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Native mycota in agricultural soils exposed to pesticides and *Aspergillus oryzae* tolerance to chlorpyrifos in microcosms assays

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Abstract

Chlorpyrifos (CPF) constitutes a class of older and riskier pesticides, with more than 50 years of use. After pesticide application, a low percentage reaches the target and the rest remain in the environment. Due to this, bioremediation strategies are being increasingly studied. The aims of the present study were to determine the competitiveness and the permanence of a non-toxigenic, CPF-degrading *A. oryzae* (AM 1) strain in agricultural soil microcosms. These microcosms were contaminated with commercial formulation of CPF (10, 20 and 50 mM) and conditioned at two water holding capacity (70 and 30 WHC). In addition, *Aspergillus* section *Flavi* counts of soils of three localities of southern Córdoba Province were evaluated, due to these soils were used to prepare the microcosms. In the microcosm's assays, together with the native mycota, *A. oryzae* was isolated from all the treatments and conditions assayed. Thus this strain was able to tolerate the different doses of CPF tested. Regarding the native mycota, *Aspergillus* sp., *Trichoderma* sp., *Penicillium* sp., *Cladosporium* sp. and *Fusarium* sp. were the most frequent genera isolated from the microcosms. The CPF treatments influenced in different ways the counts of the most frequent genera isolated. This study allowed knowing the *in situ* survival, under optimal and not optimal humidity conditions, of a CPF-degrading fungal strain in presence of the native mycota. These results are very important because the permanence in the environment of a potentially bioremediation agent is one of the main characteristic that must be evaluated.

Key words – agricultural soil microcosms – chlorpyrifos tolerance – culturable fungi

Introduction

Together with biotechnology, pesticides have become one of the tools used by producers to counter the attack of diseases produced by insects in Argentinean agricultural crops. Thus, they are key supplies in the current agricultural production model, integrating a technology package that has had a rapid development in the last ten years (Magnasco & Di Paola 2015). A significant increase in pesticides use has been registered (from 34 million liters in 1990 to more than 317 million liters in the last years) in modified genetic crops as soybean, maize and cotton. This increase is due to

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pests and weeds became resistant to pesticides (CASAFE 2014).

Chlorpyrifos (O, O–diethyl O-3, 5, 6– trichloro–2 pyridylphosphorothioate) (CPF) is a broad–spectrum, chlorinated organophosphate insecticide. It is one of the most used insecticides worldwide because their effectiveness against a wide range of insect pests in economically important agricultural crops (Fang et al. 2006). CPF applied doses depend on the crop to be protected, the insect to control and the commercial formulation. Due to the resistance developed, higher doses are being applied (CASAFE 2014).

CPF constitute a class of older and riskier pesticides, with more than 50 years of use. After pesticide application, a low percentage (less than 0.1% of the total applied) reaches the target and the rest remains in the environment (Pimentel 1995). Being the soil or water the main bodies of accumulation after the application (Kulshrestha & Kumari 2011), with risks of toxicity to aquatic biota, animals and humans (Villamil Lepori et al. 2013). Several symptoms have been associated with CPF exposition as oxidative stress in animals, bladder cancer and chromosomal damage, and hyperglycaemia in acute or subchronic exposures (Levin et al. 2002, Abdollahi et al. 2004, Giordano et al. 2007). The exposition in rodents involved disruption of neural cell development, neurotransmitter systems and synaptic formation in different brain regions (Slotkin 2004, Aldridge et al. 2005). Adversely effects on human health have been evaluated. Rauh et al. (2011) reported evidence of deficits in working memory index and full-scale intelligence quotient as a function of prenatal CPF exposure at 7 years of age. The disruptions produced by CPF have been associated with later functional impairments in learning, short term working memory and long term reference memory (Levin et al. 2002). These findings are important because they related the widespread use of CPF in agriculture and possible implications on early cognitive deficits.

The bioremediation techniques proposed actually implies the bioaugmentation of certain Indigenous Microorganisms (IMO's) which are part of the contaminated ecosystem. These competitive microorganisms able to degrade and/or remove the pollutants constitute a promising alternative in the reduction of pesticides levels in the agriculture environment (Kumar & Gopal 2015).

Although several methods have been proposed for pesticides dissipation, the biotic degradation is one of the most viable options for the remediation of CPF in soil and water. Some works have informed that the microbial degradation is a primary mechanism of pesticide dissipation from soil and aquatic environments (Awad et al. 2011, Massiha et al. 2011). In the same way, several CPF–degrading microorganisms have been informed (Chishti et al. 2013). CPF mineralization rate in soil is influenced by biotic and abiotic factors, being the environmental conditions and the microbiota those that most influence it (Chishti et al. 2013). The CPF cleavage depend on the capacity of producing pesticide degrading enzymes being the organophosphorus hydrolase (OPH) and the phosphotriesterase (PTE) which catalyzes the first step of the degradation (Singh et al. 2006). Several data establish that CPF can be metabolized as sole source of carbon, nitrogen and phosphorous by fungi and bacteria strains from contaminated environments (Singh et al. 2004, Awad et al. 2011). Most data are referred to bacteria; and limited information is available about CPF degrading fungi (Chishti et al. 2013).

