



## Dissipation and effects of tricyclazole on soil microbial communities and rice growth as affected by amendment with alperujo compost



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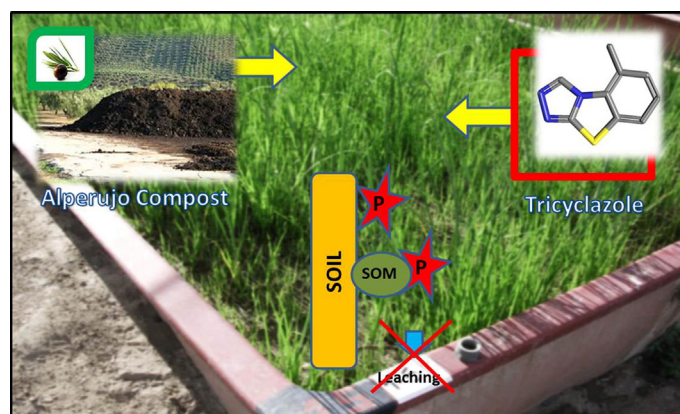
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### HIGHLIGHTS

- Soil amendment with AC has a positive effect on the photosynthesis rate of rice.
- The amendment with AC did not affect the predominant soil microbial community
- Performance of tricyclazole under flooded conditions was not affected by AC.

### GRAPHICAL ABSTRACT



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### ABSTRACT

The presence of pesticides in surface and groundwater has grown considerably in the last decades as a consequence of the intensive farming activity. Several studies have shown the benefits of using organic amendments to prevent losses of pesticides from runoff or leaching. A particular soil from the Guadalquivir valley was placed in open air ponds and amended at 1 or 2% (w/w) with alperujo compost (AC), a byproduct from the olive oil industry. Tricyclazole dissipation, rice growth and microbial diversity were monitored along an entire rice growing season. An increase in the net photosynthetic rate of *Oryza sativa* plants grown in the ponds with AC was observed. These plants produced between 1100 and 1300 kg ha<sup>-1</sup> more rice than plants from the unamended ponds. No significant differences were observed in tricyclazole dissipation, monitored for a month in soil, surface and drainage water, between the amended and unamended ponds. The structure and diversity of bacteria and fungi communities were also studied by the use of the polymerase chain reaction denaturing gel electrophoresis (PCR-DGGE) from DNA extracted directly from soil samples. The banding pattern was similar for all treatments, although the density of bands varied throughout the time. Apparently, tricyclazole did not affect the structure and diversity of bacteria and fungi communities, and this was attributed to its low bioavailability. Rice cultivation under paddy field conditions may be more efficient under the effects of this compost, due to its positive effects on soil properties, rice yield, and soil microbial diversity.

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## 1. Introduction

Rice growing techniques most widespread worldwide involve flooding processes and traditional tillage. The use of pesticides in rice farming is a high-risk scenario, resulting in numerous episodes of contamination in wetlands (Gamón et al., 2003; Padovani et al., 2006; Reichenberger et al., 2007). In Andalucía, southern Spain, nearly 40,000 ha of soil are dedicated to rice farming under flooded conditions (Aguilar, 2010). The presence of pesticides in surface and groundwater has grown considerably in the last decades as consequence of farming activity (Hildebrandt et al., 2008; Hermosín et al., 2013). Organic amendments, and their hydrosoluble fraction, have an important impact on pesticide dissipation, affecting their adsorption and transport processes through various chemical interactions (Thevenot et al., 2009). Several studies have shown the benefits of using organic amendments to prevent losses of pesticides from runoff or leaching (Cox et al., 2000; Gámiz et al., 2012; Giori et al., 2014). Barriuso et al. (2010) reported that compost addition to soil generally decreased herbicide mineralization and favored the stabilization of herbicide residues. The use of organic wastes from different origins as soil amendment has attracted the attention of researchers in recent years (Sánchez-Monedero et al., 2008). This strategy could be especially important in Mediterranean areas where the return of the organic matter to the soil will have positive effects such as the improvement of soil structure, the increase of soil fertility and the prevention of soil erosion processes (Stevenson, 1994; Mekki et al., 2013). The new technology for olive oil extraction consists of a continuous centrifuge two-phase process that generates a liquid phase (olive oil) and the organic slurry (olive mill waste) known in Spain as *alperujo*. This organic waste is considered to be a residue in the olive oil industry (Paredes et al., 1987; Della Greca et al., 2001). Over the last few years, several physical, chemical and biological processes destined to reduce the contaminant impact of olive mill waste have been proposed (Dermeche et al., 2013). Soil incorporation of these residues, with high organic matter content, increase fertility and control erosion (López-Piñero et al., 2008). In order to avoid the possible adverse effects derived of phytotoxic compounds (phenolic acids) present in that residue, *alperujo* was previously composted. Also, it has been shown the positive effects of olive mill residues after the composting process, evidencing good suppressive activity against some fungal plant pathogens (Alfano et al., 2009). Furthermore, *alperujo* compost (AC) is free of heavy metals and pathogens (Alburquerque et al., 2009). These reasons make this amendment a good candidate for improving soil properties, preventing losses of pesticides, and minimizing the risk of contamination derived of rice crop in soils with low content in organic matter.

