

Research Article

Enantioselective Degradation of Triadimefon in Green-house Soil

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Abstract: To study enantioselective degradation of triadimefon, the enantioselective degradation of triadimefon in greenhouse soil and normal soil were investigated in detail. The enantiomers of triadimefon were separated by Chiralpak AD column and determined by Liquid Chromatography Via Tandem Mass Spectrometry (LC-MS/MS). The degradation exhibited some enantioselective, resulting in a concentration order of R(-) triadimefon>S(+) triadimefon and the degradation of triadimefon in greenhouse soils with high content of organic matter was faster than normal soil.

Keywords: Enantioselective degradation, greenhouse soil, triadimefon

INTRODUCTION

Approximately 25% of the pesticides in use are chiral and only a small fraction of all are manufactured and used in the form of pure enantiomeric compounds, apparently due to economic and/or technical reasons (Pérez-Fernández *et al.*, 2011). Nevertheless, ignoring the existence of enantiomers can produce incorrect toxicological, distribution or degradation data. So it was important to know the individual properties of each enantiomer in environment (Ali *et al.*, 2003; Wong, 2006; Müller and Kohler, 2004).

Triadimefon is a 1,2,4-triazole fungicide used for the control of powdery mildews and fungi on fruits, vegetables, turf grass and other agriculture crops (Gasztonyi, 1981). Triadimefon have a chiral center and thus exists as two enantiomers (Fig. 1).

However, even though many studies have been carried out to understand the metabolism and environmental fate of triadimefon (Garrison *et al.*, 2011; Li *et al.*, 2011), essentially all of the earlier studies regarded racemic triadimefon as single compounds, failing to acknowledge potential enantioselectivity in their biologically mediated environmental processes (Bromilow *et al.*, 1996; Bromilow *et al.*, 1999; Singh, 2005). Evaluation of chiral selectivity in the degradation of triadimefon enantiomers will be of significant value for improving our understanding the enantioselective degradation in the environment.

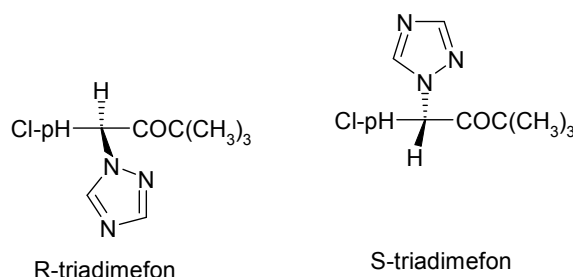


Fig. 1: The enantiomer constructor of triadimefon

Greenhouses allow for greater control over the growing environment of plant, such as temperature, levels of light and shade (Gradish *et al.*, 2010; Guo *et al.*, 2010). In the recent years, it had become an accepted and routine practice to cultivate vegetable under greenhouse condition in effect to fulfill consumer demand in off-season (Park *et al.*, 2011). The change environment of greenhouse will effect the degradation of pesticide. The dissipation rate of pesticides following application depends heavily on many factors, including chemical and photochemical degradation, climatic conditions, volatilization, cultivated species, formulation class and application mode (Sur *et al.*, 2000). Studies concerning the enantioselective degradation of triadimefon in two soils have been previously reported in the literature (Li *et al.*, 2011). However, no information is currently available

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concerning the residual status and pattern of triadimefon disappearance in greenhouse soil.

As the export base of off-season vegetable, the most technology of greenhouse practice was used in yunnan province P.R. china, expecting geographic and climatic advantage. So its potential risk to public health through the food chain, studies on enantioselective degradation of triadimefon under greenhouse soil was urgently needed. In this study, we investigated the dissipation and enantioselective degradation of triadimefon in greenhouse soil via HPLC, with confirmation conducted via tandem mass spectrometry (LC-MS/MS) and a Chiralpak AD column were used for the separation of the enantiomers of triadimefon.

MATERIALS AND METHODS

Chemicals and solvents: Racemic triadimefon 1-(4-chlorophenoxy)-3, 3-dimethyl-1-(1*H*-1, 2, 4- triazol-1-yl) butan-2-one was purchased from sigma-aldrich, solvents and other chemicals used in this study were of analytical or HPLC grade.

