

Residual dynamics and ecological risk assessment of indoxacarb in *Ipomoea aquatica*

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Abstract

The residual levels and dissipation rate of indoxacarb in *Ipomoea aquatica* were determined by UPLC-MS/MS. 30% indoxacarb suspension was sprayed at recommended doses of 45 and 67.5 g ai/ha on the leaf of *Ipomoea aquatica* at six geographically distant experimental sites, Changsha, Nanning, Jiyuan, Hangzhou, Qingdao, Shijiazhuang in China. Samples were collected randomly in triplicates at 2h 1d 3d 5d 7d 10d 14d 21d 28d intervals after insecticide application. The residues were found to have half-lives of 2.61–3.67d. Indoxacarb was detectable in *Ipomoea aquatica* harvested after intervals of 5, 7 and 10 days. We recommend that the harvest interval should be longer than 10 days after spraying 30% SC.

1. Introduction

As the global population continues to grow, so does the need for food. To meet this demand, farmers use pesticides to improve agricultural production and protect crops from damage in the process of agricultural production (Bartkiene, 2019; Skovgaard, 2017). Pesticides are substances or chemicals mainly used in agriculture, horticulture, forestry and public lands to increase crop yield, shorten the growing season of agricultural products, increase yield and reduce various crop diseases. In most cases, however, they penetrate the plant and move to the edible part of the crop. Pesticide residues in products may exceed the maximum residue limits (MRL) under current standards, resulting in potential health risks to consumers (Evrendilek, 2020; David, 2017; Kailin, 2017).

Indoxacarb (methyl-7-chloro-2,5-dihydro-2-[[[(methoxycarbonyl)[4-(trifluoromethoxy) phenyl] amino] carbonyl] indeno [1,2-e] [1,3,4]-oxadiazine-4a (3H)-carboxylate) is the first commercialized pyrazoline-type sodium-channel blocker (Lapied, 2001). It is a broad-spectrum insecticide effective against lepidopteran pests and selected sucking pests, such as flea hoppers and tarnished bugs. It will block the movement of sodium ions into certain nerve cell ion channels, and result in paralysis and death of pests (McCann, 2001).

Many methods have been used for the quantitative analysis of indoxacarb, including gas chromatography-electron capture detector (Dun-ming, 2008; Ji-Young, 2013), liquid chromatography equipped with photodiode array detector (Sun, D, 2012; Urvashi, 2012; Sdeek, F, 2018), liquid chromatography with mass spectrometry (Campbell, 2005) and liquid chromatography with tandem mass spectrometry (Dun-ming, 2008; Shao, 2013; Li, 2016). Although there has been some research on residual dynamics and dietary risk of indoxacarb in some plant or vegetable, the residual dynamics and rational use of indoxacarb in pomegranate (Soudamini, M, 2019). Someone used meteorological chromatography to detect the persistence and dietary risk assessment of the residues of indoxacarb in tomato and soil (Sakthiselvi, 2020). However, digestion dynamics and safety assessment of indoxacarb on *Ipomoea aquatica* have not been obtained by now. In this study, field trials were conducted in Hunan Changsha, Guangxi Nanning, Henan Jinan, Zhejiang Hangzhou, Shandong Qingdao and Hebei Shijiazhuang. UPLC-MS/MS was used to evaluate residue level of indoxacarb in *Ipomoea aquatica*. The dissipation and kinetics of indoxacarb were determined to provide a reference for setting the maximum safe application rate of indoxacarb for *Ipomoea aquatica* in China.

2. Materials And Methods

2.1 Chemical and reagents

Standard indoxacarb (99.0% purity) was obtained from Beijing Century Aoke Biotechnology Co. Ltd. Beijing, PRC. Indoxacarb suspension (SC, 30%) was the pesticide used and achieved from Guanlong Agrochemical Co. Ltd.

