

Effect Of Formulation On The Indoxacarb And Lufenuron Dissipation In Maize And Risk Assessment

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Abstract

The supervised field trials were conducted in maize crops using nano-microemulsion (NM) and a commercial formulation of indoxacarb and lufenuron to evaluate the effect of nano-formulation on the dissipation pattern. A modified QuEChERS-UPLC-MS/MS method was utilized for sample analysis. Results showed that the initial deposits of indoxacarb and lufenuron in plants using nano-microemulsion were 0.98 mg/kg and 8.18 mg/kg at recommended dosage while using the commercial formulation, they were 0.85 mg/kg and 5.53 mg/kg, respectively. Moreover, half-life ($t_{1/2}$) values of using nano-microemulsion were 1.25 d and 2.51 d, which were 33.16% and 16.33% shorter than indoxacarb lufenuron from the commercial formulation, suggesting that pesticide formulations have a moderate impact on the initial deposit and dissipation rate. The terminal residue test showed that indoxacarb and lufenuron residues in maize grain and maize straw were below the available maximum residue limit (MRL), suggesting 2% indoxacarb NM and 5% lufenuron NM are safe to use under the recommended dosage. The risk quotient value also revealed an acceptable risk for human consumption. These findings provide scientific evidence of the proper application of 2% indoxacarb NM and 5% lufenuron NM on maize crops.

Introduction

Maize (*Zea mays* L.) is a major annual cereal crop for humans and livestock consumption, accounting for 11% of the global cropland area (Linquist et al. 2012). China is one of the top three maize production and consumption countries, with approximately 42 million hectares of cultivated area (Wang et al. 2020). However, maize production suffers yield losses of more than 30 kinds of diseases and insect infestations, especially for the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) (Goergen et al. 2016; Jiao et al. 2019). These harmful non-native species that originate in the Americas have become a major agricultural pest globally (Tang et al. 2021). China was first invaded by the fall armyworm in 2019, with more than one million hectares of cropland suffers from this pest.

Meanwhile, the fall armyworm's multiple generations across the year, their migration, and its potential to feed on a vast range of host plants make it one of the most challenging pests to control. Currently, chemical method is the most popular way to control this species, such as indoxacarb and lufenuron recommended by the Ministry of Agriculture Rural Affairs of the People's Republic of China (2020). One of the main problems with pesticide usage is the low effectiveness with less than 42% availability. Pesticide usage poses a danger to humans and the environment (Nehra et al. 2021).

Due to their differences in the specification of the processing technology for different pesticides, a large variety of adjuvants and solvents were used. Because of this, the physicochemical features in the foliar spray dilution vary a lot. These features vary includes dilution's wetting and spreading ability, deposition, and dispersibility on the surface of the applied crop (Zheng et al. 2021). With its advantages in prolonged duration, reduced dosage, and decreased side effects, Nano-formulation is considered a promising alternative to conventional formulations (Gao et al. 2020; Lowry et al. 2019; Zhao et al. 2018; Nuruzzaman et al. 2016). As a result, nano-pesticides have attracted broad interests globally and were incorporated into the Technical Guidelines for Green Development of the Technical Guidelines for Agricultural Green

Development (2018–2030) (Ministry of Agriculture Rural Affairs of the People's Republic of China, 2018). Another available nano-enabled pesticide on the market is called microemulsion-type solvent-based pesticide, as it contains oil droplets with a majority population of less than 100 nm (Gomollón-Bel 2019). However, nano-formulations may affect the physicochemical properties (solubility, dissociation, and volatilization) of the active ingredient (Ma et al. 2010; Raliya et al. 2017), which results in different dissipation behavior and pesticide residues levels before harvest (Agathokleous et al. 2020). These bring challenges in the application of nano-formulation as its effects on the environment and human health are still unclear. Until now, relatively little research is available regarding the practicable application of nano-formulation and its risk assessment in the field.

