VERMICOMPOSTS FROM AGROINDUSTRIAL WASTES AND PESTICIDES EFFECTS ON SOIL MICROBIAL ACTITITY

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1 INTRODUCTION

Agricultural soils with low organic carbon (OC) content are common in southern Europe, where 74% of farm land is characterized by top soils with less than 2% OC content (Zdruli et al., 1999). Organic matter is added to fertile Mediterranean areas in order to improve soil quality. Pesticides are also frequently used to increase crop yield. Both practices affect soil microbial populations and alter soil-enzyme activities. Enzyme activities have been identified as a biochemical indicator of the impact of agricultural practices on the soil's biological functions. Dehydrogenase activity has been particularly useful in this regard, as it is associated with the metabolic state of microbial populations and is an effective indicator of soil microbial activity (Garcia et al., 1997).

There are abundant quantities of vine-shoot and wet olive cake or alperujo wastes in Mediterranean countries, whose disposal raises environmental and health concerns. Vermicomposting is regarded as a sustainable technology capable of transforming these residues into fertilizers. (Nogales et al., 2005; Melgar et al., 2009). However, the addition of vermicompost to soils also affects microbial communities and the impact of pesticide on this media.

In soil with low OC content, the addition of organic amendments can stimulate microbial activity due to the availability of organic molecules such as sugar and amino acids that enhance degradation and increase the retention of nutrients and contaminants (Sánchez et al., 2004). Vermicompost can influence the ecology of soil microorganisms and consequently the origin of soil enzymes. Bacteria and actinomycetes, which are primary agents in the degradation of pesticides (Boivin et al., 2005), develop much more in vermicompost-amended than in unamended soils. However, pesticide inhibits the growth of microorganisms and alters soil enzyme activities. On the other hand, it is important that the pesticide residues remaining in the ploughed layer be released in a controlled way in order to favour their microbial degradation. Vine-shoot and *alperujo* vermicomposts can sorb diuron and imidacloprid (Romero et al., 2006), its addition to the top layer can increase its persistence in the soil.

Imidacloprid (I) is a systemic insecticide widely and frequently applied in greenhouse and field crop systems. It is persistent in alkaline sandy loam soil with low organic matter content (Lopez-Capel et al., 2000). Diuron (D) is a herbicide used to control a wide variety of annual and perennial broadleaf and grassy weeds in agricultural and urban areas. Microbial degradation has been reported to be the main factor responsible for diuron dissipation in soils (Giacomazzi and Cochet, 2004).

This work studies how the application of vermicompost from vine-shoot and *alperujo* influences the dehydrogenase (Dhase) activity enzyme as a measure of soil microbial activity. The effect of this practice on the attenuation of the negative effect of D and I, pesticides frequently used in this area, was also evaluated.

2 MATERIALS AND METHODS

2.1. Chemicals

Diuron [N´-(3,4-dichlorophenyl)-N,N-dimethylurea] and Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine] of 97.5% and 99.5 % purity, respectively, were supplied by Dr. Ehrenstorfer (GmbH, Germany)). D and I have water solubility of 42 mg l^{-1} and 510 mg l^{-1}, respectively. Analytical grade sulphuric acid 96% from Panreac and HPLC-grade acetonitrile from Scharlau Chemie (Barcelona, Spain) were used.

2.2. Experimental design

Vermicomposts from *alperujo* (Va) and vine-shoot (Vs) were obtained with *Eisenia andrei* at pilot-scale and characterized following establish methods as reported Romero et al. (2006). Their main properties are shown in Table 1.

Samples from the top (20 cm) of a calcaric Cambisol soil were air dried and sieved through a <2 mm mesh. Unlike the sorption studies described in the literature, where the soil is simultaneously organically amended and treated with pesticides, the soil samples were previously 5% amended with the two assayed vermicomposts, Va and Vs, incubated for 3 months and then air-dried. This procedure was carried out twice to simulate agronomic practices. After incubation, the unamened soil (S) and vermicompost-amended soils (SVa and SVs) were analysed in triplicate using validated methods (M.A.P.A. 1986). Their main properties are shown in Table 1.

TABLE 1 **Some properties of vermicomposts, unamended soil and soils doubly amended with vermicompost**.

