

Seed Treatment with Diamide and Neonicotinoid Mixtures for Controlling Fall Armyworm on Corn: Toxicity Evaluation, Effects on Plant Growth and Residuality

Hongbo Li^{1*†}, Lei Feng^{2†}, Junhong Fu^{2†}, Ying Zhang¹, Wenyuan Huang¹, Tingting Duan¹, Yang Hu¹ and Jichun Xing^{2*}

¹Institute of Plant Protection, Guizhou Academy of Agricultural Sciences, Guiyang, China, ²Guizhou Provincial Key Laboratory for Agricultural Pest Management of the Mountainous Region, School of Agriculture, Institute of Entomology, Guizhou University, Guiyang, China

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*Correspondence:

Jichun Xing xingjichun@126.com Hongbo Li gzlhb2017@126.com

[†]These authors have contributed equally to this work

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Li H, Feng L, Fu J, Zhang Y, Huang W, Duan T, Hu Y and Xing J (2022) Seed Treatment with Diamide and Neonicotinoid Mixtures for Controlling Fall Armyworm on Corn: Toxicity Evaluation, Effects on Plant Growth and Residuality. Front. Chem. 10:925171. doi: 10.3389/fchem.2022.925171 The diamides, chlorantraniliprole (CHL) and cyantraniliprole (CYA), have been used as seed treatment agents against the fall armyworm (FAW), Spodoptera frugiperda in China. However, large-scale application of these two insecticides is prohibited because of their high cost. The neonicotinoid insecticides, clothianidin (CLO) and thiamethoxam (THI), are cheaper and widely used. In this study, we tested the efficacy of CHL + CLO and CYA + THI as seed treatment agents against FAW larvae both in laboratory and field conditions. Laboratory experiments showed that the two binary mixtures (both 240 g.a.i.100 kg⁻¹ corn seeds) caused FAW mortality exceeded 84.00% at 14 days after seedling emergence (DAE). The mortality of the binary mixtures were similar to either CHL (300 g.a.i.100 kg⁻¹corn seeds) or CYA (144 g a.i.100 kg⁻¹corn seeds), but higher than CLO (120 g.a.i.100 Kg⁻¹corn seeds) or THI (180 g a.i.100 kg⁻¹corn seeds). Two independent field experiments showed that both binary mixtures resulted in above 68.00% control efficacy at 14 DAE, suggesting that these insecticidal combinations could effectively control FAW over a relative long period. In addition, both binary mixtures showed no negative effects on the growth and development of corn seedlings. The residues of binary mixtures in corn leave were also lower at 28 DAE as compared to residues in CHL or CYA alone. Most importantly, the costs of CHL + CLO were reduced up to 50% and CYA + THI up to 20% when compared to singly used chemical. Totally, our results indicated that CHL + CLO and CYA + THI had the same control efficacy as CHL or CYA alone, but with much lower cost.

Keywords: Spodoptera frugiperda, diamide, neonicotinoid, seed treatment, combined toxicity

INTRODUCTION

Corn is a globally-important food crop, that is, consumed by approximately 4.5 billion people worldwide (Shiferaw et al., 2011). In the field, corn yield is reduced by multiple insect pests, including *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae). *S. frugiperda*, also known as the Fall Armyworm (FAW), is a native corn pest in tropical and subtropical areas of North America

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(Martinelli et al., 2006). In recent years, the FAW has invaded Africa and Asia. This pest is highly destructive due to its wide host range, robust migration ability, high fecundity and resistance to insecticides (Guo et al., 2019). In China, FAW was first found in Yunnan province in December 2018 (Sun et al., 2021); it quickly spread throughout 26 provinces and damaged approximately 65.53×10^4 hm² corn (Jiang et al., 2019). The FAW is now well-established in winter corn grown in southern China and is a dominant corn pest due to its migratory ability (Qi et al., 2020; Jiang et al., 2021).