(Henshui, PRC). Sodium chloride, magnesium sulfate, anhydrous magnesium sulphate were all AR grade and purchased from Hengxing Chemical Reagent Manufacturing Co., Ltd. (Tianjin, PRC). HPLC-grade acetonitrile (ACN) was purchased from CNW Technologies (Germany). Formic acid (FA) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Primary secondary amine (PSA, 40-60 μ m) was obtained from Agilent Technologies Palo Alto, CA, USA. Ultra-pure water was purified by a Milli-Q purification system (Bedford, MA, USA)..

Stock standard solution (100 mg/L) of indoxacarb was dissolved in HPLC-grade ACN. The standard working solutions (0.001, 0.005, 0.01, 0.05, 0.1, 0.5 and 1.0 mg/L) were prepared by gradient dilution method with blank matrix. These solutions were kept at 4 °C in a refrigerator and equilibrated to room temperature before use.

2.2 Instrumentation

UPLC-MS/MS system consist of ultra-high performance liquid chromatography equipped with RBAX Eclipse plus C18 column (2.1 $\times$ 50 mm, 1.8 μ m particle size). The binary mobile phases were (A) ultra pure water containing 0.05% FA (v/v) and (B) CAN. The gradient elution program (with respect to phase A) was set as follows: 0-1.5 min, phase A decreased from 90% to 50%; 1.5-6.0 min, phase A decreased to 20%; 6.0-6.5 min, phase A decreased to 5%; 6.50-6.60, phase A increased to 90%; 6.60-8.0, phase A kept at 90%. The flow rate was 0.3 mL/min and the injection volume was 5 μ L. The column oven temperature was held at 40 °C. Under these conditions, the retention time of indoxacarb was 6.88 min.

MS was performed by an electrospray ionization source (ESI) operated in positive mode. The 99.9% nitrogen was filled in nebulizer and the collision gas was 99.999% argon. The response reacted as following conditions: the capillary voltage, 5.0 kV; desolvation temperature, 250 °C; desolvation gas flow, 11 L/min. Selected ion monitoring (SIM) mode was used to screen ions of indoxacarb with mass-to-charge ratios (m/z) of 203 and 150.

2.4 Field experiment and sampling

From August to November in 2018, the field trials were carried out at six sites in China: Changsha, Hunan; Nanning, Guangxi; Jiyuan, Henan; Hangzhou, Zhejiang; Qingdao, Shandong; Shijiazhuang, Hebei. These trials were designed according to NY/T 788-2004 (Guideline on Pesticide Residue Trials) issued by the Ministry of Agriculture and Rural Affairs, PRC. The plot at each region was 30 m² with each treatment replicated three times (three plots). A buffer zone was set to separate plots used for different treatments in the same field. To investigate the dissipation behavior of indoxacarb in *Ipomoea aquatica* 30% indoxacarb suspension was applied at 67.5 g a.i./ha. Samples were randomly collected from each plot at 0 (2 h after treatment), 1, 3, 5, 7, 10, 14, 21 and 28 days after chemical application. In order to study the effects of the dosage and frequency of indoxacarb on its final residues in *Ipomoea aquatica*, low (45.0 g a.i./ha) and high dosage (67.5 g a.i./ha) were designed, which were sprayed one and two times, respectively, with a 7-days Interval between sprays. Samples were collected from each plot 2 h, 5 d, 7 d, 14 d and 21 d after the last spray. At the same time, collect blank samples. Sample was stored at -20 °C before analysis.

2.5 Extraction and purification procedures

5 g sample was transferred into centrifuge tube containing 10 mL acetonitrile and homogenize for 1 min. Then 3 g NaCl was added into the tube and vortex for 1 min. After centrifuged at 5000 r/min for 3 min, 3 mL supernatant was transferred into a 5 mL centrifuge tube, which contained sorbents of 0.5 g anhydrous MgSO₄ and 50 mg PSA. The tube was vortexed for 1 min and centrifuged at 5000 r/min for 5 min. The extract was filtered through 0.22 μ m filter membrane before analysis.

