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Fluorescent properties and bifenthrin binding behavior of maize (*Zea mays* L.) seedling root exudates

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ABSTRACT

The binding parameters of root exudates for organic pollutants are important for understanding the mechanisms involved in phytoavailability and phytoremediation. However, quantitative information about organic pollutant binding to root exudates is lacking. Fluorescent properties and bifenthrin (insecticide) binding behavior of root exudates from maize (*Zea mays* L.) seedlings were investigated using excitation emission matrix fluorescence spectroscopy. The protein-like fluorophores in root exudates from (*Z. mays* L.) are strong complexing ligands for bifenthrin, with logK_a in the range of 3.6 −5.7. Two protein-like fluorescence peaks were identified in the EEM spectrum of root exudates and fluorescence of both peaks could be quenched by bifenthrin. The protein-like substances in root exudates had larger binding capacity for bifenthrin under acidic condition than under neutral and basic condition. The conditional stability constants were also higher in acidic solution than in the neutral or basic solution. Similarly, more fluorescence is accessible for bifenthrin at acidic pHs. More than one binding sites are needed in root exudates for binding one bifenthrin molecule. Enough attention should be paid to the effects of root exudates on adsorption, transport and fate of pesticides in environments.

1. Introduction

Bifenthrin (422.86, 2-methyl [1,1′-biphenyl]-3-yl) methyl3-(2-chloro −3, 3,3-trifluoro-1-propenyl) −2,2-dimethylcyclopropanecar boxylate) is one of the widely used pyrethroid pesticides in agriculture, public health, homes and gardens [1]. Bifenthrin is highly immunotoxic, neurotoxic and genotoxic [2]. Bifenthrin is the most persistent synthetic pyrethroid in soil with its half-life ranging from 122 to 345 days [3]. It is frequently detected in the soil and sediment [4]. Bifenthrin pollution of soil is a growing environmental concern.

Plant root can persistently exude large amount of organic substances, which are composed of amino acids, carbohydrates, mucilage and low molecular weight secondary metabolites [5]. Root exudates can bind metals, mobilize the metals in soil and enhance the availability of heavy metals and organic pollutants to plant [6–8]. However, quantitative information about organic pollutant binding to root exudates is lacking. The binding parameters of organic pollutants to root exudates is important for understanding phytoavailability of organic pollutants.

The fluorescence excitation emission matrix (EEM) spectrometry is a rapid and sensitive technique that can quantitatively characterize the interaction of fluorescent organic matter and pollutants [9,10]. Because proteins are also key components of root exudates and they show fluorescent properties, EEM fluorescence spectroscopy should be a powerful technology for the fluorescent components in root exudates and their interaction with pollutants.

Maize (*Zea mays* L.) is a major crop in the world, and bifenthrin is one of the most used insecticides for controlling many kinds of insect pests of maize. This study aimed to quantify the binding behavior of maize root exudates for bifenthrin under various pH conditions using EEM fluorescence spectroscopy.

2. Materials and methods

2.1. Plant culture

Maize (*Z. mays* L.) seeds were collected from fields in Shihezi, Xingjiang, China. Seeds were washed with 95% ethanol and immersed for 45 min in 10% H_2O_2(v/v) solution for surface sterilization. The seeds were rinsed thoroughly with deionized water and transferred into a sterilized Petri dish lined with filter paper and wrapped with surgical gauze. The filter paper and gauze were moistened with sterilized deionized water. The seeds were incubated at 25 °C for 5 days. All the experimental materials were sterilized. Sterility was tested during seed germination [11].

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After germination, thirty seedlings were grown in six 250 mL opaque pots containing a mixture of sterilized vermiculite and perlite. The seedlings were irrigated with sterilized Hoagland solution [12] and incubated for 14 days with 70% relative humidity and 14-h photoperiod at 25°C/22 °C (day/night). The nutrient solution pH was adjusted to 6 with 0.1 M HCl and 0.1 M NaOH in all treatments.

2.2. Collection of root exudates

The procedure for the collection and preparation of root exudates was modified from the method described in literature [6,13]. The vermiculite and perlite were removed thoroughly from the roots under running deionized water and the seedlings were grown in a black cloth wrapped beaker containing 220 mL of Milli-Q water in sunlight at 25 °C. Milli-Q water was used for collection of root exudates instead of 0.1 mM $CaCl_2$ solution in order to minimize the effect of Ca^{2+} on the binding ability of root exudates to pesticide. After 3 h of growth in sunlight, the solution containing root exudates in the beaker was filtered through 0.22 μm membrane and immediately used for fluorescence titration tests. The concentration of root exudates was represented by total organic carbon (TOC) and measured with a TOC analyzer (Multi N/C 3000, Analytikjena, Germany).

2.3. Preparation of bifenthrin solution

Stock bifenthrin solution (0.1 M) was prepared by dissolving 98% bifenthrin of analytical grade (Zhongxing Inc., China) in acetone. The root exudates solution pHs were adjusted with 0.1 M HCl and 0.1 M NaOH.