Soils sample: Soil 1 were collected from the surface layer (0-15 cm) of a field plot planted with greenhouse vegetable for an extended time (about 3 years) and the normal soil 2 from outer of greenhouse vegetable in Junning country, yunnan province, China. The soil properties were as follows: soil 1, organic matter 4.89%, sand 48.7% and clay 4.3%; soil 2, organic matter 3.49%, sand 56.7% and clay 6.71%. Blank determination of the soils prior to fortification revealed no triadimefon present.

Incubation experiments: Degradation experiments were carried out with rac-triadimefon (ratio of 51:49) in the two soils. In each experiment, a portion of 10 g dry soil was transferred into 250-mL glass flasks and spiked with 0.5 mL methanol containing 50 ug triadimefon and stirred thoroughly for 10 min to allow methanol evaporated. 100g soil was added and mixed thoroughly yielding a final fortification level of 0.5 mg/kg. Then 15 mL distilled water was added to give a ~15% final moisture content. After carefully mixing, the flasks were sealed with cotton wool plugs and incubated at room temperature 10~25°C in the dark. Distilled water was added at three or four day intervals to maintain the initial moisture. The samples were removed for analysis at different time intervals and frozen at -20°C until they could be analyzed.

Extraction procedure: Five grams of soil was weighed into a 100 mL centrifuge tube and 2 g anhydrous sodium sulfate, 50 mg C18 material, 10 mL acetonitril was added. The centrifuge tube was extracted in an ultrasonic bath for 10 min. Then the mixture was centrifuged at 3000 g for 5 min. Two milliliter portions of the acetonitril layer were filtered through 0.2 μm membrane filters prior to analysis by LC-MS-MS.

LC-ESI- MS/MS: A 1290 ultra high performance liquid chromatography pump (Agilent technology, USA) equipped with auto-sampler, binary pump system, thermostated column compartment, degasser and data software was applied in the experiments. The analytical column was Chiralpak AD-RH Adrhcd-MC018 (150×2.1 mm, 5 μm) from Daicel chemical industries ld., Jap. The mobile phase was comprised of acetonitrile/water (48/52) and was delivered at a constant flow of 0.4 mL/min. The injection volume was 1 uL. MS/MS was monitored in ESI mode with an Applied Biosystem of API 4000 LC/MS/MS system. Nitrogen was employed as nebulizing gas.

Limit of Detection (LOD) defined as the concentration that produced a Signal to Noise (*S/N*) ratio of 3 and Limit of Quantification (LOQ) were set as 10:1 (*S/N*).

The accuracy of the whole method was evaluated by the development of a recovery study carried out at series of blank sample fortified with rac-triadimefon at 0.01, 0.05 and 0.1 mg/kg.

The Enantiomeric Fraction (EF) was used as a measure of the enantioselective degradation of enantiomers in soil sample. EF is defined by the following equation:

$$EF = A1 / (A1 + A2)$$

A1 and A2 are the peak areas of R(-) and S(+) triadimefon, respectively. The EF values range from 0 to 1, with EF = 0.5 representing a racemic mixture.

RESULTS AND DISCUSSION

Quality/quality assurance

Mass spectrometry allowed for the selective monitoring of the MRM transitions of (R)-, (S)- triadimefon and MS detector conditions were studied in order to obtain at least three transitions for triadimefon. The separation of S- (+)-, R(-)-triadimefon was achieved using a chiral chromatographic system. Standard solutions of triadimefon were optimized by MS/MS using ESI in positive ion mode. $[M+H]^+$ of triadimefon was 294.0, which were selected as the precursor ion (Q1 mass) and collision energies were studied in order to find the most abundant ions (Q3 mass), selecting the most sensitive ion for quantification (197.1) and a second one for qualification (225.1). Table 1 shows the MS/MS condition for quantification and qualification, as well as collision energy and collision cell exit potential values optimized for the selected compounds.

The enantiomer of triadimefon were separated by normal phase chiral column, such as OD-H, OJ-H (Li *et al.*, 2011; Gradish *et al.*, 2010) with n-hexane/isopropanl as mobile phase, which is immiscible with RP-HPLC solvent of acetonitril, water. The question can be resolved by Chiralpak AD-RH

with reversal phase material. The two enantiomers were separated completely by Chiralpark AD-RH with acetonitrile/water as mobile phase and there were no interference peaks at their times. The absolute configuration of the two enantiomers was corresponding to that reported by Li *et al.* (2012). The first eluting peak is the R-(-)-tridimefon and the other is S-(+) enantiomer.

