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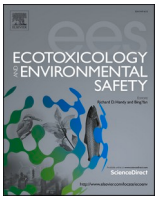

DIMBOA-induced gene expression, activity profiles of detoxification enzymes, multi-resistance mechanisms, and increased resistance to indoxacarb in tobacco cutworm, *Spodoptera litura* (Fabricius)

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ABSTRACT

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) is one of the most destructive insect pests owned strong resistance to different insecticides. Indoxacarb as a novel oxadiazine insecticide becomes the main pesticide against *S. litura*. DIMBOA [2,4-dihydroxy-7-methoxy-2 H-1,4-benz-oxazin-3(4 H)-one] is involved in important chemical defense processes in corn plants. However, the insects' adaptation mechanism to insecticides when exposed to defensive allelochemicals in their host plants remains unclear. Here, we assessed multi-resistance, and resistance mechanisms based on *S. litura* life history traits. After 18 generations of selection, indoxacarb resistance was increased by 61.95-fold (Ind-Sel) and 86.06-fold (Dim-Sel) as compared to the Lab-Sus. Also, DIMBOA-pretreated larvae developed high resistance to beta-cypermethrin, chlorpyrifos, phoxim, chlorantraniliprole, and emamectin benzoate. Meanwhile, indoxacarb (LC_{50}) was applied to detect its impact on thirty-eight detoxification-related genes expression. The transcripts of *SlituCOE073, SlituCOE009, SlituCOE074*, and *SlituCOE111* as well as *SlGSTs5, SlGSTu1*, and *SlGSTe13* were considerably raised in the Ind-Sel strain. Among the twenty-three P450s, *CYP6AE68, CYP321B1, CYP6B50, CYP9A39, CYP4L10*, and *CYP4S9v1* transcripts denoted significantly higher levels in the Ind-Sel strain, suggesting that CarEs, GSTs and P450s genes may be engaged in indoxacarb resistance. These outcomes further highlighted the importance of detoxification enzymes for *S. litura* gene expression and their role in responses to insecticides and pest management approaches.

1. Introduction

The tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), is a widely distributed destructive pest, which is distributed globally in tropical and subtropical areas of the world (Gong et al., 2022; Sun et al., 2023). As a polyphagous pest, *S. litura* attacks more than 209 plant species belonging to 99 plant families such as maize, tomato, cotton, potato, groundnut, soybean, etc., which cause significant economic losses (Ahmad et al., 2008; Tong et al., 2013). In nature, plants can synthesize a large diversity of secondary metabolites, which is often a defense response of plants to a variety of biotic (e.g. herbivores, pests, and pathogens) and abiotic (e.g. salt, light, heavy metal, cold, and drought) stresses (Agrawal and Weber, 2015; Wang et al., 2015b; Silva-Brandão et al., 2021). Plant secondary metabolites can be structurally subdivided into five major groups polyketides, isoprenoids, alkaloids, flavonoids, and phenylpropanoids (Agrawal and Weber, 2015). Benzoxazinoids are indole alkaloids found in most of the Poaceae family plants that are toxic to many chewing herbivore insects (Agrawal and Weber, 2015; Silva-Brandão et al., 2021). 2,4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA) is a benzoxazinoid found in maize (*Zea mays* L.), wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) as well as several dicot plants against resistance to herbivores (various

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lepidopteran and aphid pests) and pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Saccharomyces cerevisiae*, etc.) (Niemeyer, 2009; Rakoczy-Trojanowska et al., 2020; Silva-Brandão et al., 2021).

For decades, control of *S. litura* mostly relies on the continuous application of chemical insecticides, leading to the rapid development of resistance to almost all major classes of insecticides, including pyrethroids (such as beta-cypermethrin), organophosphates (such as profenofos), carbamate (such as methomyl), and some new chemistry insecticides (such as abamectin) (Ahmad et al., 2008; Shad et al., 2012; Tong et al., 2013; Liu et al., 2019). Accordingly, pest management of *S. litura* has become increasingly difficult all over the world (Ahmad et al., 2008; Luo et al., 2022; Shi et al., 2022).

It was reported that DIMBOA provokes variable larval responses in numerous *Spodoptera* species (Lepidoptera: Noctuidae) (Rostás, 2007; Glauser et al., 2011; Wouters et al., 2014; Guo et al., 2017; Silva-Brandão et al., 2021; Guo et al., 2022). For instance, *S. eridania* (Cramer) larvae fed on high DIMBOA concentrations display better consumption rates and growth rates. This phenomenon indicated that *S. eridania* not only owns the ability to detoxify or tolerate the DIMBOA but also utilizes the nitrogen contained therein as a nutrient (Manuwoto and Scriber, 1982). Besides, DIMBOA may inhibit the endoprotease activity in *Sesamia nonagrioides* (Lef.) whereas it reduces *S. nonagrioides* larvae growth rate (Ortego et al., 1998). *S. exigua* larvae (Hübner) acquired less biomass and prolonged development when feeding on an artificial diet containing DIMBOA (Rostás, 2007). Similarly, *S. littoralis* larvae (Boisduval) also significantly grew less under a high DIMBOA amount supplemented with the artificial diet (Glauser et al., 2011). However, DIMBOA has no significant influence on the larval development of *S. frugiperda* (Smith) however acts as a feeding stimulant that improves larval growth, particularly under low concentrations (Silva-Brandão et al., 2021).

Indoxacarb is a novel oxadiazine insecticide that acts as a more potent sodium channel blocker and exhibits excellent insecticidal activity against lepidopteran, homopteran, and coleopteran pests (Wing et al., 2000; Hou et al., 2021; Shi et al., 2022). Recently, with the extensive indoxacarb application and misuse, different caterpillar pests (such as *S. frugiperda* (J. E. Smith) and *S. litura* have developed a high level of resistance (Hafeez et al., 2022;Luo et al., 2022; Shi et al., 2022).

In insects, cytochrome P450 monooxygenases (P450s or CYPs), glutathione *S*-transferases (GSTs), and esterases (ESTs) are three important superfamilies of enzymes that participate in various plant allelochemicals and insecticide detoxification and also play important roles in insect resistance to insecticides (Feyereisen, 2006; Li et al., 2007; Tao et al., 2012; Zhao et al., 2020; Hou et al., 2021). Many studies have indicated that the induction of detoxification gene overexpression in insects is associated with the development of insecticide resistance (Hou et al., 2021; Shi et al., 2021; Shi et al., 2022). Consumption of a diet containing flavone, coumarin, DIMBOA, and visnagin significantly increased the activity of cytochrome P450 in both midgut and fat body of *H. armigera* larvae (Chen et al., 2019). Especially DIMBOA significantly increased the activity of cytochrome P450 in the fat body of *H. armigera* larvae by 4.96-fold, whereas those fed on diets containing flavone and coumarin displayed the highest activity in the midgut of *H. armigera* larvae by 2.36-fold and 2.12-fold, respectively (Chen et al., 2019). Compared with the susceptible strain, the gene expression level of *SlituCOE009*, *SlituCOE090*, *SlituCOE073*, *SlituCOE050*, *SlituCOE093,* and *SlituCOE074* were significantly upregulated in indoxacarb-resistant strain (resistance ratio = 58.39-fold) and field indoxacarb-resistant strain (resistance ratio = 40.08-fold) of *S. litura* (Shi et al., 2022). The relative transcription levels of *CYP367A1*, *CYP367B1*, *CYP341B21*, and *CYP340L2* were significantly upregulated by 2.27–4.42, 2.62–5.53, 5.22–10.06, and 2.01–4.70-fold in indoxacarb-resistant strain (resistance ratio = 58.39-fold) than in the indoxacarb-susceptible strain of *S. litura* for 6, 12, and 24 h, respectively (Shi et al., 2021). After chlorpyrifos treatment for 24 h, six cytosolic *Slgsts* (*SlGSTe1*, *SlGSTe3*, *SlGSTe10*, *SlGSTe15*, *SlGSTo2,* and *SlGSTs5*) and two microsomal *Slgsts* (*SlMGST1–2* and *SlMGST1–3*) of *S. litura* were increased by 1.59- and 5.32-fold, respectively. These consequences suggested that these GST genes might be directly responsible for the detoxification of both 12-generation-treated and susceptible strains of *S. litura* (Zhang et al., 2016).

