.) growing under natural or garden environments. The most frequently insect pathogens found were fungi (32.8% soils), being *Beauveria bassiana* (Bals.) Vuill (21% soils) the species most abundant. *Metarhizium anisopliae* (Metschn.) Sorok (6.4%) and *Lecanicillium lecanii* (Zimm.) Gams [= *Verticillium lecanii* Zimm.] (4.8%) were less frequent. *B. bassiana* also scored the highest infection percentage in a single soil sample (ca. 90% of insects infected), and was also the most frequent (77.8%) entomopathogenic fungus detected in soils under *N. oleander*.

Key words: *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii, Galleria mellonella*, biocontrol.

Resumen

Hongos entomopatógenos de suelos de la provincia de Alicante

Hemos utilizado larvas de *Galleria mellonella* (Linnaeus, 1758) como cebo para detectar patógenos de insectos en suelos de Alicante (SE España). Se recogieron muestras de suelo de 61 localidades incluyendo campos agrícolas, bosques y matorral mediterráneo (*Nerium oleander* L.). Estas últimas muestras se tomaron tanto de jardines como de formaciones de vegetación natural. Los patógenos de insectos más frecuentes (46,8% de los suelos) fueron los hongos, siendo *Beauveria bassiana* (Bals.) Vuill (21% de los suelos) la especie más abundante. También se encontraron *Metarhizium anisopliae* (Metschn.) Sorok (6,4%) y *Lecanicillium lecanii* (Zimm.) Gams [= *Verticillium lecanii* Zimm.] (4,8%), aunque con menor frecuencia. *B. bassiana* fue también el hongo con mayor porcentaje de infección en una muestra de suelo (90% de insectos infectados), y el más frecuentemente detectado (77,8%) en suelos bajo *N. oleander*.

Palabras clave: *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii, Galleria mellonella*, control biológico.

Introduction

For most soils, fungi (either saprotrophs, mycorrhizal or pathogens) are the main component of its microbiota (Gams, 1992). Soil is also the main source of fungal entomopathogens, and isolation of these organisms involves soil sampling since that is their natural habitat.

Soil factors (temperature, pH or organic content, relative moisture or mineral, organic or biotic components) can affect fungal persistence and activity (Riba *et al*., 1991; Charnley, 1997). It is therefore important to know physico-chemical properties of soils containing entomopathogens. Piralid lepidopteran *Galleria mellonella* L. larvae are a suitable bait for detection of insect antagonists (mostly microbial) present in soil samples (Zimmermann, 1986). Experiments carried out using these larvae showed that their mobility depended on soil structure and particle size (Zimmermann, 1986), factors that have been shown to influence infection of insects by microbial entomopathogens (Vänninen, 1995).

In the so-called «suppressive soils», without taking any control measures, plant diseases are not economically important. For some of these soils, natural enemies have been found to control pathogen populations by affecting several stages of its life cycle (Kerry, 1990). Chances of finding good candidates to be used as biocontrol agents in these soils

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are very high (Olivares-Bernabeu and Lopez-Llorca, 2002).

It is also interesting to compare the presence of microbial antagonists in soil environments with (agricultural soils) or without («natural» soils) human impact. A recent study of microbial entomopathogens of insect pests of apple and pear crops showed a wide variety of entomopathogenic viruses, bacteria, fungi and nematodes from soils of northern and central Europe (Cross *et al*., 1999). Bidochka *et al*. (1998) evaluated the presence in soils from Canada of cosmopolitan species of entomopathogenic fungi, such as *Metarhizium anisopliae* (Metschn.) Sorok. and *Beauveria bassiana* (Bals.) Vuill., more abundant in agricultural, and natural soils, respectively.

In the present study we have analysed the presence of entomopathogens, mainly fungi, in soils from different ecosystems of Alicante province such as forests, crop fields, natural and garden areas.