Many studies have found that the inoculation of adapted microorganisms on pesticide contaminated soils is a good option to decontaminate them (Diez 2010, Abo–Amer 2011, Massiha et al. 2011). Soil fungi have been described as the main degraders' microorganisms of complex organic matter from xenobiotics contaminated environments. They have the enzymatic capacity to degrade complex macromolecules to simple compounds (Rabinovich et al. 2004).

Aspergillus section *Flavi* is one of the most prevalent fungi isolated from maize, peanut and soybean soils in Argentina (Barros et al. 2006, Carranza et al. 2014, 2016a). In previous works, Carranza et al. (2016b) evaluated the *in vitro* tolerance, utilization and degradation of CPF by non-toxigenic *Aspergillus* section *Flavi* strains. All the strains were able to grow with high insecticide concentration that simulates an environmental spill (700 mg/L). Effective growth was observed in synthetic media supplied with CPF as carbon, nitrogen or phosphorous source. In addition, these authors observed a degradation percentage above of 75% in presence of high CPF concentrations

and under optimal growth conditions (water activity and temperature). Despite the fact that *Aspergillus* section *Flavi* species are soil-borne fungi, there is no data about the competitiveness and permanence of these strains in soil in presence of the native mycota, hydric stress and high doses of CPF. In this sense, the aims of the present study were to determine the competitiveness, permanence and CPF tolerance of non-toxigenic *A. oryzae* AM 1 strain in agricultural soil microcosms. These microcosms were contaminated with commercial formulation of CPF and conditioned at two water holding capacity. In addition, *Aspergillus* section *Flavi* counts of soils of three localities of southern Córdoba province were evaluated, due to these soils were used to prepare the microcosms.

Materials & Methods

Soil sampling

The sites used in this study were fields located in Río Cuarto department at south of Córdoba province (Sampacho 33°23′S latitude, 64°43′W longitude, 514 m altitude; Serrano 34°27′S latitude, 63°28′W longitude, 132 m altitude; Pascanas 33°33′S latitude, 62°52′ W longitude, 140 m altitude). These fields were continuously cultivated (over than 10 years) with peanut and with a rotation system for peanut and maize crops. A total of 10 soil samples of each locality were taken. Samples of about 1 kg were taken from the first 15 cm of depth. The samples were homogenized and air–dried for 1–2 days at 25–30°C. The samples were thoroughly mixed, pooled and sieved for debris separation. Soil subsamples (100 g) were chosen and stored at 4°C. The fungal isolation was performed within 2 days of collection.

Culturable mycota determination

Culturable fungi were enumerated by the plate count method. Briefly, ten grams of each soil sample were suspended in peptone water solution. From soil suspension, serial dilutions of 10^{-1} to 10^{-4} were made and aliquots were platted in triplicated on Dichloran Rose Bengal Chloramphenicol Agar (DRBC) (Pitt & Hocking 2009). Plates were incubated for 7 days at 25°C. Thus, plates containing 10–100 colonies were selected to estimate the number of cultivable fungi and the results were expressed as $\log_{10}$ CFU/g soil. Representative *Aspergillus* section *Flavi* colonies were isolated and sub-cultured on Malt Extract Agar (MEA) for subsequent morphological identification (Pitt & Hocking 2009, Samson et al. 2010). The frequency of samples in which these species were present was also determined.

Microcosm assays

The main physicochemical characteristics (Table 1) of the soil used in microcosm assays were determined (Walkley & Black 1965, Sparks 1996). All soil microcosms were prepared with 1 kg soil samples (pooled soil from Sampacho, Serrano and Pascanas localities) placed in plastic containers. Then, the microcosms were wetted with distilled water (at about 30 and 70 water holding capacity, WHC) according to the methodology described by Rivera Martínez et al. (2009). These two WHC values were chosen because they are the two extremes of WHC that usually have agricultural soils in temperate and subtropical regions.

