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Degradation of chlorpyrifos using different biostimulants/biofertilizers: Effects on soil biochemical properties and microbial community

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ABSTRACT

In this manuscript we conducted a laboratory investigation over a 120-day period studying the effect of three biostimulants/biofertilizers (BS), in a Calcaric Regosol soil, polluted with chlorpyrifos insecticide at a rate of 5 L ha^{-1} (manufactures rate recommended). The BS were manufactured by the pH-stat method, from two different types of chicken feathers (CF1 and CF2) and from sewage sludge (SS). We determined their effects on enzymatic activities and the structure of the soil microbial community by analyzing phospholipid fatty acids (PLFAs). The BS that contained higher amounts of proteins and a higher proportion of peptides, under 0.3 kDa, exerted a greater stimulation on the dehydrogenase, β-glucosidase, phosphate and arylsulfatase activities, possibly because low molecular weight proteins can be easily assimilated by soil microorganisms. The soil urease activity was not stimulated because these chemical compounds were rich in low molecular weight proteins. Soil biological parameters decreased in insecticide-polluted soil. The application of the BS in chlorpyrifos-polluted soils decreased the inhibition of the soil enzymatic activities and biodiversity, principally at 10 days into the experiment. However, this inhibition decrease was higher when CF2 was applied to soil, followed by SS and CF1, respectively. This suggested that the application of BS with higher amounts of proteins and a higher proportion of peptides under 0.3 kDa is more beneficial for remediation of soils polluted with chlorpyrifos.

1. Introduction

Chlorpyrifos [$C_9H_{11}Cl_3NO_3PS$ or *O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] is a broad-spectrum organophosphorus insecticide that is widely used for insect pest control in agriculture and for soil and foliar treatments in different crops (Korade and Fulekar, 2009; Tejada et al., 2011; Zhang et al., 2012). However, due to its intensive use and its inappropriate application, chlorpyrifos is of environmental concern as it is toxic and can cause a high contamination risk to soil and groundwater (Korade and Fulekar, 2009). Therefore, the remediation of chlorpyrifos-contaminated soils, is required in order to mitigate the hazardous effects of this insecticide.

In soil, microbes are an important biological component of the soil ecosystem and play vital roles in soil fertility through their participation in nutrient cycling and organic matter degradation (Miltner et al., 2004; Wichern et al., 2007). Consequently, a toxic effect of chlorpyrifos on soil microorganisms would be of public concern (Tejada et al., 2011). The measurement of microbial parameters, such as enzyme activities and the microbial community, may provide information on presence and the activity of viable microorganisms as well as on the effects of chlorpyrifos on soil metabolic activity. Such measurements may serve as a good index of the impact of pollution on soil health and can provide information of the resistance and dynamics of chlorpyrifos in soils (Zhang et al., 2010; Tejada et al., 2011). Subsequently, the comparison of the soil enzymatic activities and biodiversity could be helpful when evaluating the impacts of chlorpyrifos on soils.

Organic amendments play an important role in enhancing the soil fertility and microbial activity (Saviozzi et al., 1999; Fernández et al., 2009), therefore, they may also decrease the inhibitory effects of chlorpyrifos on soil microbes. In the last year, several authors have used different sources of organic matter such as agro-residues (coconut husk, peat mass, peanut shell and rice husk),

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municipal solid waste, cow manure, biogas slurry, spent mushroom compost and vermicompost, etc., in order to accelerate the degradation of chlorpyrifos in soil (Romyen et al., 2007; Tejada et al., 2011; Kadian et al., 2012).

Generally, these organic compounds contain a higher protein content of high molecular weight and therefore, the microorganisms need to employ a large amount of energy to degrade these organics. Very slowly over time, this causes the degradation of the pesticide, by soil microorganisms. Therefore, by obtaining an organic product with a high content of low molecular weight proteins which are fast and easily assimilated by soil microorganisms, without high energy consumption, this could accelerate the degradation of the contaminant in soil (Tejada et al., 2010).

In the recent years, there has been an increasing use of hydrolysates organic biostimulants/biofertilizers (BS) obtained from different organic materials by hydrolysis reactions (Parrado et al., 2008; García-Martínez et al., 2010a,b). These products are characterized by a high content of low molecular weight proteins. This aspect is of great interest, as these small proteins may be directly assimilated by soil microorganisms with lower energy expenditure. In this respect, Tejada et al. (2010) observed a rapid MCPA herbicide degradation in soils amended with different BS such as wheat condensed distillers solubles enzymatic hydrolizate, carob germ extract and rice bran enzymatic extract. These authors concluded that the molecular size of the proteins that constitute these organic materials were a critical parameter in the rapid degradation in soil MCPA. This rapid degradation of the herbicide in soil could alleviate the environmental problems caused by pesticides. The authors also emphasized that besides these low molecular weight proteins, these products are also characterized by a high content of polysaccharides, and humic-like molecules that stimulate soil microorganisms, and thus, could promote the degradation of the xenobiotic in soil.

We hypothesize that both protein hydrolysates can be very useful in the remediation of chlorpyrifos-contaminated soils. This aspect is of great environmental interest, since no studies have been reported using different BS to remediate chlorpyrifos-contaminated soil. For this reason, the objective of this study was to investigate, under laboratory conditions, the influence of different BS in a chlorpyrifos-polluted soil and its effect on soil biological properties.