Material and methods

Soil sampling

Agricultural, forest, dry river beds and garden soils were sampled in the province of Alicante (SE Spain) to survey microbial antagonists (mainly fungi) of insects (Table 1). We collected 61 samples selected to represent soil and climate variability: 12 from agricultural soils (*Prunus amygdalus* Batsch., *Citrus* sp., and other woody crops), 8 from forest soils (*Pinus halepensis* Mill. and *Quercus rotundifolia* Lam.), the rest under plants of *Nerium oleander* L., 20 from natural populations (mostly in dry river beds) and 21 from gardens.

For each sampling site, soil (1.5-2 kg) was collected from 3 points randomly selected and mixed to make an homogeneous sample. Then they were collected from 0-20 cm depth, removing for forest soils the leaflitter layer. Soil samples were homogeneized in the laboratory, 2 mm sieved and stored at 4ºC in the dark before use (less than 12 months).

Physico-chemical analysis of soils

Before analysis, soils were spread on a tray. Soil aggregates were broken by hand or using a hammer. Trays with soil were kept open until soil moisture was equilibrated with that of the laboratory.

Soil texture, pH, conductivity/salinity and carbonate content were determined for all soils collected. Protocols used were those of Anonymous (1979). Three measurements were taken per each physicochemical parameter and soil sample.

Antagonist detection. Isolation of entomopathogenic fungi

Insect antagonists in soil were detected using *G. mellonella* larvae (from Carolina Biological Supply Co., NC, USA, 27215-3398). Forty grams of soil (freshly collected or 4ºC stored for less than 12 months) were 1 mm sieved and placed in 90 mm diameter sterile Petri dishes (3 replicates per soil). Ten living L_3-L_4 *G. mellonella* larvae were buried per plate. Plates were sealed with Parafilm (American National Can.) and incubated at 25ºC for 15 days in the dark. After that time the insects were recoved from soil using a dissecting microscope at 40x magnification. Recovered insects were surface sterilised using 1% sodium hypochlorite for 1 min. The sterilizing agent was eliminated and insects were washed three times in sterile distilled water (5 min each time). Insects were finally blotted dry onto sterile filter paper and placed in moist chambers for 1 week at 25ºC in the dark. These conditions usually allowed development (and often sporulation) of insect antagonists (mostly entomopathogenic fungi).

Larvae with cottony filaments (probably fungus mycelium) were then observed with a dissecting microscope. Larvae with few hyphae on the surface were assumed to be colonized by fungal saprotrophs. On the contrary, larvae displaying abundant mycelium (i.e. in intersegmental regions) were considered infected. These larvae were plated on corn meal agar (CMA) containing 50 μg ml^{-1} penicillin, 50 μg ml^{-1} streptomycin, 50 μg ml^{-1} rose bengal and 1 mg ml^{-1} Triton X-100. Slide cultures (Gams *et al*., 1998) of fungi infecting larvae were carried out and after microscopic observation, genus was assigned according to Domsch *et al*. (1993). Species identification was determined using specialised bibliography including Lacey (1997). Entomopathogenic fungi were inoculated on CMA plates and then transferred to 10 ml universal bottles with CMA slopes and after 7-15 days under normal incubation, they were stored at 4ºC in the dark. Conidia and conidiophores of each fungal strain were measured (10 estimations per strain and structure). The size of the fun-

Table 1. Location of soil sampling sites, crop-vegetation types and physico-chemical parameters of soil samples

