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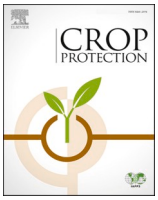

Resistance and fitness costs of the *Helicoverpa armigera* after selection with the tetraniliprole newly developed diamide insecticide

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ABSTRACT

The cotton bollworm (*Helicoverpa armigera*) (Lepidoptera: Noctuidae) is a highly destructive insect pest that feeds on more than 200 plants including cotton. Diamide insecticides are typically preferred to combat *H. armigera*. However, many reports of resistance to diamide insecticides such as chlorantraniliprole have been reported. Tetraniliprole is a novel diamide insecticide, but little is known about its resistance risk in *H. armigera*. After ten generations of treating *H. armigera* with tetraniliprole, neither the G4946 nor I4775 mutations were detected and no resistance to tetraniliprole was observed. The realized heritability (h^2) was 0.094 after ten generations of selection with tetraniliprole, suggesting a low risk of resistance in *H. armigera*. Additionally, we found that the LD_{50} of tetraniliprole (4.01 μg/g) was lower than the LD_{50} of chlorantraniliprole (5.79 μg/g) in the susceptible strain. Molecular docking analysis also found a difference between the ryanodine receptor (*RyR*) binding sites of tetraniliprole and chlorantraniliprole, the tetraniliprole free binding energy to *RyR* was −6.9 kcal/mol, which was smaller than for chlorantraniliprole (−6.4 kcal/mol). Our study reveals that there is a low resistance risk to tetraniliprole in *H. armigera*, thus suggesting that tetraniliprole can be used as a substitute for other insecticides for this pest control.

1. Introduction

Helicoverpa armigera (Lepidoptera: Noctuidae) is the agricultural pest known to harm crops in Asia, Europe, Oceania, Africa, and South America (Razaq et al., 2007; Pereira et al., 2020). It primarily damages crops in the family Gramineae, however, its polyphagous nature extends to vegetables and beans, and more than 200 plants are being inflicted (Dourado et al., 2021; Haile et al., 2021; Mironidis et al., 2010). *H. armigera* commonly affects pome fruit, stone fruit, tree nuts, small fruit vine climbing crops, fruiting vegetables, tuberous and corm vegetables, *Brassica* head and stem vegetables, and leafy vegetables (Krinski and Godoy, 2015).

Many techniques, including chemical and biological methods like *Bacillus thuringiensis* (*Bt*) have been considered for preventing attacks of *H. armigera*, but none have successfully controlled it. Due to the serious crop damage from *H. armigera*, the inclusion of the Cry1Ac gene from *Bt* in cotton was highly recommended, because it was thought cultivating *Bt* cotton would decrease *H. armigera* infestation and boost crop yields. However, *Bt* resistance has drastically increased in all places growing *Bt* cotton including Mexico. Frequent use of *Bt-based* crop varieties has led to ineffective management of lepidopteran pests including *S. frugiperda* and *H. armigera* (Kranthi and Stone, 2020; Tabashnik et al., 2012; Walsh et al., 2018; Yu et al., 2022).

The increasing global cases of insect resistance to insecticides is one of the biggest challenges facing agriculture today. *H. armigera* as the nocturnal species with the greatest number of reported incidences of resistance to insecticides globally, *H. armigera* (Hübner), has developed resistance against pyrethroids, organophosphates, carbamates, and organochlorines (Aheer et al., 2009; Jouβen et al., 2012). Moreover, *H. armigera* is involved in resistance to a wide range of chemical insecticides such as pyrethroids over a long period due to intensive and widespread agricultural use. Resistance mechanisms include target-site mutation, metabolic detoxification, and behavioural adaptation (Walsh et al., 2018). Tetraniliprole (C22H16ClF3N10O2) is a novel diamide insecticide in which the bromine atom has been replaced with a [5-(trifluoromethyl)-2H-tetrazol-2-yl] in the methyl group as well a

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molecular weight of 544.9 g/mol (Peter, 2021). The mode of action of tetraniliprole involves targeting the ryanodine receptors of insects which play a key role in muscle function and calcium regulation. A little information is available about tetraniliprole, however, there is no evidence of resistance to this insecticide in lepidopterans, supporting its potential use as an alternative pesticide for this insect.

This study aimed to investigate the response of *H. armigera* to novel tetraniliprole and determine its risk of resistance to the insecticide. The results indicated that exposing *H. armigera* to tetraniliprole affects fitness costs. Our study reveals a low risk of resistance to tetraniliprole developing in *H. armigera*.