The objectives of this study were (1) to clarify how the indoxacarb insecticide resistance and fitness traits of *S. litura* were impacted by feeding on the plant secondary metabolite, DIMBOA, (2) to explicitly assess the possible influence of DIMBOA and indoxacarb insecticide on the mechanism of three major detoxification enzymes (P450s GSTs and ESTs) activities and related genes transaction, (3) to clarify the effects of DIMBOA and indoxacarb on the multi-resistance to frequently used insecticides. The ultimate aim of the present study, therefore, was to clarify the indoxacarb resistance mechanisms and the regulatory mechanisms of plant allelochemicals that affect the tolerance of *S. litura* against commonly used insecticides.

2. Materials and methods

2.1. Insects

The strains of *S. litura* larvae were originally obtained from the Insectarium of the Institute of Entomology, Sun Yat-sen University, which was kept in the insectary (25 ± 2 °C, 70 ± 5% relative humidity with a light/dark photoperiod of 16:8 h) without exposure to any insecticides for more than five years at South China Agricultural University (Guangzhou, China). *S. litura* larvae were reared with an artificial diet containing soybean powder (100 g), wheat bran (60 g), brewer's yeast (40 g), agar (16 g), ascorbic acid (4 g), methyl p-hydroxybenzoate (2 g), sorbic acid (2 g), cholesterol (0.8 g) and water (1 L) (Chen et al., 2000; Hou et al., 2021). A 10% (*W/V*) (replaced per day) honey solution can provide sufficient nutrients for moths spawning.

2.2. Chemicals

In the present study, six chemical insecticides were used as follows: indoxacarb (90% technical) (Methyl7-chloro-2,5-dihydro-2-[N-(methoxycarbonyl)− 4-(trifluoro methoxy) anilinocarbonyl] indeno [1,2-*e*] [1,3,4] oxadiazine-4a(3*H*) carboxylate) (DuPont Agricultural Chemicals Ltd., Shanghai, China). Besides, beta-cypermethrin (95% technical) ((1 *R*)− 2, 2-Dimethyl-3*β*-(2, 2-dichloro vinyl) cyclopropane-1*β*-carboxylic acid (*S*)-*α*-cyano-3-phenoxybenzyl ester) (Jiangsu Futian Agrochemical Co., Ltd., Nanjing, China). Also, chlorpyrifos (98% technical) (*O*, *O*-Dimethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) (Hubei Sharonda Co., Ltd., Jingzhou, China). In addition, phoximb (99% technical) ((*Z*)-N Diethoxyphosphinothioyloxybenzenecarboximidoyl cyanide) (Shanghai Jiang Lai Biotechnology Co., Ltd., Shanghai, China). Moreover, chlorantraniliprole (97% technical) (3-bromo-1-(3-chloro-2-pyridyl)− 4′-cyano-2′-methyl-6′-methyl carbamoyl) pyrazole-5-carboxamide) (Shanghai Yuanye Biotechnology Co., Ltd., Shanghai, China) and 98% Emamectin benzoate ((4′R)− 4′-deoxy-4′-(methylamino) avermectin B1 benzoate) (Shanghai Yuanye Biotechnology Co., Ltd., Shanghai, China).

DIMBOA (97% technical) (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) was purchased from Shanghai Acmec Biochemical Co., Ltd. (Shanghai, China). Acetone (≥ 99.5%, analytical-grade) was purchased from Guangzhou Chemical Reagent Factory (Guangzhou, Guangdong, China). The stock solution of indoxacarb or DIMBOA was prepared in acetone at 100 mg mL^{-1} (Hou et al., 2021). Stock and working stock solutions of each chemical were stored at 4 °C and prepared immediately prior to using them. Triphenyl phosphate (TPP, 99%), diethyl maleate (DEM, 96%), and piperonyl butoxide (PBO, 90%) were purchased from Sigma (St. Louis, MO, USA). Coomassie Brilliant Blue G-250 and *α*-naphthyl acetate (*α*-NA) were obtained from Shanghai Chemical Factory, China. Nicotinamide adenine dinucleotide phosphate (NADPH), *p*-nitroanisole (*p*-NA), glutathione (GSH), and 2, 4-dinitrochlorobenzene (CDNB) were bought from Sigma (St. Louis, MO, USA).

Dithiothreitol (DTT) was purchased from Shanghai Yuanju Biotech Co., Ltd. (Shanghai, China). Ethylenediaminetetraacetic acid (EDTA) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA) was purchased from Absin (Absin Bioscience Inc., Shanghai, China) and Phenylmethanesulfonyl fluoride (PMSF) was purchased from Sino-American Biotechnology Co. Ltd. (Shanghai, China).

2.3. Preparation of insecticide and DIMBOA-supplemented diets

To prepare the DIMBOA-supplemented and insecticide diets, DIMBOA was first dissolved in 1% DMSO. 38 μg g^{-1} DIMBOA, a similar amount to that found in the induced plant (Rostás, 2007; Silva-Brandão et al., 2021), was prepared by thoroughly mixing an aqueous solution of DIMBOA with the freshly made artificial diet and transferred to plastic boxes (20 × 12.5 × 6.5 cm) before the solidification of agar (40–45 °C) (Hafeez et al., 2019). An equal volume of DMSO without DIMBOA supplementation to the artificial diet which was prepared applying the same method was employed as the control diet.

2.4. Selection of DIMBOA- and indoxacarb-resistant population

For the *S. litura* resistance selection experiments, the population was divided into two treatment groups after performing bioassays. For the DIMBOA with indoxacarb-resistant population and the indoxacarb-resistant population, early four-instar larvae were first fed on an artificial diet supplemented with 200 μg g^{-1} DIMBOA and a standard artificial diet (without DIMBOA) for 1 day, followed by a diet containing a 50% lethal concentration (LC_{50}) of indoxacarb the following day. These groups were identified as the Dim-Sel strain and the Ind-Sel strain, respectively. The number of larvae of *S. litura* employed for each generation ranged from 900 to 1200. After 3 days of insecticide exposure, the Dim-Sel strain was reared to maturity on a DIMBOA-treated artificial diet, and larvae from the Ind-Sel strain on a standard artificial diet only. A separate control population is considered as the susceptible strain (Lab-Sus). All strains were maintained in an insectary at the same condition as described above. The toxicity of deltamethrin to the Dim-Sel and Ind-Sel strains was assayed at every generation for resistance selection. Survivors of every selection were reared to obtain the next generation. Insecticide resistance levels were determined by using the resistance ratio (RR).

2.5. Bioassay, synergist experiment, and insecticide treatment

Newly molted four-instar larvae of *S. litura* were utilized for insecticide bioassays applying a diet incorporation method under laboratory conditions (Jia et al., 2009). Following the diet preparation, five doses of indoxacarb and the artificial diet (below 54 °C) were mixed thoroughly to prepare different concentrations (Gupta et al., 2005). Newly molted fourth instar larvae were first fed on an artificial diet supplemented with 200 μg. g^{-1} DIMBOA for 1 day, followed by a diet containing different concentrations of indoxacarb the following day. The artificial diet without indoxacarb was utilized as a control. After preparation, the diet was cut into small cubes (1 cm^3), transferred to 45 mL plastic jelly cups, and then covered with a fine-meshed (0.5 mm) nylon filter. After that, the fourth-instar larvae were placed individually inside the cups and fed on an adequate artificial diet with different indoxacarb doses. The diet was replaced daily to avoid any effects of spoilage throughout the experiment. A total of 540 newly molted fourth-instar larvae of *S. litura* were employed for each bioassay with each selected group including 90 control larvae and 90 newly molted fourth-instar larvae for each concentration. For each replicate, 30 individuals were studied in triplicate at each concentration.

In a synergist experiment with three different strains of *S. litura* (Lab-Sus, Dim-Sel, and Ind-Sel), the effects of indoxacarb combined with PBO (piperonyl butoxide; P450 inhibitor), DEM (diethyl maleate; GST inhibitor) and TPP (triphenyl phosphate; carboxylesterase inhibitor) were investigated. The PBO, DEM, or TPP were dissolved in acetone. Before indoxacarb exposure, newly molted fourth instar larvae of *S. litura* were pre-treated topically with 3 × 10^4 mg L^{-1} of synergist (PBO, DEM or TPP) (1 μL for each larva; 30 μg per larva) (Hou et al., 2021). This treatment corresponds to a maximal dose, which could be applied without significant larval mortality within the 96-h observation interval ($P > 0.05$).