It is generally known that pesticides-use in crop production is administrated by the Guidelines of the Good Agricultural Practices (Zhang et al. 2011). However, it is common that during the pre-harvest intervals (PHI), plant protection products may be improperly used in a high-frequency high-dosage fashion (Song et al. 2020; Gong et al. 2020). Unregistered pesticides usage also takes place sometimes in this interval (Malhat et al. 2020). Hence, monitoring residues from the field and determining the appropriate period for harvesting is essential for guaranteeing food quality and safety. Research indicated that the dissipation behavior of the pesticide depends mainly on the physicochemical factors of the dilutions of various formulations (Farha et al. 2016). For instance, Saini et al. (2015) reported that though for the commercial formulation, nano-pyridalyl in tomato fruit has a half-life ($t_{1/2}$) of 3.2 days, the half-life decreases to 2.6 days when double the recommended dose was applied (Saini et al. 2015). Similar results can also be observed in the average degradation half-life of prothioconazole with 480 g/L suspension concentrate (SC; 5.21 d), 250 g/L emulsifiable concentrate (EC; 4.78 d), 75% water dispersible granule (WG; 6.30 d), and 8% ME (4.78 d) in wheat, respectively (Gao et al. 2020). In addition, Lichiheb et al. (2015) observed that the formulated epoxiconazole has a two times higher penetration rate constant (0.47 ± 0.20) when compared to the pure epoxiconazole (0.17 ± 0.07), suggesting a strong effect of formulation on leaf penetration (Lichiheb et al. 2015). Angioni et al. (2011) suggested that although the initial deposit difference in tomato fruit and orange are negligible, the residues deposit in peach fruits is higher when applied with emulsifiable concentrates than microencapsulates wettable granules formulations (Angioni et al. 2011). All the observations suggested that the PHI and application doses recommended for other formulations are unsuitable for the nano-formulation. Thus, understanding the dissipation patterns and health risks of nano-formulation has become more and more important for establishing the guidelines for the safe use of nano-pesticides.

A nano-microemulsion (NM) of indoxacarb (2% of active ingredient) and lufenuron (5% of active ingredient) were developed by the Nanjing Scienx Ecological Technology Co., Ltd, which enhanced supposedly insecticidal activity against *S. frugiperda* compared to their water-dispersible granule (7.73%) and emulsifiable concentrate (20.80%), respectively (unpublished data). The current study studied the residues levels of indoxacarb and dissipation and lufenuron from the nano-microemulsion were studied in maize fields. The associated health risks of individual pesticides were subsequently determined after application in maize. The purpose of this research is to provide fundamental physicochemical behaviors

for developing the guidelines for the safe use of 2% indoxacarb NM and 5% lufenuron NM in the maize field.

Materials And Methods

Chemicals

Standard solutions of indoxacarb (99% purity) and lufenuron (98% purity) were acquired from the ANPEL (ANPEL Laboratory Technologies, China) and were used as external standards for the correct quantification. 2% indoxacarb NM and 5% lufenuron NM were prepared by the Nanjing ScienX Ecology Technology Co., Ltd. Commercial pesticide formulations of 30% indoxacarb water-dispersible granule (WG) and 50 g/L lufenuron emulsifiable concentrate (EC) were applied to compare the dissipation behavior pesticide residues. For the application of the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method, anhydrous magnesium sulfate (anhydrous $MgSO_4$), C18 sorbents (50 mm, 60 Å), sodium acetate trihydrate, and primary secondary amine (PSA, 40-60 mm, 60 Å) were used. All the chemicals used in the experiment were analytical reagent (AR) or better.

Standard solution preparation

Individual standard solution (~100 mg/L) was prepared by dissolving methanol. A 10 mg/L stock multi-standard solution was prepared for each pesticide by dissolving the standard solution in methanol. By proper dilution of the stock multi-standard solution using methanol or blank matrix extracts, matrix-matched calibration standards, spike solutions for solvent-only calibration standards, and intermediate solutions were prepared. Before use, all the mentioned standard solutions were stored in dark flasks at -20°C prior to use.

Sample extraction and purification

A modified QuEChERS based on European Standard EN 15662:2018 was used to extract and purify analytes (Biziuk and Stocka 2015). 2 g of homogenized acquired pesticide residues sample was extracted by adding 20 mL of cyanomethane with 1% ethanoic acid to the extraction funnel. Five minutes of vigorous shaking was performed. After the extraction, 4 g of sodium chloride was added to the solution. The solution was then centrifuged for 5 minutes at the setting of $4000 \times g$. A clean-up was applied by adding 0.10 g C18, 0.15 g $MgSO_4$, and 0.10 g PSA to the solution. 1.5 mL of the supernatant from the solution was undergone evaporation to remove the solvent. The solute was then dissolved in 1 mL of chromatography-grade methanol. Finally, the solution was filtered using a 0.22-μm syringe nylon filter and refrigerated at -20°C.