2. Materials and methods

2.1. Insect rearing

The strain used in this study was first collected in 1998 from Handan city in Hebei province, China. For more than 24 years, then has been kept alive in a laboratory without exposure to any insecticides. The strain was maintained carefully by incubating it under regulated conditions, including a photoperiod of L: D = 16: 8, a temperature of 27 ± 0.5 °C, and a humidity level of 70 ± 10%. When individuals pupated, they were kept in a cage enclosed with black cloth and covered with clean sterile white gauze. The moths were fed 10% honey water after emergence. After, moths were mated to produce successful offspring.

2.2. Preparation of artificial diet

An artificial diet was made by following the method described by (Li et al., 2021) with a little modification on diet ingredients, streptomycin sulfate was substituted by roxithromycin, the diet main ingredients such as maize-flour, soybean meal and yeast extract powder, were mixed with 350 mL of water in the cooking pan and was boiled while continuously stirring for at least 30 min. Agar strips were thoroughly dissolved in 150 mL of hot water while being mixed up until they were turned into porridge and combined with the main component. Other ingredients, including streptomycin sulfate, ascorbic acid, vitamin B and C, Chinese medicine, and citric acid monohydrate, were crushed by a mortar and pestle and then dissolved in 40 mL of hot water. After the diet was well mixed, stirred, and cooled to 60 °C. 5 mL of propionic acid was added and well stirred after the diet had been thoroughly combined, swirled, and cooled to 60 °C. After the mixture had solidified for 30 min, it was put in a refrigerator set to 4 °C.

2.3. Bioassay

To prevent larval movement during the application of tetraniliprole, spinosad, chlorantraniliprole, chlorfenapyr, beta-cypermethrin and emamectin benzoate, all of the larvae were placed in an ice box for 1 min. A 1.0 μL drop was applied on the dorsum thoracic of the 3rd instar *H. armigera* larvae. Ten larvae were chosen and placed in disposable petri dishes measuring 9 cm.

Immediately after treatment larvae were transferred into new petri dishes and fed on an artificial diet. Before applying tetraniliprole, a stock solution was made by dissolving insecticide in an acetone solvent. Then each working solution was replicated three times to reduce variation in the data; in another cohort of *H. armigera* larvae, only acetone was applied as a control. Mortality counts were recorded 72 h after application of tetraniliprole. Larvae were deemed dead if they did not show normal motion when touched with a smooth disinfected brush.

2.4. Insecticides

Insecticides used in the experiments were purchased from various companies: lambda-cyhalothrin and chlorfenapyr were from Jiangsu Fine Chemical Co. Ltd; chlorantraniliprole, emamectin benzoate, and spinetoram came from FMC Investment Co., Ltd. (China), Hebei Pharmaceutical Co., Ltd., and Kedihua Agricultural Technology Co., Ltd., respectively.

2.5. DNA extraction and PCR

A tissue/cell genome DNA isolation kit (Aidlab, China) was used to extract DNA from 60 individual *H. amigera* larvae of the F0 and F10 in each generation, following the manufacturer's instructions. DNAMAN version 8.0 (LynnonBiosoft, USA) was used to design forward and reverse primers for G4924 (5′-ACAACTCGTTCCTATACTCTC-3′ and 3′-TGTTTCCCGTTATGCGTGAC-5′) and I4775 (5′-GGAGGCACGGTGAA-GAAGACGAAG-3′ and 3′-CCTTCAAATGGTAGTACCCGATCAG-5′). PCRs using 2 μL DNA template, 2 μL reverse primer, 2 μL forward primer, 12.5 μL 2 × TaqMasterMix, and 6.5 μL ddH_2O were carried out in a final volume of 25 μL. PCR protocols included 40 cycles of denaturation at 94 °C for 15 s, annealing at 53 °C for 15 s, and extension at 72 °C; the initial denaturation phase lasted 3 min at 94 °C. Gel electrophoresis yielded fragment sizes of 150 bp and 156 bp for G4924 and I4775, respectively; PCR products were sent to Beijing Qingke Biological Co., Ltd. for sequencing.