The bioassays were performed under the same conditions described above. Mortality was assessed after 48 h of exposure to indoxacarb (Cui et al., 2018; Hou et al., 2021). Larvae were considered to be dead if they were unable to make any coordinated movement after being pushed with a probe. LC_{50} values were calculated via probit analysis using POLO-PC software (LeOra Software Inc., Berkeley, CA, USA). All these assays were conducted in three independent biological replicates with thirty larvae for each replicate.

2.6. Life table parameters and population dynamics

The life-history data of Lab-Sus, Dim-Sel, and Ind-Sel were subjected to the computer-based program software (TWOSEX-MSChart) (Chi, 2023) and analyzed using age-stage two-sex life table theory (Chi and Liu, 1985; Chi, 1988). The life tables parameters such as age-stage survival rate (s_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), age-specific net maternity ($l_x m_x$), age-stage reproductive value (v_{xj}), and life expectancy (e_{xj}), respectively, were estimated (where x is the age and j are the stages of insect). The population parameters, including the intrinsic rate of increase (r), finite rate of increase (l), net reproductive rate (R_0), and mean generation time (T) were also estimated (Chi and Su, 2006; Chi et al., 2020). Finally, the means, standard errors, and variances of the population parameters were estimated via 100,000 bootstraps (Huang and Chi, 2012), which are contained in the TWOSEX-MSChart computer program.

2.7. Enzyme assays

To assay the detoxification enzyme activities, beginning with the 18th generation, the newly molted fourth-instar individuals from the Dim-Sel population were fed first on an artificial diet supplemented with 200 μg g^{-1} DIMBOA the first day and then transferred to the artificial diet with LC_{50} of indoxacarb, respectively. Preparation of the Ind-Sel strain was conducted in the same way, the newly molted fourth-instar was fed on an artificial diet for the first day (no DIMBOA). On the third day, individuals from both treatment groups were fed on the artificial diet with LC_{50} of indoxacarb. The control group, Lab-Sus, was not exposed to any pre or post-treatment. After 24 h, whole bodies of ten fourth-instar-treated larvae of *S. litura* from different strains (Lab-Sus, Ind-Sel, and Dim-Sel) were homogenized, respectively, and the activities of P450s, GSTs, and CarEs were assayed using the method described previously (Rose et al., 1995; Yang et al., 2004; Hou et al., 2021; Luo et al., 2022). All assays for enzyme activities were assayed with four biological replicates and three technical replicates.

2.8. RNA isolation, RT-PCR, and quantitative real-time PCR analysis

To detect the detoxification gene expression in different strains, after 18 generations of selection with indoxacarb, toxicity bioassays were conducted on 1-day-old fourth instar larvae of *S. litura* of the Dim-Sel and Ind-Sel strains under laboratory conditions using a diet incorporation method (Lai et al., 2011). The lab-Sus strain without indoxacarb treatment was used as a control. The lab-Sus, Ind-Sel, and Dim-Sel strains of *S. litura* were placed individually inside the 45 mL plastic jelly cups and fed on an adequate artificial diet mixed with LC_{10} of indoxacarb at 0.062 mg L^{-1}, 2.829 mg $L^{-1,}$ and 4.251 mg L^{-1}, respectively. The surviving larvae were collected following 24 h and four individuals were pooled together as one biological sample and

immediately frozen in liquid nitrogen and stored at − 80 °C until use.

After 18 generations of selection with indoxacarb, three biological replicates of midgut and fat body tissue of six fourth instar larvae for each group were taken from the storage reagent and immediately ground separately in liquid nitrogen. After this, individual replicates were kept at − 80 °C for RNA extraction (Silva-Brandão et al., 2021). Total RNA was extracted from the lab-Sus, Dim-Sel, or Ind-Sel strains of *S. litura* larvae using a Trizol Reagent Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions protocol. RNA was monitored on 1% agarose gels and RNA quantification was performed using a NanoDrop ND2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). cDNA was synthesized from 1 μg of total RNA using the ThermoScript RT-PCR System kit according to the manufacturer's instructions (Thermo Fisher Scientific, Carlsbad, CA, USA). According to previous publications (Rehan and Freed, 2014; Wang et al., 2015a; Zhang et al., 2016; Zou et al., 2016; Wang et al., 2017a; Wang et al., 2017b; Liu et al., 2019; Xu et al., 2020; Hou et al., 2021; Shi et al., 2022), the relative expression levels of thirty-eight detoxification related genes [seven CarEs (*SlituCOE009*, *SlituCOE090*, *SlituCOE073*, *SlituCOE050*, *SlituCOE093*, *SlituCOE074*, and *SlituCOE111*), eight GSTs (*SlGSTe1*, *SlGSTe2*, *SlGSTe3*, *SlGSTe13*, *SlGSTs5*, *SlGSTt1*, *SlGSTo1*, and *SlGSTu1*) and twenty-three P450s (*CYP304F4*, *CYP321A7*, *CYP321A9*, *CYP321B1*, *CYP324A6*, *CYP4G75*, *CYP4L4*, *CYP4L10*, *CYP4M14v1*, *CYP4S9v1*, *CYP6AB12*, *CYP6AB14*, *CYP6AB31*, *CYP6AB60*, *CYP6AB61*, *CYP6AE68*, *CYP6B29*, *CYP6B47*, *CYP6B48*, *CYP6B50*, *CYP6B58*, *CYP9A39,* and *CYP9A40*)] in the whole body of 1-day-old fourth instar larvae of *S. litura* of the lab-Sus, Dim-Sel and Ind-Sel of *S. litura* larvae were measured by quantitative real-time RT-PCR (qRT-PCR), respectively. The primers employed for qRT-PCR are listed in Supplementary Table S1. The qRT-PCR analysis was performed with a MJ Research Opticon™ 2 instrument (Bio-Rad, Inc., Hercules, CA, USA) in a volume of 20 μL using SYBR Green I Master Mix (Roche Diagnostics Corp., Indianapolis, IN, USA). The PCR conditions were: 95 °C for 30 s, followed by 40 cycles of 95 °C for 10 s, and 60 °C for 30 s. The *β-actin* (*Slβ-actin*) and elongation factor 1*α* (*SlEF-1α*) genes were employed as an internal standard gene to normalize the expression levels among different samples. Relative gene expression levels for each gene were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). For each gene, three biological samples were conducted with three technical replicates.

2.9. Multi-resistance to insecticides in S. litura

Newly molted four-instar larvae of *S. litura* from the lab-Sus, Dim-Sel, and Ind-Sel strains were treated with beta-cypermethrin, chlorpyrifos, phoxim, chlorantraniliprole, and emamectin benzoate using the diet incorporation method (Jia et al., 2009) as described above to assess patterns of the cross-resistance.

2.10. Statistical analysis

Statistical analyses were performed in the SPSS 13.0 Software Package (SPSS Inc., Chicago, IL, USA). Larval mortality was measured at 48 h following treatment. All assays were done with three replicates under the rearing circumstances stated above. LC_{50} rates were estimated by probit analysis applying POLOPC software (LeOra Software Inc., Berkeley, CA, USA). Non-overlapping 95% confidence intervals were measured to imply statistically significant differences among LC_{50} rates. The chi-square value ($\chi 2$) and predicted mortality for every assay were evaluated by applying probit analysis. Resistance ratios (RRs) with 95% confidence intervals (CIs) are evaluated by the method of Robertson et al. (2007) where the resistant population LC is employed as the numerator and the susceptible population LC as the denominator. The Students'*t*-test was used to analyze data from enzyme activity tests, transcription data of selected P450, GST, and CarE genes, and toxicity experiments with indoxacarb. All data were expressed as means ± standard error (SE). Statistical differences were considered significant at $p < 0.05$. Resistance ratio (RR) = LC_{50} of resistant population/LC_{50} of sensitive population. The insecticide resistance levels were sorted to the criteria illustrated by Wang et al. (2018): susceptible (RR < 3.0), reduced susceptibility (3.0 ≤ RR < 5.0), low resistance (5.0 ≤ RR < 10.0), moderate resistance (10.0 ≤ RR < 40.0), high resistance (40.0 ≤ RR < 160.0), and extremely high resistance (RR ≥ 160.0).

