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Influence of crop species and edaphic factors on the distribution and abundance of *Trichoderma* in Alfisol soils of southern India

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Abstract – The effect of crop species and edaphic factors on the distribution of *Trichoderma* species in Alfisol soil under different agrosystems was evaluated. Each soil sample was assayed for nine abiotic factors and culturable microfungal populations. Fungal abundance was determined by dilution plate technique, and the identification of fungi was based on morphological characteristics. Pearson's correlation coefficient was used to determine the relationship of association between these factors and the presence and abundance of *Trichoderma* species in each soil type. The abundance of soil fungi ranged between 7.0×10^3 and 13.6×10^3 colony forming units (cfus) per gram of dry soil. The population densities of the two *Trichoderma* species (*T. koningii* and *T. viride*) isolated in the present study varied significantly with crop species and their abundance (varied from 0.6 to 3.6×10^3 cfus g^{-1} dry soil). Twenty-two other colony-forming fungal types with an abundance ranging between 7.0×10^3 and 13.6×10^3 cfus g^{-1} dry soil were also isolated in the present study. As soil pH negatively influenced relative abundance of *T. koningii*, soil P and relative abundance of *T. viride* were significantly and positively correlated to each other. Further, relative abundance of *T. koningii* was significantly and positively correlated to relative abundance of *Aspergillus fumigatus* but negatively correlated to relative abundance of *Stachybotrys atra.* Likewise, a significant negative correlation existed between relative abundance of *T. viride* and *Absidia glauca*.

Key words: Alfisol soil, crops, edaphic factors, fungal abundance, soil pH, *Trichoderma*

Introduction

Trichoderma are abundant free living soil fungi that are highly interactive in root, soil and foliar environments. Their significance resides in their potential for control of soil borne plant pathogens. *Trichoderma* act as biocontrol agents through competition for nutrients or space, mycoparasitism and antibiosis (WELLS 2000). Some strains also act as plant growth promoters through solubilization and sequestration of organic nutrients (HARMAN et al.

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2004a). There is a reasonable amount of information about the behavior of many soil borne plant pathogens in the soil environment. However, little attention has been given to factors affecting growth of antagonistic organisms such as *Trichoderma* in the soil (WAKELIN et al. 1999). Factors affecting the growth of *Trichoderma* under laboratory conditions have been extensively studied, but the correlation between various growth responses in *in situ* and *in vivo* conditions are dubious (COOK and BAKER 1983, PAPAVIZAS 1985). This limited knowledge on the ecology of these biocontrol agents coupled with the environment variability limits the success of *Trichoderma* as a biological control agent under field situations (LEWIS and PAPAVIZAS 1991). There is an urgent need for more information on the factors affecting the distribution of *Trichoderma*, in agro ecosystems.

The Alfisol soils that constitute around 10% of the ice-free land area are the dominant soil types in many tropical and subtropical areas, including peninsular India (RUST 1983). Alfisols are characterized by high fertility and water-holding capacity, moderate leaching and have at least 35% base saturation (SOIL SURVEY STAFF 1994). Therefore, extensive areas of Alfisols have been used for cultivation or forestry (RAO et al. 1991). As high yields and sustainable agriculture are targeted by farmers, researchers, politicians and society, knowledge of the fungal population present in the soil and their functions in the corresponding ecosystems is vital. Although some soil fungi (e.g., mycorrhizal fungi) are intensively studied, little is known about fungal community structures and community dynamics in agricultural soils. It is generally agreed that only a small percentage of the 1.5 million fungi world-wide are culturable (HAWKSWORTH and ROSSMAN 1997). Nevertheless, up to now, many studies have investigated fungal diversity by using culture–dependent approaches (ATTITALLA et al. 2012, OKOTH et al. 2009, SARIAH et al. 2005). In spite of studies examining the bioinoculant potential of *Trichoderma* against various plant pathogens in Alfisol soils (MANJULA et al. 2004, KUMAR et al. 2012), information on the survivability and proliferation of fungi in relation to soil type and factors is limited. The ability of *Trichoderma* to dispose and to colonize the rhizosphere will determine its effectiveness as a biocontrol agent. Thus, an understanding of the quantitative distribution of the fungus in different agro-ecosystems is essential before it can be developed into biological formulations for field application. The following study was undertaken with the objectives of (i) quantifying the populations of *Trichoderma* spp., from cultivated ecosystems, (ii) to determine if crop species had any significant influence on the distribution or abundance *Trichoderma* spp. and (iii) to characterize the influence of edaphic factors and co-occurring fungal species on populations of *Trichoderma.*