RT-qPCR was performed in 20 μL reactions containing 1.0 μL cDNA, 10 μL of SYBR Premix Ex Taq (Takara, China), 0.15 μL of both reverse and forward primers (10 μM), 8.3 μL nuclease-free water and 0.4 μL of Rox Reference Dye II (50 ×). Primers for *H. armigera RyR* (forward primer 5′-CAACCAGGTCAAGTCAAC-3′ and reverse primer 3′-CACAGTCGAACGCTCAGATG-5′) were designed using DNAMAN version 8.0 (LynnonBiosoft, USA). Thermal cycling conditions were: 50 °C for 2 min, 95 °C for 30 s, 40 cycles of 95 °C for 15 s, 60 °C for 34 s, and 72 °C for 30 s. Following the cycling procedure, all reactions were subjected to a melting curve analysis from 60 °C to 95 °C to confirm the presence of a single PCR product.

2.6. Resistance screening

A susceptible population of *H. armigera* was selected for laboratory tests to determine whether the population could reproduce stably under selection pressure. If the concentration of tetraniliprole was too low, it would be dissimilar from the actual situation in the field, and if the concentration was too high, the experimental population could not reproduce stably for a long time. Therefore, the mortality rate was determined at about 50%, and bioassays of tetraniliprole were conducted for each generation of *H. armigera* during the screening process. According to bioassay results, the screening concentration of this generation was determined to control the mortality within a reasonable range (Cui et al., 2018).

Resistance ratio was calculated as follows:

$$\text{Resistance ratio (RR)} = \frac{\text{LD}_{50} \text{ of the resistant strain}}{\text{LD}_{50} \text{ of the susceptible strain}}$$

2.7. Evaluation of H. armigera fitness

Separate life tables were constructed for the F0 and F10 generations. A total of 120 eggs were collected out of which 100 successfully hatched and were immediately transferred into plates with 12 wells; each larva was placed individually using a smooth brush and then fed artificial food. The food was replaced every 48 h. Virgin adult moths were mated and then females were permitted to lay eggs. The age-specific fecundity of females [f_{x7}], age-stage survival rate [s_{xj}], age-specific survival rate [l_x], age-specific fecundity [m_x], age-specific maternity [$l_x m_x$], age–-stage specific life expectancy [e_{xj}], reproductive value [v_{xj}], intrinsic rate of increase [r], finite rate [λ], net reproductive rate [R_0], and mean generation time [T] were used to construct life tables calculated using the TWO SEX-MS-Chart program (Chen et al., 2017; Jaleel et al., 2018).

Intrinsic growth rate $r_m = \ln R_0/T$

Generation mean period $T = \sum \text{xl x } m_x \Big/ R_0$

Weekly growth rate $\lambda = e^{rm}$

Population doubling time $Dt = \ln 2/r_m$

Net growth rate $R_0 = \sum l_x m_x$

where **X** represents adult individuals in a particular $\mathbf{l_x}$ Individual survival at age, $\mathbf{m_x}$ indicates the number of eggs laid by females at **x** age. (Janssen et al., 2022; Ning et al., 2017)

2.8. Realized heritability

The realized heritability of resistance is a vital index for a risk assessment of resistance development, and its calculation formula is h^2 = R/S, where R represents the selection reaction before and after the screening and was determined by R = [lg (final LD_{50}) - lg (Initial LC_{50})]/n; and S is calculated as S=I × δP, where I represents the selected strength, using formula I = 1.583-0.0193336 P+ $0.0000428p^2$+3.651941/P ($10 \leq P \leq 80$); P is calculated as P = 1 – average adjusted mortality.

Phenotypic standard deviation $\delta P = [(\text{initial slope} + \text{last generation slope})/2]^{-1}$.

2.9. Comparison of cross-sensitive reduction

To determine the insecticide resistance in both resistant and susceptible populations after LD_{50} treatment, cross-resistance was tested in five insecticides: emamectin benzoate, spinosad, chlorfenapyr, beta cypermethrin, and chlorantraniliprole. Then, the formula for cross-resistance RR (resistance ratio) = LD_{50} of resistant strain/LD_{50} of susceptible strain was used to determine whether cross-sensitive reduction occurred.

2.10. Molecular docking

AutoDock software version 4.2 (GPL-FSF) was used to conduct molecular docking (Stanzione et al., 2021). The study evaluated a specific region of the protein, whereas the other looked at the entire protein. We identified, obtained, and verified a potential ligand-binding site. Molecular protein complex coordinates were cleaned, missing hydrogens and side chain atoms were added, and the complex was divided into a macromolecule (LOCK) and a ligand (KEY).