3. Results

3.1. The resistance ratio of the Dim-Sel and Ind-Sel strains

After 18 generations of selection with indoxacarb, the Dim-Sel strain and Ind-Sel strain have developed highly resistant to indoxacarb when compared to the Ind-Sus strain, with a resistance ratio of 87.745 and 61.951, respectively (Table 1) (95% CI).

3.2. Synergistic effect of PBO, DEM, and TPP on the indoxacarb resistance in S. litura

Results displayed that compared with DEM, and TPP, the synergistic effects of PBO on indoxacarb in the fourth instar larvae in the Ind-Sel strain were obvious. PBO enhanced the toxicity of indoxacarb in larvae of the Lab-Sus, Ind-Sel, and Dim-Sel strains with synergistic ratio values of 4.422-, 5.026-and 2.567-fold, respectively (Table 2) (95% CI). These findings indicated that PBO caused obvious synergism to indoxacarb in *S. litura* and suggested that P450s in *S. litura* larvae might be involved in the metabolic resistance to indoxacarb.

3.3. P450 monooxygenases, glutathione S-transferases, and esterases activity for Dim-Sel and Ind-Sel strains

After 18 generations of selection, three major xenobiotic detoxifying enzyme mechanisms, P450s, GSTs, and ESTs in the Lab-Sus, Dim-Sel, and Ind-Sel strains were assayed. As presented in Table 3, the P450 activity of the Ind-Sel larvae (0.137 nmol/min/mg protein) was 3.34-fold higher followed by the Dim-Sel larvae which were 2.90-fold higher (0.119 nmol/min/mg protein) than the Lab-Sus strain. Similarly, the significantly elevated activities of GSTs were observed in the Dim-Sel larvae (1886.43 nmol/min/mg protein) followed by the Ind-Sel larvae (986.84 nmol/min/mg protein) as compared to the Lab-Sus larvae (885.263 nmol/min/mg protein). Compared with the Lab-Sus strain, GST activities in the Dim-Sel strain and Ind-Sel strain have increased by 2.13-fold and 1.92-fold, respectively (Table 3). In contrast, the EST activities of Lab-Sus, Dim-Sel, and Ind-Sel strains were relatively similar (Table 3).

3.4. Pre-adult developmental time for Dim-Sel and Ind-Sel strains

Comparisons of the Lab-Sus strain with Dim-Sel and Ind-Sel strains displayed fitness costs linked with the Dim-Sel resistance (Table 4). The development time for the egg stage did not differ significantly among the Lab-Sus (3.20 d), Dim-Sel (3.24 d), and Ind-Sel (3.39 d) strains, nevertheless, there were significant differences among various larval stages with extended developmental time observed in the Lab-Sus, Dim-Sel, and Ind-Sel strains. The total larval developmental time of the Ind-Sel strain (36.19 d) and Dim-Sel (35.57 d) strains was significantly longer as compared to that of the Lab-Sus strain (32.35 d) (Table 4). The prepupa and pupal developmental duration was significantly higher in the Ind-Sel strain (Prepual: 2.27 ± 0.06 d and pupal: 11.27 ± 0.13 d) as compared to the Dim-Sel (Prepual: 2.24 ± 0.07 d and pupal: 10.87 ± 0.11 d) and Lab-Sus strains (Prepual: 1.90 ± 0.08 d and pupal: 10.32 ± 0.12 d), respectively. Similarly, the pupal weight of the Ind-Sel (346.67 mg) and the Dim-Sel (379.04 mg) strains were significantly reduced as compared to the Lab-Sus strain (461.47 mg) (Table 4).

Table 1
The toxicity of indoxacarb insecticide on Dim-Sel and Ind-Sel strains of *S. litura*.

Generation	Insecticide	Slope ± SE	LC_{50} (mg L^{-1}) (95% CI)	χ^2	df	P value	RR (95% CI)[a]
Lab-Sus (G0)	Indoxacarb	2.49 ± 0.20	0.20 (0.18–0.23)	4.60	3	0.20	1.00
Dim-Sel (G0)		2.35 ± 0.19	0.21 (0.18–0.24)	3.83	3	0.28	-
Ind-Sel (G2)	Indoxacarb	1.79 ± 0.17	0.39 (0.33–0.47)	3.55	3	0.31	1.94 (1.64–2.33)
Dim-Sel (G2)		1.83 ± 0.18	0.53 (0.44–0.66)	2.15	3	0.54	2.63 (2.20–3.27)
Ind-Sel (G4)	Indoxacarb	1.81 ± 0.17	0.49 (0.42–0.58)	2.71	3	0.43	2.46 (2.08–2.91)
Dim-Sel (G4)		2.03 ± 0.17	0.56 (0.47–0.64)	2.29	3	0.51	2.72 (2.37–3.21)
Ind-Sel (G6)	Indoxacarb	1.74 ± 0.17	0.88 (0.74–1.08)	1.17	3	0.75	4.36 (3.66–5.35)
Dim-Sel (G6)		1.79 ± 0.17	1.19 (1.01–1.42)	2.04	3	0.56	5.92 (5.02–7.04)
Ind-Sel (G8)	Indoxacarb	1.75 ± 0.17	1.70 (1.42–2.01)	1.06	3	0.78	8.43 (7.06–9.97)
Dim-Sel (G8)		1.81 ± 0.17	2.39 (2.02–2.83)	4.17	3	0.24	11.82 (10.03–14.05)
Ind-Sel (G10)	Indoxacarb	1.85 ± 0.17	2.79 (2.38–3.33)	2.14	3	0.54	13.86 (11.78–16.53)
Dim-Sel (G10)		1.98 ± 0.17	3.80 (3.25–4.43)	2.34	3	0.50	18.83 (16.13–21.96)
Ind-Sel (G12)	Indoxacarb	2.19 ± 0.18	4.26 (3.70–4.92)	5.27	3	0.15	21.09 (18.32–24.37)
Dim-Sel (G12)		1.81 ± 0.17	5.58 (4.73–6.67)	3.50	3	0.32	27.62 (23.42–33.06)
Ind-Sel (G14)	Indoxacarb	1.79 ± 0.17	5.77 (4.88–6.93)	2.56	3	0.46	28.59 (24.20–34.32)
Dim-Sel (G14)		1.48 ± 0.16	8.48 (6.97–10.34)	2.42	3	0.49	41.99 (34.52–51.21)
Ind-Sel (G16)	Indoxacarb	1.60 ± 0.17	7.58 (6.25–9.54)	1.47	3	0.68	37.54 (30.99–47.23)
Dim-Sel (G16)		1.86 ± 0.17	9.97 (8.49–11.80)	0.22	3	0.97	49.38 (42.07–58.45)
Ind-Sel (G18)	Indoxacarb	1.98 ± 0.18	12.51 (10.71–14.86)	3.80	3	0.28	61.95 (53.03–73.60)
Dim-Sel (G18)		2.13 ± 0.18	17.38 (15.04–20.14)	2.37	3	0.49	87.87 (75.76–102.22)

[a] 95% CI estimated using the lethal concentration ratio significance test (Robertson and Preisler, 1992). SE, standard error; LC_{50}, the lethal concentration at 50%; CI, confidence intervals; df, degree of freedom; RR (resistance ratio) = (LC_{50} of the Dim-Sel or Ind-Sel strain)/ (LC_{50} of the Lab-Sus Strain).

Table 2
Effects of indoxacarb and synergists on survival of fourth-instar larvae from the Lab-Sus, Ind-Sel, and Dim-Sel strains of *S. litura*.