Spraying chemical insecticides remains as the most effective measure for controlling this pest. However, there are several factors reducing the efficacy of insecticide spraying against FAW. For example, the efficacy of insecticidal sprays is largely effected by weather and subject to dilution by rain and wind (Zheng et al., 2006; Ranabhat and Wang, 2020). The efficacy of insecticides is also impacted by larval behavior; this is especially relevant for FAW since larvae hide inside the maize whorl, which reduces their exposure to insecticides (Muraro et al., 2020). A further barrier is the labor shortage, which can delay spray applications and reduce efficacy. Finally, the overuse of chemical insecticides contaminates the environment and has impacts on mammals and nontarget arthropods (Lahm et al., 2007).

Seed treatment with systemic insecticides has been used to control pests on many crops (Taylor et al., 2001). In general, treating seeds with chemicals requires less insecticide than spray application and reduces environmental contamination and exposure of nontarget organisms (Schemeer et al., 1990; Nault et al., 2004). Consequently, seed treatment is popular in integrated pest management programs (Zhang et al., 2011). Previous studies demonstrated that seed treatment with carbofuran or thiamethoxam (THI) was ineffective for controlling early larval stages of FAW on corn (Azevedo et al., 2004). Chlorantraniliprole (CHL) and cyantraniliprole (CYA) are anthranilic diamides that target insect ryanodine receptors and disrupt the functioning of calcium channels (Lahm et al., 2007). These two compounds control insect pests by directly killing individuals and inhibiting their feeding, development and reproduction (Huang et al., 2016; Lutz et al., 2018). Previous studies demonstrated that these insecticides showed excellent control efficacy, particularly when used for lepidopteran pests such as FAW (Wang et al., 2019a; Pes et al., 2020). Recently, CHL and CYA were labeled as seed treatments for the control of FAW larvae (Muraro et al., 2020; Pes et al., 2020) because of their low LogPow (octanol/water partition coefficient) and high solubility in water (Selby et al., 2017).; unfortunately, the cost of CHL and CYA is much more expensive than traditional insecticides.

The combined application of two different insecticides could improve control of target pests (Thrash et al., 2013; Carscallen et al., 2019; Muraro et al., 2020). The dual application of anthranilic diamides with other insecticides for FAW control is under-investigated but clearly needed to reduce the cost of control. Thus, the objective of this study was to compare the efficacy of corn seed treatments with CHL, CYA, clothianidin (CLO), THI and the binary mixture of CHL + CLO and CYA + THI for control of FAW larvae in laboratory and field conditions. A recent study suggested that the application of selected insecticides as seed treatments inhibited crop growth because of prolonged, high residue level (Abdu-Allah and Hashem, 2017). Thus, we also evaluated the effects of the above-mentioned chemicals on growth of corn plants and determined insecticide residue levels.

MATERIALS AND METHODS

Insects

FAW populations for laboratory experiments were collected in 2019 from corn fields located at the Guizhou Academy of Agricultural Sciences in Guizhou province, China. The larvae were reared on a corn-based artificial dietat $25 \pm 1^{\circ}$ C and 70% relative humidity (RH) with a 16:8 h (L:D) photoperiod (Wang et al., 2019b). Third instar larvae were used in the laboratory experiments.

Corn Seeds and Insecticides

Corn seeds of the cultivar Jinyu 818 were provided by Guizhou Jinlong Technology Co., Ltd. Insecticides were sourced from the following companies: chlorantraniliprole (CHL, 50% FSC), DuPont Crop Protection (United States); cyantraniliprole + thiamethoxam (CYA + THI, 60% FSC) and cyantraniliprole 40% (CYA, FSC), Syngenta AG (Switzerland); chlorantraniliprole + clothianidin (CHL + CLO, 40% FSC), Guangdong Kairuifeng Technology Co., Ltd. (China); clothianidin (CLO, 20% FSC), Hebei Lishijie Technology Co. (China); and thiamethoxam (THI, 30% FSC), Bayer Crop Science LP, Monheim (Germany).

Laboratory Experiments

Laboratory experiments were conducted from May to July in 2021 and consisted of the following seven treatments (concentrations in g a.i.100 kg⁻¹corn seeds): 1) CHL, 300; 2) CYA, 144; 3) CHL 60 + CLO 180; 4) CYA 120 + THI 120; 5) CLO, 120; 6) THI, 180; and 7) untreated control. The concentration of each insecticide was chosen based on recommended field rates.