2.11. Statistical analysis

Bioassay results were processed by POLO Plus software version 2 (LeOra Software, Berkeley, CA, USA). LD_{50} value was considered to be significantly different if the 95% confidence intervals of the ratio were on either side of value 1. The biological characteristics of *H. armigera*, such as its developmental period, pupal and feathering rates, and egg-laying capacity, were assessed using Student's *t*-tests in SPSS 28.0 (IBM, USA).

3. Results

3.1. Screening for tetraniliprole resistance in H. armigera

The drop method was used to select a susceptible population of *H. armigera* to test tetraniliprole resistance, with a selection pressure of about 50%. The sensitivity of the chosen population was 3.63 times greater than that of the susceptible population after ten generations of resistance breeding. LD_{50} increased in the susceptible population from 1.10 μg/g to 4.01 μg/g. During the breeding process, LD_{50} values were low and fluctuated in the 1st – 4th generations, however were quick in the 5th generation it is quick (Table 1).

3.2. Realized heritability of resistance of H. armigera

The broad trait analysis method was used to calculate the realized heritability (h^2) of the population to tetraniliprole after ten generations of breeding at a tetraniliprole selection strength of 0.83. The specific results (Table 2) indicated that *H. armigera* has a low risk of developing resistance to tetraniliprole with realized heritability (*h2*) of 0.094.

3.3. Effect of tetraniliprole on life table and fitness cost to H. armigera

The net fertility rate, internal growth rate, and weekly growth rate of the control and treated cohorts were computed in accordance with the survival rate and egg laying rate at an average time of 35.60. Results revealed that the control cohort was larger than the treated one. Compared with the control cohort, *R0*, r_m, and λ of the treated cohort were reduced, and *T* and *Dt* were increased indicating that tetraniliprole inhibits the growth and development of the cotton bollworm (Table 3).

The pupation rate, emergence rate, and adult life span of the selected population were lower after ten generations of breeding *H. armigera* resistance to tetraniliprole than in the susceptible population, but the difference was not statistically significant. The resistant strain of *H. armigera* had a population trend index after selective breeding of 86.73, which was lower than the susceptible population, and a relative fitness of 0.51, making it less fit than the susceptible strain (Table 5).

3.4. Comparison of cross-sensitivity reduction to tetraniliprole

A cross-sensitivity reduction test was conducted using five insecticides (chlorantraniliprole, emamectin benzoate, spinosad, chlorfenapyr, and beta cypermethrin on the *H. armigera* strain. Screening for chlorantraniliprole revealed low levels of cross-sensitivity reduction with other insecticides and a positive interaction with resistance of 2.3-fold, followed by beta-cypermethrin resistance of 1.6-fold (Table 4).

Table 1
Selection of resistance to tetraniliprole in *H. armigera*. LD_{50} increased in the susceptible strain from 1.10 μg/g to 4.01 μg/g. In the breeding process, LD_{50} increased was slow and fluctuated in the 1st – 4th generations, however was quick in the 5th generation.

Generation	LD_{50} (95% CL) (μg/g)	Slope + SE	χ^2 (df)	Resistance ratio
F_0	1.10 (0.64 2.86)	1.061 ± 0.19	5.66 (18)	1.0
F_1	–	–	–	–
F_2	0.82 (0.62–1.08)	1.415 ± 0.17	11.55 (25)	0.74
F_3	0.97 (0.77–1.32)	1.626 ± 0.21	10.20 (22)	0.88
F_4	1.23 (0.77–1.32)	1.588 ± 0.18	10.00 (26)	1.18
F_5	1.64 (1.24–2.21)	1.325 ± 0.14	15.81 (26)	1.49
F_6	2.75 (1.89–4.38)	1.011 ± 0.13	10.73 (26)	2.50
F_7	2.91 (2.02–4.60)	1.058 ± 0.14	12.17 (26)	2.64
F_8	3.15 (2.3–4.39)	1.207 ± 0.14	14.38 (26)	2.87
F_9	3.44 (2.40–5.49)	1.325 ± 0.147	10.73 (26)	3.12
F_{10}	4.01 (3.18–5.20)	1.530 ± 0.14	16.80 (30)	3.63

Note: Resistance ratio = LD_{50} of the selected population/LD_{50} of the control population.

Table 2
Estimation of realized heritability of resistance to tetraniliprole in *H. armigera*. Tetraniliprole resistance anticipated realized heritability (h^2) was 0.094 with low h^2 indicating that it is difficult to develop tetraniliprole resistance.