Strains	Compound	Slope ± SE	LC_{50} (mg L^{-1}) (95% CI)	χ^2 (df)	P value	SR (95% CI)[a]
Lab-Sus	Indoxacarb	2.49 ± 0.20	0.20 (0.17–0.22)	4.60 (3)	0.20	1
	Indoxacarb + PBO	1.68 ± 0.22	0.04 (0.03–0.06)	3.35 (3)	0.34	4.12 (3.02–6.31)
	Indoxacarb + DEM	1.81 ± 0.16	0.24 (0.20–0.28)	3.02 (3)	0.39	0.84 (0.714–0.99)
	Indoxacarb + TPP	1.88 ± 0.16	0.21 (0.17–0.24)	3.49 (3)	0.32	0.96 (0.82–1.13)
Ind-Sel	Indoxacarb	1.98 ± 0.17	12.51 (10.71–14.86)	3.80 (3)	0.28	1
	Indoxacarb + PBO	1.66 ± 0.19	2.49 (1.82–3.12)	3.79 (3)	0.29	5.03 (4.00–6.85)
	Indoxacarb + DEM	1.96 ± 0.17	8.40 (7.19–9.82)	3.26 (3)	0.35	1.49 (1.27–1.74)
	Indoxacarb + TPP	1.80 ± 0.16	11.14 (9.44–13.32)	1.34 (3)	0.72	1.12 (0.94–1.33)
Dim-Sel	Indoxacarb	2.13 ± 0.17	17.72 (15.34–20.53)	2.11 (3)	0.55	1
	Indoxacarb + PBO	1.74 ± 0.18	6.90 (5.45–8.35)	3.63 (3)	0.31	2.57 (2.12–3.25)
	Indoxacarb + DEM	2.04 ± 0.19	7.79 (6.45–9.14)	1.45 (3)	0.69	2.28 (1.94–2.75)
	Indoxacarb + TPP	1.96 ± 0.18	9.13 (7.61–10.71)	4.68 (3)	0.19	1.94 (1.66–2.33)

PBO, piperonyl butoxide; DEM, diethyl maleate; TPP, triphenyl phosphate; SE, standard error; LC_{50}, lethal concentration at 50%; CI, confidence intervals; df, degree of freedom; SR, synergism ratio = LC_{50} without synergist/LC_{50} with synergist. a 95% CI estimated using the lethal concentration ratio significance test (Robertson and Preisler, 1992).

Table 3
Activities of detoxification enzymes in fourth-instar larvae from different *S. litura* strains.

Enzyme	Population	Enzyme activity (nmol min^{-1} mg^{-1} protein)	Ratio
P450s	Lab-Sus	0.04 ± 0.01	1
	Dim-Sel	0.12 ± 0.003	2.90 ± 0.33 **
	Ind-Sel	0.15 ± 0.01	3.58 ± 0.18 **
GSTs	Lab-Sus	885.26 ± 26.83	1
	Dim-Sel	1886.43 ± 33.62	2.13 ± 0.04 **
	Ind-Sel	1696.86 ± 29.03	1.92 ± 0.09 **
CarEs	Lab-Sus	1040.06 ± 33.93	1
	Dim-Sel	1637.24 ± 27.73	1.57 ± 0.04 **
	Ind-Sel	1285.39 ± 31.96	1.24 ± 0.06 * *

Data are presented as mean ± SE obtained from four independent replicates. Asterisks indicate a significant difference in enzyme activity between the Lab-Sus and Dim-Sel or Lab-Sus and Ind-Sel population (Student's t-test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

3.5. Adult longevity and growth metrics of S. litura strains

Our results presented that the longevity of female and male adults was significantly different, with shorter longevity of females in the Ind-Sel strain (10.52 d) followed by the Dim-Sel strain (11.45 d) as compared to the Lab-Sus strain (12.79 d). Whereas the total longevity of male adults in the Ind-Sel strain (7.18 d) and the Dim-Sel strain (7.05 d) was significantly shorter as compared to the Lab-Sus strain (8.16 d) (Table 4). Similarly, significantly reduced longevity of all adults was observed in the Ind-Sel strain (8.64 d) and the Dim-Sel strain (8.44 d) was significantly shorter as compared to the Lab-Sus strain (9.65 d) (Table 4). The adult pre-oviposition (APOP) and the total pre-oviposition period (TPOP) were comparatively extended in the Ind-Sel and Dim-Sel strains whereas significantly shorter as compared to the Lab-Sus strain (Table 4). Similarly, significantly shorter oviposition days were observed in the Ind-Sel strain (5.10 d) followed by the Dim-Sel strain (6.54 d) as compared to the Lab-Sus strain (8.15 d). A similar trend in the total number of eggs laid per female was noted in the Ind-Sel strain (759.05) followed by the Dim-Sel strain (1200.00) strain as compared to the Lab-Sus strain (1430.52).

3.6. Fitness comparison

The population parameters indicated significant differences among

Table 4
Adult longevity, adult pre-oviposition period (APOP), total pre-oviposition period (TPOP), oviposition days, fecundity, Intrinsic rate of increase, finite rate of increase, net reproductive rate and mean generation time of the Lab-Sus, Dim-Sel, and Ind-Sel strains of *S. litura*.

Parameter	Lab-Sus	Dim-Sel	Ind-Sel
Egg (d)	3.20 ± 0.04 b	3.24 ± 0.05 a	3.38 ± 0.05 a
First instar larva (d)	3.12 ± 0.06 b	3.35 ± 0.05 a	3.41 ± 0.07 a
Second instar larva (d)	2.70 ± 0.10 b	2.83 ± 0.05 b	3.38 ± 0.08 a
Third instar larva (d)	2.48 ± 0.05c	2.85 ± 0.05 b	3.03 ± 0.04 a
Fourth instar larva (d)	2.73 ± 0.05c	3.14 ± 0.05 a	2.98 ± 0.06 b
Fifth instar larva (d)	2.68 ± 0.05 b	3.29 ± 0.06 a	3.33 ± 0.10 a
Sixth instar larva (d)	3.21 ± 0.05 b	3.56 ± 0.08 a	3.42 ± 0.08 a
Prepupa (d)	1.90 ± 0.08 b	2.24 ± 0.07 a	2.27 ± 0.06 a
Pupa (d)	10.32 ± 0.12c	10.87 ± 0.11 b	11.27 ± 0.13 a
Pupal weight (mg)	461.47 ± 5.31 a	379.04 ± 4.36 b	346.67 ± 4.78c
Adult longevity (d)	9.65 ± 0.32 a	8.44 ± 0.28 b	8.64 ± 0.28 b
Longevity (female) (d)	12.79 ± 0.14 a	11.45 ± 0.14 b	10.52 ± 0.15c
Longevity (male) (d)	8.16 ± 0.22 a	7.05 ± 0.213 b	7.18 ± 0.21 b
APOP (d)	1.10 ± 0.07c	1.25 ± 0.24 b	1.81 ± 0.15 a
TPOP (d)	33.90 ± 0.40c	37.35 ± 0.42 b	40.00 ± 0.34 a
Oviposition days (d)	8.15 ± 0.30 a	6.54 ± 0.23 b	5.10 ± 0.30c
Fecundity (eggs/female)	1430.52 ± 81.54 a	1200.00 ± 24.77 b	759.05 ± 46.90c
Intrinsic rate of increase (r) (day^{-1})	0.15 ± 0.01 a	0.14 ± 0.01 b	0.12 ± 0.01c
Finite rate of increase (λ) (day^{-1})	1.16 ± 0.01 a	1.15 ± 0.01 b	1.13 ± 0.01c
Net reproductive rate (R_0)	317.88 ± 65.26 a	266.67 ± 52.79 a	177.11 ± 35.33 a
Mean generation time (T) (days)	38.08 ± 0.39c	41.27 ± 0.49 b	43.62 ± 0.36 a
Adult longevity (d)	9.65 ± 0.32 a	8.44 ± 0.28 b	8.64 ± 0.28 b
Longevity (female) (d)	12.79 ± 0.14 a	11.45 ± 0.14 b	10.52 ± 0.15c
Longevity (male) (d)	8.16 ± 0.22 a	7.05 ± 0.213 b	7.18 ± 0.21 b
APOP (d)	1.10 ± 0.07c	1.25 ± 0.24 b	1.81 ± 0.15 a
TPOP (d)	33.90 ± 0.40 c	37.35 ± 0.42 b	40.00 ± 0.34 a
Oviposition days (d)	8.15 ± 0.30 a	6.54 ± 0.23 b	5.10 ± 0.30 c
Fecundity (eggs/female)	1430.52 ± 81.54 a	1200.00 ± 24.77 b	759.05 ± 46.90c

All data are presented as means ± SE. Means followed by the same letters within rows are not significantly different based on the paired bootstrap test at the 5% significance level. Ninety larvae of *S. litura* were used for each treatment.

strains. The intrinsic rate of increase (*r*) and finite rate of population increase in the Ind-Sel strain (0.12 and 1.13) was significantly reduced as compared to the Dim-Sel strain (0.14 and 1.15) and Lab-Sus strain (0.15 and 1.16). Similarly, mean generation time (*T*) was noticeably escalated in the Ind-Sel (43.62 d) and Dim-Sel (41.27 d) strains compared to the Lab-Sus strain (38.08 d). While the Net reproductive rate (R_0) was not significantly different among the Ind-Sel, Dim-Sel, and Lab-Sus strains (Table 4).

