Journal of Agricultural Science; Vol. 7, No. 12; 2015
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Comparative Uptake of Chlorantraniliprole and Flubendiamide in the Rice Plant

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Received: September 8, 2015 Accepted: October 25, 2015 Online Published: November 15, 2015

doi:10.5539/jas.v7n12p238 URL: http://dx.doi.org/10.5539/jas.v7n12p238

Abstract

Chlorantraniliprole and flubendiamide as novel commercial insecticides could control almost all economically important lepidopteran insects and a number of key pests in vegetables and rice. At present, no researches were focused on the comparative uptake of chlorantraniliprole and flubendiamide in protected rice plants. Uptake of chlorantraniliprole or flubendiamide was investigated through hydroponic experiment or foliar absorption experiments. The results indicated that chlorantraniliprole had uptake character in rice plants and could only transport upward in the rice plant while flubendiamide had no uptake character in rice plants.Their uptake characteristic are a factor in determining their actions and toxicology,enrichment parts of the protected rice plants, persistence, metabolic processes and residual dynamics in protected rice plants.

Keywords: chlorantraniliprole, flubendiamide, uptake

1. Introduction

Chlorantraniliprole and flubendiamide are novel commercial insecticides which can control almost all economically important lepidopteran insects and a number of key coleopteran, dipteran, hemipteran, and isopteran pests in fruits, vegetables and rice (Lahm et al., 2005, 2007, 2009; Hannig et al.,2009). The intended use of chlorantraniliprole or flubendiamide as insecticides included the *Ostrinia furnacalis*, *Cnaphalocrocis medinalis*, *Chilo suppressalis*, *Tryporyza incertulas*, *Helicoverpa armigera* and others (Hoffmann et al., 2009; Ioriatti et al., 2009; Rhainds & Sadof, 2009; Spomer et al., 2009; Cao et al., 2010). At present, researches of chlorantraniliprole or flubendiamide focus on chemical synthesis routes of chlorantraniliprole or flubendiamide (Liu et al., 2010), field control efficacy of chlorantraniliprole or flubendiamide preparations, and its insecticidal spectrum (Cao et al., 2009). Moreover, some studies focus also on the residual effects of chlorantraniliprole or flubendiamide in some fruits, vegetables or water which includes the residues and degradation dynamics of chlorantraniliprole or flubendiamide in soil or other environmental media (Caboni et al., 2008; Bu et al., 2008; Grant et al., 2010; Qin et al., 2010; Gaddamidi et al., 2011; Chen et al., 2012, 2014).

The most concerning problem on pesticide preparation was the uptake characteristic of pesticides in protected plants when pesticide preparations were used to control plant insects or diseases. This is a significant factor in determining the actions and toxicology of pesticide, enrichment parts of the protected plants, persistence, metabolic processes and residual dynamics in protected plants. On the other hand, researches of uptake of chlorantraniliprole in the velvetleaf were reported by Chen (Chen et al., 2013), and no researches were focused on the comparative uptake of chlorantraniliprole and flubendiamide in protected plants such as rice plants. The objective of this study was to comparatively evaluate the uptake and translocation of chlorantraniliprole and flubendiamide in the rice plant through hydroponic experiment or smeared on the rice leaves. Findings from this study may be used to prioritize chlorantraniliprole and flubendiamide for future evaluations, and to improve understanding of the pesticides utilization efficiency.