Aliquots of aqueous solution of CPF (Hor–tal®, commercial formulation) were added to each container to achieve final concentrations of 10, 20 and 50 mM, while control microcosms received an equal amount of sterile deionized water. The first two concentrations are the doses commonly used in the fields, whereas 50 mM is more than twice of the doses recommended. Each microcosm was inoculated with 10^6 spores/ml of *Aspergillus oryzae* AM 1 strain. This species is considered GRAS (Generally Regarded as Safe) by the FDA (Food and Drug Administration) in USA (Tailor & Richardson 1979, Blumenthal 2004, Abe et al. 2006). It was isolated previously from agricultural soils (Carranza et al. 2016a) and besides being non-toxigenic; it showed the best growth parameters in culture media with CPF (Carranza et al. 2016b). The nucleotide sequences for the calmodulin and ß– tubulin gene of AM 1 strain were deposited in GenBank under accession numbers

KX298157– KX306816, respectively. Control microcosm without *A. oryzae* AM 1 strain inoculation was included. Forty eight containers of soil for each experiment (3 insecticide levels and control x 2 WHC conditions x 3 replicates) were prepared.

Soil moisture content was maintained throughout the incubation by weighing and correcting for any weight loss, using distilled water. Microcosms were incubated in greenhouse at 18-28ºC for 60 days. The experiment was repeated twice.

Table 1 Soil physicochemical characteristics.

Organic Matter (%)	Nitrate's Nitrogen (ppm)	Nitrates (ppm)	Phosphorous (ppm)	Humidity (%)	pH	Electrical conductivity (dS/m)	Sulfates (ppm)	Calcium (cmol/Kg)	Magnesium (cmol/Kg)	Sodium (cmol/Kg)	Potassium (cmol/Kg)	CEC (cmol/Kg)
2.95	23.00	101.9	56.20	5.00	7.57	0.50	3.80	15.25	4.25	0.52	2.10	22.70

CEC: Cation Exchange Capacity.

Enumeration of culturable mycota from microcosm

From each microcosm, soil samples were collected at 0, 10, 20, 30, 40, 50 and 60 days of incubation for mycological analysis. Culturable fungi were enumerated by the plate count method described above. *A. oryzae* AM 1, predominant genera and total fungal counts were evaluated along the incubation time. The results were expressed as $\log_{10}$ CFU/g of soil.

Statistical analysis

Data analyses were performed by analysis of variance. All data were transformed to $\log_{10}$ (x + 1) to obtain the homogeneity of variance. Means were compared by Fisher's protected LSD test to determine the significant differences between the fungi counts. The analysis was conducted using PROC GLM in SAS (SAS Institute, Cary, NC).

Results

The percentage of samples containing *Aspergillus* section *Flavi* varied according to the locality considered. The highest percentage of samples contaminated with this fungi were from Serrano, with mean fungal counts of 2.82 $\log_{10}$ CFU/g (SD: 0.75); followed by Sampacho (30%) and Pascanas (20%) with mean counts of 3.79 ± 0.49 and 2.30 ± 0.75 $\log_{10}$ CFU/g, respectively (Fig. 1). The fungal total counts ranged from 4.23 to 5.49; 4.00 to 5.67; 4.69 to 5.66 $\log_{10}$ CFU/g from Pascanas, Sampacho and Serrano samples, respectively (Data not shown).

Figs 2 & 3 show the native mycota vs. *A. oryzae* counts in control and three CPF treatments at 30 and 70 WHC along the 60 days of incubation. Together with the native mycota, *A. oryzae* was isolated from all the treatments and conditions assayed. In general, the native mycota count increased with increasing CPF concentration at 70 WHC (Fig. 2A). The *A. oryzae* strain was able to tolerate the different doses of the pesticide tested. A significant decrease in this fungi count was only observed with 10 and 50 mM (Fig. 2A).

At 70 WHC in general, the counts of both native mycota and *A. oryzae* were similar comparing to the respective control in the different treatments along time (Fig. 2B). With 50 mM of CPF, *A. oryzae* counts were lower than native mycota counts at 10 days of incubation. At 10, 50 and 60 days of incubation, *A. oryzae* counts were higher in control treatment than in all the pesticide treatments; whereas with 50 mM of pesticide a significant increase in *A. oryzae* count with respect to control treatment was observed at 20, 30 and 40 days ($p<0.0001$).