3.7. Survival rate and fecundity of the Dim-Sel and Ind-Sel populations of *S. litura*

Results indicated that the lowest age-specific survival rate of the eggs occurred in the Ind-Sel and Dim-Sel strains whereas the highest rate was observed in the Lab-Sus (Fig. 1A, B, and C). Similarly, the Survival rate (S_{xj}) for the larval and the pupal stages was decreased in the Ind-Sel and Dim-Sel strains whereas the highest rate was recorded in the Lab-Sus strain (Fig. 1A, B, and C). The values of survival rate significantly differed across the different developmental stages among the treatments, which proposed that the growth rates changed among the individuals. The survival curves of different age stages of *S. litura* larvae overlap, and larvae completed development at 22 and 25 ds in the Ind-Sel and Dim-Sel strains, respectively as compared with the Lab-Sus strain (28 d) (Fig. 1A, B, and C). Conversely, there was shorter survival of male and female adults in the Ind-Sel strain (male: 14 and female: 19 d) and Dim-Sel strain (male: 15 and female: 20 d) as compared to the Lab-Sus strain (male: 15 and female: 20 d) (Fig. 1A, B and C). The survival rate (l_x) of *S. litura* on the Ind-Sel strain (49 d) and Dim-Sel strain (51 d) was shorter as compared to the Lab-Sus strain (53 d) (Fig. 1D, E, and F). Similarly, lower age-specific fecundity (l_xm_x) of *S. litura* was observed in the Ind-Sel strain (56 d) and indicated a downward trend in the Ind-Sel strain (95) and Dim-Sel strain (100 d) as compared to the Lab-Sus strain (122 d). Thus, the present study outcomes advocate that the selection pressure of insecticide and phytochemicals was not in favor of the survival and reproduction of *S. litura* (Fig. 1D, E, and F).

3.8. Reproduction value and life expectancy of *S. litura*

Significantly lower reproductive value (v_{xj}) of *S. litura* on the Ind-Sel strain and Dim-Sel respectively (Fig. 1G, H, and I). The peak value of the v_{xj} curve was observed on the Ind-Sel strain followed by the Dim-Sel strain as compared to the Lab-Sus strain (Fig. 1J, K, and L). Figs. 1 A, B, and C present the life expectancy (the time that an individual of *S. litura* of age x and stage j is expected to live) (e_{xj}) of *S. litura* at different treatments. The (e_{xj}) of *S. litura* gradually decreased as age increased. Similarly, the e_{xj} of female adults of *S. litura* on the Ind-Sel and Dim-Sel strains was higher than male adults during the whole development stage as compared to the Lab-Sus strain. The e_{xj} curve decreased synchronously in both males and females in the Ind-Sel and Dim-Sel strains (Figs. 1 A, B, and C).

3.9. Responses of P450s, GSTs, and CarEs genes to Indoxacarb treatment in the Lab-Sus, Dim-Sel, and Ind-Sel strains

To identify specific P450s, GSTs, and CarEs genes contributing to detoxification indoxacarb or DIMBOA, thirty-eight *S. litura* detoxification-related genes (seven CarEs, eight GSTs, and twenty-three P450s) potentially involved in insecticide resistance or plant secondary metabolite for expression analysis. RNA was isolated from fourth-instar larvae of the Lab-Sus, Ind-Sel, and Dim-Sel population reared on an artificial diet (with LC_{10} indoxacarb). The synthesized cDNA was then applied to determine relative transcription levels of the P450s, GSTs, and CarEs genes by RT-qPCR (Fig. 2).

As illustrated in Fig. 2A, compared with the Lab-Sus strain, transcripts of *SlituCOE009*, *SlituCOE073,* and *SlituCOE111* were significantly more highly expressed in the Dim-Sel and Ind-Sel strains. *SlituCOE073* was 1.69-fold higher in the Dim-Sel strain and 4.15-fold higher in the Ind-Sel strain compared with the Lab-Sus strain. While *SlituCOE009* was 1.81-fold higher in the Dim-Sel strain and 3.33-fold higher in the Ind-Sel strain compared with the Lab-Sus strain (Fig. 2A). The transcription of *SlituCOE073*, *SlituCOE009*, *SlituCOE074*, and *SlituCOE111* were significantly increased after indoxacarb treatment in the Ind-Sel strain as compared to the Lab-Sus, and Dim-Sel strains (Fig. 2A).

The transcripts of *SlGSTe1*, *SlGSTe2*, *SlGSTe3*, and *SlGSTe3* were 2.73-, 1.65-, 2.26- and 1.56-fold higher in Dim-Sel strain when compared with the Lab-Sus strain. Compared to the Lab-Sus, and Dim-Sel strains, the transcripts of *SlGSTs5*, *SlGSTu1*, and *SlGSTe13* were significantly higher in the Ind-Sel strain. Meanwhile, the transcripts of *SlGSTe1*, *SlGSTe3*, and *SlGSTe2* were significantly higher in the Dim-Sel strain compared with those in the Lab-Sus, and Ind-Sel strains (Fig. 2B). While, the transcripts of *SlGSTt1*, and *SlGSTo1* displayed maximum levels in the Lab-Sus strain than those in the Ind-Sel, and Dim-Sel strains (Fig. 2B).

Among the twenty-three P450s, the transcripts of *CYP6AB14*, *CYP324A6*, *CYP321A9*, *CYP6AB12*, *CYP4L4*, *CYP9A40*, *CYP6B48*, *CYP6AB60*, *CYP4G75*, *CYP321A7*, *CYP6B58*, and *CYP6B47* were significantly greater in the Dim-Sel strain than in the Lab-Sus, and Ind-Sel strains (Fig. 2C). Meanwhile, the transcripts of *CYP6AE68*, *CYP321B1*, *CYP6B50*, *CYP9A39*, *CYP4L10*, and *CYP4S9v1* indicated significantly higher levels in the Ind-Sel strain compared with those in