Sample	Sampling site (UTM)	Crop-vegetation	pH (in H_2O)	Conductivity CS (meq l^{-1})	Carbonates (% $CaCO_3$)	Texture
1	Maigmó (30SYH141544)	Almond trees	8.93 ± 0.04	142.93 ± 5.94	52.28 ± 5.04	Loamy sand
2	Maigmó (30SYH078668)	Almond trees	8.77 ± 0.03	99.45 ± 22.13	55.03 ± 2.40	Loamy sand
3	Tárbena (30SYH521876)	Almond trees	8.30 ± 0.145	96.40 ± 1.35	36.78 ± 17.07	Loamy sand
4	Callosa (30SXH902425)	Lemon trees	8.39 ± 0.017	166.18 ± 36.87	54.82 ± 8.90	ND
5	Orihuela (30SXH789237)	Lemon trees	8.40 ± 0.051	1012.4 ± 185.7	41.26 ± 6.03	Loamy-Clay-silt
6	Benjúzar (30SXH902171)	Lemon trees	8.61 ± 0.055	158.50 ± 21.53	41.31 ± 1.61	Loam
7	Benferri (30SXH786262)	Orange trees	8.67 ± 0.025	104.96 ± 7.83	67.44 ± 19.83	ND
8	Almoradí (30SXH935189)	Orange trees	8.85 ± 0.023	117.67 ± 9.08	46.41 ± 3.11	ND
9	Gata (31SBC488968)	Orange trees	8.19 ± 0.040	78.33 ± 13.03	61.04 ± 13.39	Loamy-Clay-silt
10	Denia (31SBD451025)	Orange trees	8.16 ± 0.081	116.37 ± 6.37	55.67 ± 12.33	Loam
11	Salinas (30SXH821649)	Apple trees	8.76 ± 0.026	157.65 ± 21.32	41.58 ± 8.60	ND
12	Ibi (30SYH175798)	Apple trees	8.57 ± 0.055	77.33 ± 5.73	56.92 ± 3.57	ND
13	Pla de Girau (30SYH101611)	*Pinus halepensis*	8.43 ± 0.025	135.04 ± 4.48	135.04 ± 4.48	ND
14	Maigmó (30SYH076647)	*Pinus halepensis*	8.41 ± 0.040	120.51 ± 8.53	120.51 ± 8.53	Loamy sand
15	Castalla (30SXH971745)	*Pinus halepensis*	8.62 ± 0.040	113.92 ± 23.90	113.92 ± 23.90	Loamy sand
16	Sax (30SXH935728)	*Pinus halepensis*	8.82 ± 0.130	108.07 ± 4.08	108.07 ± 4.08	ND
17	Orba (30SYH554951)	*Pinus halepensis*	8.34 ± 0.060	74.73 ± 5.07	74.73 ± 5.07	ND
18	Ibi (30SYH136787)	*Pinus halepensis*	8.27 ± 0.035	131.84 ± 10.47	131.84 ± 10.47	Sandy loam
19	Font Roja (30SYH18)	*Quercus rotundifolia*	7.60 ± 0.011	214.18 ± 33.66	214.18 ± 33.66	Loamy sand
20	Font Roja (30SYH127818)	*Quercus rotundifolia* and *P. halepensis*	8.19 ± 0.060	123.41 ± 3.98	123.41 ± 3.98	Loam
21	Aguas de Busot (30SYH277597)	*N. oleander* (N)	8.67 ± 0.025	167.68 ± 19.32	51.10 ± 5.88	Loamy sand
22	La Vila (30SYH368642)	*N. oleander* (N)	8.44 ± 0.034	98.38 ± 0.57	40.67 ± 5.79	ND
23	Finestrat (30SYH431699)	*N. oleander* (N)	8.18 ± 0.030	915.62 ± 69.94	43.65 ± 17.03	ND
24	Benidorm (30SYH433686)	*N. oleander* (N)	8.22 ± 0.047	313.38 ± 21.26	46.52 ± 2.40	ND

Table 1 (cont.)