2.6 Linearity and recovery

The linearity of pesticide was evaluated by linear regression analysis of standard calibration curves at seven concentration levels, ranging from 0.001 to 1.0mg/L. Eligible linearity was acquired when correlation coefficient (R^2) was higher than 0.99. The recovery of the method was validated by recovery assays. Five replicates of spiked samples at four concentration levels (0.01, 0.10, 1.00, 5.00mg/kg) were prepared on different days. The limit of quantification (LOQ) was defined as the lowest concentration in the calibration curve with acceptable accuracy and precision.

2.7. Dissipation and Residue Assessment.

The dissipations and residues assessment of indoxacarb were figured out by the HPLC –MS/MS method. The exponential function $C=C_0e^{-kt}$ was used as mathematical expressive model for pesticide dissipations, where C_0 (mg/kg) represents the initial residue concentration; C_t (mg/kg) represents the residue concentration at time t (d) after pesticide application; $k(d^{-1})$ is the dissipation rate constant. Pesticide persistence was expressed as half-life ($T_{1/2}$), and the calculated value was $0.693/k$. Mathematical curve fitting was identified by computer-associated calculation on the basis of measured data.

3 Results And Discussion

3.1. Linearity and recovery

The analytical method was validated on the basis of criteria set in the single laboratory method validation approach[?], as shown in the curve(Fig. 1). Indoxacarb exhibited accepted linearity($R^2 > 0.999$)with a wide range of concentration, as plotted in the calibration curve(Fig. 2). The recoveries and relative standard deviation(RSD) were evaluated to validate the method by spiking target compounds in blank samples ((0.01, 0.10, 1.00, 5.00mg/kg), with five replicates for each spiking level.

Results(Table 1) showed that the average recovery rate in water spinach plants was 74.99%-111.29%, and the RSD was 3.69%-8.66%. Under the conditions of liquid chromatography-mass spectrometry, limit of quantification (LOQ) can reach as low as $0.01mg \cdot kg^{-1}$. The LOQ value is below the maximum residue limits (MRL) (GB 2763 – 2021, issued by the Ministry of Agriculture of the People’s Republic of China) of 0.1mg/kg on vegetables. The results demonstrated that the developed method is suitable for analysis of indoxacarb in Ipomoea aquatica.

Table 1
Average recoveries and RSDs of indoxacarb in Ipomoea aquatica (n = 5)

Fortified concentration / (mg·kg ⁻¹)	Recovery rate / %						RSD(%)
	1	2	3	4	5	average value	
0.01	102.98	119.85	111.4	120	102.24	111.29	8.66
0.1	72.56	73.07	74.91	77.18	77.21	74.99	2.2
1	78.65	83.08	83.44	85.28	88.8	83.85	3.69
10	94.81	89.79	82.02	94.47	92.38	90.69	5.25

3.3 The results of loss data

Dissipation pattern along with regression equations is presented in Table 2. According to the time-varying concentration of indoxacarb, curve fitting was detected for data analysis. Residues of indoxacarb in *Ipomoea aquatica* decreased gradually and continuously over time after application (Fig. 3). The initial residue of in *Ipomoea aquatica* grown in Hangzhou was 3.357 mg/kg with a half-life of 3.67d; 97% of this initial residue had dissipated after 14d. The initial of the pesticide in *Ipomoea aquatica* grown in Changsha was 8.073mg/kg with a half-life of 2.61d; 99% of this initial residue had dissipated after 10d. Equation ($C = 3.303e^{0.2655x}$) and equation ($C = 1.641e^{0.189x}$) were used to describe the kinetic equations of Changsha in Hunan province and Hangzhou in Zhejiang Province, whose coefficients were 0.8165 and 0.8240, respectively (Table.2). The difference of the dissipation mode is due to the climate conditions that the plants are grown in.