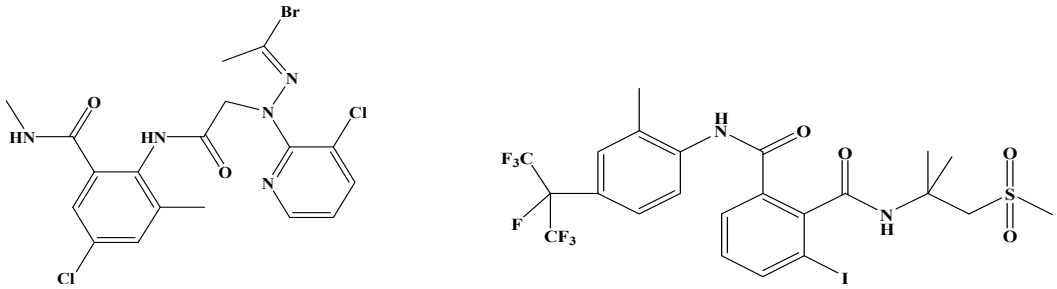

Figure 1. Chemical structure of compound chlorantraniliprole (left) and flubendiamide (right)

2. Materials and Methods

2.1 Chemicals and Materials

The instruments were BS210S electronic balance, SB-1000 rotary vacuum evaporator and THZ-82A vibrating machine made by Sartorius company of Germany, Eyela company of Japan and FuHua Company of Jiangsu province, China, respectively. Agilent 1200 Series HPLC system equipped with an Agilent 6460 Triple Quadrupole LC/MS system was made by Agilent Co. (USA).

Chlorantraniliprole (purify 99.0%) and flubendiamide (purify 98.52%) were purchased from Chemservice, USA, Fluka Company (USA), respectively. Dehydration magnesium sulfate, N-propyl ethylenediamine (PSA) and C_{18} were obtained from DIMA Technology Inc. HPLC grade solvents acetonitrile were obtained from Tedia Inc. USA. Water was used as the mobile phase in HPLC and was redistilled in a glass apparatus. Sodium chloride was obtained from Tianjin Chemical Reagent Factory and was analysis reagent.

2.2 Rice Seeds and Rice Nutrient Solution

Full rice seeds (Zhenjiang 2) were selected and soaked in the water for 12 h and the seeds were disinfected with sodium hypochlorite for 20 min. Then the seeds were rinsed with water and put in the shade at 35 °C to pregermination. When the rice seedlings had grown and some rice seedings was transplanted into the nutrient solution. The composition of rice nutrient solution (consisted of major element and minor elements): major elements in nutrient ($mg \cdot L^{-1}$): $Ca(NO_3)_2 \cdot 2H_2O$ 89, $MgSO_4 \cdot 7H_2O$ 250, $(NH_4)_2SO_4$ 49, KH_2PO_4 34, $FeCl_3$ 3; minor elements in nutrient($\mu g \cdot L^{-1}$): H_3BO_3 2.86, $ZnSO_4 \cdot 7H_2O$ 0.22, $MnC1_2 \cdot 4H_2O$ 1.81, $CuSO_4 \cdot 5H_2O$ 0.08, $H_2MoO_4 \cdot H_2O$ 0.02, citric acid 2.00 and so on.

2.3 Experiment Methods

2.3.1 Determinations of Recoveries Chlorantraniliprole or Flubendiamide in Rice Plants

Rice leaf or rice stem samples (5 g) were weighed separately in a homogenate cup, and 30 mL of acetonitrile was added. After mashing the samples by a high-speed homogenizer for 3 min, chlorantraniliprole or flubendiamide was added to the samples until the final concentrations of chlorantraniliprole or flubendiamide were 5.00, 1.00, and 0.20 $\mu g\ g^{-1}$, respectively. A blank control group was also set up, and all experiments were repeated thrice. Then, 1.5 g of sodium chloride and 6 g of anhydrous magnesium sulfate were added in the samples prior to 5 min agitation in a rotary shaker at 4000 r min^{-1}. About 2 mL of the upper mixture was collected, added to the centrifuge tubes containing $MgSO_4$ (150 mg), PSA (25 mg), and C_{18} (25 mg), and agitated for 5 min in a rotary shaker at 4000 r min^{-1}. The sample solution (0.60 mL) was diluted to 1.0 mL of distilled water. The sample solution was filtered through a 0.22 μm membrane and subjected to LC-MS/MS chromatographic analysis in MRM mode. Three replicates for each level were analyzed by LC-MS/MS.