One day prior to sowing, seeds and pesticides were placed in plastic bags, sealed, shaken until insecticides coated the seed surface, and allowed to dry overnight. Fifty corn seeds were sown in individual containers $(30 \times 20 \times 20 \text{ cm})$ containing sand (40%), clay (40%) and organic matter (20%) at 25°C, 70% RH and a 14:10 (L:D) photoperiod. Water was provided during seed emergence and growth as necessary, and the emergence rate of seeds in each treatment was recorded. At 3, 7, 14, 21, 28 days after seedling emergence (DAE), twenty newly-molted 3rd instar larvae were collected, starved for 2 h, and then transferred to corn plants. Finally, the plants together with FAW larvae were placed in nylon cages to prevent escaping. Each potting container was considered as one replication, and each treatment had four replications. Larval mortality was recorded after 3 days transferring, and larvae were considered dead if there was no response to stimulation with a moist brush.

Field Experiments

Two identical field experiments were conducted to evaluate the efficacy of six insecticidal formulations for FAW larval control on corn seedlings in Luodian county (106.63°E, 25.62°N), Guizhou province, in July and September of 2021. In the two field experiments, 28 plots were arranged in a randomized complete block design with seven treatments and four replications. Insecticide-treated corn seeds were sown on 23 June and 23 August 2021. Each plot was 30 m^2 (5 × 6 m) and consisted of 10 rows separated by 60 cm of uncultivated ground. The emergence rate of corn seeds was recorded by counting the number of emerged plants in each plot. A five-point sampling method where each point consisted of 10 plants was used to record the number of FAW larvae on corn in each plot at 7, 14, 21, and 28 DAE. The damage rate caused by FAW and the control efficacy of tested insecticides were calculated by the following formulas:

Percentage of corn seedlings with FAW damage (%)

$$= \frac{\text{#corn plant with FAW damage}}{\text{#investigated corn plants}} \times 100\%$$
(1)
control efficacy (%) =

С

(2)#FAW in untreated plot – #FAW in treated plot ×100% #FAW in treated plot

Effect of Insecticides on Fall Armyworm **Emergence and Corn Growth**

The emergence rate of corn seeds treated with the six insecticidal formulations were recorded at 7 DAE in both laboratory and field experiments. The impact of the six insecticides on corn grown was evaluated by random selection of 20 plants in each plot at 14 DAE. Plant height, root length, and above-ground and underground fresh weight were measured in July and September of 2021.

Determination of Residual Insecticides in Corn Leaves

Corn leaves were randomly selected from field plots at 3, 7, 14, 21, and 28 DAE in July 2021 and stored at -20°C until needed for analysis. Homogenized corn leaves $(5.0 \pm 0.1 \text{ g})$ were weighed in 50 ml Teflon centrifuge tubes, and water (5 ml) and 10 ml acetonitrile with 1% (ν/ν) methanol (HOAc) were added to samples. Sample tubes were shaken vigorously for 10 min, allowed to stand for 30 min, and NaCl (3 g) and MgSO₄ (4 g) were added. Tubes were capped, mixed for 1 min and centrifuged for 5 min at 3216 \times g. The upper acetonitrile layer (1.5 ml) was transferred into 2.0 ml tubes containing 50 mg octadecylsilane (C18), 15 mg graphitized carbon black (GCB) and 150 mg MgSO4. The tubes were vortexed for 30 s and centrifuged for 5 min at 2233 \times g. The resulting supernatants were subjected to ultrafiltration (0.22 µm nylon filter) and then loaded into autosampler vials for UHPLC-MS/MS analysis.