	Sand/Silt /Clay g kg^{-1}	***pH***	***TOC g kg^{-1}***	***TEC g kg^{-1}***	***AH g kg^{-1}***	***C/N***	***HR %***	***Dhase µgINTF g^{-1}h^{-1}***
Va		8.6	292	201	75	15	69	49
Vs		7.3	295	247	146	11	84	19
S	111 / 489 /401	8.4	14	6.2	4.9	5.5	44	1,75
SVa		8.2	33	10.6	6.9	9.8	32	3,61
SVs		7.9	37	10.5	6.8	6.7	28	2,40

HR : humification rate = (TEC/TOC)x100

Unamended (S) and doubly amended soil samples (SVa and SVs) were treated with the same dosage of diuron (D) and imidacloprid (I) (3µg g^{-1}) and moistured at 80% of field capacity. After 24 h equilibration, the soil samples were incubated at 20ºC in a thermostatic chamber for 90 days. Following a drying period (30 days), a second pesticide treatment (3µg g^{-}1) and incubation procedure (an additional 90 days) were carried out under similar conditions. . Triplicate soil samples were collected at different times (5, 10, 30, 60 and 90 days) during each incubation period, and soil dehydrogenase (DHase) activity was analyzed. 1 g of wet soil sample was incubated for 20 h at 25ºC with 0.2 mL of 0.4% 2-p-iodophenyl-3-p-nitrophenyl-5-tetrazolium chloride (INT). The iodonitrotetrazolium formazan (INTF), produced in the reduction of INT, was extracted with acetone:tetrachloroethylene (1.5:1) and measured in a spectrophotometer at 490nm (Garcia et al., 1993). Assays in soils without INT were simultaneously carried out as controls.

3 RESULT AND DISCUSSION

The effects of imidacloprid (I) on dehydrogenase activity in the unamended soil and soil amended twice with alperujo (Va) and vine shoot (Vs) vermicompost are shown in Figure 1. The application of imidacloprid to soil S+I increased Dhase activity during the first 30 days of the incubation period compared with the untreated soil (S). Thereafter, Dhase activity values were similar for both treatments. The second imidacloprid addition did not affect Dhase activity, whose values were generally lower (0.74-1.48 µg INTF g^{-1}h^{-1}) than those recorded at the end of the first incubation period.

In the soil amended twice and incubated previously with vermicompost from *alperujo*, the application of imidacloprid (SVa+I) significantly increased soil Dhase activity, which peaked after 30 days of incubation (4.43 µgINTF g^{-1}h^{-1}). Later, Dhase activity decreased abruptly, and after 90 days, levels were similar to those recorded in S and S+I. The reapplication of imidacloprid stimulated soil Dhase activity, which increased throughout the second incubation period, reaching a maximum value of 6.34 µgINTF g^{-1}h^{-1} at the end of this period, which was 4 times greater than that recorded in the unamended soil (S+I) and S2VaI .

In the soil amended twice and incubated previously with vermicompost from vine-shoots, application of imidacloprid (SVs+I) also increased Dhase activity in relation to the unamended soil (S). However, the increases were less marked than those observed when the insecticide was applied to the unamended soil (S+I) or when amended with vermicompost from alperujo (SVa+I). As observed in SVa+I, the second application of Imidacloprid

enhanced soil Dhase activity, which, however, after 210 days, was significantly lower (4.23 µgINTF $g^{-1}h^{-1}$) than the level observed in the soil amended with alperujo vermicompost.

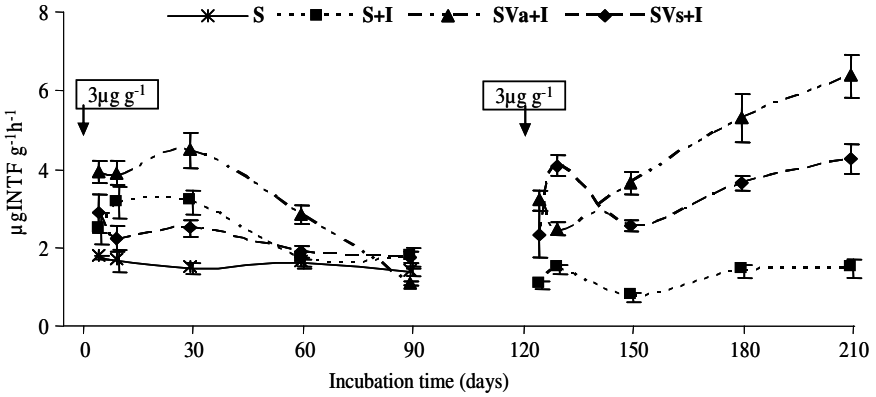

FIGURE 1 **Effect of Imidacloprid on dehydrogenase activity in unamended soil (S+I) and soil amended twice with vermicomposts from alperujo (Sva+I) and from vine-shoots (SVs+I).**

