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Enantioselective phytotoxicity of metolachlor against maize and rice roots

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ABSTRACT

Rac-metolachlor, a widely used chloracetanilide herbicide, is now being replaced by *S*-metolachlor in many countries. The enantioselective effects of *rac*- and *S*-metolachlor on root growth of maize and rice was studied in hydroponics. Visible morphological changes in root growth were observed after treatment with *rac*- or *S*-metolachlor. The main root and lateral roots were shorter in length, and the number of lateral roots was reduced. The half inhibition ($IC_{50,5d}$) values for root length of *rac*- and *S*-metolachlor were 18.86 and 10.61 μM, respectively, for maize, and 7.33 and 5.35 μM, respectively, for rice. The root system activity after treatment with *rac*- or *S*-metolachlor was lower than that of the control, while the root membrane permeability was higher. The activities of superoxide dismutase, peroxidase, and catalase in the roots were lower after *rac*- or *S*-metolachlor treatment compared to those of the control, while the malondialdehyde content was higher. After rice was treated with 3.1 μM *rac*- or *S*-metolachlor, the cell wall separated from the cell membrane, and some destruction of nuclei and organelles was observed. The entire cell was destroyed after treatment with 12.4 μM *rac*- or *S*-metolachlor. The results showed that *S*-metolachlor has stronger effects than *rac*-metolachlor on crop roots.

1. Introduction

More than 30% of currently used pesticides are chiral compounds [1], including synthetic pyrethroids, organophosphorus insecticides, imidazolinones and chloracetanilide herbicides. The percentage of chiral pesticides is increasing with the introduction of more complex structures [2]. The assessment of enantiomer selectivity in both exposure and effects is required for comprehensive risk assessments [3]. An increasing number of studies have investigated the environmental fate and microbial transformation of chiral pesticides [4,5]. The toxicities of chiral pesticides and their metabolites against non-target animals and human cancer cell lines have also been studied [6,7]. However, the enantioselective ecological effects and toxicities of chiral herbicides against plants have not received as much attention as those observed in animals [8]. As important ecological receptors in the ecosystem, plants are immobile and cannot avoid harmful effects. Therefore, the enantioselective phytotoxicity of herbicides against plants merits more attention.

Metolachlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide, is one of the most important herbicides used for the selective weed control of more than 70 crops worldwide [9]. Metolachlor has two chiral elements: a chiral axis and a stereogenic center, leading to four stereoisomers [10]. Metolachlor was introduced into the market in 1976 as a racemic product, which contains two *R*- and *S*-enantiomers that occur in an equal ratio. In 1982, it was determined that about 95% of the herbicidal activity of metolachlor resides in the two (1′*S*) diastereomers [11], and since 1997, metolachlor (*rac*-metolachlor) has been replaced by *S*-metolachlor in a number of countries. A content ratio of about 90% 1′*S* -isomers has the same biological effect at 65% of the racemic dosage [9] has been accomplished (e.g., United States in 1997, Switzerland in 1997, Canada in 1998, South Africa in 1998 and Australia in 1999) [12,13].

Metolachlor inhibits the synthesis of proteins and chlorophyll in plants, but it has a low toxicity in mammals. The sorption and desorption behaviors of metolachlor in the soil has been studied [14], along with its dissipation properties, metabolites and effects on organisms [15–17]. The enantioselectivity of *rac*- and *S*-metolachlor to *Daphnia magna* and *Chlorella pyrenoidosa* have been compared [10,18], and the stereoselective degradation of metolachlor has been studied [19,20]. However, relatively few studies have examined the enantioselectivity of *rac*- and *S*-metolachlor against plants [21], and there is a dearth of information on the effects of *rac*- and *S*-metolachlor on root growth in maize and rice.