Chromatographic separation of CLO, THI, CHL, CYA, and J9Z38 (metabolite of CYA) was performed using a Dionex Ultimate 3000 UHPLC system and a Syncronis C18 column (100 mm $\,\times\,$ 2.1 mm, 1.9 $\mu m)$ (Thermo Fisher Scientific, United States) at 40°C with a 5 µl injection volume. The mobile phases consisted of solution A (H₂O containing 0.1% ν/ν formic acid) and solution B (methanol); the flow rate was $0.25 \text{ ml} \cdot \text{min}^{-1}$. The elution program was: 25% solvent B from 0 to 1.0 min; 25%-85% solvent B from 1.0 to 1.5 min; 85% solvent B from 1.5 to 6 min; 85%-25% solvent B from 6 to 6.5 min; and 25% solvent B for 1.5 min. Qualitative and quantitative analysis of CLO, THI, CHL, CYA, and J9Z38 were obtained with a triple-quadrupole mass spectrometer (TSQ Vantage) equipped with an ESI interface (Thermo, San Jose, CA, United States). Nitrogen was used as the sheath and auxiliary gas at 30 and 10 PSI, respectively. The vaporizer and capillary temperatures were both 330°C, and the spray voltage was 3.2 kV. The pressure of argon in the collision cell was 1.5 mTorr. The MS/MS conditions were optimized to acquire satisfactory sensitivity and resolution using the parameters listed in Supplementary Table S1. First-order kinetic and bi-exponential models were used to analyze the dissipation curves of CLO, THI, CHL, CYA, and J9Z38. Analysis of t kinetics was performed in KinGUIIv2.1 (BASF Corporation) as follows:

$$C = C_0 e^{-kt}$$
(3)

$$T_{1/2} = \ln 2/K$$
 (4)

Statistical Analysis

Data associated with mortality were arcsine-transformed before statistical analysis. One-way ANOVA was used to determine statistical significance among treatments, followed by a Tukey's HSD method. Results were considered significant at p < 0.05. Statistical analyses were performed in DPS v. 17.0 (Tang and Zhang, 2013).

RESULTS

Efficacy of Insecticidal Seed Treatments in Laboratory Experiments

In laboratory experiments, the percentage of corn seedlings damaged by FAW in CHL + CLO, CYA + THI, CHL, and CYA ranged from 15.35 to 45.13% at 3-28 DAE, and these levels were significantly lower than the untreated control where damage was 52.03%-86.70% (Figure 1). The percentage of corn seedlings with FAW damage in CLO or THI alone was generally either slightly lower or not significantly different from the untreated control (Figures 1A-E).

The corrected mortality of FAW larvae fed on corn plants treated with the six insecticides declined as days after seed emergence increased (Table 1). At DAE 3, 7, and 14, CHL, CYA, CHL + CLO and CYA + THI treatments resulted in similar levels of mortality and ranged from 72.00% to 94.44%; furthermore, FAW mortality in these four treatments was significantly higher than in larvae exposed to CLO and THI treatments. At 21 or 28 DAE, mortality in the CHL, CYA, CHL + CLO and CYA + THI treatments ranged from 60.00% to 72.97% and remained significantly higher than mortality in the CLO or THI treatments.



cyantraniliprole (CYA), clothianidin (CLO), thiamethoxam (THI), CHL + CLO and CYA + THI; CK is the untreated control. Damage is shown on corn seedlings subjected to FAW larvae at 3 panel (A), 7 (B), 14 (C), 21 (D), and 28 (E) days after emergence (DAE). Columns labeled with different letters indicate significant differences among treatments with Tukey's HSD test at p < 0.05.

TABLE 1 | The corrected mortality (±SE) of FAW larvae fed on corn plants subjected to insecticidal seed treatments in laboratory experiments.

Treatments	Dosage (ga.i.100 kg ⁻¹ seeds)	Corrected mortality (%)					
		3 DAE	7 DAE	14 DAE	21 DAE	28 DAE	
CHL	300	88.89 ± 2.27a	78.08 ± 2.24b	72.00 ± 1.33b	63.51 ± 1.35a	61.43 ± 2.74a	
CYA	144	90.28 ± 3.21a	89.04 ± 2.24a	78.67 ± 5.33ab	66.22 ± 1.35a	60.00 ± 6.17a	
CHL + CLO	240	94.44 ± 1.39a	90.41 ± 2.62a	89.33 ± 4.87a	71.62 ± 2.59a	64.29 ± 5.89a	
CYA + TH	240	94.44 ± 2.27a	93.15 ± 1.37a	84.00 ± 2.18ab	72.97 ± 6.62a	61.43 ± 2.74a	
CLO	120	65.28 ± 4.74b	58.90 ± 1.58c	54.67 ± 1.54c	35.14 ± 6.24b	17.14 ± 1.65b	
THI	180	58.33 ± 3.59b	43.84 ± 4.11d	41.33 ± 3.77d	40.54 ± 2.21b	18.57 ± 1.43b	
F	-	14.12	50.47	14.14	14.84	25.72	
df	-	23.5	23.5	23.5	23.5	23.5	
p	-	<0.001	<0.001	<0.001	<0.001	<0.001	

Values followed by different letters indicate significant differences among treatments with Tukey's HSD test at p < 0.05.





