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Research Article

The Efficacy of Chlorantraniliprole as a Seed Treatment for *Mythimna separata* (Walker) (Lepidoptera: Noctuidae)

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The oriental armyworm (OAW), *Mythimna separata* (Walker) (Lepidoptera: Noctuidae), is an important pest in China and causes serious economic losses in corn. The anthranilic diamide, chlorantraniliprole (CHL), has been widely used as a seed treatment to control corn pests; however, no information is available on the efficacy of this insecticide as a seed treatment for OAW. In this study, the efficacy of seed treatment with CHL alone and CHL combined with the neonicotinoid insecticide clothianidin (CHL + CLO) was evaluated for controlling OAW larvae in the laboratory and field conditions. Pot experiments demonstrated that seed treatment with CHL and CHL + CLO (both 240 g a.i. 100 kg^{-1} seeds) resulted in >79% mortality of OAW larvae and a damage rate <20% in corn at 14 days after seed emergence (DAE). Similar to results obtained in pots, the residual toxicity of CHL and CHL + CLO to OAW larvae in the field declined with DAE and larval development. The control efficacy of field plots treated with CHL and CHL + CLO was >70% within 14 DAE, which was significantly higher than CLO alone. These results suggest that CHL and CHL + CLO as seed treatments could effectively reduce OAW larval infestation in corn. This study validates the effectiveness of corn seed treatment for OAW as an alternative to conventional foliar applications.

1. Introduction

The oriental armyworm (OAW), *Mythimna separata* (Walker) (Lepidoptera: Noctuidae), is a polyphagous pest on various crops in Asia and Australia [1–3]. In China, OAW has a long history and occurs throughout the country. OAW larvae feed on multiple grain crops, including corn, wheat, and rice [3, 4]. In corn, larvae feed on leaves and strip them to the midrib, which reduces yield [5, 6]. Damage occurs as a result of adult moth migration, and there are four or more migration events per year in China [3]. Approximately 20 OAW outbreaks occurred from 1950 to 2014 [7], the area was affected, and the amount of damage was staggering. For example, the OAW occurrence area in corn was about 7.00×10^7 hm^2 in 2013, and the estimated yield losses were approximately 9.92×10^5 tons [7].

For many years, the management of OAW relied on spraying chemical insecticides [8–10]; however, *M. separata* has developed resistance to several conventional insecticides, including lambda-cyhalothrin, chlorfenapyr, phoxim, and chlorpyrifos [11–13]. A further study showed that lambda-cyhalothrin significantly stimulated reproduction of OAW moths, thus promoting population growth in the field [4]. Furthermore, the biological characteristics of OAW, especially larval feeding inside the corn whorl, hinder control by spraying foliage. Therefore, the management of OAW requires novel pesticides with a more precise application strategy.

Chlorantraniliprole (CHL) is an anthranilic diamide insecticide that targets insect ryanodine receptors (RyRs) channels and affects the functioning of calcium channels [14]. This insecticide can control a wide range of sucking and chewing insects and is particularly effective against lepidopteran pests, including *Spodoptera frugiperda* [15, 16], *Helicoverpa zea* [17], *Mythimna unipuncta* [18], *Spodoptera exigua* [19], and *Athetis lepigone* [20].

Treatment of seeds with insecticides facilitates the precise targeting of pests [21]. In China, chlorantraniliprole has been widely used as a seed treatment to control corn pests, including *Agrotis ipsilon*, *Pleonomus canaliculatus*, and

Anomala corpulenta [21, 22]; however, no information is available on the efficacy of chlorantraniliprole as a seed treatment for controlling OAW larvae. In this study, we evaluate the efficacy of seed treatments with CHL alone and in combination with neonicotinoid insecticide clothianidin (CHL + CLO). We examined the effects of CHL and CHL + CLO on mortality of OAW larvae and determined residual toxicity of the insecticides to OAW in the laboratory. Finally, we evaluated the effect of these insecticides on corn growth and efficacy for controlling OAW in the field.