Materials and methods

Sampling

Alfisol soil samples were collected from agrosystems within a 2 km radius of Coimbatore (11°04' N and 76°92' E, altitude 426.7–550 m a.s.l.), Tamil Nadu, India. These included soil samples under ten different crop species: tomato (*Lycopersicon esculentum*, soil 1), coconut (*Cocus nucifera*, soil 2), sorghum (*Sorghum bicolor*, soil 3), ground nut (*Arachis hypogea*, soil 4), maize (*Zea mays*, soil 5), sugarcane (*Saccharum officinarum*, soil 6), rice (*Oryza sativa*, soil 7), turmeric (*Curcuma longa*, soil 8), areca nut (*Areca catechu*, soil 9) and papaya (*Carca papaya*, soil 10).

Ten undistributed soil cores measuring 3 cm diameter were randomly taken from the depth of 0–15 cm of the top horizon adjacent to the plants, and combined. The composite samples were placed in airtight sterile Ziploc polythene bags and transported to the laboratory. Half of the soil samples used to assess the soil microfungal populations was stored at 4 °C, while the other half was air dried, passed through a 2-mm screen to remove coarse debris and plant materials, and used to analyze the physico-chemical properties of the soil.

Determination of soil characteristics

The composite soil sample from each agrosystem was divided into five equal parts and assessed for various properties. One hundred mL of sterile distilled water was added to 100 g of soil sample, thus yielding a 1:1 soil suspension. The samples were stirred briefly and allowed to equilibrate for 1h. The pH of each sample was then measured using a digital pH meter (ELICO LI 610, Hyderabad, India) by immersing the electrode in the sediment at the bottom of the soil slurry. One hundred mL of distilled water was added to 10 g of dry soil to make a suspension of 1: 10 (W/V) dilution. The electrical conductivity EC was measured using a digital EC TDS analyzer (ELICO CM 183, Hyderabad, India). The total nitrogen (N) and phosphorus (P) in the soils were determined by micro-Kjeldahl and molybdenum blue methods respectively (JACKSON 1971). Soil K was estimated by the flame photometric method after extraction in ammonium acetate solution (JACKSON 1971). Other mineral (Fe, Zn, Cu and Mn) element composition was also analyzed according to JACKSON (1971).

Evaluation of soil micro fungal population

Soil dilution technique was used to evaluate the total number of fungal colony forming units (cfus) in the soil samples and to isolate pure cultures (DANIELSON and DAVEY 1973). Each composite soil intended for the assessment of micro fungal populations was divided into five equal parts. Five grams (on dry weight basis) of the composite soil subsample were added to 250 mL sterile conical flasks containing 100 mL of sterilized distilled water. Flasks were shaken vigorously for five minutes. One mL aliquots of the $1:10^3$ dilutions were spread evenly on each of the five Petri dishes (100 × 15 mm) containing 20 ml of agar rose Bengal medium (MARTIN 1950) supplemented with 0.06 g L^{-1} of streptomycin sulphate for each composite soil subsample. Petri plates were incubated at room temperature (26–30 °C) till the development of fungal colonies. After four days, all fungal colonies were counted and the plates were incubated again for another three days. Many colonizers sporulated after seven days, so at that time individual colony types were counted, including those of *Trichoderma.* For each of the colony types counted, representative colonies were isolated and brought under pure culture for identification. Results were recorded as the number of colonies per plate. Averages of composite soil subsamples were used to determine the abundance and frequency of soil micro fungal populations.