Efficacy of Insecticidal Seed Treatments in Field Experiments

In July 2021, the percentage of corn seedlings damaged by FAW in insecticide-treated field plots ranged from 19.50% to 63.00% at 7 DAE, which was significantly lower than the control (80.00%) (**Figure 2A**). At 14 DAE, the value in insecticide-treated plots showed a substantial increase but were still significantly lower than the untreated control (**Figure 2B**). At 21 and 28 DAE, the value in treated plots were not significantly different from the control (**Figures 2C,D**). The percentage of corn seedlings with

FAW damage in September 2021 at all four sampling times were similar to results in July (**Figures 2E–H**).

In July, CHL, CYA, CHL + CLO and CYA + THI treatments resulted in 79.84%–87.88% control efficacy at 7 DAE, which were significantly higher than those in the CLO and THI treatments (**Table 2**). Furthermore, control efficacy in the CYA + THI treatment was higher than in corn treated with CYA alone. At 14 DAE, control efficacy in the CHL, CYA, CHL + CLO and CYA + THI treatments were higher than CLO and THI treatments; however, the efficacy of the CHL, CYA, CHL + CLO and CYA +

Experimental time	Treatments	Dosage (ga.i.100 kg ⁻¹ seeds)	Control efficacy (%)			
			7 DAE	14 DAE	21 DAE	28 DAE
July	CHL	300	84.15 ± 2.82ab	76.42 ± 2.22ab	55.79 ± 4.37ab	40.94 ± 2.45b
	CYA	144	79.84 ± 3.45b	68.93 ± 3.82b	49.54 ± 4.76bc	14.09 ± 7.02d
	CHL + CLO	240	87.88 ± 1.83a	77.94 ± 3.25a	48.61 ± 1.71bc	32.21 ± 4.57b
	CYA + THI	240	86.71 ± 1.92a	71.46 + 1.51ab	44.68 ± 2.34c	19.46 ± 1.10cd
	CLO	120	65.97 ± 2.20c	57.09 ± 4.14d	57.87 ± 1.39a	31.54 ± 4.17bc
	THI	180	72.49 ± 2.20c	62.35 ± 1.99cd	58.33 ± 2.17a	53.69 ± 2.29a
	F	-	14.68	7.27	3.36	12.34
	df	-	23.5	23.5	23.5	23.5
	p	-	<0.001	<0.001	0.03	< 0.001
September	CHL	300	84.43 ± 1.09a	74.79 ± 3.12a	61.23 ± 5.64ab	66.79 ± 4.20a
	CYA	144	87.00 ± 1.92a	70.15 ± 1.67a	62.28 ± 2.90a	63.38 ± 1.78a
	CHL + CLO	240	85.53 ± 1.85a	68.33 ± 0.74a	57.56 ± 2.91ab	66.43 ± 3.46a
	CYA + THI	240	87.73 ± 0.92a	72.31 ± 1.06a	50.00 ± 5.56b	64.63 ± 1.56a
	CLO	120	41.94 ± 3.63b	58.54 ± 2.79b	51.02 ± 2.13ab	59.43 ± 3.60a
	THI	180	42.67 ± 2.21b	61.19 ± 2.43b	57.8 ± 2.51ab	62.84 ± 2.61a
	F	-	113.42	8.77	3.73	0.80
	df	-	23.5	23.5	23.5	23.5
	p	-	<0.001	<0.001	0.02	0.56

TABLE 2 | Control efficacy (mean ± SE) of six insecticidal seed treatments for control of FAW on corn in the field.

Values with different letters indicate significant differences with Tukey's HSD test at p < 0.05.



THI treatments decreased dramatically at 21 and 28 DAE (**Table 2**). In September, CHL, CYA, CHL + CLO and CYA + THI treatments resulted in control efficacy ranging from 84.43% to 87.73% and 68.33%–74.79% at 7 and 14 DAE, respectively, and the values were significantly higher than those in the CLO and THI treatments. Similar to results obtained in July, the efficacy in CHL, CYA, CHL + CLO and CYA + THI treatments decreased at 21 and 28 DAE and were not significantly different from the CLO and THI treatments (**Table 2**).