2. Materials and Methods

2.1. Insect Rearing. *M. separata* was originally collected in June 2014 from infested corn fields in Qianxi City (27.00°N, 106.03°E), China. Insects were maintained at the Institute of Plant Protection, the Guizhou Academy of Agricultural Sciences. Larvae were reared on a diet of corn leaves at 25°C under a 16 : 8 h light : dark photoperiod as described previously [23]. Second, third, and fourth instar larvae were used in this study.

2.2. Seed Treatments. Seeds of corn cv. Jinyu 818 (non-transgenic) were provided by Guizhou Jinlong Technology Co., Ltd. Seeds were treated with recommended rates (Table 1) of CHL, CHL + CLO, and CLO as described previously [21]; untreated seeds were used as a control. Fifty treated seeds per treatment were sown in plastic pots (30 × 20 × 20 cm) containing a mixture of sand/clay/organic matter (4 : 4 : 2) in a climate-controlled room at 25°C, 70% RH with a 14 : 10 h (L : D) photoperiod. Water was provided during seed emergence and growth, as necessary.

2.3. Pot Experiments. Laboratory assays were conducted in April 2020. At 3, 7, 14, 21, and 28 d after seedling emergence (DAE), 20 newly molted 3rd instar larvae of *M. separata* larvae were transferred to corn plants. To prevent escape, corn plants and larvae were placed in nylon cages (60 × 60 × 60 cm). Each cage was considered as one replication, and each treatment included four replications. After 3 d, the number of dead OAW larvae and the proportion of corn plants damaged by OAW larvae (defined as the damage rate) were recorded. Plants were considered damaged if the feeding spot caused by FAW larvae was found on corn leaves. Larvae were considered dead if they failed to move when stimulated with a moist brush.

2.4. Residual Toxicity of Insecticides to OAW. Seeds were planted in fields located at the Guizhou Academy of Agricultural Sciences (26.48°N, 106.65°E) on 23 May 2020; the treatment area was 20 m^2. Plants were watered as needed and fertilized with a controlled release fertilizer. To prevent feeding by other insects, pots were caged with a nylon net after seedling emergence. Residual toxicity was measured on treated corn plants collected at 3, 7, 14, 21, and 28 DAE.

Twenty newly molted 2nd, 3rd, and 4th instar larvae were collected, transferred to plastic containers (diameter, 12 cm; height, 6 cm), and starved for 2 h prior to experiments. The larvae were fed on insecticide-treated corn leaves at each sampling date. Larval mortality was evaluated after three days, and larvae were considered dead if they were unable to move when stimulated with a moist brush. Each treatment was replicated four times.

2.5. Field Experiments. Field studies were conducted in Luodian County (25.62°N, 106.63°E) in 2021. Sixteen plots were arranged in a randomized, complete block design with four treatments and four replications. Treated corn seeds were sown on June 3, 2021, and a controlled release fertilizer was applied. 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 at 7 DAE, and plant height was determined by measuring random plants ($n = 20$) in each plot at 14 DAE. Each treatment included four replications.

The density of *M. separata* larvae in the field was generally low; therefore, the efficacy of insecticidal formulations was also evaluated by inoculating field-grown corn with larvae reared in the laboratory. A single newly molted 3rd instar larva was inoculated into corn whorls at 7 and 14 DAE, respectively, and fifty individuals were included in each plot. The plot was caged with nylon netting to prevent escape. Each plot was considered as on replication, and each treatment included four replications. The damage rate of corn and the number of survived larvae were recorded 3 d after inoculation.

2.6. Statistical Analysis. The damage rate of corn, corrected mortality, and control efficacy were calculated as follows:

$$\text{Damage rate (\%)} = \frac{\text{number of corn with OAW damage}}{\text{total number of investigated corn plants}} \times 100, \quad (1)$$

$$\text{Corrected mortality (\%)} = \frac{\text{mortality in treatment} - \text{mortality in control}}{100 - \text{mortality in control}} \times 100, \quad (2)$$

$$\text{Control efficacy (\%)} = \frac{\text{\#OAW in control plot} - \text{\#OAW in treated plot}}{\text{\#OAW in control plot}} \times 100. \quad (3)$$

Variables evaluated include the damage rate of corn, corrected mortality of larvae, seed emergence rate, plant height, and control efficacy of insecticides. One-way ANOVA was used to determine the statistical difference among treatments at each sampling date, followed by Tukey's test.