Sample	Sampling site (UTM)	Crop-vegetation	pH (in H_2O)	Conductivity CS (meq l^{-1})	Carbonates (% $CaCO_3$)	Texture
25	Altea (30SYH524758)	*N. oleander* (N)	8.08 ± 0.035	156.37 ± 15.09	47.03 ± 5.63	Loamy sand
26	Calpe (30SYH577776)	*N. oleander* (N)	7.95 ± 0.025	665.81 ± 56.29	24.72 ± 2.31	Loamy sand
27	Benisa (30SYH419837)	*N. oleander* (N)	8.24 ± 0.075	124.58 ± 2.66	49.04 ± 7.96	Loamy sand
28	Fonts de l'Algar (30SYH7956)	*N. oleander* (N)	8.11 ± 0.03	114.56 ± 2.79	39.28 ± 2.58	Sandy
29	Tibi (30SYH116683)	*N. oleander* (N)	8.25 ± 0.07	139.95 ± 4.11	49.99 ± 7.28	Loam
30	Elda (30XH915635)	*N. oleander* (N)	8.44 ± 0.04	153.39 ± 3.52	62.89 ± 8.93	Sandy loam
31	Alcoy (30SYH105802)	*N. oleander* (N)	8.36 ± 0.07	129.71 ± 7.42	68.33 ± 14.01	Sandy loam
32	Líber (30SBC605926)	*N. oleander* (N)	8.24 ± 0.03	114.56 ± 7.27	37.93 ± 2.26	Sand
33	Jalón river (30SYH599928)	*N. oleander* (N)	7.97 ± 0.01	161.07 ± 15.36	64.51 ± 9.41	Sand
34	Parcent (30SYH556926)	*N. oleander* (N)	8.24 ± 0.01	146.13 ± 7.74	31.54 ± 1.38	Sandy loam
35	Vall de Laguart (30SYH505972)	*N. oleander* (N)	8.08 ± 0.01	74.88 ± 11.21	35.37 ± 4.87	ND
36	Fuente Baladrar (30SYH546997)	*N. oleander* (N)	7.66 ± 0.05	128.64 ± 2.31	24.65 ± 1.65	ND
37	Pego (30SYJ40606)	*N. oleander* (N)	8.15 ± 0.04	141.01 ± 10.52	26.61 ± 2.37	ND
38	Cocentaina (30SYJ0343)	*N. oleander* (N)	8.16 ± 0.07	123.01 ± 0.97	ND	ND
39	Callosa (30SXH861246)	*N. oleander* (N)	8.78 ± 0.01	84.26 ± 5.33	69.42 ± 5.12	ND
40	Callosa (30XH932216)	*N. oleander* (N)	8.59 ± 0.01	164.91 ± 29.53	36.78 ± 1.79	ND
41	El Palmeral (30SYH17402)	*N. oleander* (A)	8.61 ± 0.04	176.21 ± 6.66	62.38 ± 8.26	Loam
42	Univ. Alicante (30SYH173502)	*N. oleander* (A)	8.30 ± 0.02	153.60 ± 3.20	71.87 ± 9.63	Loamy sand
43	Guardamar (30SYH41179)	*N. oleander* (A)	8.52 ± 0.03	391.68 ± 20.23	33.67 ± 4.50	Clay
44	Gran Alacant (30SYH161353)	*N. oleander* (A)	8.03 ± 0.03	250.03 ± 4.49	37.02 ± 2.37	Loam
45	Guardamar (30SYH051188)	*N. oleander* (A)	8.93 ± 0.01	351.36 ± 2.31	62.07 ± 10.40	Loam
46	Guardamar (30SYH066188)	*N. oleander* (A)	8.11 ± 0.01	114.56 ± 8.10	ND	ND
47	Ibi (30SYH118785)	*N. oleander* (A)	8.48 ± 0.06	151.68 ± 12.85	62.48 ± 9.39	ND
48	Castalla (30SYH317749)	*N. oleander* (A)	8.14 ± 0.02	825.17 ± 13.80	62.32 ± 7.96	Loamy sand

Table 1 (cont.)