The triadimefon enantiomer peaks were nominally of equal area, indicating the expected racemic form of the compound; the Enantiomer Fraction (EF) was 0.51, as quantified by the following equation, where brackets indicate enantiomer concentrations:

The LOD and LOQ values for R-(-) and S-(+) triadimefon were 0.001 and 0.005 mg/kg, respectively. All the method blank were less than 5% of the lowest residues values of the samples and the pesticide concentration reported were not corrected by method blank. Spike recovery experiment with three concentrations, which were >85%, were estimated by comparing the peak area of the extracted analyte to that of an equivalent amount of the standard solution.

Enantioselective degradation of racemic triadimefon in soil:

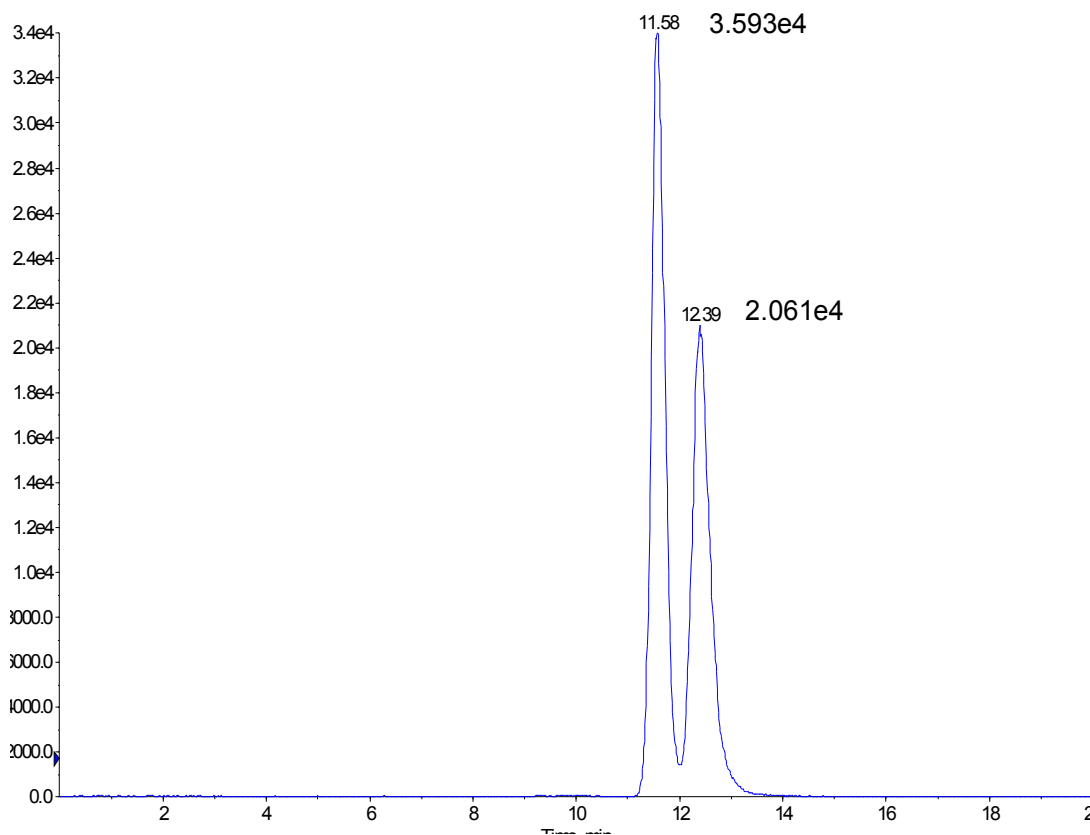
In two soils, the loss of triadimefon is enantioselective; the S-(+) -enantiomer always reacted faster than the R-(-) -one., because it was the case of significant conversion of S-(+)-triadimefon to R-(-) one (Li *et al.*, 2011), the degradation exhibited some enantioselective, resulting in a concentration order of R-(-) triadimefon>S-(+) triadimefon. Figure 2 showed chromatogram of triadimefon in two soil incubation. Figure 3 illustrated the change of EF value in 30 days. The EF value changed gradually from initial 0.51 to 0.65 over 30 days period in greenhouse soil and EF value changed from 0.51 to 0.56 in normal soil. The result showed a relatively higher enantioselectivity in greenhouse soil than in normal soil.