UPLC-MS/MS analysis

Processed pesticide residues sample was analyzed using a XEVO Triple Quad mass spectrometry system (Waters Co., Milford, MA, USA) with a UPLC® BEH C18 column (2.1×50mm, 1.7 μm; Waters). The isocratic elution profile of water was set at a flow rate of 0.2 mL/min containing cyanomethane (15/85, v/v) and 0.1% methanoic acid. The column oven temperature was set to 50°C with the injection volume at 50 μL.

The operating mode of the system was set to multiple reaction monitoring (MRM) using electrospray positive ionization (ESI+, 3.0 kV), values were m/z 528.10 > 293.15 (quantitative ion) and m/z 528.10 > 249.10 for indoxacarb, as well as m/z 509.10 > 339.10 (quantitative ion) and m/z 509.10 > 326.10 for lufenuron. For the parameters of the ionization source, desolvation temperature was at 500°C; source temperature was at 150°C; cone voltage was set to 50 volts; the cone gas flow was 150 L/h; desolvation gas flow is at 1000 L/h.

Quality assurance and quality control (QA/QC)

Five critical aspects of pesticide analysis QA/QC process were performed: accuracy, linearity, precision, sensitivity, and recovery. For the precision and accuracy aspect, the analysis was examined by reproducibility and recovery experiments. Five repeated spiked samples at different concentration levels (0.05, 0.2, 1 mg/kg) have been experimented, and the relative standard deviations (RSDs) were calculated. For linearity assessment, an array of standard solutions at different concentration levels (2, 10, 20, 50, 100, 500 µg/L) were evaluated, and the result data were analyzed using statistical method. For the sensitivity and precision aspects, the control group was prepared with the same extraction process using verified pesticide-free samples. In addition, the matrix effect (ME) of the target analytes were also evaluated, as the combined interference of components in the extract can lead to an inaccurate result of the target analytes in both qualitative and quantitative forms.

Field experiment

Field trials of the pesticide residues were executed in Linquan county (33°06' N, 115°25' E) of Anhui province, China, including terminal residue and dissipation behavior experiments. The experiments were designed according to the *Guideline on Pesticide Residue Trials* issued by the Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA), People's Republic of China. Selected field trial plots were 30 square meters in area with no indoxacarb or lufenuron application history. Four plots were arranged for each treatment, with three duplicated experiment plots and one control plot. To avoid cross-contamination, each plot was separated at a 1-m distance from the other.

The dissipation experiments in maize (*Zea mays* Linn.) crop was carried out on different plots with a recommended dosage (45 g a.i./hm² for 2% indoxacarb NM and 90 g a.i./hm² for 5% lufenuron NM, respectively) by spraying on the surface of plants. In addition, the commercial formulations of 30% indoxacarb WG and 50 g/L lufenuron EC were compared with the corresponding nano-microemulsion, respectively. The process was repeated for each treatment and on each triplicate experiment plot. After two h and 1, 3, 5, 7, 10, 14, 21, and 28 d of spraying, a 2.0 kg plant sample was collected for each experiment plot.

The terminal residues experiments were taken place in separate matrices. One dose of treatment was applied to both the maize straw and full-grown maize grain. 2 kg of full-maize grain and 2 kg of maize straw were collected randomly from the matrices after 45 d for the terminal residues experiments. Collected samples were divided into quarters and stored at -20°C.

Chronic dietary intake risk assessment

For the safe application of 2% indoxacarb NM and 5% lufenuron NM on maize crop, risk assessment compared to pesticide toxicity was performed by calculating the risk quotient (RQ) by dividing the national estimated daily intake (NEDI, mg) with the acceptable daily intake (ADI, mg/kg bw). The NEDI of the target analytes and the corresponding RQ were calculated as follows:

$$NEDI = \sum STMR_i \times F_i RQ = \frac{NEDI}{ADI \times bw} \times 100\%$$

Where $STMR_i$ (mg/kg) is the median residue data from supervised trials.

The corresponding maximum residue limits (MRLs) were taken for intake calculations in the absence of relevant $STMR_i$.