Table 2
The dissipation of indoxacarb in *Ipomoea aquatica* from experimental sites at Changsha and Hangzhou

Experimental site	Regression equation	R ²	T _{1/2}
Changsha, Hunan	Ct = 3.302e ^{- 0.2655t}	0.8165	2.61
Hangzhou, Zhejiang	Ct = 1.640e ^{- 0.1889t}	0.8240	3.67

Indoxacarb was detectable in *Ipomoea aquatica* harvested after intervals of 5, 7 and 10 days (Table 3). After intervals of 5 days, the residual amount of indoxacarb in *Ipomoea aquatica* were between 0.063 and 6.489 mg/kg. After intervals of 7 days, the residual amount of indoxacarb was between 0.37 and 4.186mg/kg. At the time of 10d, the residual amount of indoxacarb was between 0.037 and 4.049mg/kg. The residues of indoxacarb in Hunan samples were lower than samples in other sites. FAO/WHO has only established MRL for indoxacarb in Fruiting vegetables and cucurbits group. In some countries, such as Japan, USA and China, the MRL of indoxacarb in cabbage, cruciferous vegetables have stipulated to be 0.6-12mg/kg. Our results provide a quantitative basis for the reasonable application of this pesticide to vegetables. We recommend that the harvest interval should be longer than 10 days after spraying 30% SC.

Table 3
The average residue amount of indoxacarb in *Ipomoea aquatica* (n = 3)

Application dose/g·hm ²	Application times	Sampling interval time	Residual amount of indoxacarb					
			Huanan	Guangxi	Henan	Shandong	Hebei	Zhejiang
45	1	5	0.738	1.511	0.339	1.487	2.073	0.729
		7	0.636	0.461	0.523	1.165	1.86	0.715
		10	0.104	0.173	0.213	1.07	1.33	0.536
	2	5	1.01	1.929	0.794	3.006	4.376	0.815
		7	0.165	0.887	0.599	2.453	3.321	1.105
		10	0.101	0.192	0.163	1.634	3.031	0.404
67.5	1	5	0.605	3.142	1.656	2.073	3.165	1.256
		7	0.461	0.593	0.851	1.86	2.702	0.53
		10	0.066	0.649	0.374	1.33	2.596	0.392
	2	5	0.063	6.489	2.768	4.376	4.633	2.569
		7	0.37	1.341	0.721	3.321	4.186	2.123
		10	0.037	0.714	0.289	3.031	4.049	0.734

4. Conclusions

A UPLC-MS/MS method was used for analysis of indoxacarb in this study of *Ipomoea aquatica*. The dissipation rates of indoxacarb at two experimental sites were different, suggesting that degradation of the pesticide was affected by local climate. Indoxacarb was detectable in *Ipomoea aquatica* harvested after intervals of 5, 7 and 10 days. Although indoxacarb can easily degrade in *Ipomoea aquatica*, the observed residual levels suggest that the pesticide should be applied at reasonable dosage. We recommend that the harvest interval should be longer than 10 days after spraying 30% SC.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical statement

No human or animal was involved for experimental purpose

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information file.

Author Contribution Rong Li: Conceptualization, Validation, Methodology, Investigation, Writing – original draft. Zongyuan Zhang: Investigation, Revision, review, editing. Fangfan Hu: Investigation, Writing – review & editing. Qiang Fu: Investigation, Writing – review & editing. Jia Zhao: Conceptualization, Supervision, Writing – review & editing. Daoxin Gong: Supervision, Writing – review & editing.