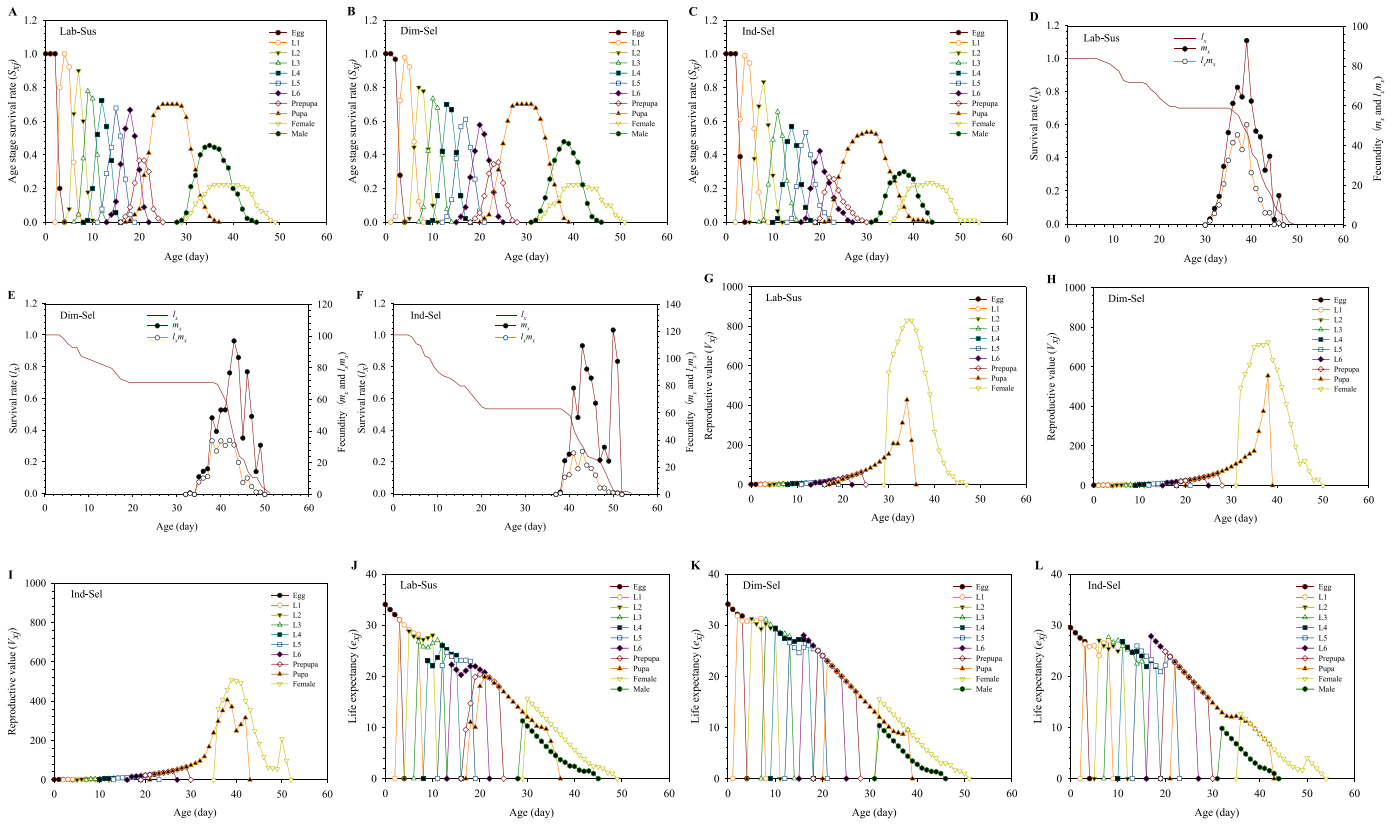

Fig. 1. Age-stage specific survival rate (s_{xj}) of the Lab-Sus (A), Dim-Sel (B), Ind-Sel (C), Age-specific survival rate (l_x), age-specific fecundity (m_x), and net maternity (l_xm_x) of the Lab-Sus (D), Dim-Sel (E), Ind-Sel (F), Age-stage specific life expectancies (v_{xj}) of the Lab-Sus (G), Dim-Sel (H), and Ind-Sel (I), Life expectancy (e_{xj}) values of the Lab-Sus (J), Dim-Sel (K), and Ind-Sel (L) strains of *S. litura*.

the Ind-Sel and Dim-Sel strains (Fig. 2C). Compared to the Lab-Sus strain, the transcripts of eighteen P450 genes significantly elevated in the Dim-Sel strain, especially *CYP6AB14* (11.13-fold). The transcripts of nine P450 genes significantly elevated in the Ind-Sel strain, however, the transcripts of *CYP6AE68* (15.52-fold) were significantly higher in larvae of the Ind-Sel strain as compared to the Lab-Sus strain (Fig. 2C).

3.10. Multiple insecticide resistance of S. litura to different insecticides

As presented in Table 5, the resistance ratios of beta-cypermethrin, chlorpyrifos, phoxim, chlorantraniliprole and emamectin benzoate to the Ind-Sel strain of *S. litura* were 2.64-, 4.13-, 3.20-, 1.92- and 1.15-fold, respectively. Therefore, no obvious multiple insecticide resistance was observed. Whereas, the resistance ratios of beta-cypermethrin, chlorpyrifos, phoxim, chlorantraniliprole and emamectin benzoate to the Dim-Sel strain of *S. litura* were 95.37-, 92.01-, 85.94-, 66.82- and 57.89-fold, respectively. These outcomes implied that following selection with indoxacarb insecticides on DIMBOA-pretreated larvae for 18 generations, the Dim-Sel strain developed high resistance to beta-cypermethrin, chlorpyrifos, phoxim, chlorantraniliprole, and emamectin benzoate, respectively.

4. Discussion

S. litura is one of the most destructive agricultural pests and has developed strong resistance to several kinds of chemical insecticides. Indoxacarb is a novel oxadiazine insecticide that has good field activity against *S. litura*. Due to the intensive use of indoxacarb, *S. litura* develops a high level of resistance to indoxacarb in field populations (Ahmad et al., 2008; Shad et al., 2012; Tong et al., 2013; Hou et al., 2021; Shi et al., 2022). As a highly polyphagous pest, *S. litura* encounters an array of allelochemicals in its host plants, DIMBOA is the dominant hydroxamic acid in corn plants (Niemeyer, 2009; Rakoczy-Trojanowska et al., 2020; Silva-Brandão et al., 2021). Thus, clarifying disintoxication plant allelochemicals and delaying indoxacarb resistance evolution in field populations of *S. litura* is very important to develop sustainable integrated pest management strategies in practice. After 24 generations of selection, *S. frugiperda* larval resistance to indoxacarb was increased by 472.67-fold as compared to the Ind-Unsel strain and the Ind-Sel strain of *S. rugiperda* showing high cross-resistance to deltamethrin (31.23-fold) and very low or negligible cross-resistance to chlorfenapyr (3.24-fold), spinosad (2.65-fold), chlorantraniliprole (1.89-fold), emamectin benzoate (1.98-fold) and methoxyfenozide (1.59-fold) (Hafeez et al., 2022). Compared to control groups of *H. armigera* larvae fed on a diet with dimethyl sulfoxide (DMSO), larvae fed on diets containing flavone, coumarin, DIMBOA, and visnagin demonstrated significantly lower mortality to methomyl by 47.6%, 34.9%, 23.8%, and 31.7%, respectively (Chen et al., 2019). The indoxacarb-resistant strain (RR = 1069.3) of *Plutella xylostella* showed cross-resistance to beta-cypermethrin (8.7-fold), metaflumizone (20.9-fold), and chlorfenapyr (8.3-fold), but no significant resistance to cyantraniliprole, chlorantraniliprole, abamectin, chlorfluazuron, spinosad and diafenthiuron compared with the susceptible strain (Zhang et al., 2017). Our results indicated that the Dim-Sel strain of *S. litura* illustrated high cross-resistance to beta-cypermethrin (95.37-fold), chlorpyrifos (92.01-fold), phoxim (85.94-fold), chlorantraniliprole (66.82-fold) and emamectin benzoate (57.89-fold), respectively, while the Ind-Sel strain indicated no significant resistance to those insecticides. The present outcomes indicate that DIMBOA is one of the effective plant allelochemicals for inducing insecticide tolerance in *S. litura*.

The present study aimed to investigate the effects of indoxacarb and DIMBOA on the biological characteristics and fitness cost. In the present study, we observed that the fitness cost decreased the pre-adult developmental time of the individuals, adults' longevity, and female