Semi-permanent slides were prepared by mounting fungi in lactophenol containing 0.5% cotton blue. The characterization of *Trichoderma* isolates into species aggregates were based on cultural and morphological characters (BISSETT 1984, 1991, 1992; BISSETT et al. 2003; CHAVERRI et al. 2001, 2004; CHAVERRI and SAMUELS 2002, 2003; RIFAI 1969; WEBSTER 1964; WEBSTER and RIFAI 1968). The other co-existing soil fungi were also identi-

fied according to the descriptions by BARRON (1972), DOMSCH et al. (1980), ELLIS (1971), NELSON et al. (1983), PITT (1979), and SUBRAMANIAN (1970).

Abundance (isolates of a species as percent of total isolates) and frequency of occurrence (percent from total plate samples) for each soil was calculated using the formulae:

$$\text{Abundance} = \frac{\text{Number of colonies of a particular fungus in the sample}}{\text{Total number of fungal colonies of the sample}} \times 100$$

$$\text{Frequency} = \frac{\text{Number of agrosystems in which a particular fungus was present}}{\text{Total number of agrosystems examined}} \times 100$$

Statistical analysis

Data on soil characteristics and microfungal populations were subjected to Analysis of Variance and the means were separated using Duncan's Multiple Range Test (DMRT) (SPSS for windows, Version 9). The relationships between soil factors and microfungal populations and among fungal populations were analyzed by Pearson's correlation. Data were log transformed prior to statistical analysis.

Results

Soil factors

Measurement of pH, EC, soil N, P, K, Fe, Zn, Mn, Cu and Fe are presented in table 1. The soils in the present study were near neutral to alkaline with a pH range of 7.23 to 8.23. The EC ranged from 0.245 to 1.05 dSm^{-1}. The soils were nutrient deficient with various nu-

Tab. 1. Characteristics of the Alfisol soils used in the study.

Soil	pH	EC ($dS\ m^{-1}$)	Soil nutrients ($mg\ kg^{-1}$)						
			Nitrogen	Phospho-rus	Potassium	Copper	Iron	Zinc	Manga-nese
S1	7.23 a	0.245 a	9.8 b	0.25 a	42.00 c	1.41 a	26.44 f	1.24 d	8.24 c
S2	7.64 b	0.636 f	10.1 b	0.75 b	50.00 c	1.31 a	10.42 d	1.52 f	2.84 a
S3	7.93 c	0.299 b	14.0 e	1.15 c	40.00 c	1.21 a	5.44 a	1.28 de	4.24 b
S4	7.89 c	0.536 e	11.5 c	0.26 a	51.00 d	1.80 b	8.32 c	0.86 b	8.22 c
S5	8.23 e	0.315 b	6.4 a	0.28 a	42.00 c	1.24 a	6.78 b	0.76 ab	4.24 b
S6	8.10 d	0.302 b	14.0 e	0.45 a	18.00 a	3.64 e	10.60 d	1.48 f	18.60 e
S7	7.74 b	1.050 g	12.6 d	1.20 c	51.00 d	2.82 c	8.50 c	1.08 c	7.06 c
S8	8.12 d	0.408 c	10.4 b	1.55 d	31.00 b	4.06 f	14.80 e	0.64 f	15.56 d
S9	7.99 c	0.448 d	10.6 b	1.30 c	19.00 a	3.08 d	9.41 cd	1.40 ef	16.20 d
S10	7.93 c	0.548 e	14.0 e	1.35 c	51.00 d	4.24 f	10.36 d	1.60 f	20.24 f

Means in a column followed by same letter(s) are not significantly ($p > 0.05$) different according to Duncan's multiple range test. Soil types S1–S10 are explained in Methods.

Tab. 2. Distribution and frequency (percent from total plate samples) of soil microfungi associated with different crop species. Soil types S1 – S10 are explained in Methods.