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Figures

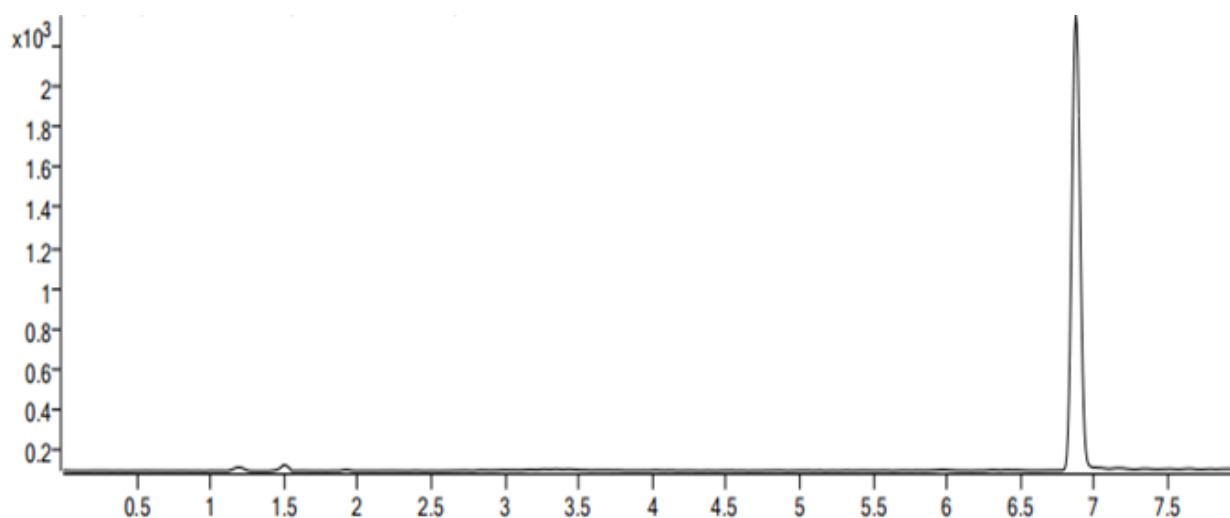


Figure 1

Chromatogram of 0.01 mg/L indoxacarb

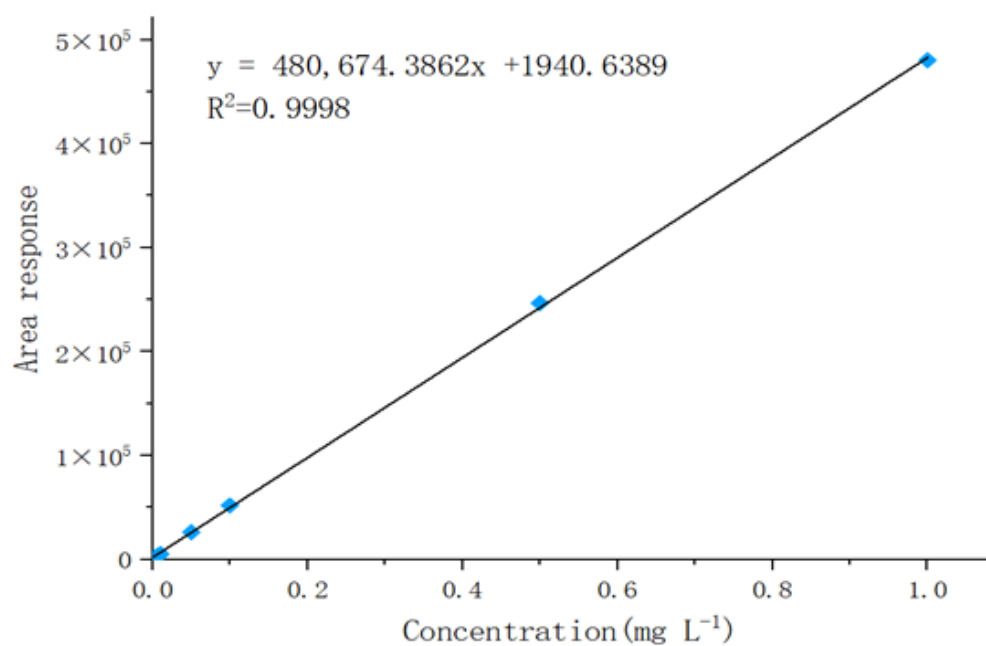


Figure 2

Standard curve of Indoxacarb (0.001mg/L~1.00mg/L).