Effect of Insecticides on Emergence Rates and Corn Growth

The emergence rate of corn treated with the six different insecticide formulations exceeded 90% in both laboratory and field experiments

with no significant differences between treatments (**Figure 3**). In July 2021, corn seedlings in field plots treated with CHL + CLO and CYA + THI were significantly taller than seedlings in other treatments (**Figure 4A**). Root length and fresh weight of aboveground tissue were generally higher in seedlings treated with CHL + CLO and CYA + THI as compared to the other four treatments, although these differences were not always significant (**Figures 4B,C**). The underground fresh weights in CHL + CLO and CYA + THI treatments were significantly higher than those in CLO treatment and control (**Figure 4D**). In September, data points for plant height and fresh weight of above-ground and underground tissues were similar to those recorded in July for all treatments (**Figures 4E,G,H**). Unlike July, the root length data in September was not significantly different among treatments (**Figure 4F**).



Insecticide Residues in Treated Corn Plants

Residues levels of the six insecticidal formulations and their metabolites gradually decreased in corn plants throughout the sampling period (**Figure 5**). For example, in the CHL + CLO treatment, the CHL residues declined from 0.29 mg·kg⁻¹ at 3 DAE to 0.02 mg·kg⁻¹ at 28 DAE, while the CLO residues declined from 11.44 mg·kg⁻¹ at 3 DAE to 0.12 at 28 DAE (**Figure 5E**). The half-life ($t_{1/2}$) of CHL in corn plants treated

with CHL + CLO was 2.32 days, which was shorter than that in the CHL treatment alone (4.35 days) (**Supplementary Table S2**). The half-life of CLO in CHL + CLO treated corn plants was 2.26 days, which was similar to that in the CLO treatment alone (2.15 days) (**Figure 5A**, **Supplementary Table S2**).

In the CYA + THI treatment, residues of CYA and its metabolite J9Z38 degraded from 1.58 to $0.04 \ mg \cdot kg^{-1}$ and



plants. Panels: (A) CHL residue in CHL treated leaves; (B) CYA residues in CYA treated leaves; (C) J9Z38 residues in CYA treatedleaves; (D) CHL residues in CHL + CLO treated leaves; (F) CYA residues in CYA + THI treated leaves; (G) J9Z38 residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residue in CYA + THI treated leaves; (I) CLO residue in CYA + THI treated leaves; (I) CLO residue in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residue in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residues in CYA + THI treated leaves; (I) CLO residue in CYA + THI treated leaves; (I) CLO residue in CYA + THI treated leaves; (I) CLO residue in CYA + THI treated leaves; (I) CLO residue in CYA + THI treated leaves; (I) CLO residue in CYA + THI treated leaves

0.03–0.00 mg·kg⁻¹, respectively, during 3–28 DAE period (**Figures 5F,G**). Residues of THI and its metabolite (CLO) degraded from 3.23 to 0.02 mg·kg⁻¹ and 0.68 to 0.03 mg·kg⁻¹, respectively, during the same period (**Figures 5H,I**). The estimated half-life of the CYA, J9Z38, THI and CLO in CYA + THI combination were 3.07, 7.76, 1.99 and 2.67 days, respectively, most of which were low when compared to the CYA or THI treatment alone (**Figures 5B,C,K,L**, **Supplementary Table S2**).

Comparison of the Cost of the Insecticides

As shown in **Supplementary Table S3**, the cost of both CHL + CLO and CYA + THI were below $50.00 \text{ } \text{ } \text{ha}^{-1}$, which lead to reduced cost of $42.52 \text{ } \text{ } \text{ha}^{-1}$ and $11.34 \text{ } \text{ } \text{ha}^{-1}$ when compared to CHL ($85.04 \text{ } \text{ } \text{ha}^{-1}$) and CYA ($56.69 \text{ } \text{ } \text{ha}^{-1}$), respectively.