In the present study, the enantioselectivity of two commercial products, *rac*- and *S*-metolachlor, on root growth in maize and rice was evaluated under hydroponic conditions. The enantioselective

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effects of *rac*- and *S*-metolachlor on root growth were compared. The half inhibition ($IC_{50,5d}$) values of *rac*- and *S*-metolachlor were calculated. Root system activity and root membrane permeability treated with *rac*- and *S*-metolachlor after 24 h were measured. The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) of maize and rice roots were examined. The malondialdehyde (MDA) content of maize and rice roots were determined. In addition, the effects of *rac*- and *S*-metolachlor on the ultrastructure of rice root cells were studied by transmission electron microscopy (TEM). An attempt was made to compare the effects of *rac*- and *S*-metolachlor and to reveal possible effects of chiral differences on the root toxicities of these two herbicides.

2. Materials and methods

2.1. Chemicals

Rac-metolachlor (97% chemical purity) was obtained from the Qingfeng Pesticide Company (China), and *S*-metolachlor (96% chemical purity) was obtained from Syngenta (Switzerland). All other reagents used in this study were analytical reagents.

2.2. Plant culture

Maize seeds (Yedan 13, *Zea mays* L.) and rice seeds (II You 92, *Oryza sativa* L.) were submersed in tap water for 24 h, sterilized in a 3‰ (v/v) Clorox solution for 5 min, washed several times with sterilized deionized water, and germinated in the dark for 48 h at 25–30 °C. Uniformly germinated seedlings were selected and placed in growth media for 5 days at 25–30 °C with a 16 h light/8 h dark cycle. The growth medium for maize was a nutrient solution containing 0.25 mM KH_2PO_4, 0.1 mM KCl, 0.6 mM $MgSO_4$, 1.0×10^{-3} mM H_3BO_3, 4.0×10^{-3} mM $FeCl_3$, 1.0×10^{-3} mM $ZnSO_4$ and 1.0×10^{-4} mM $CuSO_4$. The growth medium for rice was a modified Hoagland nutrient solution with a pH of 5.0–5.1, and contained 914 mg/L NH_4NO_3, 403 mg/L $NaH_2PO_4{\cdot}2H_2O$, 714 mg/L K_2SO_4, 3240 mg/L $MgSO_4{\cdot}7H_2O$, 886 mg/L $CaCl_2$, 800 mg/L Na_2SiO_4, 3 mg/L $MnCl_2{\cdot}4H_2O$, 0.15 mg/L $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1.87 mg/L H_3BO_3, 0.07 mg/L $ZnSO_4{\cdot}7H_2O$, 0.06 mg/L $CuSO_4{\cdot}5H_2O$, and 15.40 mg/L $FeCl_3{\cdot}6H_2O$.

2.3. Growth inhibition tests

Rice growth inhibition tests were performed according to the OECD guidelines for chemical testing [22]. Ten seedlings were tested for each replicate. Three replicates were tested for each treatment. *Rac*- and *S*-metolachlor were dissolved in methanol to produce stock solutions of 10,000 mg/L, and the methanol concentration was 0.02% (v/v) or less in the treatment conditions. *Rac*- and *S*-metolachlor were added to attain the concentrations of 0, 6.2, 18.6, 37.2, 74.4, 93, and 124 μM for maize and 0, 1.55, 3.1, 6.2, 12.4, 24.8, and 31 μM for rice. The roots were scanned by a stereomicroscope (LEICA MZ9.5 Germany). The mean root lengths of the seedlings were measured, and the relative inhibition rates of maize and rice root elongation caused by *rac*- or *S*-metolachlor in each concentration were calculated after 5 days of treatment. The relative inhibition rate was calculated as follows:

$$\mathrm{RI}\,(\%) = \frac{(X_0 - X_n) \times 100\%}{X_0} \quad (1)$$

where X_0 represents the average root length of CK and X_n represents the average root length of each treatment. The concentration of each herbicide that caused a 50% inhibition (IC_{50}) of maize and rice root elongation was determined from the dose–response regression curve using a logarithmic model.