Table 1: Insecticides and dosages used in this study.

Treatment	Insecticide	Dose (g a.i.100 kg^{-1} corn seed)	Producer
CHL	Chlorantraniliprole	CHL 240	DuPont Crop Protection (USA)
CHL + CLO	Chlorantraniliprole + clothianidin	CHL 60 + CLO 180	Guangdong Kairuifeng Technology Co., Ltd. (China)
CLO	Clothianidin	CLO 120	Hebei Lishijie Technology Co., Ltd. (China)
CK	Control	—	—

Results were considered significant at $P < 0.05$. All statistical analyses were performed using DPS v. 17.0 software [24].

3. Results

3.1. Efficacy of CHL and CHL + CLO Seed Treatments for OAW Larvae in Pot Experiments. Overall, the corrected mortality of OAW larvae fed on corn plants treated with CHL and CHL + CLO declined with increasing days of seed emergence (Figure 1(a)). At 3 and 7 DAE, OAW mortality in CHL and CHL + CLO treatments exceeded 90%, which was significantly higher than mortality in CLO treatment. Beginning at 14 DAE, OAW in CHL and CHL + CLO treatments began to decline and was 79.74% and 79.55%, respectively; at 21 DAE, mortality in CHL and CHL + CLO treatments was below 60% but remained higher than CLO. At 28 DAE, OAW mortality in CHL, CHL + CLO, and CLO treatments was below 30.00% with no significant difference among three treatments.

The percentage of corn plants damaged by OAW in the untreated control was about 80% and remained stable and high throughout the experiment (Figure 1(b)). From 3 to 14 DAE, damage rates in CHL and CHL + CLO treatments ranged from 11.00 to 17.10%, respectively, and values in the CLO treatment ranged from 49.00 to 63.50%. All damage rates were lower than the control at each sampling date. Beginning at 21 DAE, damage rates in CHL and CHL + CLO treatments increased but remained lower than the CLO treatment and untreated control. Damage rates of corn plants in CLO and control treatments were not significantly different at 21 and 28 DAE (Figure 1(b)).

3.2. Determination of Residual Insecticide Toxicity. The residual toxicity of CHL and CHL + CLO treatments to *M. separata* gradually declined with the number of days after seed emergence (Figure 2). Mortality of 2nd and 3rd instar larvae in CHL and CHL + CLO treatments exceeded 67% when OAW fed on plants at 3–14 DAE. Although there was no significant difference in mortality rates between CHL and CHL + CLO treatments, they were, generally, higher than mortality in CLO treatment. As corn plants grew, mortality in CHL and CHL + CLO treatments decreased; for example, mortality of 2nd and 3rd instar larvae was below 50% beginning at 21 DAE (Figures 2(a) and 2(b)). Furthermore, OAW mortality declined as larvae developed; for example, mortality of 2nd and 3rd instar larvae in CHL and CHL + CLO treatments ranged from 82.35 to 89.24% at 3–7 DAE, but was only 70.01–73.16% in 4th instar larvae for the same time period (Figure 2(c)). Similarly, CHL and CHL + CLO treatments resulted in over 67% mortality in the 2nd and 3rd instar larvae at 14 DAE and declined to less than 48% in 4th instar larvae (Figures 2(a)–2(c)).