Fungal species	Soils										Frequency (%)
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	
Absidia glauca Hagem	+	+		+		+	+	+		+	70
Aspergillus candidus Link ex. Fr.	+	+	+			+		+	+	+	70
Aspergillus flavipes (Bain. et Sart.) Thom et Chruch	+			+	+	+	+	+	+		70
Aspergillus fumigatus Fresen.	+	+	+	+	+		+				60
Aspergillus sp.				+		+	+	+	+		50
Aspergillus pulverulentus (McAlpine) Thom.		+			+		+	+	+	+	60
Aspergillus terreus Thom.	+	+	+	+		+	+	+	+	+	90
Cladosporium cladosporioides (Fres.) de Vries.			+	+	+	+	+			+	60
Curvularia lunata (Wakker) Boedijn.		+	+		+					+	40
Drechslera halodes (Drechsler) Subram. et B.L. Jain	+		+	+			+	+	+		60
Mucor sp.		+	+		+		+	+		+	60
Fusarium coeruleum (Lib.) Sacc.		+			+	+		+	+		50
Fusarium solani (Mart.) Sacc.	+		+	+	+			+	+	+	70
Humicola grisea Traeen	+			+		+		+	+		50
*Nigrospora sphaerica (*Sacc.) E.W. Mason		+	+	+	+						40
Penicillium adametzi Zaleski	+	+		+	+		+	+			60
Penicillium citrinum Sopp		+			+	+			+	+	50
Penicillium rubrum Stoll	+	+	+	+		+	+	+		+	80
Rhizopus stolonifer (Ehrenb.) Vuill.	+	+		+		+	+	+	+	+	80
Scopulariopsis brevicaulis (Sacc.) Bainier	+		+	+	+	+				+	60
Stachybotrys atra Corda.			+	+	+	+	+	+	+	+	80
Trichoderma koningii Oudem	+	+		+	+	+	+	+	+		80
Trichoderma viride Pers. ex. Fries	+	+	+		+	+	+	+	+	+	90
Verticillium albo-atrum Reinke et Berth		+	+						+	+	40

trient levels ranging as follows: 6.4 to 14.0 mg Kg^{-1} of total N, 0.25 to 1.55 mg Kg^{-1} of total P, 18 to 51 mg Kg^{-1} of exchangeable K, 5.44 to 26.44 mg Kg^{-1} of Fe, 0.60 to 1.60 mg Kg^{-1} of Zn, 2.84 to 20.24 mg Kg^{-1} of Mn and 1.21 to 4.24 mg Kg^{-1} of Cu.

Trichoderma populations

Trichoderma was isolated from all the Alfisol soils (Tab. 2). These include *Trichoderma koningii* Oudem and *Trichoderma viride* Pers. ex. Fries. The average population density of *T. koningii* ranged between 0.6×10^3 cfus and 3.6×10^3 cfus g^{-1} of soil, *T. koningii* was not found in *S. bicolor* and *C. papaya*-cultivated soils. *Trichoderma viride* average populations ranged between 0.6×10^3 cfus and 1.6×10^3 cfus g^{-1} of soil. *Trichoderma viride* was absent from *A. hypogea* soil. Populations of both *T. koningii* ($F_{7,36} = 2.45$, $p < 0.05$) and *T. viride* ($F_{8,36} = 2.31$, $p < 0.05$) significantly varied with plant species. Based on the overall occurrence in different soils, *T. viride* was the most frequent *Trichoderma* species occurring in 90% of the soil samples examined.

Co-occurring microfungal population

The 22 colony-forming soil microfungi, apart from *T. koningii* and *T. viride* were classified into 20 species belonging to 14 genera and two could not be identified to species level (Tab. 2). Total fungal colonies significantly varied among crop species ($F_{9,40} = 4.485$, $p < 0.001$) and were maximum in *S. bicolor* soil (14×10^3 cfu g^{-1} soil) and minimum in *S. officinarum* soil (7×10^3 cfu g^{-1} of soil) (Fig.1). *Aspergillus terreus* and *T. viride* occurred in the soils of most crop species examined, whereas *Nigrospora sphaerica* and *Verticillium albo-atrum* were present in a minimum number of soil samples (Tab. 2). Among the 10 soil samples examined *Trichoderma* species (*T. koningii*) was abundant only in three soils (*A. hypogea*, *C. nucifera*, and *L. esculentum* soils) (Tab. 3, Fig. 2). Relative abundance of other soils was shared by *Aspergillus terreus* (*S. officinarum*, *A. catechu* and *C. papaya* soils), *Stachybotrys atra* (*S. bicolor* soil), *Drechslera halodes* (*C. longa* soil), *Mucor* sp. (*Z. mays* soil) and *Rhizopus stolonifer* (*O. sativa* soil). *Aspergillus* was the most diverse genus in the present study with six species followed by *Penicillium* (three species).