F_i is the average daily consumption of certain foods in the general population (kg/d). bw was a bodyweight (the average weight of a Chinese adult is 63 kg). ADI is obtained from the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). RQ values < 1 are considered safe for human health, while higher than 1 indicates unacceptable risk for humans.

Statistical analysis

The experiments mentioned were repeated more than three times, and the results were collected and shown in average and error represented by the relative standard deviation (RSD). ANOVA and t-test were used provided by IBM SPSS Statistics 22.0 (SPSS, Inc., Chicago, IL, USA). Results with a p-value less than 0.05 were considered significant. GraphPad Prism 7 (GraphPad Software, Inc., USA) was used to generate the figures.

Matrix effects (ME) was calculated based on the following equation:

$$ME = \left(\frac{dMMS}{dSS} - 1 \right) \times 100\%$$

Where dMMS represents the slope of the matrix-matched standard and dSS represents the slope of the solvent standard. If $|ME| < 20\%$, effects on enhancing or suppressing signal remain low. $20\% < |ME| < 50\%$ stands for moderate effects, $|ME| > 50\%$ stands for severe effects on the signal (Zhang et al. 2015).

Results And Discussion

Method validation

Methods were validated with matrix effect (ME), recovery, method quantification limits (MQLs), method detection limits (MDLs), and linearity. Results are shown in Table 1. The indoxacarb and lufenuron separation process was done in less than 2 mins. From the matrices, no interference was observed. For the verification of linearity, calibration curves were established by a gradient of diluted, mixed stock solution with the range from 0.05 to 1.00 mg/L. Good linear regression coefficients ($r > 0.9982$) were obtained for

the target analytes. The definition of the MDL is set to three times the background noise signal produced by the control sample. MQL is defined by spiking the control sample at the lowest concentration for each enantiomer with all validation criteria were met (Tong et al. 2014). MDL was from 1.02 to 5.08 µg/kg, and MQL was from 2.00 to 10.00 µg/kg, respectively. Matrix effects showed that combined interferences from the environment remain low to moderate. Method recovery test was performed by spiking mixed stock solution at the concentration levels of 0.05, 0.2, and 1 mg/kg into substrate blank (in quintuplicate). The average recoveries for indoxacarb lie between 68.72% and 95.61%, with RSD < 9.62%. Meanwhile, the RSD values for lufenuron were lower than 8.27%, with satisfactory recoveries over the range of 76.52-119.55%, proving excellent reproducibility. All the results suggested that the designed method for the experiment was reliable for detecting and quantifying indoxacarb and lufenuron in maize plant, maize grain, and maize straw at the same time.

Table 1
Validation results for indoxacarb and lufenuron in maize at three concentration levels (n=5)

Analytes	Matrix	r	MDL/MQL (µg/kg)	Average recovery ± RSD (%)			ME (%)
				0.05 mg/kg	0.2 mg/kg	1 mg/kg	
Indoxacarb	maize plant	0.9995	1.11/2.00	86.00±6.29	79.70±1.87	86.33±3.48	2.84
	maize grain	0.9999	1.02/2.00	73.30±9.62	77.89±3.58	90.92±3.22	16.19
	maize straw	0.9985	1.08/2.00	87.81±6.18	70.72±1.60	95.61±2.20	23.78
Lufenuron	maize plant	0.9982	4.19/10.00	106.00±6.65	105.68±4.89	119.55±2.14	34.28
	maize grain	0.9992	5.08/10.00	112.85±8.27	97.96±2.97	100.28±5.59	20.55
	maize straw	0.9992	4.06/10.00	101.60±2.67	76.52±3.00	90.38±4.03	15.40

Effect of formulations on pesticide dissipation in maize plant

The permitted safe interval from harvest to last pesticide application is influenced by various biotic and abiotic factors such as pesticide formulations. One critical step to the pesticide application is to estimate the dissipation of pesticides in agricultural products. In the present study, dissipation residues of indoxacarb and lufenuron NM in maize plants were investigated under field conditions. As shown in Figure 1, a fast decrease in persistence was observed within the first seven days after application followed by gradual dissipation at a slower rate. The initial deposits of the active ingredient of 2% indoxacarb NM and 5% lufenuron NM in the maize plant were found to be 0.98 mg/kg and 8.18 mg/kg, respectively, and the residues were gradually reduced over time. After application of 21 days, almost 99% of the residues had dissipated from the indoxacarb and lufenuron treated with the recommended dosage, respectively, and the