The aim of this work was to assess, under flooded conditions, the effect of AC on soil properties, microbial diversity and on dissipation of the highly persistent fungicide tricyclazole in soil, surface and drainage water. Additionally we compared several physiological parameters of rice plants growth in amended and unamended soils.

## 2. Materials and methods

### 2.1. Soil, organic amendment and pesticide

Soil used in this study was taken from an area traditionally devoted to rice crops in the Guadalquivir valley. Soil texture was determined by sedimentation using the pipette method (Gee and Bauder, 1986; Sheldrick and Wang, 1993). Clay mineralogy was studied by X-ray diffraction on oriented specimens (Jackson, 1975). The organic matter content was determined according to Walkley and Black (1934) and the total nitrogen by the Kjeldahl method (Benton, 1991). Soil pH and electrical conductivity were measured in a 1:2.5 (w/v) soil/deionized water mixture. All the measurements were carried out in triplicates.

Samples of undisturbed soil were collected from each experimental pond, before and after rice cultivation. These samples were dried at

80 °C for 24 h, and pore size distribution (from  $1 \times 10^5$  to 1 nm) was determined by mercury intrusion porosimetry using an Autopore 9510 mercury intrusion porosimeter (Micromeritics, Georgia, USA). The surface tension of mercury was assumed as  $0.48 \text{ Nm}^{-1}$  and a mercury solid contact angle of  $141.3^\circ$ , using the Washburn equation (Washburn, 1921) for the calculation of the pore size distribution.

The specific surface area of the same samples was measured by nitrogen surface sorption, using a Carlo Erba Sorptomatic 1900 (Fisons Instruments, UK). Isotherms were interpreted by the Brunauer–Emmett–Teller (BET) equation as described by Brunauer et al. (1938).

*Alperujo*, which is the residue of olive oil production, was composted with straw and sheep manure. This *alperujo* compost (AC) was produced in the research station IFAPA-Centro Venta del Llano, Jaén (Spain) in 2012.

Commercial Tricyclazole (Sapac Agro,  $\geq 75\%$  purity) was used in field assays. Analytical grade tricyclazole ( $\geq 97\%$  purity), used to prepare the initial pesticide solutions employed as external standards for pesticide analysis, was provided by Dr. Ehrenstorfer GmbH (Augsburg, Germany). Water solubility was  $596 \text{ mg L}^{-1}$  (20 °C), and molecular mass  $189.24 \text{ g mol}^{-1}$ .

### 2.2. Phytotoxicity studies and experimental rice ponds

In order to check if AC was detrimental to rice germination, rice seeds were planted in flowerpots (12 L) with the collected soil, and exposed to different doses of AC (ranging from 145 kg N/ha to 1172 kg N/ha). Field study was conducted in 6 m<sup>2</sup> ponds (2 m × 3 m), filled with the collected soil and under flooded conditions. The superficial water layer was around 5 cm during the rice growth. The assay was carried out in the experimental farm IFAPA-Las Torres, Sevilla, (Spain, 37° 30' 45" N; 05° 57' 50" W). Three different treatments were assayed: soil without any organic amendment (T1), soil amended with AC at a rate of 1% (w/w) (T2) and at a rate of 2% (w/w) (T3). Every treatment was run in triplicates. An aleatory distribution was used for the ponds placement. All the ponds were additionally amended with a standard dose of inorganic fertilizer (urea), in order to avoid possible nitrogen stress in the plants growth without AC. Rice was transplanted to the ponds at the beginning of the tillering phenological stage, 21 days after seeding. The population density was 22 plants m<sup>-2</sup>.

### 2.3. Fungicide application, monitoring and analysis

Foliar application of tricyclazole was made with single-nozzle hand-carried spray boom operated from a knapsack sprayer. Tricyclazole was applied two months after rice transplant. Spray volume was 1500 L/ha. Rates and intervals were executed as were recommended by the manufacturer. To motorize tricyclazole concentration in soil and water, after its application (0.6 kg/ha) in amended and unamended soils, samples were collected periodically. Sampling was prolonged one and a half months after its application. Triplicate samples of soil were collected from the surface (0–10 cm) of each pond, put in plastic receptacles, and stored at  $-20^\circ \text{C}$  until use. Water samples were collected on the same dates as the soil, by duplicate, from the surface layer and drainage water and stored at  $4^\circ \text{C}$  until analysis. Soil samples were extracted with methanol in a ratio 1:2 (w/v). The flasks were shaken orbital over night at  $20^\circ \text{C}$  and centrifuged at 3000 rpm for 10 min, then filtered through pre-rinsed  $0.45\text{-}\mu\text{m}$  cellulose-acetate filters and kept at  $4^\circ \text{C}$  till analysis, before than 24 h. Tricyclazole recoveries were close to 100%. Tricyclazole from soil extracts and surface and drainage water was analyzed by HPLC using a Waters 600E chromatograph coupled to a Waters 996 diode-array detector. Conditions were as follow: Nova-Pack C18 column of  $150 \times 3.9 \text{ mm}$ , 20:80 acetonitrile/water eluent mixture, flow rate of  $1 \text{ mL min}^{-1}$  under isocratic conditions, UV detection at 230 nm, and injection volume  $25 \mu\text{L}$ .

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