3.3. Efficacy of CHL and CHL + CLO Seed Treatments for OAW Larvae in the Field. The emergence rates of corn seeds were above 90% in the four treatments, and no significant differences were observed among treatments (Table 2). At 14 DAE, corn plants in plots treated with CHL + CLO and CLO were significantly taller than in plots treated with CHL and CK treatments. The percentage of corn seedlings with OAW damage in CHL and CHL + CLO treatments was below 20% at both 7 and 14 DAE, and these values were significantly lower than CLO and CK treatments. At 7 DAE, control efficacy of CHL and CHL + CLO treatments was 86.59% and 84.91%, respectively, and was significant than CLO treatment (36.87%). At 14 DAE, control efficacy of CHL (72.14%) and CHL + CLO (74.56%) decreased but remained higher than CLO treatment (34.91%).

4. Discussion

Insecticide resistance is a challenge in integrated pest management [25]. Although seed treatments with insecticides are excellent choices for pest control, the efficacy of seed treatments for OWA has not been previously reported. Our study clearly shows that seed treatments with CHL and CHL + CLO can reduce OAW-mediated damage to corn plants and can result in control levels exceeding 70% up to 14 DAE. These results indicate that CHL alone or in combination with CLO can effectively control early stage OAW larvae on corn seedlings and has relatively good persistence.

Insecticide treatments can effectively reduce feeding injuries inflicted by insects [18]. The percentage of corn plants with OAW damage in CHL and CHL + CLO treatments was less than 20% up to 14 DAE and was significantly lower than damage in CLO and CK treatments. These results are consistent with a prior study conducted on seed treatments for *M. unipuncta* [18]. Prior studies have shown that CHL can result in rapid feeding cessation of several insect species; for example, the lepidopteran species, *Plutella xylostella*, *Trichoplusia ni*, and *H. zea*, stopped feeding within 30 min after exposure to plants grown from CHL-treated seed [26]. The damage rate in CHL-treated plants was reduced by 50–99% [18,26]. Similarly, *Coptotermes gestroi* feeding stopped within five minutes after exposure to CHL-treated plant materials [27]. In our study, CHL treatment alone or in combination with CLO reduced the damage rate caused by OAW larval feeding.

In previous studies, CHL showed a systemic insecticidal activity against various pests when applied as a seed

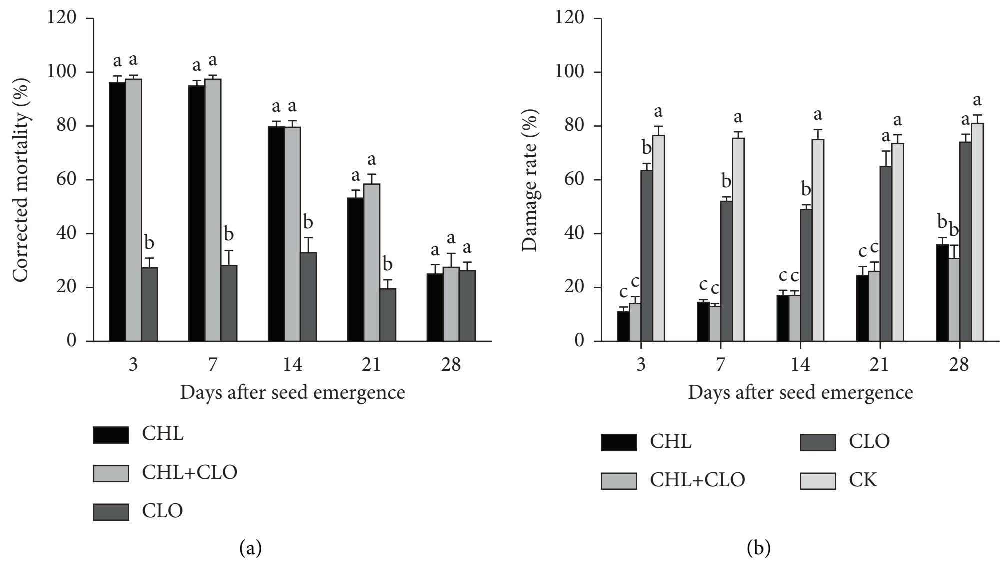

Figure 1: Corrected mortality and damage rates of OAW fed on corn plants treated with CHL, CHL + CLO, and CLO. (a) Corrected mortality. (b) Damage rate. CHL, chlorantraniliprole; CHL + CLO, chlorantraniliprole + clothianidin; CLO, clothianidin; CK, untreated control. All data are expressed as mean ± SE. Different letters above bars indicate significant difference by Tukey's test ($P < 0.05$).

