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Review

Enantioselective phytotoxicity and bioacitivity of the enantiomers of the herbicide napropamide

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ABSTRACT

Enantioselectivity of chiral pesticide enantiomers should be taken into consideration in pesticide application and environmental risk assessment. The phytotoxicity of the enantiomers of napropamide to cucumber, soybean, and the bioactivity to the target weeds *Poa annua* and *Festuca arundinacea* have been studied in this work. To the non-target crops, the influences of napropamide on the root, shoot, fresh weight, chlorophyll, superoxide dismutase (SOD) and catalase (CAT) activities and membrane lipid peroxides have been studied. (−)-Napropamide was more toxic than the racemate and (+)-napropamide to soybean and cucumber in terms of root, shoot and fresh weight. The content of chlorophyll was not affected by napropamide. The impacts on the activities of SOD, CAT and membrane lipid peroxides showed that napropamide could induce the oxidative stress and rac-napropamide caused a stronger oxidative damage to cucumber and soybean than (−)-napropamide and (+)-napropamide. For the target weeds, the influences of napropamide on root, shoot and fresh weight have been studied. (−)-Napropamid was more active to *P. annua*, while rac-napropamide was more active to *F. arundinacea*. To reduce environmental pollution and improve the effectiveness of chiral pesticide, single enantiomer should be developed and produced. This work may provide evidence for developing optical pure product.

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1. Introduction

Pesticides have played very important roles in agriculture, in which chiral pesticides account for more than 40% of the commonly used pesticides [1], and this percentage is increasing as compounds with more complex structures are introduced into use [2]. Although the enantiomers of a chiral pesticide have identical physical and chemical properties, they usually display different physiochemical and biochemical properties [3]. It is very common that only one enantiomer of a chiral pesticide shows the desired effect to a target species, while the other enantiomer is less active, even inactive, but which may not be less toxic

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to the nontarget organisms and cause less impact to the environment [4]. Therefore, it is significant to investigate the enantioselectivity of chiral pesticides, which may promote the use of pure active enantiomers, cut down the dosage of pesticides applied and reduce the burden of pesticides on the environment.

Napropamide [N,N-diethyl-2-(1-naphthalenyloxy)propanamide] (Fig. 1) belongs to the amide herbicide family [5], which is one of the most commonly used pre-emergence herbicide for fruits, vegetables and crops to control broadleaf weeds. Napropamide is polar and slightly soluble in water. Commercial napropamide can easily pass into the tissues of organisms and soil layer [6]. There are reports about the environmental behavior of napropamide, such as degradation in soil, tea, alfalfa [6–8] and photolysis [9]. Enantioselective degradation in soil, cumber, cabbage, rape and tomato has also been reported, and the results showed the degradation of napropamide enantiomers was nearly not enantioselective [10]. Previous research has studied the impacts of the enantiomers of napropamide on the dry weight of *Digitaria sanguinalis* (crabgrass), *Setaria glauca* (foxtail), and *Echinochloa crusgalli* (watergrass), and based on the dry weight it was found D-(−)-enantiomer was about eightfold more active than L-(+)-enantiomer [11]. Development of pesticide should not only consider its activity, but also consider its toxicity to nontarget organisms.

In the external environment, plants are under various abiotic and biotic stresses. When plants are exposed to pesticide or others contaminants, plants can response to the stress by producing reactive oxygen species (ROS), like superoxide radical ($O2^{\bullet -}$), hydroxyl radical (•OH), alkoxy radical (•RO), singlet oxygen ($^1O2$), and toxic hydrogen peroxide (H_2O_2), which can damage biological cells [12–15]. To prevent the oxidative damage initiated by ROS, plants have protective mechanisms, including enzymatic and nonenzymatic scavenging systems with antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX), and nonenzymatic scavengers such as ascorbate and glutathione [16–18]. If there is high abundance of ROS in plant cell, it may cause oxidative damage to macromolecules such as biological membrane, proteins, lipids and nucleic acids [19,20]. Napropamide can induce lipid peroxidation and oxidative damages in plant system and act as catalysts in ROS production [8].

In this work, the phytotoxicity of napropamide enantiomers to cucumber, soybean, *Festuca arundinacea* and *Poa annua* was investigated. The impacts of the enantiomers and the racemate of napropamide on the target weeds and nontarget crops were studied at the plant morphological level and physiological level. This work was helpful for evaluating the chiral pesticide environmental risks and providing more evidence to develop optical pure products.