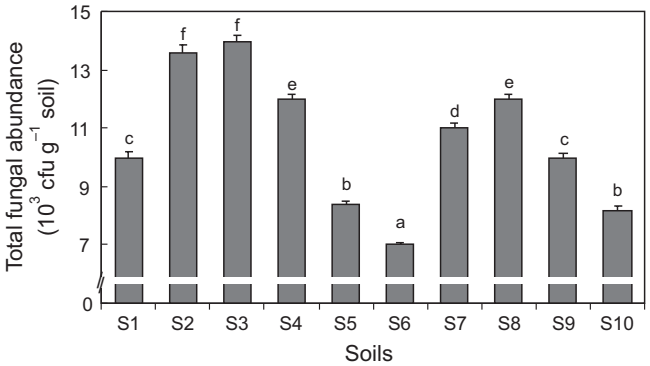

Fig. 1. Total soil micro fungal populations in different agrosystems. Vertical bars indicate ± 1 S.E. Bars bearing same letter(s) are not significantly different according to Duncan's multiple range test ($p > 0.05$). Soil types S1–S10 are explained in Methods.

Tab. 3. Abundance of soil microfungal species (isolates as a percent of total isolates isolated from agrosystems of different crop species). Abundant species in each agrosystem is presented in bold. Soil types S1 – S10 are explained in Methods.

Fungus name	Soils									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Absidia glauca	4.00	8.82		1.67		2.86	1.82	1.67		4.88
Aspergillus candidus	6.00	2.94	2.86			2.86		5.00	2.00	4.88
Aspergillus flavipes	2.00			3.33	2.38	5.71	7.27	5.00	10.00	
Aspergillus fumigatus	12.00	13.24	4.29	5.00	7.14		12.73			
Aspergillus pulverulentus		4.41			7.14		1.82	6.67	2.00	2.44
Aspergillus sp.				6.67		5.71	1.82	6.67	6.00	
Aspergillus terreus	14.00	17.65	8.57	16.67		**17.14**	9.09	5.00	**20.00**	**17.07**
Cladosporium cladosporioides			2.86	3.33	7.14	11.43	1.82			7.32
Curvularia lunata		1.47	2.86		2.38					2.44
Drechslera halodes	2.00		8.57	1.67			1.82	**15.00**	2.00	
Fusarium coeruleum		1.47			4.76	2.86		1.67	4.00	
Fusarium solani	2.00		11.43	10.00	7.14			8.33	14.00	14.63
Humicola grisea	2.00			6.67		5.71		1.67	6.00	
Mucor sp.		1.47	7.14		**16.67**		5.45	3.33		4.88
Nigrospora sphaerica		2.94	2.86	5.00	4.76					
Penicillium adametzi	18.00	1.47		8.33	2.38		3.64	5.00		
Penicillium citrinum		1.47			2.38	8.57			2.00	4.88
Penicillium rubrum	2.00	5.88	14.29	3.33		2.86	5.45	5.00		4.88
Rhizopus stolonifer	4.00	2.94		1.67		2.86	**14.55**	6.67	8.00	7.32
Scopulariopsis brevicaulis	2.00		5.71	5.00	9.52	11.43				2.44
Stachybotrys atra			**14.29**	5.00	9.52	2.86	7.27	3.33	6.00	7.32
Trichoderma koningii	**22.00**	**26.47**		**16.67**	7.14	8.57	12.73	8.33	4.00	
Trichoderma viride	8.00	4.41	11.43		9.52	8.57	12.73	11.67	12.00	12.20
Verticillium albo-atrum		2.94	2.86						2.00	2.44