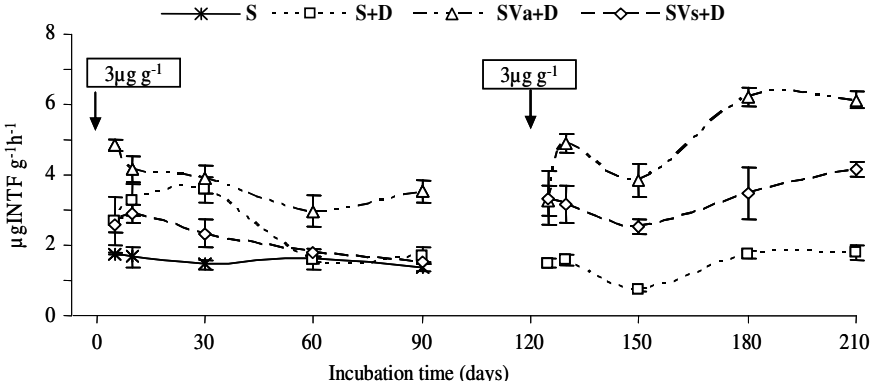

FIGURE 2 **Effect of Diuron on dehydrogenase activity in unamended soil (S+I) and soil amended twice with vermicomposts from alperujo (Sva+I) and from vine-shoots (SVs+I)**

The diuron (D) effects on dehydrogenase activity in the unamended soil and soil amended twice with the vermicomposts from alperujo (Va) and from vine-shoots (Vs) are shown in Figure 2. In general, soil Dhase activity during both incubation periods followed a similar pattern to that recorded for the Imidacloprid both in the unameded soil (S+D) and the soils amended with vermicompost from *alperujo* (SVa+D) and vine-shoots (SVs+D). In comparative terms, Dhase activity values for all soil treatments were slightly higher when treated with Diuron, being this effect more apparent in the soil amended with *alperujo*-vermicompost (SVa+D).

These results reveal that the application of Imidaclorpid and Diuron over a short time period (first incubation period) led to important changes in dehydrogenase activity and, consequently, in soil microbial activity. It is widely known that although pesticides can destroy some microbial populations, resulting in lower Dhase activity, they can also enhance the growth of other populations and their capacity to degrade pesticides. In our study, both pesticides assayed initially stimulated soil dehydrogenase activity. However, this activity later decreased and tended to reach levels similar to those for the unamended and untreated soil (S). Similar increases during the first 14 days of incubation were observed when the soil was treated with diuron and/or amended with spent grape marc vermicompost (Romero et al., 2010). In comparative terms, the effect of both types of pesticide was similar, meaning that their different chemical characteristics scarcely affected soil Dhase activity.

The larger increases recorded in the soil doubly amended with alperujo vermicompost (SVa) could be explained by the capacity of this organic amendment and the vine–shoot vermicompost (to a lesser extent for the latter), to provide microorganisms, enzymes and nutrients for soil microbiota and to change the soil's innate dehydrogenase activity. In addition, these changes could also be due to the less stable nature of the alperujo-vermicompost (higher C/N and HR) and its higher dehydrogenase activity (Table 1).

Further additions of Imidacloprid and Diuron during the second incubation period increased soil Dhase activity in the organically amended soils, though not in the unamended soils, where Dhase activity was scarcely affected or reduced. Thus, the vermicompost-amended soils have created or increased a pool of stabilized organic matter, as well as a tolerant microbial community resistant to the further addition of the pesticides. These tolerant or adapted microorganisms, while degrading these pesticides, may also use them as a nutritional source, thus increasing their number and activity, as reflected in the growth of soil dehydrogenase activity.

4 CONCLUSIONS

Unlike the unamended soils, successive applications of Imidacloprid and Diuron to the soils doubly amended with alperujo and vine shoot vermicompost do not inhibit soil microbial activity, as measured in terms of dehydrogenase activity. The addition of soil organic amendments such as *alperujo* and vine-shoot vermicompost could therefore represent a sustainable waste management procedure, which could help to minimize the negative side effects of pesticide on microbial communities and to maintain soil quality. Under field conditions, improvements in the attenuation effects induced by these vermicomposts could depend on the frequency and dosage of pesticide applications as well as the humification rate and microbiological activity of the vermicomposts.

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