2.3.2 Investigation of Uptake of Chlorantraniliprole or Flubendiamide through Hydroponic Experiment

Standard stock solutions of chlorantraniliprole or flubendiamide (2000 $\mu g \cdot mL^{-1}$) were prepared in N,N-dimethyl formamide by weighing chlorantraniliprole (0.1010 g) or flubendiamide (0.1015 g) of the analytic reagent-grade N,N-dimethyl formamide into a 50 mL volumetric flask and diluting to volume, respectively. The solutions were stored in the dark at 4 °C until use. Solutions of chlorantraniliprole or flubendiamide (200, 50 $\mu g \cdot mL^{-1}$) were prepared in nutrient solution by measuring 10, 2.5 mL standard stock solutions of chlorantraniliprole or flubendiamide (2000 $\mu g \cdot mL^{-1}$) with 0.5 mL Tween-80 into a 100 mL volumetric flask and diluting to volume with nutrient solution, respectively. The nutrient solution for control treatment had the same volume of N,N-dimethyl formamide and Tween-80.

Some rice plants were planted in the nutrient solution of the rice for uptake experiments through hydroponic

experiment. The rice plants were then transferred to a 500-mL glass jar filled with nutrient solution consisting of different concentration of chlorantraniliprole or flubendiamide (200, 50 μg·mL^{-1}), respectively. The rice roots were completely submerged in solution. After treatment the seedlings were cultivated under normal conditions until sampling. The rice plants were harvested after the rice plants that had grown in the rice nutrient solution for 24, 48 and 72 h. After sampling the roots were washed with ultrapure water and dried with absorbent paper.

The whole rice plant was separated into two parts (the root of rice plant part and the rice plant above the liquid level of the rice nutrient solution part). And then each part sample (5 g) was weighted and extracted with acetonitrile, treated according to the method of sequence 1.3.1 and content of chlorantraniliprole or flubendiamide was determintated in each part by LC-MS/MS. Each experiment repeated for three replicates.

2.3.3 Investigation of Uptake of Chlorantraniliprole or Flubendiamide through Smeared on the Rice Plant Leaves

Solutions of chlorantraniliprole or flubendiamide (100 μg·mL^{-1}) were prepared in nutrient solution by measuring 5 mL standard stock solutions of chlorantraniliprole or flubendiamide (2000 μg·mL^{-1}) with 0.5 mL Tween-80 into a 100 mL volumetric flask and diluting to volume with nutrient solution, respectively.

2.3.3.1 Determination of the Uptake of Chlorantraniliprole or Flubendiamide Smeared on the Leaves near the Roots through Foliar Absorption Experiments

After germination described previously, the seedlings of rice were transferred to plastic pots containing loamy soil and cultivated under the same conditions described. One seedling was planted into each container.

The leaves near the roots was evenly smeared with 5 mL of solution containing chlorantraniliprole or flubendiamide solution (100 μg·mL^{-1}) and covered by a vent bag to prevent other blades from becoming contaminated. At the same time, in order to prevent the droplets from dropping into other leaves or the soil, plastic was used to cover the leaves and the soil. When the droplets on the leaves were dried, the plastic was taken away. The rice leaves were harvested after treated for 24, 48 and 72 h. The rice leaves were separated into two parts (the leaves near the roots part and above the leaves near the roots part). And then each part sample (5 g) was weighted and extracted with acetonitrile, treated according to the method of sequence 2.3.1 and content of chlorantraniliprole or flubendiamide determintated in each part by LC-MS/MS. Each experiment repeated for three replicates.

2.3.3.2 Determination of Uptake of Chlorantraniliprole or Flubendiamide Smeared on the Stem Leaves through Foliar Absorption Experiments

The stem leaves were evenly smeared with 5 mL of solution containing chlorantraniliprole or flubendiamide solution (100 μg·mL^{-1}) and covered by a vent bag to prevent other blades from becoming contaminated. Treated methods were according to the method of sequence as above 2.3.3.1. The content of chlorantraniliprole or flubendiamide was determintated in each part by LC-MS/MS. Each experiment repeated for three replicates.