value is below MQLs on 28 days after application. A notable exception is 50 g/L lufenuron EC, in which residue of 0.31 mg/kg was detected at the same harvesting time. The rate kinetics of indoxacarb and lufenuron in maize plant followed first-order kinetics, with $C_t = 0.9673e^{-0.5588t}$ ($R^2 = 0.9955$) and $C_t = 7.823e^{-0.2772t}$ ($R^2 = 0.9837$). The corresponding half-lives ($t_{1/2}$) were 1.25 days and 2.51 days, suggesting that the 2% indoxacarb NM and 5% lufenuron NM can be dissipated rapidly in the maize plant after field application which indicates appropriate stability and safety.

By comparison, two active ingredients of commercial formulation had lower initial deposits and longer half-lives than that of the nano-microemulsion formulation. Briefly, the initial deposits of commercial formulation of indoxacarb and lufenuron were 0.85 mg/kg and 5.53 mg/kg, respectively, which was 14.84% and 48.01% lower in residue levels compared with the in the case of the nano-microemulsion formulation. The degradation trends of commercial formulation of indoxacarb and lufenuron in maize plants also followed first-order kinetics, with half-lives of 1.87 days and 3.00 days, suggesting that the nano-microemulsion formulation can increase the speed of pesticide dissipation. However, no significant differences were observed for the dissipation rates after 21 days of application. Higher droplet density and coverage for nano-microemulsion formulation could be the reason for the high deposition and shorter $t_{1/2}$ ¹¹. The data collected are consistent with the results of Yu et al. (2019) and Cui et al. (2018), who reported that the retention rates of abamectin nano pesticides on the foliage were remarkably enhanced by more than 50%, compared with unmodified nano pesticides (Yu et al. 2019; Cui et al. 2018). Studies of 2% indoxacarb NM and 5% lufenuron NM application are thus crucial for improving its effectiveness.

Additionally, pesticide property (Zhu et al. 2018), application methods (Xiao et al. 2020), crop characteristics (Yang et al. 2012), and various complex environmental factors may affect pesticide deposition and persistence (Kumar et al. 2019). Further study of these parameters could help provide a scientific basis for setting a safe application mode and proper PHIs.

Terminal residue of pesticides in maize grain and straw

The maximum residue limit (MRL) of both indoxacarb and lufenuron in maize was set to 0.01 mg/kg by the European Union standard to ensure food consumption safety. The terminal residues of indoxacarb and lufenuron in maize grain and maize straw on days 45 after the last application are shown in Table 2. In this work, the residue levels of lufenuron at harvest time in the maize grain and straw samples were undetectable (below LOD), significantly lower than the MRL. For indoxacarb, the terminal residues of commercial formulation in maize grain and maize straw were below the MQL (0.002 mg/kg), whereas the corresponding residues of NM in maize grain were at 0.01 mg/kg and in maize straw at 0.0098 mg/kg. Despite a relatively high residue level of 2% indoxacarb NM, it didn't exceed the MRL (0.01 mg/kg). According to the experiment result, applying the formulation at the recommended dosage would be considered acceptable. The experiment result gave us the quantitative information for applying the nano-microemulsion formulation of indoxacarb and lufenuron to maize crops.

Table 2

Terminal residues of indoxacarb and lufenuron from applications of nano-microemulsion and commercial formulations in maize grain and maize straw

pesticide	Application doses (g a.i./ha)	matrix	Residues(mg/kg)
2% indoxacarb NM	45	maize grain	0.01
		maize straw	0.0098
30% indoxacarb WG	45	maize grain	<0.002
		maize straw	<0.002
5% lufenuron NM	90	maize grain	<0.01
		maize straw	<0.01
50 g/L lufenuron EC	90	maize grain	<0.01
		maize straw	<0.01