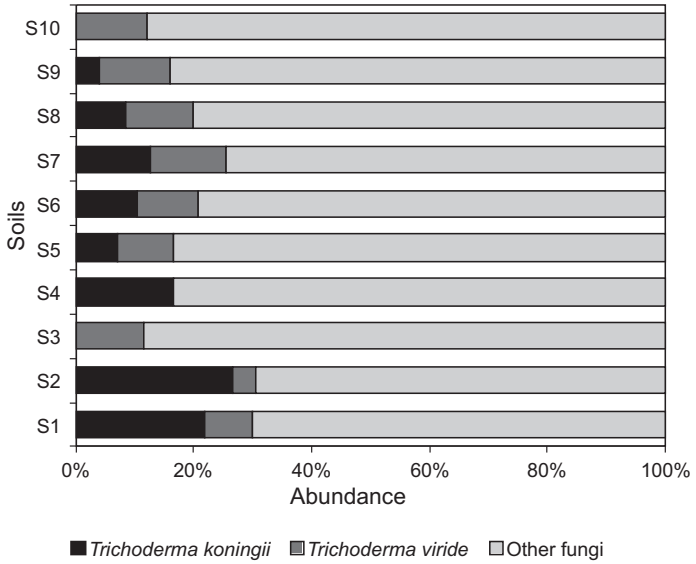

Fig. 2. Percent abundance (%) of *Trichoderma koningii* and *Trichoderma viride* in relation to other soil fungi in different agrosystems. Soil types S1 – S10 are explained in Methods.

Relationship between soil factors and microfungal populations

Correlation analysis revealed an absence of significant influence of soil factors on total soil microfungal populations. But abundance of *T. koningii* was significantly and negatively correlated to soil pH ($r = –0.670$; $p < 0.05$; $n = 10$) and relative abundance of *T. viride* was significantly and positively correlated to soil P ($r = 0.697$; $p < 0.05$; $n = 10$). Further, relative abundance of *T. koningii* was significantly and positively correlated to relative abundance of *A. fumigatus* ($r = 0.749$; $p < 0.05$; $n = 10$), but was significantly and negatively correlated to *Stachybotrys atra* ($r = –0.766$; $p < 0.05$; $n = 10$). Relative abundance of *T. viride* was significantly and negatively correlated to relative abundance of *Absidia glauca* ($r = –0.727$; $p < 0.05$; $n = 10$). Relative abundance of *T. koningii* and *T. viride* was significantly and negatively correlated to each other ($r = –0.666$; $p < 0.05$; $n = 10$).

Among the co-occurring fungi, relative abundance of *Penicillium adametzi* was significantly and negatively correlated to soil pH ($r = –0.712$; $p < 0.05$; $n = 10$). In contrast, a significant positive correlation existed between soil EC and relative abundance of *Rhizopus stolonifer* ($r = 0.756$; $p < 0.01$; $n = 10$). As soil Cu was significantly and negatively correlated to relative abundance of *Aspergillus fumigatus* ($r = –0.678$; $p < 0.05$; $n = 10$), soil Fe was significantly and positively correlated to relative abundances of *Aspergillus candidus* ($r = 0.713$; $p < 0.05$; $n = 10$) and *Penicillium adametzi* ($r = 0.826$; $p < 0.001$; $n = 10$).

The relative abundance of the unidentified *Aspergillus* sp. was significantly and positively correlated to relative abundances of *Aspergillus flavipes* ($r = 0.683$; $p < 0.05$; $n = 10$) and *Humicola grisea*, ($r = 0.817$; $p < 0.01$; $n = 10$) and negatively to relative abundance of *Curvularia lunata* ($r = –0.708$; $p < 0.01$; $n = 10$). Likewise, relative abundance of *Cladosporium cladosporioides* was significantly and positively correlated to relative abundances of *Penicillium citrinum* ($r = 0.841$; $p < 0.01$; $n = 10$) and *Scopulariopsis brevicaulis* (r =

0.844; $p < 0.001$; $n = 10$). As the relative abundance of *Curvularia lunata* was significantly and positively correlated to the relative abundance of *Mucor* sp., ($r = 0.669$; $p < 0.05$; $n = 10$), it was significantly and negatively correlated to the relative abundance of *Humicola grisea* ($r = –0.648$; $p < 0.05$; $n = 10$). A significant positive correlation existed between relative abundances of *Mucor* sp. and *Stachybotrys atra* ($r = 0.637$; $p < 0.05$; $n = 10$). In contrast, a significant negative correlation existed between the relative abundances of *Rhizopus stolonifer* and *Scopulariopsis brevicaulis* ($r = –0.632$; $p < 0.05$; $n = 10$).