2. Materials and methods

2.1. Materials

Racemic napropamide (Rac-napropamide) standard (98.0% purity) was provided by the Institute for the Control of Agrochemicals, Ministry of Agriculture, China. The single enantiomer of napropamide ((−)- or (+)-napropamide) was prepared by HPLC with a Chiralpak IC chiral column. All the reagents were of analytical grade from Beijing Chemical Reagent Company (Beijing, China). Working standard solutions were prepared by dilutions of the stock solution with acetone. All plant seeds in the experiments were purchased from Beijing Zhongnongbaihe Technology and Development Company Limited (Beijing, China). The soil was collected from the surface layer (0–20 cm) of an uncontaminated field in the Experimental Station of China Agricultural University. Physicochemical properties of the soil were as follows: organic matter (OM), 71.07 g/kg; clay, 25%; sand, 57%; silt, 18%; and pH, 7.85.

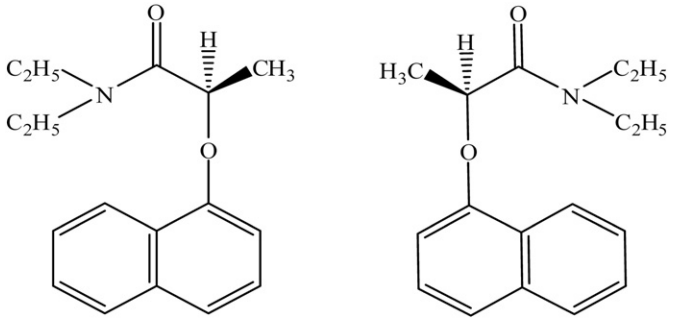

Fig. 1. Chemical structure of napropamide enantiomers.

2.2. Plant culture

The bioactivity to the target weeds *F. arundinacea* and *P. annua* and the toxicity to the non-target crops cucumber and soybean were investigated. According to the OECD guideline 208 [21], the seeds were soaked in 5% hypochlorite solution, rinsed by running water, dried, and then sown in plastic pot (10 * 10 cm). The spiked concentrations of racemic or enantiomerically pure napropamide in the soils were 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/kg for the bioactivity determination (for *F. arundinacea* and *P. annua*), and 0.5, 2.5, 5, 10, and 20 mg/kg for toxicity determination (for cucumber and soybean).

The experiments were carried out in a controlled environment (light intensity: 400 μmol m^{-2} s^{-1}, photoperiod: 16/8 h light/dark cycle, temperature: 25 °C, relative humidity: 60%) [22] for 9 or 14 days. The plant tissues were separately harvested and measured.

All experiments were carried out in triplicate. In the mean time, control groups were treated.

2.3. Determination of chlorophyll

Chlorophyll content in the leaves of cucumber and soybean was determined based on the procedure described in the previous research [23]. An aliquot of 0.1 g of the fresh leaf samples was homogenized with 5 mL of 80% acetone (pH 7.8 adjusted with sodium phosphate buffer) in ice bath, followed by centrifugation at 10,000 rpm for 10 min, and the supernatant was subjected to spectrophotometric assay, and the total chlorophyll was calculated.

2.4. Determination of TBARS

Thiobarbituric acid reactive substance (TBARS) was a biomarker for membrane lipid peroxides. Accumulation of lipid peroxides in tissues was measured according to the method of Wang [24]. Fresh tissues (0.5 g) were ground in 5 mL of ice-cold phosphate buffer solution (0.05 mM, pH 7.8) in an ice bath. The homogenate was centrifuged at 10,000 rpm for 10 min to obtain supernatant. Then 2 mL of the supernatant was mixed with 2 mL of thiobarbituric acid (TBA) (0.5% TBA, 20% TCA). The mixture was heated at 100 °C for 30 min, cooled to room temperature, and centrifuged at 10,000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm, 660 nm and 450 nm.

2.5. Enzyme extraction and assays

Fresh leaves (0.5 g) were ground as mentioned above, the homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant was used for assays of the enzyme activity.

The activity of superoxide dismutase (SOD) in the supernatant was determined using its capacity of inhibiting the photochemical reduction of nitroblue tetrazolium (NBT). One unit of SOD activity was defined as the enzyme inhibiting 50% of the photoreduction of NBT to blue formazan. The assay was performed with illumination for 20–30 min at 28 ± 2 °C in the phosphate buffer (pH 7.8,50 mM, 3 mL), containing methionine (130 mM), riboflavin (0.02 mM), NBT (0.75 mM), ethylenediamine tetraacetic acid disodium salt (0.1 mM) and 100 μL