2.4. Root system activity and root membrane permeability

Maize plants were treated with 0, 18.6, 37.2 or 74.4 μM *rac*- or *S*-metolachlor, Rice plants were treated with 0, 1.55, 3.1 or 6.2 μM *rac*- or *S*-metolachlor.

Root system activity was determined using the triphenyl tetrazolium chloride (TTC) method [23]. Maize and rice roots after 24 h of treatment were washed with distilled water, and the root tips of the main roots were cut into small pieces of 0.5 cm in length. A 0.2 g portion of each root tip sample was placed into a beaker, and 5 mL of 0.4% TTC and 0.1 M phosphate buffer solution (pH 7.0) were added and allowed to react for 3 h at 37 °C. The root tips turned red, and 2 mL of 1 M H_2SO_4 was added to stop the reaction. The tips were dried with filter paper and transferred to tubes with stoppers. Then 20 mL of methanol was added to turn the tip color white (approximately 3–5 h). The root activity was expressed as the amount of triphenyl formazan (TPF) that was deoxidized by TTC.

The root membrane permeability was determined by a method modified from Alpaslan and Gunes [24]. Maize and rice roots after 24 h of treatment were washed with distilled water and dried, and then, the roots were cut into small pieces that were 2 cm in size. A portion of fresh material (1 g) was placed into test tubes containing 20 mL of deionized, distilled water. The test tubes were vortexed for 5 s, and the solution was assayed for initial electrical conductivity (EC_0). The test tubes were immersed at 30 °C for 12 h and then assayed for EC_1. After boiling the samples for 10 min, their conductivity was measured, when the solution was cooled to room temperature (EC_2). The percent of root membrane permeability was calculated as

$$\mathrm{EC}\,(\%) = \frac{\mathrm{EC}_1 - \mathrm{EC}_0}{\mathrm{EC}_2 - \mathrm{EC}_0} \times 100\% \quad (2)$$

where EC_1 and EC_2 represent the electrolyte conductivities measured before and after boiling, respectively.

2.5. SOD, POD, and CAT activities and MDA content

Maize plants were treated with 0, 18.6, 37.2 or 74.4 μM *rac*- or *S*-metolachlor. Rice plants were treated with 0, 1.55, 3.1 or 6.2 μM *rac*- or *S*-metolachlor.

Roots (7.5 g fresh weight) that were subjected to different treatments were used for enzyme extractions and analyses after 24 h of treatment. Roots were homogenized in a cooled mortar with quartz and 20 mL of 0.1 M phosphate-buffered solution (PBS) (pH 7.5) containing 1% (w/v) polyvinylpyrrolidone (PVPP). The homogenate was filtered through three layers of Miracloth and centrifuged at 12,000 × *g* for 20 min at 4 °C. The supernatant was collected, and the samples were stored at −80 °C for the determination of antioxidant enzyme activities. The protein content was determined according to the method of Bradford [25] using bovine serum albumin (BSA) as a standard. Three replicates were performed for each treatment. All experiments were conducted out at 4 °C.

The activity of SOD was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), a method that was modified from Giannopolitis and Ries [26]. The assay was performed with illumination for 20–30 min at 25–35 °C in a 5 mL cuvette containing 0.4 mL of 130 mM L-Met, 0.4 mL of 750 μM NBT, 80 μL of 500 μM EDTA-2Na, 0.4 mM of 100 μM vitamin B2, 0.5 mL of protein suspension and 2.22 mL of PBS (pH 7.6) against a blank with no protein suspension. One unit of SOD activity was defined as the amount of enzyme that was required to cause 50% inhibition of the reduction of NBT at 560 nm.

The activity of POD was determined by the oxidation of guaiacol in the presence of H_2O_2. One unit of POD activity was defined as the amount of enzyme that was required to oxidize guaiacol in 1 min at 470 nm and at 20 °C [27]. The reaction mixture contained 50 mL of