DISCUSSION

The diamide insecticides kill insect pests by targeting their ryanodine receptor channels (RyRs) that cause muscle contraction and death (Lahm et al., 2007), while the neonicotinoid insecticides kill insect pests by targeting their nicotinic acetylcholine receptors (nAChRs) (Matsuda et al., 2020). In previous laboratory studies, the diamide insecticides such as CHL and CYA were highly toxic to FAW larvae with LC_{50} values of 0.01 and 0.25 µg ml⁻¹, respectively (Bolzan et al., 2019; Zhou et al., 2020). These two insecticides were recently labeled as a seed treatment for controlling FAW and exhibited a high level of control efficacy against this species (Zhang P. et al., 2016; Pes et al., 2020). Neonicotinoid insecticides such as CLO and THI have been widely used as seed treatments in multiple crops and

exhibit good activity against a broad range of pests including aphids (Zhang P. et al., 2016; Zhang Z. et al., 2016), whiteflies (Zhang et al., 2011), and thrips (Reisig et al., 2012; Ding et al., 2018). To the best of our knowledge, there are no prior studies assessing the efficacy of diamides in combination with neonicotinoids for FAW larval control as seed treatments.

CHL + CLO, CYA + THI, CHL, and CYA were effective at reducing percentage of corn seedlings with FAW damage in the laboratory. The percentage of corn seedlings with FAW damage was similar among the four treatments, suggesting that CHL + CLO and CYA + THI were comparable to CHL and CYA treatment alone. Prior studies reported that CHL can rapidly inhibit the feeding of some lepidopteran pests. For example, the feeding behavior of *Trichoplusia ni*, *Plutella xylostella* and *Helicoverpa zea* ended within 30 min after exposure to CHL, and damage decreased by 90%–99% (Hannig et al., 2009). Therefore, diamides alone or in combination with neonicotinoid insecticides reduce damage by causing early mortality, inhibiting feeding, and disrupting larval development, which is similar to results reported elsewhere (Hannig et al., 2009; Carscallen et al., 2019).

The percentage of corn seedlings with FAW damage in our two field experiments was generally higher than in the laboratory, which may be attributed to one or more of the following reasons, Firstly, 3rd instar larvae were used in the lab studies, and this life stage is relatively stable and uniform. Secondly, the high damage rate in the field is likely related to FAW behavior and environmental conditions; for example, FAW adults randomly deposit eggs on corn and the newly-hatched larvae are disseminated by wind, thus increasing range and the damage rate of corn. Therefore, damage rates may not be a suitable index for evaluating the efficacy of insecticides when applied as seed treatments in the field. Recently, an injury score rating was used to evaluate the control efficacy of seed coatings by measuring the feeding area in some lepidopteran species (Carscallen et al., 2019; Wu et al., 2020). It is unclear whether this index is suitable for FAW and further study is needed.

Our lab experiments showed that application of CHL + CLO and CYA + THI to corn resulted in high FAW mortality (84.00%-94.44%) at 14 DAE, which indicates that the combined application of insecticides was an effective control strategy. Our results were similar to those reported for application of CHL and CYA alone in one study (Wu et al., 2020), but were much higher than results reported by Thrash et al. (2013). These disparate outcomes may be caused by variability in plant hosts, FAW populations and insecticide doses. The effectiveness of diamide insecticides alone or in combination with neonicotinoids has also been reported for Mythimna unipuncta (Carscallen et al., 2019). Furthermore, as corn plants grew larger in the present study, the corrected mortality of FAW larvae in all treatments decreased; this is likely due to the decline in insecticidal residues and increase in body size of larvae over time. We also observed that the CLO and THI treatments caused mortality from 41% to 65.28% at 14 DAE, which indicates that these two insecticides are somewhat effective in controlling FAW larvae. These data are consistent with previous results reported for Agrotis ipsilon (Zhang et al., 2019), Ostrinia nubilalis, and Plodia interpunctella (Yue et al., 2003).