Sample	Sampling site (UTM)	Crop-vegetation	pH (in H_2O)	Conductivity CS (meq l^{-1})	Carbonates (% $CaCO_3$)	Texture
49	Onil (30SYH024784)	*N. oleander* (A)	8.58 ± 0	173.44 ± 2.93	60.32 ± 11.90	Loamy sand
50	Novelda (30SXH959516)	*N. oleander* (A)	8.55 ± 0.03	229.33 ± 1.95	52.73 ± 19.04	Loamy sand
51	Aspe (30SXH958459)	*N. oleander* (A)	8.29 ± 0.02	483.62 ± 7.02	57.82 ± 6.67	Sandy loam
52	Elche (30SYH032382)	*N. oleander* (A)	7.92 ± 0.02	696.11 ± 41.26	59.69 ± 6.41	Loamy sand
53	Alcoy (30SYH109803)	*N. oleander* (A)	7.92 ± 0.05	182.19 ± 5.52	40.00 ± 2.42	Loamy sand
54	Cocentaina (30SYH203904)	*N. oleander* (A)	8.14 ± 0.05	151.89 ± 9.26	46.75 ± 9.68	Sandy loam
55	La Vila (30SYH6642)	*N. oleander* (A)	8.77 ± 0.02	293.12 ± 13.32	78.26 ± 4.74	ND
56	Benidorm (30SYH502698)	*N. oleander* (A)	8.25 ± 0.04	200.53 ± 4.50	ND	Loamy sand
57	Altea (30SYH509703)	*N. oleander* (A)	8.25 ± 0.05	331.73 ± 10.73	38.96 ± 5.31	Loamy sand
58	Calpe (30SYH061081)	*N. oleander* (A)	8.44 ± 0.04	222.08 ± 4.43	69.78 ± 2.95	Sandy loam
59	Alcalalí (30SYH566932)	*N. oleander* (A)	8.35 ± 0.04	138.02 ± 3.75	ND	Sandy loam
60	Fuente Baladrar (30SYH548998)	*N. oleander* (A)	8.21 ± 0.02	58.88 ± 4.83	21.40 ± 1.85	ND
61	Callosa (30SXH861218)	*N. oleander* (A)	8.45 ± 0.01	164.91 ± 29.53	64.73 ± 10.89	ND

UTM: universal transverse mercator. N: natural. A: artificial. ND: not determined.

gal structures was estimated as the average of the measurements taken.

Data analysis

Statistics were applied to analyse differences with respect to the isolation of entomopathogenic fungi in the environments surveyed. For soils, when checking the homogeneity of variances Levene's statistic, we found that they were non homogeneous within the four populations (agricultural, forest and natural or gardens *N. oleander* soils) tested. We could not then apply an ANOVA test, since the four populations did not show internal variations. The alternative was to apply a Kruskall-Wallis test, used when differences are non-significant, and we carried out an analysis of possible interpopulation differences.

Results

Physical and chemical characterization of soils

Table 1 indicates physico-chemical characteristics of soil samples. Most soils collected displayed in water a basic pH (more than 8). Conductivity was moderate for most soils (over 100 meq l^{-1}). However some soil samples taken in *Nerium oleander* sites had a high conductivity (more than 600 meq l^{-1}). The highest value of conductivity (over 1000 meq l^{-1}) was recorded from a lemon tree field. Carbonate content was also moderate (40-60%) for most soils and high (100%) for soil samples collected under forests. Texture was very variable, being most of the classes represented.

For the variable soil pH we found a Kruskall-Wallis value of H [60,20]=0.026 with P>0.05. This indicates

Table 2. List of entomopathogenic fungi isolated from soil samples and their morphological data for conidia and phialides (conidiophores)