Kinetics degradation of racemic triadimefon in soil:

In all soils, the degradation rate of triadimefon was the first order reaction (Fig. 4 and 5). In greenhouse soil, the rate constants of racemic triadimefon, R-(-) and S-(+) - enantiomers were -0.087, 0.0795, -0.0976/d,

Table 1: API 3200 LC/MS/MS condition

Ion spray voltage	Curtain gas	Collision gas	Ion source gas1	Ion source gas2	Temperature
5000 V	25 psi	5 psi	65 psi	60 pai	650°C
Q1 mass	Q3 mass	Declustering potential	Entrance potential	Collision energy	Collision cell exit potential
294.0	225.1	30.0 V	7.0 V	14 V	4.3 V
	197.1			20 V	4.1 V



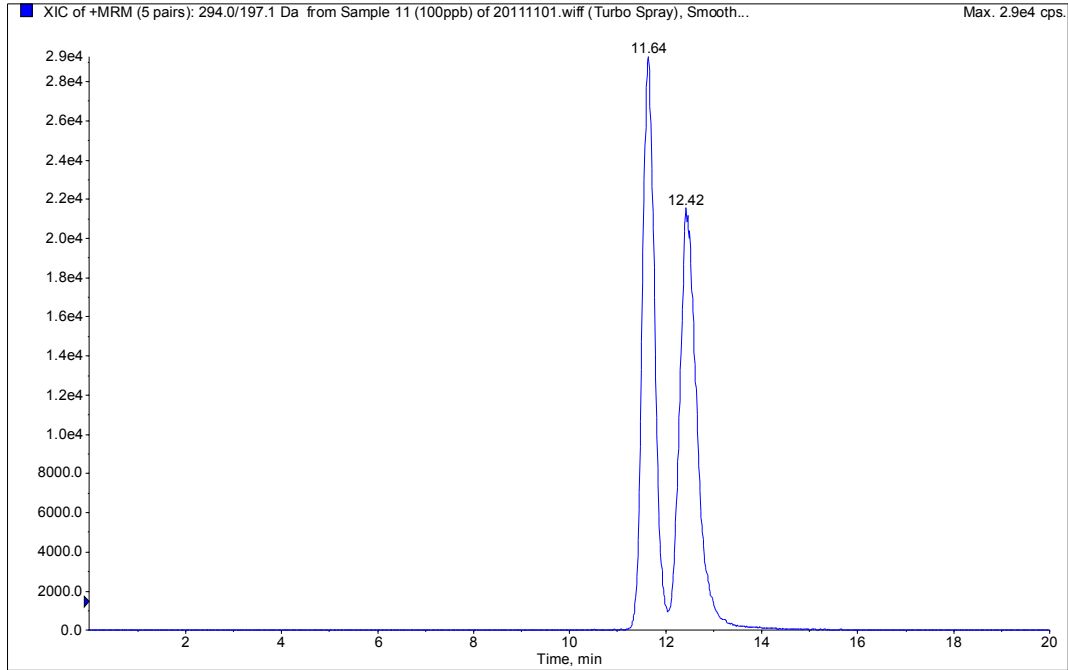


Fig. 2: LC-MS-MS of S-(+)- and R-(-) enantiomeric triadimefon in greenhouse soil and outer soil over 30 days

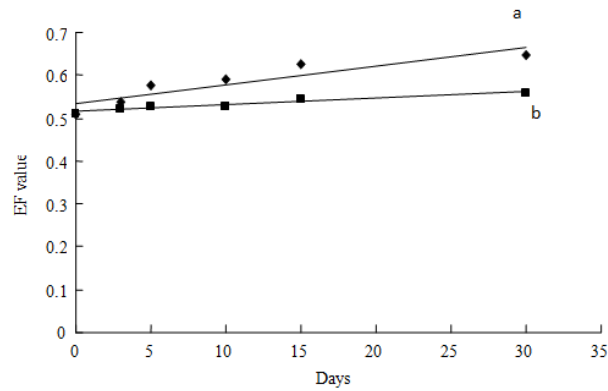


Fig. 3: Enantiomer Fraction (EF) vs time for change of triadimefon in greenhouse soil (a) and outer soil (b)