Generations of selection	Initial LC_{50}	Final LC_{50}	R	Survival %	Intensity of selection	δp	Selection differential	h^2
10	1.10	4.01	0.0602	47.41	0.83	0.77	0.64	0.094

Table 3
Effects of the LD_{50} of tetraniliprole on life table parameters of *H. armigera*. Results showed that the control cohort was larger than the treated in parameter of net fertility rate, internal growth rate, and weekly growth rate additionally, doubling time and generation average time in control cohort were smaller than treated cohort.

Life table parameter	Control	Treated
Net growth rate R_0	339.8 ± 29.59	160.2 ± 19.36*
Weekly growth rate λ	1.19 ± 0.059	1.15 ± 0.041*
Internal growth rate r_m	0.17 ± 0.005	0.14 ± 0.035*
Doubling time D_T	3.96 ± 0.114	4.86 ± 0.122*
Generation average time T	33.59 ± 0.70	35.60 ± 0.10*

Note: Data in the table are means ± standard errors. * Indicates significant differences between the two strains (Student's *t*-test, $P < 0.05$).

Table 4
Cross-sensitivity reduction of the tetraniliprole-resistant H. armigera population to other insecticides. In screening chlorantraniliprole showed a positive interaction with resistance of 2.3-fold, followed by beta-cypermethrin resistance of 1.6-fold, and low levels of cross-sensitivity reduction with other insecticides.

Insecticide	Slope + SE	χ^2 (df)	LD_{50} (95%CL) (μg/g)	Strain	Resistance ratio
Emamectin benzoate	1.96 ± 0.37	4.89 (16)	11.07 (8.35–14.68)	S	1.0
	1.92 ± 0.26	7.44 (16)	12.34 (9.31–16.49)	R	1.3
Spinosad	1.30 ± 0.16	13.66 (22)	20.51 (4.58–28.73)	S	1.0
	1.48 ± 0.19	8.55 (22)	26.88 (19.72–41.02)	S	1.1
Chlorantraniliprole	1.31 ± 0.17	5.79 (19)	2.32 (1.64–3.26)	S	1.0
	1.12 ± 0.29	6.76 (19)	5.37 (3.65–8.79)*	R	2.3
Beta-cypermethrin	1.80 ± 0.31	8.47 (22)	127.7 (99.42–163.7)	S	1.0
	1.69 ± 0.22	10.73 (22)	191.8 (147.3–256.1)	R	1.6
Chlorfenapyr	1.74 ± 0.22	7.25 (22)	42.41 (32.91–54.91)	S	1.0
	1.73 ± 0.26	9.26 (22)	53.25 (41.31 69.88)	R	1.3

Note: Data in the table are means ± standard error, * indicates a significant difference between control and treatment (Student's *t*-test, $P < 0.05$).

3.5. Effect of tetraniliprole on expression of RyR gene and mutation detection in H. armigera

Resistance mutations were detected by collecting samples of 3rd instar *H. armigera* individuals from the tetraniliprole resistant and susceptible populations. RNA was extracted and qRT-PCR was conducted to detect ryanodine receptor (RyR) gene expression levels in the two populations of *H. armigera*. Gene expression of 3rd instars from the susceptible population was set to 1. Expression of the *RyR* gene in the *H. armigera* population of tetraniliprole screening was significantly higher than that in the susceptible population (Fig. 1a).

Table 5
Effect of tetraniliprole treated for the 10th generation continuously on the development of *H. armigera*. The pupation rate, emergence rate, and adult life span of the selected strains were lower after ten generations of breeding *H. armigera* resistance to tetraniliprole than in the sensitive strain, but the difference was not statistically significant.

Parameter	Susceptible strain	Resistant strain
Eggs/d	2.52 ± 0.05	2.57 ± 0.13
1st instar larvae/d	2.98 ± 0.12	3.07 ± 0.33
2nd instar larvae/d	3.18 ± 0.04	3.18 ± 0.11
3rd instar larvae/d	3.02 ± 0.67	3.25 ± 0.48
4th instar larvae/d	3.28 ± 0.66	3.60 ± 0.88
5th instar larvae/d	3.32 ± 0.53	3.87 ± 0.62
6th instar larvae/d	3.10 ± 0.43	3.42 ± 0.49
Pupation rate/%	84.01 ± 2.10	78.41 ± 10.86
Pupa/d	9.09 ± 0.53	9.84 ± 0.85*
Emergence rate/%	92.26 ± 2.69	83.3 ± 7.91
Number of eggs laid by single female	712.1 ± 100.1	509.7 ± 60.6*
Adult life span/d	8.28 ± 0.73	7.06 ± 0.81*
Hatchability/%	75.1 ± 1.68	70 ± 11.7
Number of female moths (sex ratio 1:1)	32	24
Predict the number of first-hatched larvae in the next generation	17,110	8673
Population trend index	171.10	86.73
Relative fitness	1	0.51

Note: Data in the table are means ± standard error, * indicates a significant difference between susceptible and resistant strain (Student's t-test, $P < 0.05$).