Sample	Entomopathogenic fungus	Morphological data	
		Phialide size (µm)	Conidium size (µm)
1	*Metarhizium anisopliae*	24.53 ± 7.39 × 1.6	8 ± 1.30 × 2.88 ± 0.55
2	*Metarhizium anisopliae*	20.8 ± 4.52 × 1.3 ± 0.44	7.36 ± 0.82 × 3.2 ± 0.01
2	*Beauveria bassiana*	21.33 ± 7.89 × 1.6	2.56 ± 0.82 × 1.68 ± 0.25
3	*Beauveria bassiana*	12.08 ± 4.52 × 1.6	2.64 ± 1.06 × 1.84 ± 0.53
7	*Lecanicillium lecanii*	36 ± 3.06 × 3.2	6.88 ± 1.31 × 2.88 ± 0.55
11	*Beauveria bassiana*	7.46 ± 5.60 × 1.6	2.16 ± 0.75 × 1.92 ± 0.67
12	*Metarhizium anisopliae*	13.6 × 2.4	9.12 ± 0.77
13	*Lecanicillium psalliotae*	15.2 ± 9.28 × 1.6	8.96 ± 1.54 × 2.88 ± 0.67
15	*Beauveria bassiana*	19.2 ± 6.4	2.8 ± 1.26 × 1.6 ± 0.03
16	*Metarhizium anisopliae*	12.8 ± 0.03 × 2.4 ± 0.04	7.52 ± 1.07 × 3.2 ± 0.07
17	*Beauveria bassiana*	6.4 × 2 ± 0.56	4.16 ± 1.34 × 2.56 ± 0.82
20	*Beauveria bassiana*	16 ± 5.6 × 1.6	2.16 ± 0.65 × 1.68 ± 0.25
22	*Beauveria bassiana*	6.4 × 2 × 0.56	4.16 ± 1.34 × 2.56 ± 0.82
27	*Beauveria bassiana*	15.6 ± 5.7 × 1.6	4.8 ± 1.25 × 2 ± 0.40
28	*Lecanicillium lecanii*	30.4 ± 2.49 × 1	10.6 ± 0.28 × 1
48	*Lecanicillium lecanii*	29.2 ± 0.55 × 1	11 ± 0.79 × 1
49	*Beauveria bassiana*	21.60 ± 3.27 × 1	7.2 ± 0.82 ×1
31	*Beauveria bassiana*	23.80 ± 1.74 × 1	7.76 ± 0.94 × 1
56	*Beauveria bassiana*	18.8 ± 1.67 × 1	4.64 ± 0.44 × 1
32	*Beauveria bassiana*	19.6 ± 1.64 × 1	7.2 ± 1.78 × 1
36	*Beauveria bassiana*	24.0 ± 3.32 × 1	5.6 ± 0.57 × 1

that no differences on pH were found regarding the environment or population type. For the rest of the variables, such as soil conductivity (H [60,20]=0.03) and carbonate content (H [60,20]=0.00), similar results were found.

Presence of entomopathogenic fungi in soils

As already described, *G. mellonella* larvae were used as bait for microbial antagonists of insects in the soils collected. At 32.8% of soils (20 out of 61), entomopathogenic fungi were the most frequent insect pathogens. Results of isolations of fungal strains and the morphological data for conidia and phialides (values are averages for 10 measurements carried out at random) are recorded in Table 2.

Species of entomopathogenic fungi isolated included the Hyphomycetes *Beauveria bassiana* (Balsamo) Vuillemin in 13 soil samples (21% of soils), *Metarhizium anisopliae* (Metschnikoff) Sorokin (6.4% of soils), *Lecanicillium lecanii* (Zimmerman) Viegas (=*Verticillium lecanii*) (4.8%), and finally *Lecanicillium psalliotae* Treschow (= *Verticillium psalliotae*) (only 1.6% soils) (Figs. 1 and 2).

In Fig. 3, data are given on the abundance of entomopathogenic fungi in the soils where they were detected. Values are the percentages of larvae developing a given fungus. *B. bassiana* was also the pathogen which scored the highest infection percentage in a single soil sample (no. 27, from a natural population of *N. oleander* with ca. 90% of insects infected). Only one soil sample (no. 2), presented two species (*B. bassiana* and *M. anisopliae*) of entomopathogenic fungi (Table 2).

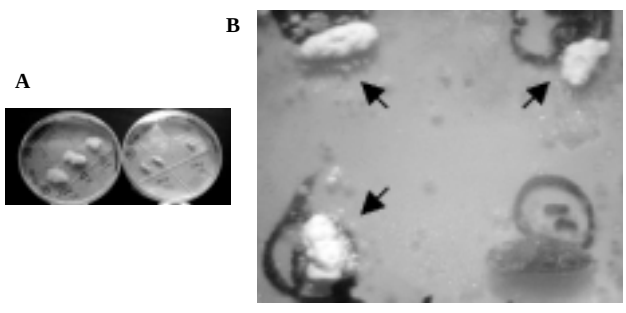

Figure 1. A) *Galleria mellonella* L. larvae developing *Beauveria bassiana* (Bals.) Vuill. (moist chamber). B) *G. mellonella* on rose bengal medium after developing *B. bassiana* (arrows).