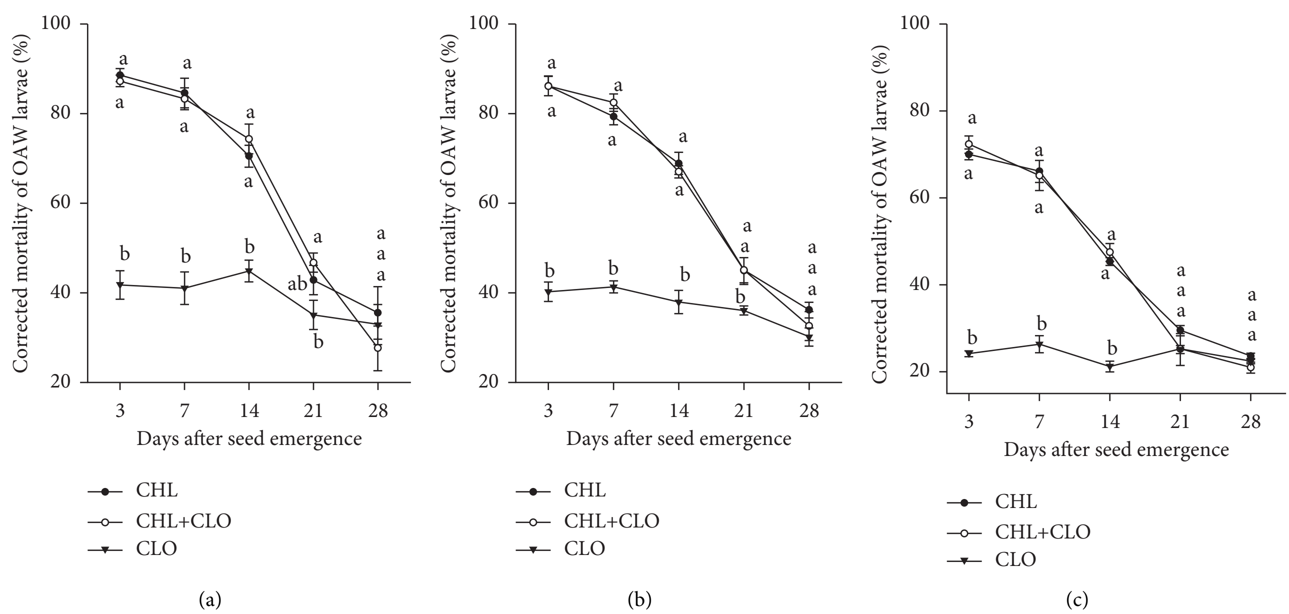

Figure 2: Mortality of OAW larvae fed on field-grown plants treated with CHL, CHL + CLO, and CLO. Panels show mortality for 2nd (a), 3rd (b), and 4th (c) instar larvae. CHL, chlorantraniliprole; CHL + CLO, chlorantraniliprole + clothianidin; CLO, clothianidin. Data are analyzed using one-way ANOVA followed by Tukey's test at each sampling date, and different letters indicate significance at $P < 0.05$.

Table 2: Control efficiency and growth indices of corn seedlings treated with CHL, CHL + CLO, and CLO in the field*.