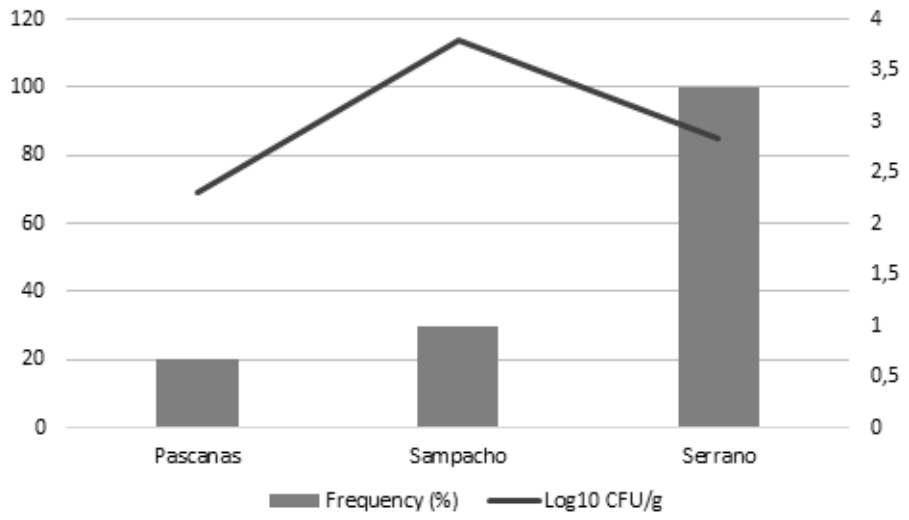

Fig. 1 Frequency of samples containing *Aspergillus* section *Flavi* and mean counts from agricultural soil of three localities of Córdoba province

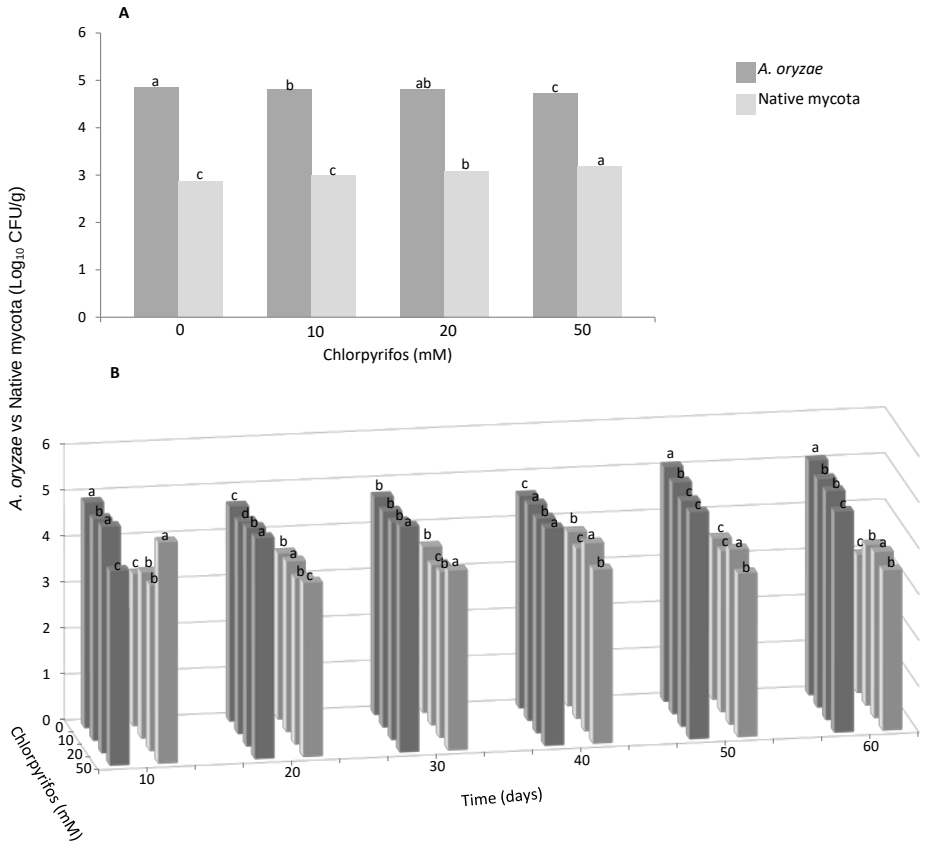

Fig. 2 *A. oryzae* and native mycota counts isolated from microcosms conditioned at 70 WHC and different chlorpyrifos concentrations (10, 20 and 50 mM). The statistical analysis was done comparing the data from each treatment with its respective control. Media values for each treatment (A) and data from each sample period for each treatment (B) were considered. Data with different letters are significantly different ($p<0.0001$) according to LSD test. (S.E. ± 0.01)

At 30 WHC, both *A. oryzae* and native mycota counts had a similar behavior pattern. The highest counts were registered in the control treatment and the lowest ones with 20 mM of CPF (Fig. 3A). At 20, 30 and 40 days of incubation, unlike the observed at 70 WHC, the highest *A. oryzae* and native mycota counts were registered in the control treatment and not with the highest pesticide concentration. The lowest counts were observed in the control treatment at 50 days of incubation (Fig. 3B).