Discussion

The frequent occurrence of *Trichoderma* species in Alfisol soils under different crop species is in accordance with the fact that species of *Trichoderma* are an abundant and biologically important component of soil micro-flora, though differences in their abundance can occur (SAMUELS 2006). In contrast, ATTITALLA et al. (2012) isolated *Trichoderma harzianum* only from the five of the 23 soil samples they examined from Al-Jabal AL-Akhdar region of Libya. The population of *Trichoderma* in the present study varied from 0.6 to 3.6×10^3 cfus g^{-1} of soil, which is in agreement with published ranges. Nearly all temperate and tropical soils contain *Trichoderma* populations of $10^1 – 10^3$ cfus g^{-1} of soil (see HARMAN et al. 2004a, b and references therein). Studies have shown that short-term changes in *Trichoderma* populations tend to occur in response to temporary changes in soil moisture, nutrient availability or soil temperature (KREDICS et al. 2003; WAKELIN et al. 1999). In the present study, populations of *Trichoderma* significantly varied with crop species. However, to our knowledge, there is only one previous study (OKOTH et al. 2009) that has examined the role of crop species on *Trichoderma* populations in addition to the substrate specificity of a few *Trichoderma* species. Nevertheless, the influence of plant species on *Trichoderma* has been mostly restricted to endophytic species. For example, *Trichoderma stromaticum* is found only in association with *Theobroma* species in tropical America (SAMUELS et al. 2000). It has been shown that plant species play a major role in determining the occurrence and distribution of fungal species (GRAYSTON et al. 1998; IBEKWE et al. 2002). In spite of their coexistence in 70% of the soil samples, populations of both the *Trichoderma* spp. were negatively related to each other. This suggests that both these soil fungi compete in the soil environment or the fungi may be reacting variedly to the given environmental conditions. The first view is supported by the fact that strains of *T. viride* are known to be highly competitive and are known to proliferate best in the presence of healthy plant roots, whereas, *T. koningii* tends to occur in diverse soil conditions (HJELJORD and TRONSMO 1998; SAMUELS 2006). The second view is supported by the fact that soil pH has been thought to be of great importance to the activity and occurrence of *Trichoderma* species (KREDICS et al. 2003, KÜÇÜK and KIVANÇ 2003, HARMAN et al. 2004a). KREDICS et al. (2003) showed that species of *Trichoderma* grow optimally around pH 4.0 to 5.0, and exhibit little or no growth below pH 2.0 or above pH 6.0. Nevertheless, the pH of the soils in the present study varied between 7.23 and 8.23, and *T. koningii* was abundant in soils with a pH of 7.23 and 7.89. This corroborates the findings of GHERBAWY et al. (2004) reporting that *Trichoderma* was isolated from the Nile Valley soil in Egypt with a pH range of 7.4 to 8.4. In the present study, soil pH was found to have significant influence on the relative abundance of *T. koningii* but not *T. viride*. This clearly shows that even the taxa within a genus can respond differently to the same environmental factors. EASTBURN and BUTTER (1988) also reported that soil pH

had no significant influence on the distribution of *Trichoderma harzianum* in a wide pH range of 6.2 to 7.9. Application of ammonia to the soil was found to increase the populations of *Trichoderma* spp. (SIMON et al. 1988). However, no significant relationship existed between soil nutrient contents and populations of *Trichoderma* spp. indicating that the occurrence of *Trichoderma* spp. is not strongly influenced by these soil nutrients, within the range encountered. This is in line with OKOTH et al. (2009) who also failed to find any relationship between *Trichoderma* spp., and soil nutrients in different land use types of Kenya. GHERBAWY et al. (2004) also reported a lack of correlation between the abundance of *Trichoderma* to the physical and chemical properties of the soils or pH. It is therefore, possible to infer that the indigenous *Trichoderma* spp. in the present study might have adapted to the changes in the physical and chemical properties of these cultivated soils.