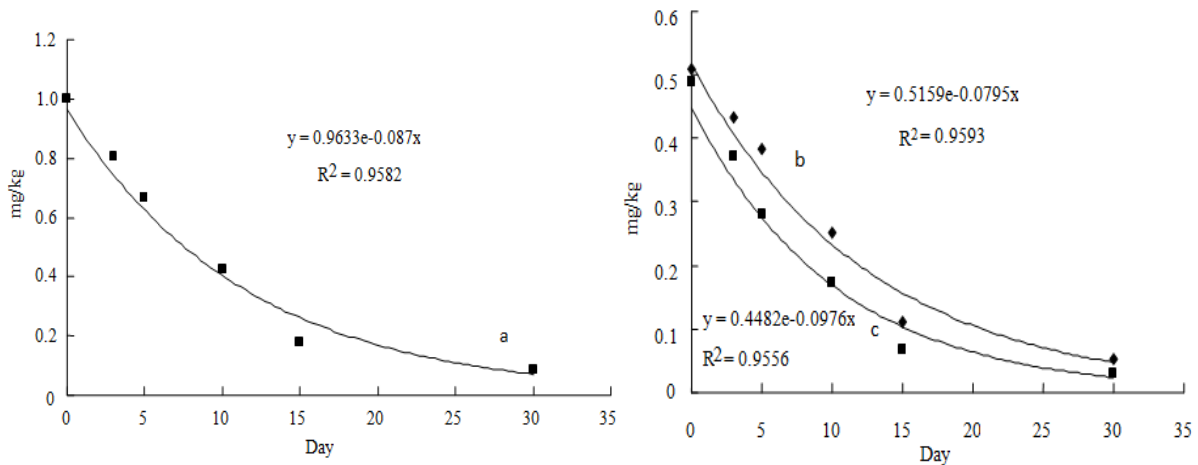


Fig. 4: Kinetics of first order degradation of total triadimefon (a), R-(-)-triadimefon; (b), S-(+)-triadimefon (C) in greenhouse soil

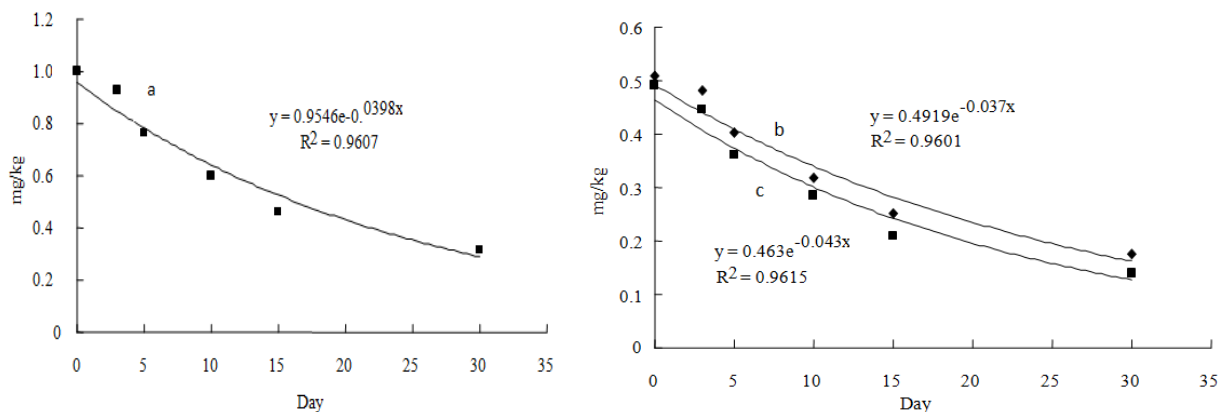


Fig. 5: Kinetics of first order degradation of total triadimefon (a), R(-)-triadimefon (b), S(+)-triadimefon (C) in normal soil

respectively corresponding to half-lives $T_{1/2}$ of 7.5, 9.1 and 5.9 days. The dissipation of triadimefon under greenhouse soil was faster than the literature data (Li *et al.*, 2011). It reported that half-lives $T_{1/2}$ for dissipation of triadimefon in baoding alkaline and wuhan acidic soil were 10.49 and 19.04 days.

In the field, physical and chemical factors, including light, heat, pH and as well as organic matter factors may perform a pivotal role in the degradation of pesticide residues (Khay *et al.*, 2006). The results indicated the degradation of triadimefon in greenhouse soils with high content of organic matter was faster than normal soil.

ACKNOWLEDGMENT

We were grateful for support funded the science and technology department of Yunnan province (project No: 2008 CD181, 2012CA 005), the risk of food safety in agro-production (GJFP2014001) (2014GNA001).

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