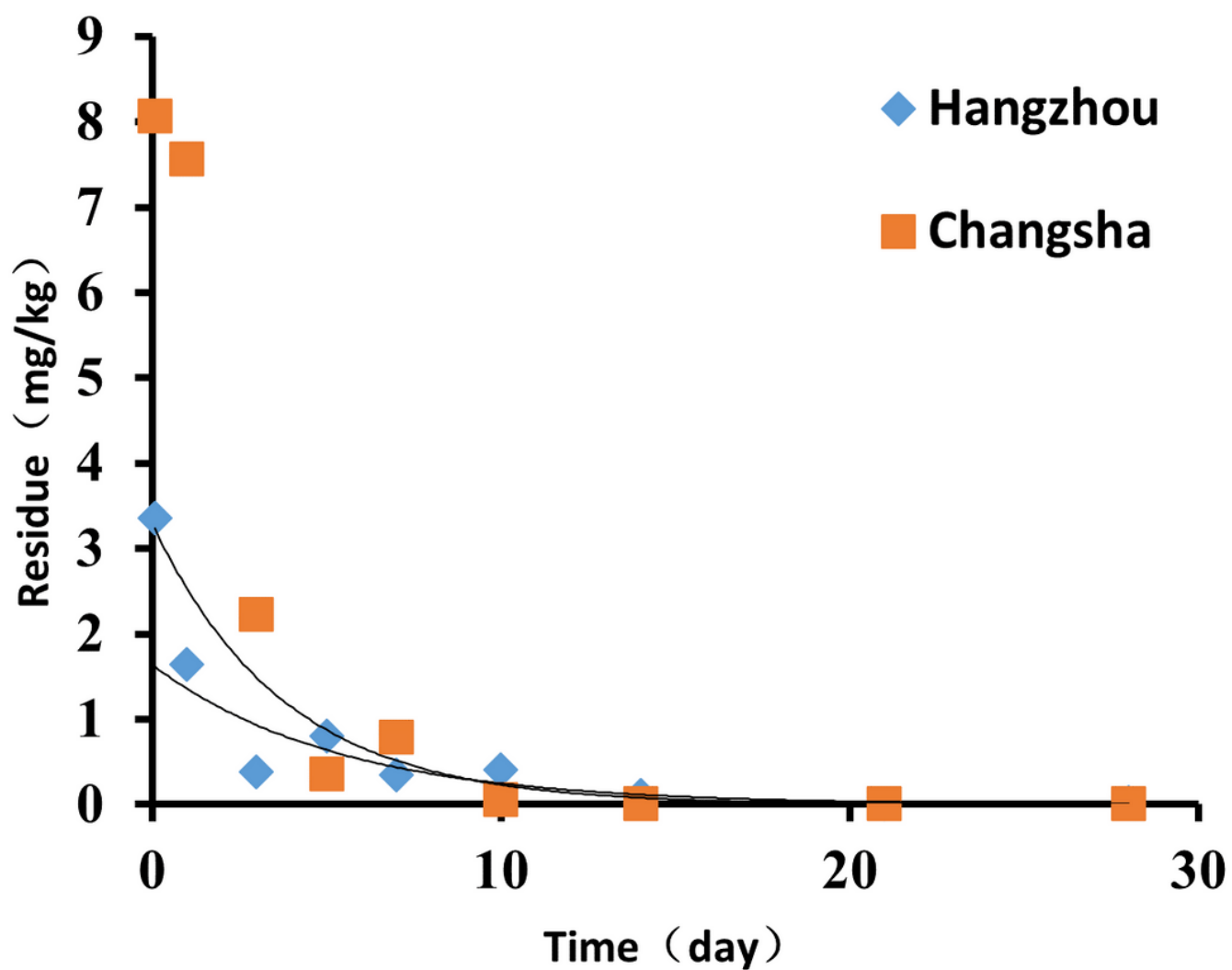


Figure 3

Dissipation of indoxacarb residue in *Ipomoea aquatica* grown in Hangzhou and Changsha