2. Material and methods

2.1. Soil, BS and insecticide characteristics

The soil used in this experiment is a Calcaric Regosol (FAO, 1989). Soil samples were collected from the 0–25 cm surface layer. The main soil characteristics are shown in Table 1. Soil pH was determined in distilled water with a glass electrode (soil:H_2O ratio 1:2.5 w/v). Soil texture was determined by Robinson's pipette method (Avery and Bascomb, 1982). N-Kjeldahl was determined by the MAPA (1986) method. Soil organic-C was determined by the method of Yeomans and Bremner (1988).

The insecticide used in this experiment was chlorpyrifos. The commercial formulation Senator® 48 (48% chlorpyrifos) was purchased from Bayer CropScience (Madrid, Spain). The recommended dose for soil application of chlorpyrifos is 5 L ha^{-1} which, according to Giménez et al. (2004), caused toxic effects on soil enzyme activities.

Three BS were used: (1) BS derived from sewage sludge (SS) by enzymatic hydrolysis process, and (2) two BS derived from chicken feathers (CF1 and CF2) also obtained by enzymatic hydrolysis. The differences between CF1 and CF2 are a consequence of the different origin of this organic material. Sewage sludge and both feathers were hydrolysed according to the pH-stat method (Adler-Nissen, 1977), using an endoprotease obtained by liquid fermentation of *Bacillus licheniformis* ATCC 21415 as the hydrolytic agent in a bioreactor operating under controlled temperature and pH, agitation and NaOH consumption (Parrado et al., 2008).

The BSs were chemically analyzed (Table 2). Organic matter content was determined by combustion at 550 °C for 6 h. Phosphorus and sulfur were determined after combustion and analyzed by an inductively coupled plasma atomic emission spectrometry (ICP-AES) using a Fisons-ARL 3410 sequential multielement instrument equipped with a data acquisition and control system. Summarized standard operational conditions of this instrument are: argon, the carrier, coolant, and plasma gas at 80 psi of pressure, the carrier gas flow rate is 0.8 L min^{-1}, the coolant gas flow rate is 7.5 L min^{-1}, the plasma gas flow rate is 0.8 L min^{-1}, and the integration time is 1 s. One mini-torch consumes argon gas at a radio-frequency power of 650 W. Crude fat was determined gravimetrically after extraction with hexane for 12 h in a soxhlet extractor (Clemente et al., 1997). Total nitrogen was determined by the Kjeldahl method (AOAC, 1990).

The molecular mass distribution of protein in the samples was determined by size-exclusion chromatography using an ÄKTA-purifier (GE Healthcare) and a Superdex Peptide™ 10/300GL column (optimum separation range 300–10,000 Da) (Table 3). Samples were centrifuged at 12,000 × *g* for 30 min at 4 °C to remove insoluble molecules; the supernatant was passed through a 0.2 μm filter and loaded into a 0.1 mL loop connected to an ÄKTA-purifier system. The column was equilibrated, and eluted with 0.25 M Tris–HCl buffer (pH 7.0) in isocratic mode, at a flow-rate of 0.5 mL min^{-1}, and proteins/peptides were detected at 280 and 215 nm with a GE Healthcare UV900 module coupled to the column elution.

2.2. Biostimulation experiment design

Five hundred grams of soil were pre-incubated at 25 °C for 7 days at 30–40% of their water-holding capacity according to Tejada (2009), prior to the treatments. After this pre-incubation period, soil samples were mixed with chlorpyrifos.

Three days after applying insecticide to soil, the three BS were also applied to the soil. Soil samples were mixed with SS at a rate of 0.50%, or CF1 at a rate of 0.8% or CF2 at a rate of 0.65%, in order to applying to the soil the same amount of organic matter with each

Table 1
Characteristics of the experimental soil (mean ± standard error). Data are the means of three samples.

pH (H_2O)	7.9 ± 0.2
Coarse sand (g kg^{-1})	486 ± 49
Fine sand (g kg^{-1})	130 ± 25
Silt (g kg^{-1})	123 ± 29
Clay (g kg^{-1})	260 ± 35
N-Kjeldahl (g kg^{-1})	0.93 ± 0.08
Organic C (g kg^{-1})	17 ± 1

Table 2
Chemical composition of the three biostimulants/biofertilizers. Data are the means of three samples. Rows (mean ± S.E.) followed by the same letter(s) are not significantly different ($p > 0.05$).

	SS	CF1	CF2
Organic matter (g kg^{-1})	773b ± 21	463a ± 48	550a ± 39
N-Kjeldhal (g kg^{-1})	34.9c ± 2.3	14.1b ± 1.6	9.8a ± 2.7
Total carbohydrates (g kg^{-1})	42a ± 19	65a ± 11	73a ± 18
Total P (g kg^{-1})	2.9a ± 0.1	27c ± 8	11b ± 3
Total S (g kg^{-1})	5.9a ± 1.6	19b ± 4	11b ± 2
Fat (g kg^{-1})	18a ± 3	20a ± 2	281b ± 10