2.4. Fluorescence quenching titration

The fluorescence spectra of root exudates were recorded with a fluorescence spectrophotometer (F-7000, HITACHI, Japan) [5]. A 450-W Xenon lamp was used as the excitation source. EEM were collected every 5 nm over an excitation range of 200–400 nm, with an emission range of 200–550 nm by 2 nm. Both the excitation and emission slits were set to 5 nm of band-pass. Scan speed was 1200 nm min^{-1}. The temperature of the solutions was controlled at 25 ± 0.1 °C in a thermostat water bath. The fluorometer's response to a Milli-Q water blank solution was subtracted from the fluorescence spectra recorded for samples containing root exudates and bifenthrin under the same conditions. The bifenthrin solution (0.1 M) was added incrementally to root exudates (12.5 mg L^{-1}), and the fluorescence intensity of peaks A and B was recorded by EEM fluorescence spectroscopy after 15-min reaction. The root exudates solution was titrated with 0.1 M bifenthrin solution at pH 5, 7 and 9, respectively.

2.5. Data analysis

All the experiments were triplicated and the mean values were used. The fluorescence data were processed with the software Origin 6.0. The quenching data was analyzed using the modified Stern–Volmer equation [14]:

$$F_0/\Delta F = F_0/(F_0 - F) = 1/(f_a K_a[\text{bifenthrin}]) + 1/f_a \quad (1)$$

Where K_a is the conditional stability constant for the accessible fluorophores. Conditional stability constant are concentration quotients which are not true equilibrium constants but can be derived from them. The value of the conditional stability constant indicates the stability of the complex. f_a is the fraction of accessible fluorescence [bifenthrin], concentration of bifenthrin.

The binding constant (K_b) and the numbers of binding sites (n) were determined by the Hill equation [15]:

$$((F_0 - F)/F) = \log K_b + n \log[\text{bifenthrin}] \quad (2)$$

3. Results and discussion

3.1. EEM spectra of root exudates

Two fluorescent peaks were identified in the EEM spectra of maize root exudates. Peak A was at Ex/Em = 225/338–340 nm and peak B at Ex/Em = 285–290/318–324 nm. Fluorescence of peaks A and B could be attributed to the protein-like fluorescence [6,16]. This was similar to those of root exudates from halophyte *Salicornia europaea* L., whose position was at Ex/Em = 225/336–340 nm and Ex/Em = 275/326–336 nm [5].

3.2. Fluorescence quenching titration

Generally, the fluorescence intensities of peaks A and B decreased with increasing bifenthrin concentration at different pHs, indicating that fluorescence of peaks A and B were quenched by bifenthrin under acidic, neutral and basic conditions. Fluorescence intensities of peaks A and B decreased in lower rates under basic condition than under acidic and neutral conditions.

3.3. Conditional stability constants

Good linear relationship was observed between $F_0/(F_0-F)$ and 1/[Q] for peaks A and B at various pHs (R^2 = 0.921–0.986). logK_a for peaks A and B was similar and in the range of 3.64–5.73(Table 1). The values of logK_a were close to those of Cu(II) complexation of root exudates from *S. europaea* L [5]. logK_a for peaks A and B showed a decreasing trend with increasing pH, suggesting that the complexes formed between maize root exudates and bifenthrin are more stable under acidic or neutral conditions than under basic condition. The values of f_a for peak A were in the range of 0.57–0.88, implying that more than half of the fluorescence was accessed by bifenthrin. For peak B, more than half of the fluorescence was inaccessible for bifenthrin ($f_a < 0.5$). In addition, the values of f_a were also bigger under acidic condition than under neutral and basic conditions. This

Table 1
The conditional stability constant (logK_a) and the fraction of accessible fluorescence (f_a) for peaks A and B derived from modified Stern–Volmer equation, and the binding constants K_b and binding site number n at various pHs.

	pH	Modified Stern–Volmer equation					Hill equation				
		logK_a	f	R^2	SD	p	K_b (L/mol)	n	R^2	SD	p
Peak A	5	5.73	0.8752	0.922	0.0069	0.0095	6.02	0.193	0.984	0.0078	8.78E-4
	7	4.28	0.5715	0.976	0.1601	0.0016	0.58	0.568	0.917	0.0543	0.0103
	9	3.65	0.6141	0.967	0.0012	0.1170	1.09	0.180	0.856	0.0348	0.2471
Peak B	5	4.12	0.434	0.984	0.2106	<0.0001	81.60	0.606	0.986	0.0237	<0.0001
	7	4.23	0.371	0.994	0.1188	<0.0001	30.73	0.508	0.985	0.0211	<0.0001
	9	3.64	0.183	0.921	0.5415	1.61E-4	15.18	0.605	0.769	0.1095	0.0043

suggests that more fluorescence can be accessed for bifenthrin in acidic solution. The reason might be that some fluorophores are uncoiled under acidic condition but masked under basic condition.

3.4. Binding constant and number of binding sites

There was good linear relationship for plots of $\log[(F_0-F)/F]$ versus log[bifenthrin] and the calculated parameters at various pHs were listed in Table 1. It was found that the values of K_b for peaks A and B under acidic condition were much greater than those under neutral and basic conditions, indicating that fluorophores in root exduates have bigger binding ability to bifenthrin in acidic solution. The values of n at three pHs were all less than 1, implying that more than one binding sites are required in root exudates to bind one bifenthrin molecule.

4. Conclusions

The protein-like fluorophores in maize root exudates are strong complexing ligands for bifenthrin. The protein-like substances in root exudates had larger binding capacity for bifenthrin under acidic conditions than under neutral and basic conditions. The conditional stability constants were also higher in acidic solution than in the neutral or basic solutions. More fluorescence is accessed by bifenthrin under acidic conditions. Binding one bifenthrin molecule needs more than one binding sites. Enough attention must be paid to their influence on environmental behavior of pesticides.

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