Treatment	Concentration (g a.i.100 kg^{-1} corn seed)	Emergence rate (%)	Plant height (cm)	7 DAE Damage rate (%)	7 DAE Control efficacy (%)	14 DAE Damage rate (%)	14 DAE Control efficacy (%)
CHL	240	91.62 ± 2.33^{a}	59.22 ± 3.90^{b}	$11.62 + 1.52^{c}$	86.59 ± 2.04^{a}	16.84 ± 1.50^{c}	72.19 ± 2.02^{a}
CHL + CLO	240	90.38 ± 0.97^{a}	69.30 ± 0.81^{a}	12.76 ± 1.93^{c}	84.91 ± 2.30^{a}	15.15 ± 1.75^{c}	70.56 ± 2.44^{a}
CLO	120	92.47 ± 1.56^{a}	68.91 ± 0.25^{a}	39.29 ± 3.37^{b}	36.87 ± 3.34^{b}	43.36 ± 2.90^{b}	34.91 ± 2.46^{b}
CK	—	90.89 ± 1.64^{a}	56.61 ± 0.68^{b}	73.16 ± 4.74^{a}	—	71.50 ± 2.98^{a}	—

*CHL, chlorantraniliprole; CHL + CLO, chlorantraniliprole + clothianidin; CLO, clothianidin; CK, untreated control. Data are analyzed using one-way ANOVA followed by Tukey's test, and different lowercase letters indicated significance at $P < 0.05$.

treatment. For example, seed treatment with CHL at 25 μg a.i. seed^{-1} resulted in 90.4% suppression of immature water weevils approximately 25 d after flooding [28]. A corn seed treated with CHL at 2 g a.i. kg^{-1} provided 76.02% control efficacy against *A. ipsilon* larvae at 19 DAE [21]. Similarly, Pes et al. found that CHL applied at 45 g a.i. per 60,000 corn seeds significantly reduced infestation by *S. frugiperda* larvae up to 30 d after planting [15]. The current study indicated that seed treatments with CHL and CHL + CLO caused systemic insecticidal activity against OAW larvae. High mortality rates (70%) were observed up to 14 DAE and began to decline thereafter, suggesting that the efficacy declined due to the dilution of active ingredients. Furthermore, the control efficacy decreased in the 4th instar larval stage, which suggests that insecticide concentrations were insufficient to kill older larval instars.

Interestingly, CHL and CHL + CLO treatments showed similar efficacy for controlling OAW larvae, although the concentration of CHL in the CHL + CLO treatment was lower than the concentration of CHL alone. In this respect, our results were similar to those reported previously [18], where CHL and CHL + THI (thiamethoxam) had similar efficacy in reducing foliar injury to corn by *M. unipuncta* [18]. Our results suggests that CHL and CLO function synergistically when combined, although the underlying mechanism is unclear and warrants a further study. Potential synergism could reduce both the amount and cost of pesticide applications and may delay the onset of insecticide resistance.

In recent years, the subterranean pests *A. ipsilon*, *Proxenus lepigone*, *P. canaliculatus*, and *A. corpulenta* caused serious damage to corn seedlings in China, and diamide insecticides have been used to manage these pests as seed treatments [21, 22, 29]. Neonicotinoid insecticides, such as CLO, are considered effective for controlling aphids and thrips on various crops [30–32]. Thus, the use of CHL and CHL + CLO as seed treatments can control both OWA and other target pests on corn. Furthermore, seed treatment reduces insecticide exposure to nontarget organisms and has fewer side effects to nontargets residing on corn plants and in soil. Finally, we found that treating a corn seed with CHL + CLO promotes corn growth, which is consistent with previous studies [21, 33].

5. Conclusion

In summary, our results indicate that CHL and CHL + CLO seed treatments effectively control OAW larvae on corn seedlings up to 14 DAE. The OAW-induced damage rate was low in both CHL and CHL + CLO treatments as compared to the untreated control. Thus, the use of CHL and CHL + CLO as seed treatments offers potential alternatives to conventional foliar sprays in the management of OAW on corn.

Data Availability

The data used to support this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

H.L. conceptualized, supervised, validated, visualized, and investigated the study, performed data curation, formal analysis, funding acquisition, and project administration, collected resources, and developed software. H.L. and C.D. performed the methodology. H.L. and Y.H wrote and reviewed the article. All authors have read and agreed to the published version of the manuscript and contributed equally to this study.

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