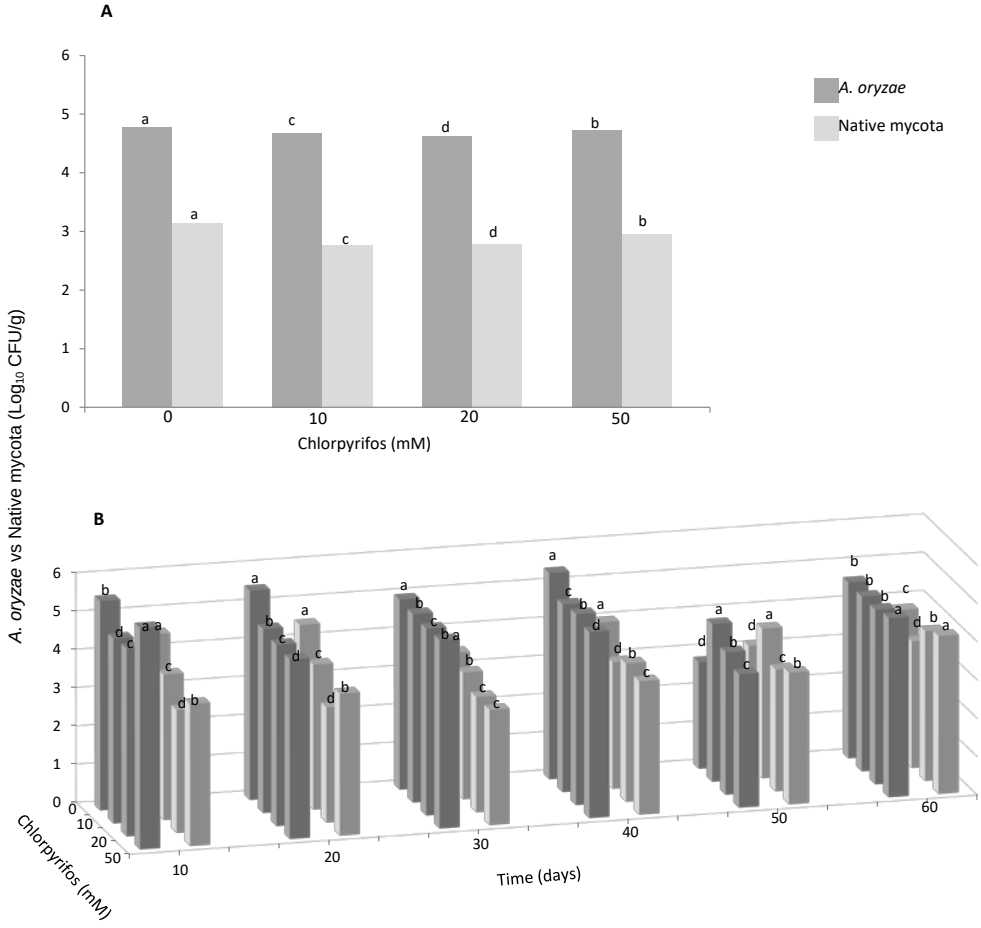

Fig. 3 *A. oryzae* and native mycota counts isolated from microcosms conditioned at 30 WHC and different chlorpyrifos concentrations (10, 20 and 50 mM). The statistical analysis was done comparing the data from each treatment with its respective control. Media values for each treatment (A) and data from each sample period for each treatment (B) were considered. Data with different letters are significantly different ($p<0.0001$) according to LSD test. (S.E. ± 0.01)

The main genera isolated from the microcosms were *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp. and *Fusarium* sp. Fig. 4 shows the native mycota counts of prevalent genera isolated from the microcosms along the incubation period. *Trichoderma* sp. counts (Fig. 4A) varied from 3.0 to 3.5 and 3.0 to 4.4 $\log_{10}$ CFU/g at 70 and 30 WHC, respectively. In general, the counts of this genus increased at 10 and 20 days of incubation in all treatments. At 30 WHC with 20 and 50 mM of CPF, no species of *Trichoderma* sp. were detected. With 10 mM of pesticide at both WHC, the values registered were similar or higher than the respective control at 10 and 20 days of incubation. At 30 days, this fact was only observed at 70 WHC (Fig. 4A).