To validate findings in the laboratory, we conducted two field experiments in July and September of 2021, respectively. Our results showed that CHL + CLO and CYA + THI treatments resulted in control efficacy of 79.84%-87.88% at 7 DAE and 68.93%-77.94% at 14 DAE. These results were similar to the use of diamides alone and were consistent with laboratory results. Thus, CHL + CLO and CYA + THI treatments exhibited control efficacy equivalent to CHL and CYA treatment alone; however, it is important to mention that the dosage of diamides in the combined treatments was lower than the usage of CHL and CYA alone. These results suggest that synergistic action are present when the diaminde and neonicotinoid insecticides are simultaneously used. The snyergistic effect may be caused by the following two reasons: firstly, neonicotinoid insecticides block the metabolic systems of FAW that would break down diaminde molecules; secondly, neonicotinoid insecticides interfere with the detoxication of diamindes insecticides through their action on polysubstrate monooxygenases (PSMOs) and other enzyme systems (Bernard and Philogène, 1993). Most importantly, application of CHL + CLO and CYA + THI reduce the control cost when they are used to manage FAW larvae. For example, the control cost of CHL + CLO is 42.52 \$ per hectare, which reduced 42.52 \$ when compared to CHL (85.04 \$ ha⁻¹). Similarly, control cost of CYA + THI is 45.35 \$ per hectare, which reduced 11.34 \$ per hectare when compared to CYA (56.69 ha⁻¹). Therefore, this strategy is helpful for large-scale application of these insecticides as seed treatments.

Moreover, the control efficacy of the four treatments in field experiments was lower than laboratory studies, which may be attributed to the rapid degradation of insecticide residues in fieldgrown corn. Weather conditions, application time, insecticide characteristics, and translocation within plants can influence the persistence of insecticides and may impact efficacy (Pfeil, 2014; Teló et al., 2015). Reduced control efficacy in the field can be impacted by: 1) environmental factors such as UV irradiation and rain (Lim et al., 1990; Lanka et al., 2014); 2) translocation to plant tissues where active ingredients may be diluted (Zhang et al., 2019); and 3) insecticide resistance. In addition, we observed that the control efficacy at 21 DAE or later was lower in summer months as compared to autumn. This may be caused by higher soil temperatures or elevated moisture levels during seedling emergence in summer, which may influence microbial activity and further contribute to insecticide degradation (Zhang et al., 2019).

To evaluate the effects of CHL + CLO and CYA + THI as seed treatments in corn, we measured growth indicators and evaluated residue concentrations and dynamics. Firstly, the emergence rates of corn seeds treated with CHL + CLO and CYA + THI were not different from other treatments and the untreated control both in laboratory and field experiments, which suggests that the combined insecticides had no adverse effects on emergence. Secondly, the CHL + CLO and CYA + THI treatments had stimulatory effects on measured parameters. Our results were different from those reported by Abdu-Allah and Hashem (2017) and Huang et al. (2015) but consistent with studies showing that

neonicotinoid seed treatments could promote seed germination and increase primary root length, weight, and height of corn seedlings (Horii et al., 2007; Duan et al., 2012; Zhang et al., 2015). The stimulation of growth parameters may be the result of improved activity of antioxidants and stress-related enzymes such as guaiacol peroxidase and glucose-6-phosphate dehydrogenase (Ding et al., 2018). Previous studies have shown that insecticides, especially neonicotinoids, cause a decline in natural enemies and pollinators (Moser and Obrycki, 2009; Bredeson and Lundgren, 2018). Therefore, further studies are needed to explore the effects of CHL + CLO and CYA + THI on nontarget insects in the corn field. Finally, we observed that insecticide residues in the CHL + CLO and CYA + THI treatments gradually declined, and the half-life of the combined residues was relatively low when compared that of individual, single applications. Collectively, our results suggest that CHL + CLO and CYA + THI are relatively safe insecticides when applied to seeds and had no negative effect on corn growth.

CONCLUSION

In summary, our results indicate that the application of CHL + CLO and CYA + THI as a corn seed treatment effectively controls FAW larvae on seedlings up to 14 DAE without compromising plant growth and development. Thus, CHL + CLO and CYA + THI are effective, environmentally-friendly insecticidal formulations that reduce the cost associated with single applications of CHL and CYA.

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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LF: methodology, software, data curation, and analysis. JF: data curation. YZ: residues data analysis; WH: residues determination. TD: UHPLC-MS/MS equipment. YH: draft manuscript review and editing. JX: supervision. HL: writing—review and editing, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2022.925171/full#supplementary-material

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