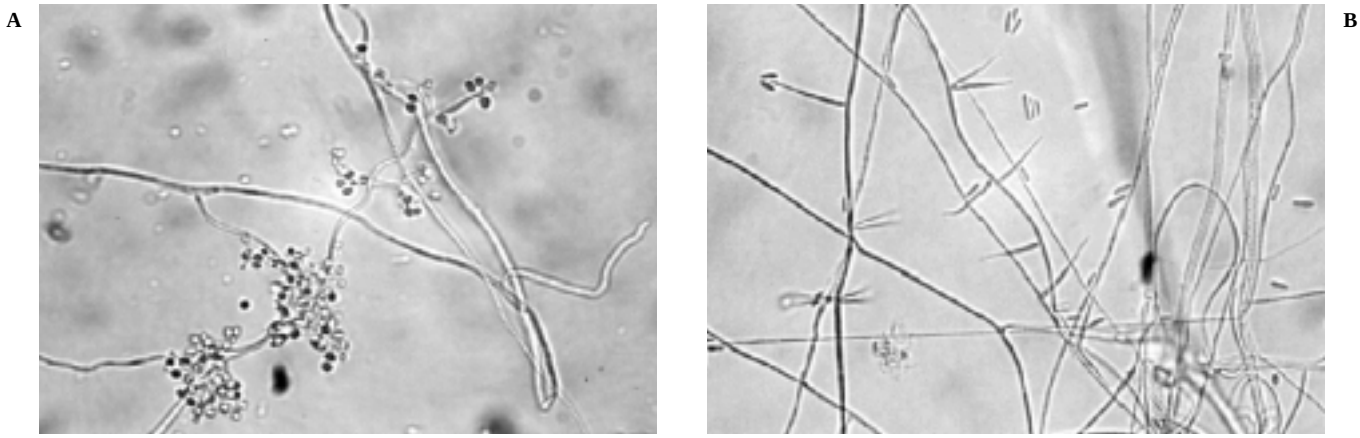

Figure 2. Entomopathogenic fungi isolated: A) *Beauveria bassiana* from soil sample 50. B) *Lecanicillium lecanii* (= *Verticillium lecaniii)* from soil sample 29.

Only three species of entomopathogenic fungi were found in both agricultural and forests soils. On the contrary, *B. bassiana* was nearly the only entomopathogenic fungus detected in soils under *N. oleander* (77.8%).

A higher number of forest soils analysed (62.5%) displayed entomopathogenic fungi respect to agricultural soils (50%). In the case of soils under *N. oleander,* this number was higher in soils under natural vegetation (30%) than in those from gardens (14%).

In accordance to this, *B. bassiana* was mostly isolated from forest soils, and least from garden soils. On the contrary, *M. anisopliae* was mainly found in agricultural soils, and not found under *N. oleander*. *L. lecanii* was equally found in *N. oleander* soils (both natural and from gardens) and agricultural soils, but not in forest soils. We found *L. psalliote* in only one forest soil, but with a high (>80%) infection percentage.

Apart from fungal entomopathogens, we also detected colonisation of *G. mellonella* larvae by common soil fungi (Zygomycetes and Deuteromycetes or Mi-