Dietary intake risk assessment on maize

Various agricultural foods can cause humans to be exposed to pesticide residues (Li et al. 2021).³ Currently, the dietary risk of pesticides in the crop has been thoroughly studied, while studies of risk from other food sources were still lacking (Damalas et al. 2011). In this study, the risk probability of the dietary intake was calculated using risk quotients (RQ) based on the Chinese dietary pattern. From the Chinese national nutrition and health survey in 2002, F_i of ordinary healthy Chinese people was obtained shown in Tables 3. The reference residue limits were based on the supervised trials median residue (STMR) of food classifications. MRL value was used if the STMR was missing. As MRL standards are different in each country, a priority order was set for choosing the MRL, which was China > CAC > the USA > Australia > Korea > European Union > Japan (Details in Table S1). Maize is classified as other grains in China; thus, the value of F_i was 0.0233 kg. Experiments showed that the STMR of indoxacarb and lufenuron in maize grain was 0.002 mg /kg, respectively. After calculation, the total NEDI of indoxacarb and lufenuron for an average Chinese person was 0.1118 mg and 0.0297 mg, respectively. The ADI of indoxacarb and lufenuron were 0.01 and 0.02 mg/kg bw, with the RQ equal to 17.7% and 2.4%, according to the JMPR report. These results suggest that the application of the formulation at the recommended dosage would be considered acceptable. Thus, maize crops treated with 2% indoxacarb NM and 5% lufenuron NM at the dosage of 45 g a.i./ha and 90 g a.i./ha with spray once and PHI at harvest time was recommended.

Table 3
Dietary exposure assessment of indoxacarb and lufenuron in maize

Food classification	Fi (kg)	Indoxacarb			Lufenuron		
		Reference residue limits (mg/kg) and their sources	NEDI (mg)	RQ (%)	Reference residue limits (mg/kg) and their sources	NEDI (mg)	RQ (%)
Rice and its products	0.299	0.01(MRL, Rice, EU)	0.002399	17.7	0.01(MRL, Rice, EU)	0.002399	2.4
Flour and its products	0.1385	0.01(MRL, Wheat, EU)	0.001385		0.01(MRL, Wheat, EU)	0.001385	
Other grains	0.0233	0.01	0.000233		0.002	0.000047	
Tubers	0.0495	0.01(STMR, Potato, JMPR-Evaluation 2005)	0.000495		0.01(STMR, Potato, JMPR-Evaluation 2015)	0.000495	
Dried beans and their products	0.016	0.02(STMR, Mung bean (dry), JMPR-Evaluation 2005)	0.000320		0.01(MRL, Soybean, JMPR-Evaluation 2015)	0.000160	
Dark vegetables	0.0915	0.11(STMR, Tomato, JMPR-Evaluation 2005)	0.010065		0.08(STMR, Tomato, JMPR-Evaluation 2015)	0.007320	
Light vegetable	0.1837	0.435(STMR, Cabbages (Head), JMPR-Evaluation 2005)	0.079910		0.02(STMR, Melons, JMPR-Evaluation 2015)	0.003674	
Pickles	0.0103		0.000000		0.05(MRL, Gherkin, China)	0.000515	
Fruits	0.0457	0.21(STMR, Apples, JMPR-Evaluation 2005)	0.009597		0.15(MRL, Apple, EU)	0.006855	
Nuts	0.0039	0.02(MRL, Pecans, EU)	0.000078		0.01(MRL, Nut, EU)	0.000039	
Livestock and poultry	0.0795	0.01(STMR, Meat, JMPR-Evaluation 2005)	0.000795		0.012(STMR, Meat, JMPR-Evaluation 2015)	0.000954	
Milk and its products	0.0263	0.048(STMR, Milk, JMPR-	0.001262		0.066(STMR, Milk, JMPR-	0.001736	

		Evaluation 2005)			Evaluation 2015)
Egg and its products	0.0236	0.01(STMR, Eggs, JMPR-Evaluation 2009)	0.000236	0.01(STMR, Eggs, JMPR-Evaluation 2015)	0.000236
Fish and shrimp	0.0301		0.000000		0.000000
Vegetable oil	0.0327	0.018(STMR, Soybean refined oil, JMPR-Evaluation 2005)	0.000589	0.01(MRL, Oilseeds, EU)	0.000327
Animal oil	0.0087	0.44(STMR, Mammalian fat, JMPR-Evaluation 2005)	0.003828	0.3(STMR, Mammalian fats, JMPR-Evaluation 2015)	0.002610
Sugar, starch	0.0044	0.1(MRL, Sugar beet roots, EU)	0.000440	0.01(MRL, Sugar canes, EU)	0.000044
Salt	0.012		0.000000	0.01(MRL, Teas, EU)	0.000840
Soy sauce	0.009	0.02(MRL, Garlic, EU)	0.000180	0.01(MRL, Garlic, EU)	0.000090
Total	1.0286	/	0.111812		0.029726