2.4 LC-MS/MS Detection Parameters of Chlorantraniliprole or Flubendiamide

The samples above were determinated and analyzed by LC-MS/MS. Determination method of chlorantraniliprole or flubendiamide in the rice plant was described by some literatures (Caboni et al., 2008; Chen et al., 2012). LC analysis was performed with an Agilent 1200 HPLC system equipped with a binary pump, auto plate-sampler, column oven, and diode-array detector. Separation was performed on Agilent Eclipse Plus chromatographic columns C_{18} (4.6 mm × 150 mm (i.d.), 5 μm) at 20 °C, with mobile solvents consisting of methanol: ammonium acetate with 1% 5 mmol L^{-1} acetic acid = 60:40 (V:V), isocratic at 1 mL min^{-1}. Aliquots of 5 μL were injected directly to the LC-MS/MS system to test flubendiamide and quantified with external standard peak area. Mass spectra was recorded on an Agilent 6460 triple quadrupole (QQQ) mass spectrometer equipped with an ESI source. System control and data acquisition were controlled by Agilent Mass Hunter software. Detailed MS conditions were: cluster voltage: -120 V; gas temperature: 300 °C, gas flow 10 L min^{-1}, nebulizer pressure: 15 psi, sheath gas temperature: 250 °C; sheath gas flow: 7 L min^{-1}, capillary voltage: 4 kV, nozzle voltage: 500 V. ESI was operated in the negative ion mode in the MRM (multiple reaction monitoring). Full-scan spectra were obtained in the ranges of 0-500 for chlorantraniliprole or flubendiamide.

2.5 Statistical Analysis

All data were analyzed by analysis of Duncan multiple comparison. All the experiments were repeated three as means ± standard error of mean. Different uppercase letters after the number was 1% significant difference.

3. Results

3.1 LC-MS/MS Optimal Detection Condition of Chlorantraniliprole or Flubendiamide Standard

Chlorantraniliprole or flubendiamide standard solution was injected into the instrument directly and the parent ion of the chlorantraniliprole or flubendiamide was determined by full-scan. And then the parent ion was scanned by MS and fragment ions were obtained. Qualitative and quantitative ion pairs were identified by multiple reaction ion monitoring the relative abundance of high ions (Table 1). MS/MS figure was obtained by optimizing the conditions such as the voltage and collision voltage and other parameters. For chlorantraniliprole, we observed intense fragmentation ions at m/z 453, 286 and 177. Selected reaction monitoring of the precursor-product ion transition was m/z 681/453 for chlorantraniliprole for the quantitative ion pair. The second and third transitions were used for confirmatory purposes for samples (Figure 2). LC-MS/MS parameters and spectrum for flubendiamide was shown in Table 1 and Figure 3.

Table 1. The LC-MS/MS parameters for chlorantraniliprole and flubendiamide

Analyte	Ion pairs	Collision energy (eV)	Note
chlorantraniliprole	484/453	14	Quantitative ion pair
	484/286	10	Qualitative ion pair
	484/177	50	Qualitative ion pair
flubendiamide	681/254	-22	Quantitative ion pair
	681/272	-10	Qualitative ion pair
	681/274	-10	Qualitative ion pair
	681/214	-48	Qualitative ion pair