Regarding *Aspergillus* sp. the counts ranged from 3.0 to 4.5 $\log_{10}$ CFU/g in all treatments tested (Fig. 4B). It is important to highlight that the species of this genus did not belong to the

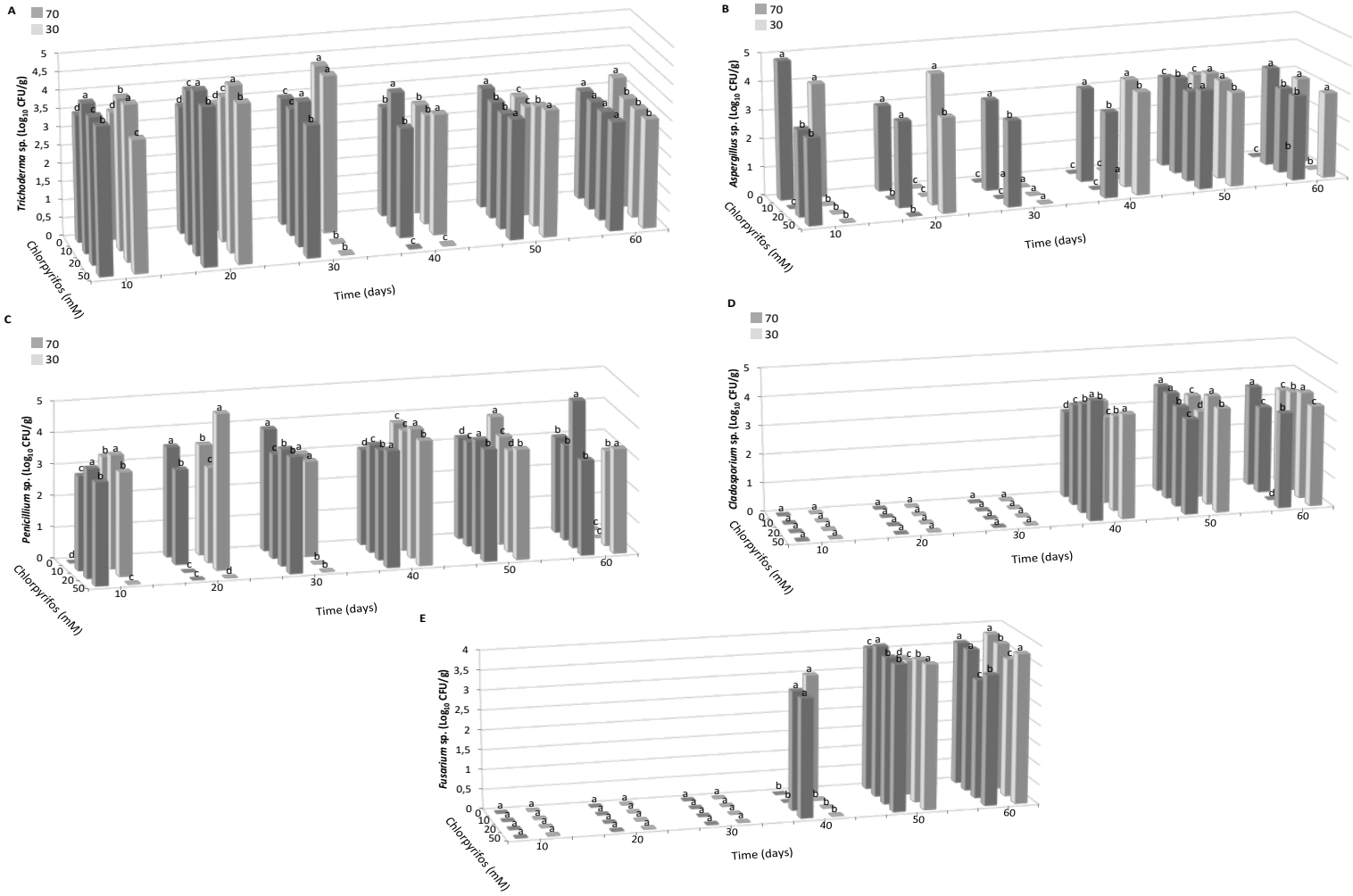

Fig. 4 *Trichoderma* sp. (A), *Aspergillus* sp. (B), *Penicillium* sp. (C), *Cladosporium* sp. (D) and *Fusarium* sp. (E) counts (Log_{10} CFU/g) isolated from microcosms conditioned at 70 and 30 WHC and different chlorpyrifos concentrations (10, 20 and 50 mM). The statistical analysis was done comparing the data from each treatment with its respective control. Data with different letters are significantly different ($p<0.0001$) according to LSD test. (S.E. ± 0.01)

section *Flavi*. The highest values were found at 10 days of incubation in the control treatment at both WHC. From the 20th day of incubation, the counts observed in the pesticide treatments were similar or higher than the ones registered in the respective control. At 70 WHC with 10 mM of CPF the counts increased along the incubation time; whereas with 20 and 50 mM of pesticide, in general, the *Aspergillus* sp. counts remained constant.

