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MINERALISATION OF ^{14}C-LABELLED METALAXYL FUNGICIDE IN BRAZILIAN SOILS

ANDREA M. SPESSOTO[1a], ITAMAR S. MELO[1a], REGINA T. R. MONTEIRO[b]

[a] Laboratory of Microbiology, Embrapa Environment, Jaguariúna-SP, Brazil

[b] Laboratory of Ecotoxicology, Cena-USP, Piracicaba-SP, Brazil

ABSTRACT

Laboratory incubation experiments were carried out to estimate the mineralisation of metalaxyl ^{14}C {N-(2-6 dimethyphenyl)-N-(methoxyacetyl) alanine methyl ester} in four Brazilian soils with different physico-chemical properties, at 3 and 30 µg a.i. g^{-1}. In the Petrolina sandy soil the mineralisation presented higher $^{14}CO_2$ production rates, at two essayed concentrations, after 70 days. Microbiological studies were done to determine the numbers of bacteria, actinobacteria and fungi (CFU g^{-1} soil). In relation with other microbial community, bacterial population demonstrated to be a major component of the cultivable heterotrophic community after the application.of the compound. No detectable metabolites were found in this study. The results suggest that soil properties and application history may have a strong influence on the fungicide behavior in these soil samples.

1. INTRODUCTION

Metalaxyl is a systemic benzenoid fungicide used in mixtures as a foliar spray for tropical and subtropical crops, as a soil treatment for control of soil-borne pathogens and as a seed treatment to control downy mildews. Several authors observed that metalaxyl might be degraded by microorganisms. However, the role of microorganism in the dissipation of metalaxyl has not been well understood .Droby & Coffey (1991)(1) demonstrated that soil fungi, bacteria and actinobacteria were able to break down the metalaxyl. Di *et al.* (1998)(2) found that the degradation of this compound in sandy soil by natural microbiota varies with soil depth.

Adsorption and mobility of pesticides in the environment have been directly correlated to the rates of degradation. Kookana *et al*. (1995)(3) and Peng *et al*. (1995)(4) observed that these phenomena may change metalaxyl available to soil microorganisms. Studies have indicated that metalaxyl is adsorbed onto clay particles and organic matter of soils, showing low mobility under these conditions (Sukop & Cogger, 1992(5); Sharma & Awasthi, 1997(6)).

Metabolites from metalaxyl degradation in soil samples have been detected and identified. Musumeci & Ruegg (1984)(7) observed two metabolites of the degradation of metalaxyl after 60 days of incubation. However, only N-(2-methoxyacetyl)-N-(2,6 xylyl)-DL-alanine was determined. Droby & Coffey (1991)(1) found one acid metabolite as major product of the fungicide degradation

The aims of the present work were to quantify ^{14}C labeled metalaxyl mineralisation in four Brazilian soils with and without metalaxyl application history; and determine changes in microbial enzymatic activity in soils by the FDA method after metalaxyl addition.

2. MATERIAL AND METHODS

2.1. Soils

Soil samples collected in Aguaí (Ag) (Lat. 22° 03’ 45” S; Long. 46° 56’ 15” W), Estiva Gerbi (Es) (Lat. 22° 18’ 45” S; Long. 46° 56’ 15” W) and Jaguariúna (Ja) (Lat. 22° 41’ 15” S; Long. 46° 56’ 15” W) sites in São Paulo State and Petrolina (Pe)) (Lat. 9° 26’ 15” S; Long. 40° 33’ 45” W) sites in the Pernambuco State were used in the present work. Soil samples were taken from the 0-15 cm depth and held to the laboratory at 4 °C under moisture conditions similar to those in the.

2.2 Fungicide

Technical grade ^{14}C-ring labeled metalaxyl {N-(2-6 dimethyphenyl)-N-(methoxyacetyl) alanine methyl ester}, tested and confirmed to have a radiochemical purity of 98 % and specific activity of 11. 1 MBq mg^{-1} and analytic standard metalaxyl with 99.6 % purity, were kindly supplied by Norvatis® (Basle – Switzerland). The supplementation solution was prepared using both labeled and unlabeled chemical, which each 1 g of soil (dry weight) received 5 KBq radioactivity.

2.3 Metalaxyl ^{14}C mineralisation

Fifteen grams (dry weight) of each soil sample (4 replicates) were placed into biometric flasks, and metalaxyl was added at final concentration of 3 and 30 µg a.i. g^{-1} metalaxyl and thoroughly mixed. The moisture was adjusted to 75% of field capacity and the flasks were incubated in dark room at 28 °C. Vial containing 10 mL of 0.2N NaOH were

placed in each flask to trap CO_2 evolved during the incubation. After 7, 14, 21, 28, 35 and 70 days the flasks were opened, and the CO_2 traps were removed for analysis and replaced. Deionized and sterilized water were added to the flasks the during incubation period to maintain humidity A 1 mL sample was removed from the CO_2 traps and mixed with 10 mL of scintillation cocktail (Sigma-Aldrich). The ^{14}C in the samples was counted for 15 min with a Beckman LS 0025 auto scintillation counter.