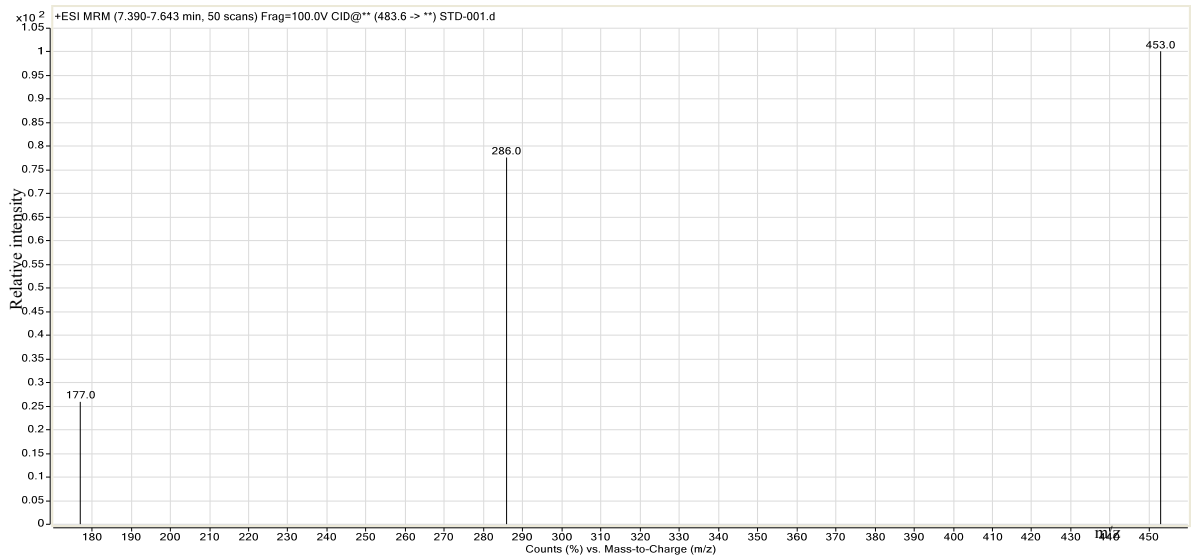

Figure 2. MS-MS spectrum of chlorantraniliprole standard by MRM mode

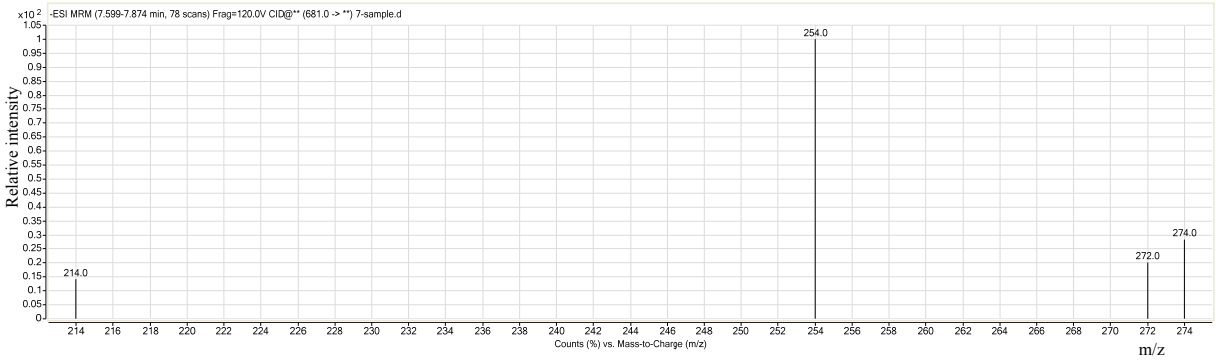

Figure 3. MS-MS spectrum of flubendiamide standard by MRM mode

3.2 Recoveries of Chlorantraniliprole or Flubendiamide from the Rice Plant

To know the efficiency of extraction and clean-up procedures, recovery experiments were carried out at different levels to establish the reliability and validity of analytical method. The samples were spiked at 5, 1.0, and 0.2 μg g^{-1} respectively followed by methodology as described above. The average recoverie of chlorantraniliprole was above 80%. The coefficients of variation were 1.04% to 3.54% and 1.74% to 5.44%, respectively.The average recoverie of flubendiamide was above 80%. The coefficients of variation were 2.69% to 4.59% and 1.95% to 5.14%, respectively (Table 2). These values were in accordance to the pesticide residue criterion at different fortification levels.