Species of the genus *Penicillium* sp. were isolated from all the treatments assayed (Fig. 4C). Significant differences were observed comparing the counts registered in control and CPF treatments ($p<0.0001$). The highest *Penicillium* sp. counts were detected at 20 days at 30 WHC (4.9 $\log_{10}$ CFU/g) and at 60 days at 70 WHC (4.6 $\log_{10}$ CFU/g) with 20 mM of CPF. With 10 mM of CPF at 70 WHC, *Penicillium* sp. counts increased from the 20^{th} to 40^{th} day, being only significantly higher than the counts of the control treatment at 40 days of incubation. With the highest pesticide concentration tested (50 mM) and the lowest WHC (30), *Penicillium* sp. was isolated from the 40 days of incubation. The counts were similar or lower that the counts observed in the control treatment, except at 40 days.

Cladosporium sp. was isolated from both the control and pesticide treatments from 40 days of incubation (Fig. 4D). At 70 WHC a significant increase in this genus counts with increasing CPF concentrations was observed at 40 days of incubation. The highest values were registered with 50 mM (4.3 $\log_{10}$ CFU/g). On the contrary, at 50 and 60 days of incubation the counts decreased with increasing CPF concentration. The highest counts were observed in the control treatment. This behavior pattern was not observed at 30 WHC. The highest *Cladosporium* sp. counts were observed with 50 mM at 40 days of incubation and with 20 mM at 50 and 60 days.

Similarly, *Fusarium* spp. was isolated from 40 days (Fig. 4E). Regarding the pesticide treatments, at 70 WHC, this genus was only isolated with 20 and 50 mM at 40 days. Similar values were found at 50 days of incubation among the control and pesticide treatments at both WHC tested. At 70 WHC with 10 and 20 mM of CPF, *Fusarium* sp. counts were significantly higher that the respective control at 50 days. While at 30 WHC this behavior was observed with 20 and 50 mM also at 50 days. On the other hand, at 30 WHC, the counts registered in the CPF treatments were similar or lower than the counts in the control treatment at 60 days.

Discussion

In the soil samples the *Aspergillus* section *Flavi* counts changed according to the locality sampled. The frequency of isolation was 100% in Serrano, while in the other two places this frequency was lower than 50%. *A. flavus* was the most frequent species isolated. These results are comparable to others informed previously in different soils of Córdoba province. Barros et al. (2003, 2005) and Alaniz Zanon et al. (2013) also reported that *A. flavus* was the dominant species, being isolated in 90% of soils samples from peanut fields. Carranza et al. (2014), also informed that *A. flavus* was the most frequent species isolated from fields from the south of Córdoba. The isolation percentages were 87.5%, 50% and 67% from maize, soybean and soybean-maize rotation respectively. These results shows that *Aspergillus* section *Flavi* species are one of the most frequent fungi isolated as native mycota of agricultural soils of the studied region.

The microcosm's assays showed that the *A. oryzae* strain isolated from agricultural soils is tolerant to the CPF concentrations tested at 70 and 30 WHC. It is important to highlight that the CPF doses applied were higher than the doses commonly used to control insects on fields. In addition, this strain remained viable and its count was not affected by the presence of the native mycota. These facts suggest that *A. oryzae* AM 1 has the capacity to efficiently compete in this ecological niche.

Several studies have proposed the use of non-toxigenic *Aspergillus* section *Flavi* strains as biological control agents to prevent aflatoxins contamination on crops through competitive exclusion of toxigenic strains during infection (Tsitsigiannis et al. 2012, Alaniz Zanon et al. 2013). However few bioremediation studies were done with *Aspergillus* section *Flavi* strains.

Most microcosms' studies were done with microbial communities or bacteria (Qureshi et al. 2009, Grenni et al. 2012, Giri & Rai 2012, Zhang et al. 2015). There is little information about the

in situ tolerance or degradation of pesticides by fungi (Serrano Silva et al. 2009). Filamentous fungi are an important component of the soil microbiota. They evolved to use efficiently several solid substrates. Because their primary metabolism, fungi are able to secrete enzymes that transform complex macromolecules into smaller compounds and then use them for their growth and metabolism (Rabinovich et al. 2004, Singh & Singh 2014).

Kengara et al. (2010) proposed that the lack of native contaminant degrading microorganisms in an ecosystem can be overcome by the inoculation of foreign microorganisms in the same system. This strategy is usually described as "bioaugmentation" and it involves the inoculation of a strain or a microbial consortium with degradation capacity in the contaminated system (Wang et al. 2010). Studies carried out in Brazil with forest soil microcosm's bioaugmentated with *Aspergillus* sp. showed the removal of benzo-anthracene and to a lesser extent of benzopyrene (Serrano Silva et al. 2009). Zhang et al. (2015) evaluated the removal of an organochlorine pesticide (0.1 and 1.0 mg/Kg of endosulfan) in agricultural soil microcosms. More than 50% of endosulfan removal after 42 days was observed and the microbial community only changed with the highest pesticide concentration. These last results are comparable with the results of the present study because the fungal count was significantly lower with the highest pesticide concentrations tested (20 and 50 mM). In another study, Grenni et al. (2012) demonstrated that the presence of the native microbial community promoted the degradation of several compounds (organochlorine pesticides and pharmaceutical products) on soil and water compared to the microcosms with sterile soil. In the same way, Qureshi et al. (2009) observed 90% of reduction of an organochlorined contaminant in soil microcosms treated with a bacterial consortium. On the contrary, Giri & Rai (2012) informed that the highest endosulfan percentage of degradation was observed in the *P. fluorescens*-inoculated sterile soil microcosm.