2.5. Microbial activity

Microbial activity in the soils at 0 and after 70 days of incubation in biometric flasks was determined by fluorescein diacetate method (FDA). The values were expressed as μg FDA hydrolyzed g^{-1} soil dry min^{-1} using a spectrophotometer Beckman DU-8B at a wavelength of 490nm.

3. RESULTS

3.1. Mineralisation of metalaxyl ^{14}C

The $^{14}CO_2$ data presented in Table 1 enable comparison of the rates of mineralisation in non-sterilized and sterilized soils. Mineralisation was higher in Pe soil (53 % $^{14}CO_2$) than in the others soils with application history of fungicide (Ag and Es) at 3 μg a.i. g^{-1} metalaxyl. At 30 μg a.i. g^{-1} metalaxyl, Pe also showed similar rates of $^{14}CO_2$ production than compared to 3 μg a.i. g^{-1} metalaxyl concentration.

However, in Ag, Es and Ja soil samples, the mineralisation rates metalaxyl decreased according to increment of fungicide application. These phenomenon's were positively correlated with application history, soil characteristics and supplementation doses ($P \leq 0.05$).

The rate of mineralisation of metalaxyl in the soils suggests a possible microbial adaptation. Thus,in the Brazilian soils with repeated metalaxyl application, the data showed an increase the mineralisation rates, phenomenon known as enhanced biodegradation, suggesting the previous presence of microorganisms able to metabolize the fungicide

Table 1. $^{14}CO_2$ percentages accumulated after 70 days incubation with 3 and 30 μg a.i. g^{-1} metalaxyl.

	Incubation Time (days)						
	7	14	21	28	35	70	
Treatments[1]	**$^{14}CO_2$ percentages accumulated per week (mean ± S.E.)**						**% $^{14}CO_2$ total**
Ag 3 μg a.i. g^{-1}	0.58(±0.14)	2.03(±1.16)	6.02(±2.16)	9.19(±0.69)	11.81(±1.12)	13.60(±1.12)	43.23
Es 3 μg a.i. g^{-1}	0.65(±0.16)	4.92(±0.16)	6.32(±0.35)	9.12(±1.02)	12.2(±1.04)	14.03(±1.21)	47.24
Ja 3 μg a.i. g^{-1}	0.32(±0.80)	0.63(±0.16)	0.76(±0.18)	7.32(±0.36)	10.02(±1.10)	11.90(±1.04)	30.95
Pe 3 μg a.i. g^{-1}	1.37(±0.75)	6.54(±0.33)	8.19(±0.46)	10.24(±1.14)	12.71(±0.99)	14.60(±1.07)	53.65
Ag 30 μg a.i. g^{-1}	0.41(±0.12)	1.52(±0.75)	5.75(±0.29)	7.24(±0.34)	9.01(±1.01)	10.43(±0.98)	34.36
Es 30 μg a.i. g^{-1}	0.49(±0,13)	2.66(±0.22)	6.41(±0.30)	8.23(±0.40)	9.95(±1.07)	11.21(±1.11)	38.95
Ja 30 μg a.i. g^{-1}	0.27(±0.04)	0.42(±0.12)	0.58(±0.14)	5.03(±2.67)	8.29(±1.98)	9.40(±0.87)	23.99
Pe 30 μg a.i. g^{-1}	0.57(±0.13)	2,89(±0.72)	8.14(±0.46)	12.55(±0.67)	13.85(±1,13)	15.23(±1.20)	52.66
Pe 3 μg a.i. g^{-1} sterelised	0.05(±0.01)	0.04(±0.02)	0.04(±0.01)	0.04(±0.02)	0.05(±0.01)	0.04(±0.01)	0.22

1-the values are presented to four replicates

3.3. Microbial enzymatic activity

After 70 days incubation of the fungicide it was observed an increase of microbial When compared with the control (measured at the beginning of the experiment) the enzymatic activity was significantly increased.(Figure 1).

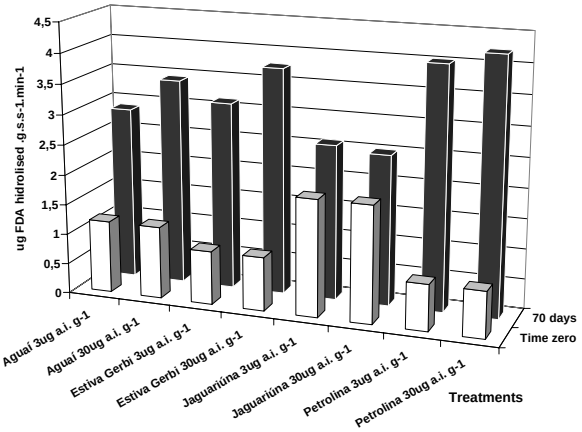

Figure 1. Microbial activity of Brazilian soils supplemented with metalaxyl evaluated by FDA method.

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