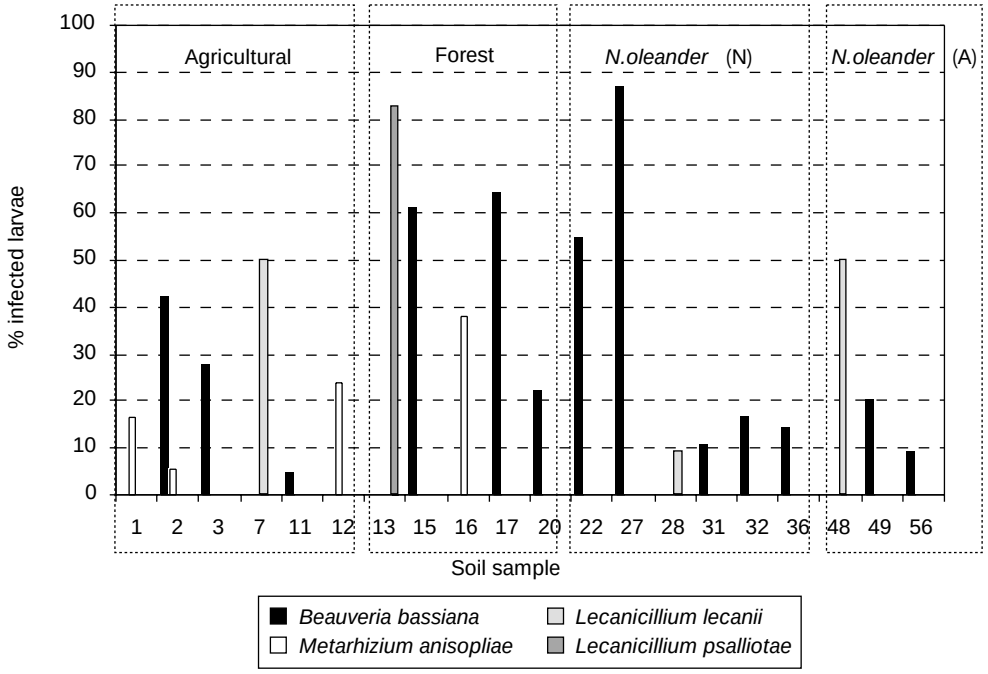

Figure 3. Abundance of entomopathogenic fungi in different soil samples, grouped by their environmental origin. N: natural. A: artificial.

tosporic fungi). The most common were *Mortierella* sp., *Rhizopus* sp., *Penicillium* sp. and *Aspergillus* sp., although other genera such as *Fusarium* sp. were also found.

Discussion

In our study we have isolated entomopathogenic fungi from several soil environments, including garden soils with a large antropic influence, although less fungi were recovered from these. This indicates that entomopathogenic fungi can be naturally found from environments close to host plants (either cultivated or growing naturally) that harbour phytophagous insects.

We have not found significative differences in the physico-chemical conditions among soils sampled in our survey. This seems logical since most of soils sampled are basic with a high carbonate content. We have found several species of entomopathogenic fungi, although the most common was *B. bassiana*.

Tarasco *et al*. (1997) found *B. bassiana* and *M. anisopliae* to be the most abundant entomopathogenic fungi in Southern Italy. However, they never recorded simultaneously two species in the same soil sample. In Canadian soils, the most frequent species were also *M. anisopliae* and *B. bassiana* (Bidochka *et al*., 1998). The latter was majority in soils from colder areas. These soils also had larvae infected with *Paecilomyces* spp.

Diverse factors may affect survival of entomopathogenic fungi in soil. Lingg and Donaldson (1981) found that survival of *B. bassiana* conidia is dependent on temperature and soil water content. Raid and Cherry (1992) reported that *M. anisopliae* conidia were pathogenic on the sugar cane pest *Lygirus subtropicus* under a wide range of soil temperatures and moistures. Moreover, in general terms, *M. anisopliae* is able to resist in soil longer than *B. bassiana*, because the latter seems more sensitive to soil microbiota (Bidochka *et al.*, 1998).

We have found entomopathogenic fungi from nearly 15% of the *N. oleander* soils tested. Natural *N. oleander* populations develop under mediterranean conditions in streams with very irregular water supply, therefore with poor soils, little profiled and with low organic matter. The presence of entomopathogenic fungi in those soils shows that these antagonists are able to cope with high environmental stress.

Our study confirms that soils are an important reservoir of strains of entomopathogenic fungi, potential antagonists for controlling insect pests. The strains isolated in this study are being further tested for development as biocontrol agents.

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