Although there is reasonable information about the behavior of many soil fungi in their soil environment, little attention has been given to factors affecting the growth of *Trichoderma* in field soils. Factors affecting growth of *Trichoderma* spp. on artificial cultured media have been extensively studied, but the correlation between various growth responses on agar and in soil is dubious (COOK and BAKER 1983). Though most of the abiotic factors studied did not significantly affect the abundance of *Trichoderma* spp. except soil pH and P, some biotic factors were found to be important in determining the distribution of both *T. koningii* and *T. viride*. Although the occurrence of *T. koningii* and *T. viride* was not significantly influenced by changes in the total fungal populations, the populations of the two *Trichoderma* spp. were associated with changes in certain fungal species like *Absidia glauca*, *Aspergillus fumigatus* and *Stachybotrys atra*. The existence of an inverse relation between *Trichoderma* spp. abundance and populations of *Absidia glauca* and *Stachybotrys atra* is interesting as these fungi may be competing with *Trichoderma* spp. for nutrients or space (CALVET et al. 1992). There have been relatively few studies examining the competition of fungal species for nutrients, space or infection sites in the rhizosphere. Competition for carbon, N and Fe has been shown to be a mechanism associated with suppression of *Fusarium* wilt by *Trichoderma* species (WHIPPS 2001). Further, antibiotic production by isolates of *Trichoderma / Gliocladium* has been reported (HOWELL 1998). EASTBURN and BUTLER (1988) indicated species of *Aspergillius* (*A. ustus, A. tamarii*) and *Penicillium* (*P. citrinum, P. chrysogenum, P. grieoroseum*) to be positively related with a population of *T. harzianum* suggesting the tendency for the co-existence between these species. The relative abundance of *T. koningii* had a positive influence on the relative abundance of *Aspergillius fumigatus* in the present study. This positive relation between populations of *T. koningii* and *A. fumigatus* could be due to the lack of antagonism or synergism among these fungi or it could be an indirect response resulting from the similar response of the two organisms to certain environmental conditions.

An understanding of the organisms occupying the same microsites as *T. koningii* and *T. viride* might give us insight into what types of habitats these *Trichoderma* spp. prefer. However, the varied responses of *T. koningii* and *T. viride* to the same environmental factors clearly explain how a species within the same genus can respond diversely to similar environmental conditions. Unfortunately, there is little information available on the habitat requirements of the co-occurring species of *Trichoderma,* especially *Aspergillus* and *Penicillium* especially at microsite level. The frequent occurrence of the co-occurring *A. fumigatus* is in agreement with ATTITALLA et al. (2012) who also found *Aspergillus* spp. to be more fre-

quent in cultivated and non-cultivated soils of Libya. Interestingly, fast-growing Zygomycetes were not frequent in the present study. This corroborates the general observation that fast-growing Zygomycetes are not frequently isolated from agricultural soil in general, and from arable soils, in particular (HAGN et al. 2003).

Trichoderma species are known to increase the concentration of a variety of nutrients under axenic conditions. However, the population of neither *T. koningii* nor *T. viride* was related to any soil nutrients except soil P. Evidence does exist that *Trichoderma* species can solubilize various plant nutrients such as rock phosphate, Fe^{3+}, Cu^{2+}, Mn^{4+} and Zn, that can be unavailable to plants in certain soils (HARMAN et al. 2004a). Though there may be important abiotic or biotic factors, which were not accounted for in this study, the establishment and wide-spread occurrence of *Trichoderma* sp., appears to be more directly determined by the environment. A similar conclusion was reached by WIDDEN (1984, 1987) and EASTBURN and BUTLER (1988). They found that the environment had the greatest effect on the densities of several species of *Trichoderma*. Thus, it appears that in soils where the abiotic environment is not greatly limiting, the factors of primary importance are those involving competition, antagonism and synergism.

In conclusion, our study clearly showed that crop species could influence the structure of the soil fungal communities. The results of the present study also indicated that the fungal populations in the studied soils are very stable and are not easily influenced by edaphic factors. However, further studies involving a dual approach of culture-dependent and -independent techniques would throw more light on our understanding on the actual fungal diversity of the studied soils. Furthermore, such a study would also help us to deduce the influence of agricultural management practices on shifts in the indigenous fungal community structure.

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