The head of *H. armigera* was used to extract DNA was extracted for PCR detection, and used for multi-sequence comparison with *Plutella xylostella*. Mutation sites of *H. armigera* were sequenced and the sequencing peak plot, with gene frequencies of the resistant and susceptible populations after tetraniliprole screening. The I4775 gene of *H. armigera* corresponded to *P. xylostella* I4790, and G4924. I4775 wild type is isoleucine (Ile) with codon ATA, and is located close to the transmembrane domain TM3. G4924 wild type is glycine (Gly), and is located in T of *H. armigera* between M4 and TM5. After multi-sequence comparison of the resistant and susceptible populations after tetraniliprole screening, no mutations in I4775 and G4924 were found in either the resistant or susceptible population (Fig. 1b).

3.6. Homologous modelling results of C-terminal transmembrane structure of H. armigera RyR

Using the amino acid sequence of *RyR* (PDB:5goA) of rabbits as a template (Fig. 2A), Discovery Studio was used to construct the *H. armigera RyR*. The C-terminal structure model scores the protein model by saving and selecting the best model for subsequent docking experiments. The Raman diagram of the model shows that 93% of the C-terminal structure of the *RyR* protein of *H. armigera* was present. The average 3D-1D score of amino acid residues was ≥0.2; the proportion of amino acid residues in the best position was 86%; and the proportion of amino acid residues in the allowable region was 10.1%. Only 2.1% of the residues were in the uninhibited region, so the model is reasonable and

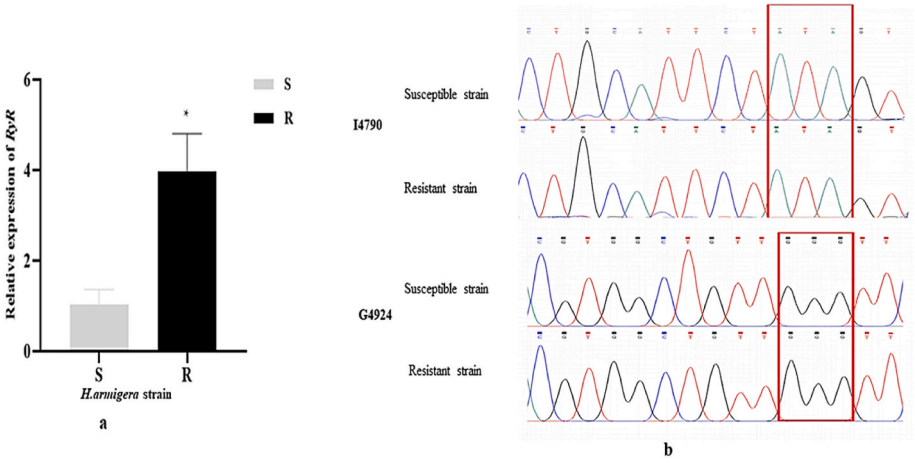

Fig. 1. a Expression of *RyR* in tetraniliprole susceptible and resistant populations of *H. armigera*. Data are mean ± standard errors, with * indicating a significant difference between control and treatment (*t*-test, $P < 0.05$). R = resistant strain and S = susceptible strain **b** Sequencing peaks of *RyR* gene resistance-related mutation sites of *H. armigera*.