Currently, food consumption cluster diets in China are still in their early stage with incomplete food categories. To improve the estimation, more detailed data should be used. In addition, the isomers of chiral pesticides have different dissipation and toxicity. For example, Zhang et al. (2015) mentioned that the (*R*)-flutriafol exhibited 2.17–3.52 times higher acute toxicity to earthworms and *Scenedesmus obliquus* than (*S*)-flutriafol (Zhang et al. 2015). Wang et al.(2021) reported that the *S*(-)-pydiflumetofen was degraded faster in bok choy, but *R*(+)-pydiflumetofen preferentially dissipate in soil under field conditions (Wang et al. 2021). A similar phenomenon may occur with chiral pesticide indoxacarb and lufenuron, leading to increased uncertainty in the risk assessment. Based on the factors mentioned, a more comprehensive risk assessment should be taken on the 2% indoxacarb NM and 5% lufenuron NM formulation.

Conclusions

This study demonstrated that indoxacarb and lufenuron in maize were rapidly degraded following first-order kinetics models with a shorter dissipation half-life of 1.25-3.00 days. Formulation of nano-microemulsion could increase droplet deposition and shorten the half-life of indoxacarb and lufenuron compared to their commercial formulation. The terminal residues of analytes in maize grain and straw were all below MRL value under the designed dosages with spray once and PHI at harvest time. The risk

quotient in this experiment suggested that the indoxacarb and lufenuron residues in maize are safe for human consumption. These results contribute to the residue behavior study of nano pesticides in plants and thus make practical application of nano pesticides achievable in the future.

Declarations

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Data availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Ethical approval: Not applicable

Consent to participate: Not applicable

Consent to Publish: Not applicable

Competing interests: The authors declare no conflicts of interest.

Authors Contributions: Haiqun Cao and Jinjing Xiao designed the experiments; Xi Cheng, Yuanhui Liu, Qun Gao, and Qingkui Fan carried out the experiments; Bing Min Liao, Liang and Zhendi H contributed to supervision; Xi Cheng and Jinjing Xiao analyzed the experimental data and wrote the manuscript. All authors read and approved the final manuscript.

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Figures

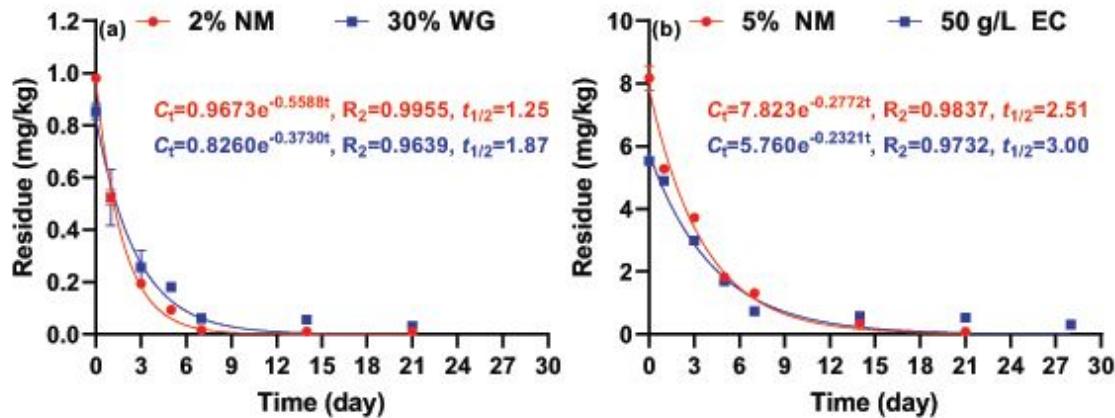


Figure 1

Average initial residues and residue dynamics of indoxacarb (a) and lufenuron (b) between nano-formulation and commercial formulation. NM, WG, and EC are the abbreviation of nano-microemulsion, water-dispersible granule, and emulsifiable concentrate, respectively.

Supplementary Files

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- TableS1.xlsx