Table 2. The recoveries of chlorantraniliprole and flubendiamide from the rice roots and rice leaves

Insecticides	Sample	Fortification level (μg g^{-1})	Average recovery (%)	Standard deviation	Coefficent of variance (%)
Chlorantraniliprole	Rice roots	5.0	90.47	0.0469	1.04
		1.0	90.34	0.0311	3.44
		0.2	85.45	0.0061	3.54
	Rice leaves	5.0	90.53	0.1083	2.39
		1.0	89.57	0.0156	1.74
		0.2	84.57	0.0092	5.44
Flubendiamide	Rice roots	5.0	91.95	0.1235	2.69
		1.0	85.39	0.0272	3.19
		0.2	81.87	0.0075	4.59
	Rice leaves	5.0	94.14	0.0916	1.95
		1.0	92.49	0.0208	2.25
		0.2	86.01	0.0088	5.14

Note. Mean value of three replicates.

3.3 Uptake Results of Chlorantraniliprole or Flubendiamide in Rice Plant through Hydroponic Experiment

When rice roots were incubated in chlorantraniliprole solution at a concentration 50 μg·mL^{-1} through hydroponic experiment, concentration of chlorantraniliprole in the rice plant above the liquid level part was 5.13, 9.17 and 12.86 μg·g^{-1} after treatment for 24, 48 and 72 h, respectively. When rice roots were incubated in chlorantraniliprole solution at a concentration 200 μg·mL^{-1} through hydroponic experiment, concentration of chlorantraniliprole in the rice plant above the liquid level was 13.87, 19.62 and 22.51 μg·g^{-1}after treatment for 24, 48 and 72 h, respectively. Flubendiamide was not detected in the rice plant above the liquid level of nutrient solution at a concentration 50 and 200 μg·mL^{-1} after treatment for 24, 48 and 72 h (Table 3).

Table 3. The content of chlorantraniliprole or flubendiamide in rice plant above the liquid level of nutrient solution after pesticides treated through hydroponic experiment[1),2)]

Insecticides	Treatment concentration (μg mL^{-1})	Detected time (h)	Content of pesticides in the plant (μg g^{-1})	
			Treated root	plants above the liquid level part
Chlorantraniliprole	50	24	16.89±0.42a	5.13±0.12c
		48	13.84±0.58b	9.17±0.09b
		72	9.33±0.79c	12.86±0.27a
	200	24	24.88±0.65a	13.87±1.14c
		48	18.97±0.47b	19.62±0.56b
		72	12.04±0.28c	22.51±1.10a
Flubendiamide	50	24	17.65±0.15c	ND
		48	18.29±0.41b	ND
		72	18.50±0.36a	ND
	200	24	27.91±0.52b	ND
		48	29.86±0.44a	ND
		72	30.27±0.63a	ND

Note. [1)] Mean value of three replicates; [2)] "ND" means no detected in the detection limit.

3.4 Uptake Results of Chlorantraniliprole or Flubendiamide in Rice Plants after Smeared on Leaves through Foliar Absorption Experiments

3.4.1 Uptake Results of Chlorantraniliprole or Flubendiamide Smeared on Leaves near the Roots through Foliar Absorption Experiments

When chlorantraniliprole was smeared on the leaves near the roots through foliar absorption experiments at a concentration of 100 μg·mL^{-1}, chlorantraniliprole was found in stem leaves and its concentration was 3.41, 4.18 and 4.92 μg·g^{-1} after treatment for 24, 48 and 72 h, respectively. Flubendiamide was not detected in the stem leaves at a concentration 100 μg·mL^{-1} after treatment for 24, 48 and 72 h, respectively (Table 4).

Table 4. The content of chlorantraniliprole or flubendiamide in stem leaves after pesticides smeared on leaves near the roots through foliar absorption experiments[1),2)]

Insecticides	Detected time (h)	Content of pesticides in the rice plant (μg g^{-1})	
		Treated leaves	Stem leaves
Chlorantraniliprole	24	34.56±0.21a	3.41±0.37c
	48	25.88±0.17b	4.18±0.28b
	72	17.63±0.27c	4.92±0.41a
Flubendiamide	24	35.81±0.33a	ND
	48	33.89±0.38b	ND
	72	32.67±0.16c	ND

Note. [1)] Mean value of three replicates; [2)] "ND" means no detected in the detection limit.