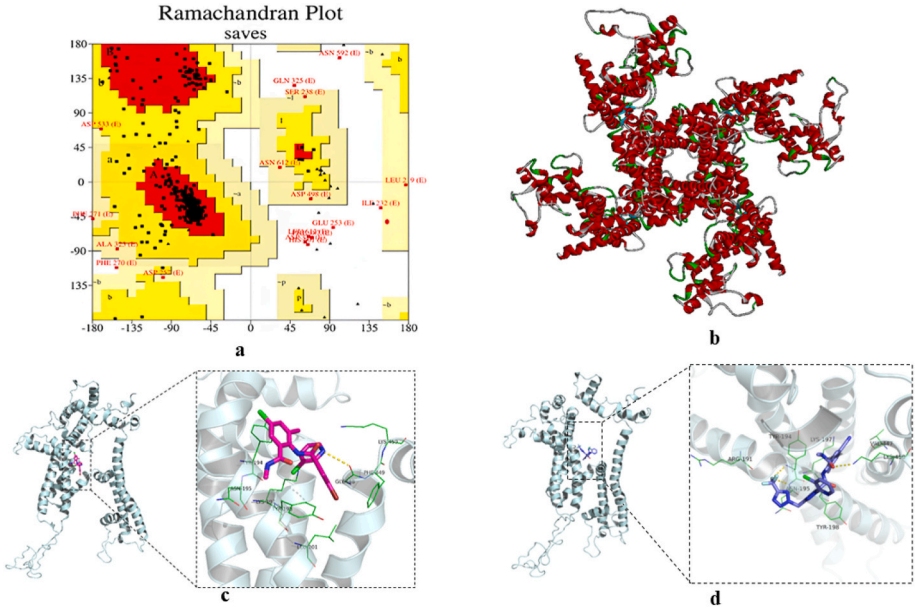

Fig. 2. Representation of the binding on *RyRs* of *H. armigera* by chlorantraniliprole and tetraniliprole. **a** Ramachandran plot of *H. armigera* RyR. **b** C domain models *H. armigera RyR*. **c** Graphical representation of the binding on *RyRs* of *H. armigera* by chlorantraniliprole. **d** Graphical representation of the binding on *RyR* of *H. armigera* by tetraniliprole.

can be used for the next docking experiment.

Docking studies on the C-terminal structural model of the *H. armigera RyR* receptor were conducted using the molecular docking software AutoDock version 4.2 (GPL-FSF) (Fig. 2b). For affinity prediction, tetraniliprole and chlorantraniliprole were tested as ligands. The preferred conformation was selected based on the principle of the lowest binding energy after predicting and examining the docking structure, obtaining a number of conformations (Fig. 2a). Tetraniliprole had a small free binding energy to the *RyR* protein of −6.9 kcal/mol, while the chlorantraniliprole free binding energy to the *RyR* protein was −6.4 kcal/mol (Table 6).

Table 6
Molecular docking results of *RyRs* of *H. armigera* and insecticide ligands. Tetraniliprole had a small free binding energy to the *RyR* protein while the chlorantraniliprole free binding energy to the *RyR* protein was high.

Receptor	Ligand	Binding free energy	Hydrogen Bond	Bond length (Å)	Bond strength
H. armigera RyR receptor	Tetraniliprole	−6.9 kcal/mol	Asn 4674-F_2	3.02	medium
			Tyr 4673- F_2	3.20	medium
			Lys 4929-OH	3.20	medium
	Chlorantraniliprole	−6.4 kcal/mol	Gly 4927-N_4	3.33	medium

Note: The hydrogen bond with a ligand-acceptor distance of 2.5–3.2 Å is medium intensity, mainly electrostatic force, and the hydrogen bond with a donor-receptor distance of 1.6–2.5 Å is stronger and mostly covalent bonds.

4. Discussion

Many conventional insecticides including organophosphorus, beta cypermethrin, deltamethrin, and chlorpyriphos are no longer effective against *H. armigera* due to resistance, which has been measured at 10.1 g/g, 10.5 g/g, 24.1 g/g, and 16.5 g/g LC_{50}, respectively. Beta cypermethrin, deltamethrin, chlorpyriphos, and organophosphorus each contribute resistance ratios of 5.2-, 6.7-, 8-, and 5.5-fold, respectively (Achaleke and Thierry, 2010). Moreover, chlorantraniliprole has developed resistance based on an LC_{50} of 9.6 μg/g in *H. armigera* (Kliot and Ghanim, 2012; Ma et al., 2019; Dourado et al., 2021; Tang et al., 2021). This has caused ineffective control against *H. armigera*, threatening food security.