The microcosm studies allowed confirming the hypothesis that the native *A. oryzae* AM 1 strain is tolerant to CPF, hydric stress conditions and is able to remain in the soil with the presence of the native mycota. In a previous study, Carranza et al. (2016b) observed that two *A. oryzae* strains (AM 1 and AM 2) were able to remove *in vitro* about 75% of CPF at 0.98 and 0.95 of a_W. Since this insecticide is widely used to control plagues in our region, it is essential to create strategies to reduce the residues in soil. More microcosms' studies are needed to evaluate the degradation rate of this strain and its potential application as bioremediation agent.

Regarding the native mycota, *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp. and *Fusarium* sp. were the most frequent genera isolated from the microcosms. These results partially agree with previous studies, where the first three genera were isolated in high frequency of agricultural soils of the central region of Córdoba followed by *Cladosporium* sp., *Paecilomyces* sp. (Carranza et al. 2014), *Fusarium* sp. and *Alternaria* sp. (Nesci et al. 2006).

The results showed that CPF concentration, WHC and incubation time affect the soil native mycota. Some genera like *Trichoderma* sp., *Aspergillus* sp. and *Penicillium* sp. were isolated from all the treatments. Whereas *Cladosporium* sp. and *Fusarium* sp. were isolated from 40 days of incubation. It seems that, in the first days after the CPF application, there is a selection of those genera tolerant to the pesticide. After some time, other fungi may be adapt to the new environment and are able to colonize again the niche. The WHC was another factor that affected some genera count. For example, *Trichoderma* sp. was not isolated from the treatments with 20 and 50 mM of CPF at 30 WHC. *Cladosporium* sp. count increased with increasing CPF concentrations only at 70 WHC. Recent studies informed several changes in microbial community structure exposed to pesticides (Zhang et al. 2015, Álvarez Martin et al. 2016, Huang et al. 2016). These works used molecular biology techniques to evaluate the changes on microbial structure and diversity after the application of pesticides on soil. Although these authors used other techniques to evaluate the mycota, the results are comparable to the obtained in the present work. Zhang et al. (2015) observed a significant change in the structure of fungal community only when the highest endosulfan concentration was applied. The fungal count significantly decreased after the first day of treatment, and then the values were similar to the count of the control along the incubation time. In the present study significant changes on the count of the prevalent genera (*Trichoderma* sp.,

Aspergillus sp. and *Penicillium* sp.) were also detected with the highest pesticide concentration applied. This behavior was also observed in other studies with endosulfan (Adebayo et al. 2007, Joseph et al. 2010) and it was attributed to a mycota adaptation to the pesticide.

In the present study, the fungal count was not only affected by the CPF concentration applied. Several changes along the incubation time were also observed. This fact is also comparable with results informed by Álvarez Martin et al. (2016). These authors found differences in the soil microbial community structure comparing its composition after the application of a fertilizer and other pesticides as azoxystribin and pirimicarb along the incubation time.

In another study, Huang et al. (2016) evaluated the CPF effect (50-59 mg/Kg) on the abundance and structure of the fungal community from an agricultural soil by quantitative polymerase chain reaction (qPCR) and denaturing gradient gel electrophoresis (DGGE). A significant reduction of fungal diversity and abundance when the insecticide was applied was observed. They also observed a change in the community composition between the CPF-treated soils and control soils (without pesticide). Like in the present work, *Fusarium* sp. was one of the genera most affected, since their abundance remained constant in control soils and then decreased with the CPF application. After the incubation time, *Fusarium* sp. abundance increased in the CPF-treated soils being even higher than the abundance observed in the control soils. This is in agreement with the *Fusarium* sp. count observed in the microcosm assay, since this genera was only isolated from the 40 days of incubation and the values registered was similar or even higher than the count observed in the control treatment (without pesticide).

This study allowed knowing the *in situ* survival, under optimal and not optimal humidity conditions, of a CPF-degrading fungal strain in presence of the native mycota. These results are very important because the permanence in the environment of a potentially bioremediation agent is one of the main characteristic that need to be evaluated. In future studies the *in situ* CPF removal by *A. oryzae* will be evaluated under the same WHC conditions.

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