3.4.2 Uptake Results of Chlorantraniliprole or Flubendiamide Smeared on the Stem Leaves through Foliar Absorption Experiments

When chlorantraniliprole was smeared on the stem leaves through foliar absorption experiment at a concentration of 100 μg·mL^{-1}, chlorantraniliprole was found in the stem leaves and its concentration was 41.88, 39.56 and 37.72 μg·g^{-1} after treatment for 24, 48 and 72 h, respectively; while no chlorantraniliprole was detected in the leaves below the stem leaves. Flubendiamide was not detected in the leaves below the stem leaves at a concentration 100 μg·mL^{-1} after treatment for 24, 48 and 72 h (Table 5).

Table 5. The content of chlorantraniliprole or flubendiamide in the leaves below the stem leaves after pesticides smeared on the stem leaves through foliar absorption experiments[1),2)]

Insecticides	Detected time (h)	Content of pesticides in the rice plant (μg g^{-1})	
		Treated leaves	Below stem leaves
Chlorantraniliprole	24	41.88±0.39a	ND
	48	39.56±0.54b	ND
	72	37.72±0.27c	ND
Flubendiamide	24	42.79±0.94a	ND
	48	40.23±0.83b	ND
	72	38.76±0.70c	ND

Note. [1)] Mean value of three replicates; [2)] "ND" means no detected in the detection limit.

4. Discussion

In present, chlorpyrifos, profenofos, abamectin, hexaflumuron and other pesticides that were used to control *Cnaphalocrocis medinalis* does not exhibit uptake character. Hence, these not be transported effectively in the plants of rice, and the efficiency of protection of new leaves were limited. Therefore, these pesticides have to be sprayed repeatedly in the rice field which can be attacked frequently by adult insects for a long period (Cao et al., 2010; Shao et al., 2011; Shen et al., 2011). Hence, some excellent insecticides were urgently developed to control rice pests or uptake characters or action mechanisms of some insecticides on the rice insects need be clarified. If chlorantraniliprole preparations could be transported in rice plants and the retention could last for 20 days, then spraying once is enough for controlling each generation of *Cnaphalocrocis medinalis*. Based on the current experiment, chlorantraniliprole could be detected in a conjoined stem and leaf sheath on the treated leaf when it was applied on the leaves of rice plant. However, no chlorantraniliprole was detected in other adjacent leaves. The reason for this may be that chlorantraniliprole could penetrate in rice leaves and is transported for a short distance, moving to the stem or the leaf sheath of the treated leaves. Thus, it could not be transported from one leaf to the other. It may indicate that chlorantraniliprole has limited penetrated ability, and could not be transported between leaves.

In order to improve the absorption efficiency of chlorantraniliprole in rice, enough volume of the solution should be sprayed so that it could flow onto the rice stems along the leaf sheaths. The results indicated that chlorantraniliprole had uptake in rice plants and could be transported from the bottom to the top of the plant. Therefore, it could not only be sprayed on rice leaves which could not work its potency. In order to control insect integrity, chlorantraniliprole preparations such as WG, granular formulation, water dispersible granule, could be considered for manufacturing.

5. Conclusions

As shown by our results, it indicated that chlorantraniliprole had uptake character in rice plants and could only transport upward in the rice plant while flubendiamide had no uptake character in rice plants.

Acknowledgements

We thank the Natural Science Foundation of Jiangsu Province (BK20130443) and Key Research and Development Program of Jiangsu province (BE2015342), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (13KJB210010) , the Social Development Science and Technology Projects of Yangzhou City, China (2012110) and Graduate students' Practice Innovation Training Program of Higher School (KYLX15_1373, 201411117048Y) for providing financial support.

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