Assessing resistance risk is a crucial step in managing resistance because it models how resistance might emerge in the field under indoor conditions. Insecticide resistance is a serious problem in pest management, and researchers have initiated several measures to mitigate it, including developing new types of insecticides. A new tetraniliprole product has not been widely used to control lepidopteran pests, so there are no reports of tetraniliprole resistance risk. However, it is extremely important to closely monitor the resistance of pests to tetraniliprole (Hawkins et al., 2019; Dourado et al., 2021; Tang et al., 2021). In our study, tetraniliprole resistance in *H. armigera* reached 3.63-fold after 10 generations, and the anticipated realized heritability (h^2) was 0.094. This low h^2 indicates that it is difficult for *H. armigera* to develop tetraniliprole resistance. This h^2 is higher than broflanilide in *P. xylostella*; after 10 generations of selection h^2 was 0.033, meaning that *P. xylostella* is unlikely to develop resistance to broflanilide (Sun et al., 2022; Cui et al., 2018). Furthermore, Roy et al. (2023) found that *S. frugiperda* collected from the field after sequential selection of ten generations through continuous exposure to fluxametamide had an h^2 of 0.084.

Moreover, our results indicated that exposure to tetraniliprole inhibits egg production of *H. armigera* compared with the control cohort. The *R0*, r_m, and λ of the treated cohort were reduced, and the *T* and *Dt* were increased (Table 3), indicating retarded growth and development of *H. armigera*. Additionally, the survival rate, egg laying capacity and number of eggs laid at a specific time, the net fertility rate, internal growth rate, and weekly growth rate of the control and the treated cohorts indicated that the control cohort was larger than the treated cohort. This finding is similar to a study on the effects of chlorantraniliprole in the F1 generation of *P. xylostella*, which prolonged the length of the 4th instar larva to pupal phase by 4.27 days compared to 3.34 days in the control, pupal weight was reduced by 3.58 mg compared to 4.17 mg in the control, and adult fecundity decreased by 42% (Zhang et al., 2013). Tetraniliprole affects the insect reproductive system destroying cells, tissues, and sexual desires of insects. In this study, the treated cohort had fewer eggs than the control cohort signifying that novel tetraniliprole can be used to reduce the population of *H. armigera*.

Our study revealed that tetraniliprole had a binding energy of −6.9 kcal/mol, while chlorantraniliprole had higher binding energy of −6.4 kcal/mol in the *RyR* protein, demonstrating that novel tetraniliprole performs better in the *RyR* protein of *H. armigera* than chlorantraniliprole. Tetraniliprole binds effectively and more quickly to the insect *RyR* protein structure than chlorantraniliprole, suggesting that tetraniliprole provides better control of *H. armigera* (Hasenbein et al., 2018; Stinson et al., 2022). On *H. armigera* tested in this study, tetraniliprole had an LD_{50} of 4.01 g/g while chlorantraniliprole had an LD_{50} of 5.79 g/g.

Our findings also showed that the two insecticides exhibited different hydrogen bond strengths: tetraniliprole bound to Asn4674 and Tyr4673 with respective strengths of 3.02 and 3.20, while chlorantraniliprole bound to Lys4929 and Gly4927 with respective strengths of 3.20 and 3.33. It may therefore be concluded that tetraniliprole and chlorantraniliprole both have the ability to create hydrogen bonds with particular *RyR* amino acid residues. The presence of specific amino acid residues such as Asn4674, Tyr4673 (for tetraniliprole), Lys4929, and Gly4927 (for chlorantraniliprole) suggests the possibility of hydrogen bonding interactions between these residues and the respective insecticides.

Another study revealed that the G4946E mutant exhibited significantly reduced sensitivity to diamides and affinity for other ligands, including ryanodine. In *S. frugiperda*, flubendiamide and chlorantraniliprole indicated the presence of I4743M and G4946 E/V mutations with binding affinities on membranes reduced by nearly 450 and 159 times, respectively (Huang et al., 2021).

5. Conclusion

Following ten generations of treatment, neither the I4775 nor the G4924 mutations were detected. This finding implies that tetraniliprole resistance is unlikely to develop in *H. armigera*, suggesting that tetraniliprole is a promising alternative insecticide to control *H. armigera*. At present, there is low resistance to tetraniliprole in *H. armigera*, and thus tetraniliprole can be used as a substitute for other insecticides for controlling this pest.

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CRediT authorship contribution statement

Amosi Leonard Mbuji: Conceptualization, Investigation, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing, Formal analysis, Methodology, Software. **Zhaoxiang Xue:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization. **Mingcheng Guo:** Data curation, Validation, Visualization, Writing – review & editing. **Mingjian Li:** Conceptualization, Data curation, Formal analysis, Validation, Visualization, Writing – original draft. **Shenglan Lv:** Conceptualization, Data curation, Investigation, Methodology, Resources, Visualization, Writing – original draft. **Lei Zhang:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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