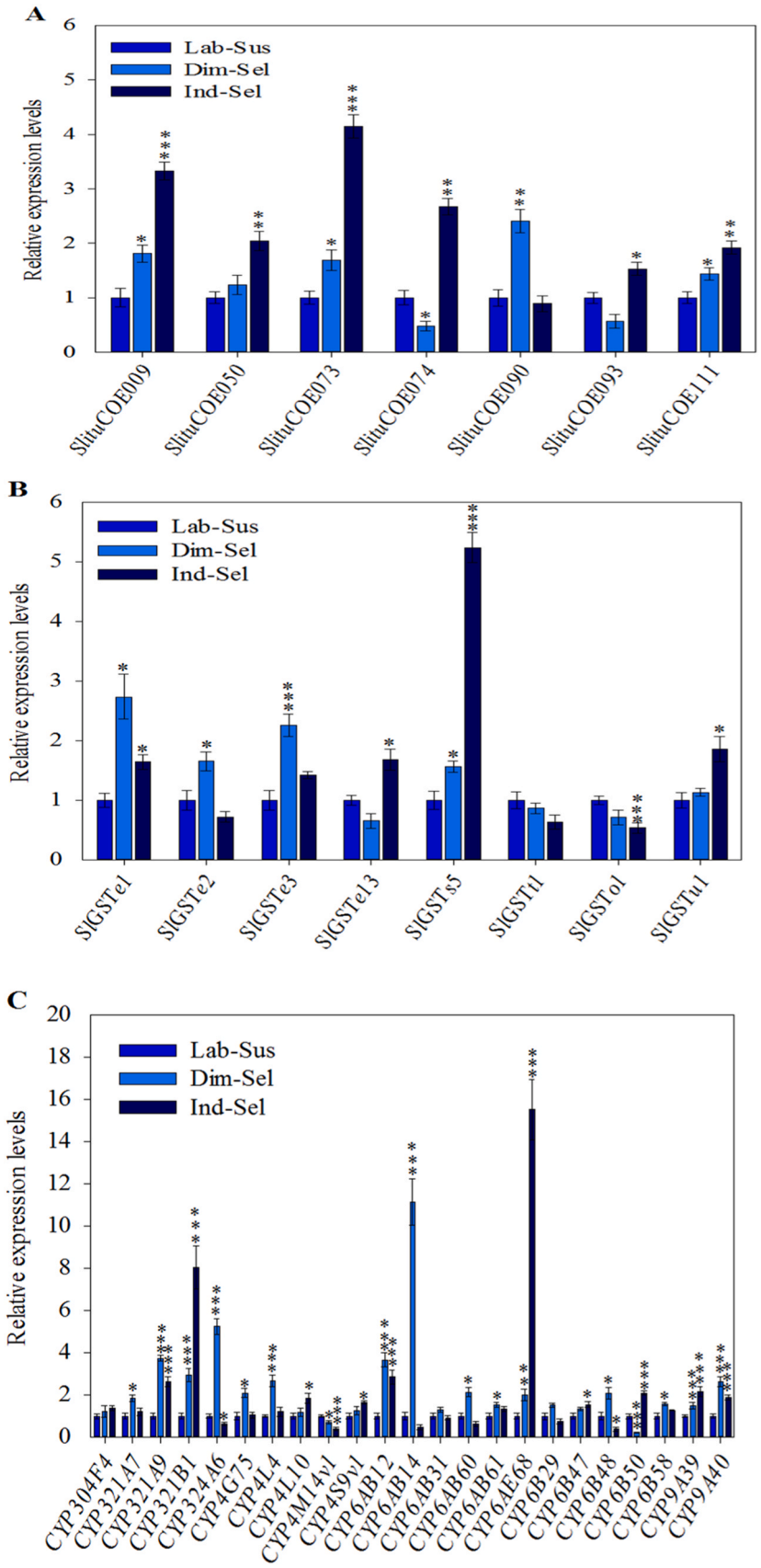

Fig. 2. RT-qPCR analysis of the transcription levels of thirty-eight detoxification-related genes [seven CarEs (A), eight GSTs (B), and twenty-three P450s (C)] in the Lab-Sus, Dim-Sel, and Ind-Sel strains of *S. litura* after treatment with indoxacarb (LC_{10}) for 24 h. Data are presented as mean ± SE from three independent replicates (3 RNA extractions). Asterisks indicate significant differences between the Lab-Sus and Dim-Sel or Lab-Sus and the Ind-Sel strains (Student's *t*-test; *P< 0.05, **P< 0.01, ***P< 0.001).

Table 5
Bioassay results of beta-cypermethrin, chlorpyrifos, phoxim, chlorantraniliprole, and emamectin benzoate to the Lab-Sus, Dim-Sel, and Ind-Sel strains of *S. litura*.

Insecticides	Strains	Slope ± SE	LC_{50} (mg L^{-1}) (95% CI)[b]	χ^2 (df)	P value	RR (95% CI)[a]
Beta-cypermethrin	Lab-Sus	1.99 ± 0.19	0.26 (0.22–0.31)	1.83 (3)	0.61	1
	Dim-Sel	1.84 ± 0.18	24.79 (20.86–30.39)	4.10 (3)	0.25	95.37 (80.22–116.90)
	Ind-Sel	2.02 ± 0.08	0.69 (0.59–0.81)	3.96 (3)	0.27	2.64 (2.27–3.09)
Chlorpyrifos	Lab-Sus	2.25 ± 0.18	1.22 (0.83–1.73)	6.60 (3)	0.09	1
	Dim-Sel	2.32 ± 0.20	111.79 (98.05–128.06)	1.10 (3)	0.78	92.01 (80.69–105.40)
	Ind-Sel	1.29 ± 0.17	5.02 (3.77–7.64)	1.59 (3)	0.66	4.13 (3.10–6.28)
Phoxim	Lab-Sus	2.10 ± 0.19	0.19 (0.16–0.22)	3.85 (3)	0.28	1
	Dim-Sel	1.93 ± 0.19	16.33 (13.27–19.40)	3.91 (3)	0.27	85.94 (69.84–102.12)
	Ind-Sel	1.93 ± 0.18	0.61 (0.52–0.73)	1.18 (3)	0.76	3.20 (2.73–3.82)
Chlorantraniliprole	Lab-Sus	2.04 ± 0.19	0.11 (0.09–0.13)	2.20 (3)	0.53	1
	Dim-Sel	1.98 ± 0.18	7.55 (6.47–8.96)	4.36 (3)	0.23	66.83 (57.21–79.27)
	Ind-Sel	2.14 ± 0.18	0.22 (0.18–0.25)	5.08 (3)	0.17	1.92 (1.66–2.22)
Emamectin benzoate	Lab-Sus	1.75 ± 0.133	0.03 (0.02–0.03)	3.71 (3)	0.29	1
	Dim-Sel	2.05 ± 0.18	1.56 (1.35–1.82)	3.58 (3)	0.31	57.89 (49.82–67.29)
	Ind-Sel	1.86 ± 0.14	0.03 (0.03–0.04)	3.36 (3)	0.30	1.15 (0.96–1.41)

SE, Standard error; LC_{50}, the lethal concentration at 50%; CI, confidence intervals; df, degree of freedom; RR (resistance ratio) = (LC_{50} of the Dim-Sel or Ind-Sel strain)/(LC_{50} of the Lab-Sus strain). a 95% CI estimated using the lethal concentration ratio significance test (Robertson and Preisler, 1992).

fecundity of *S. litura* after exposure to indoxacarb and DIMBOA (97%). Similar influences were reported in the former studies where fitness cost decreased the developmental time, fecundity, and longevity of various insects after exposure to different insecticides (Cui et al., 2018; Okuma et al., 2018; Hafeez et al., 2019; Padovez et al., 2022; Hua et al., 2023). This study indicated that insecticides, whether used alone or in combination with phytochemicals, have a negative impact on the fitness cost of insects that come into contact with them. This finding has implications for the effectiveness of integrated pest management (IPM) programs that aim to control insect pests through multiple approaches. Similar to previous results, our findings indicate that, under constant selection pressure from indoxacarb and DIMBOA (97%), the costs associated with larval growth, adults' longevity, and female fecundity were different for the Dim-Sel strains and Ind-Sel strain populations (Saska et al., 2016; Hafeez et al., 2019; He et al., 2019; Gul et al., 2021). Life table analysis enables the calculation of the population for insects in specific conditions. One crucial parameter within life tables is the intrinsic rate of increase, which provides a comprehensive and intuitive description of the population potential for insects reared under specific conditions. This parameter can be derived from data on developmental time, fecundity, longevity, sex ratio, and survivorship (Chi et al., 2020). In this study, the life table parameters of *S. litura* were found to be adversely affected by the Dim-Sel and Ind-Sel treatments, which reflect the adverse effects on population growth traits. Compared with the Lab-Sus and Dim-Sel strains, S_{xj} was significantly decreased in the Ind-Sel strain as were l_x and l_xm_x, which demonstrated a marked reduction in the Ind-Sel strain. Similarly, former reports have disclosed similar impacts at the demographic level on other insect pests (Saska et al., 2016; Hafeez et al., 2019; He et al., 2019; Iftikhar et al., 2020 Gul et al., 2021). Knowledge of the resistance to indoxacarb induced by DIMBOA (97%) may help to elucidate the mechanisms leading to plasticity in fitness costs.

Almost all phytophagous insects utilize diverse mechanisms of metabolic detoxification to evade the toxicity of plant secondary metabolites and synthetic insecticides. Overexpression of these detoxifying enzymes, capable of metabolizing insecticides and phytotoxins, can result in higher levels of metabolic tolerance/resistance to synthetic insecticides and plant secondary metabolites (Dawkar et al., 2013; Beran and Petschenka, 2022). Our results show that the activities of P450, EST, and GST might play a key role in the development of resistance to indoxacarb after ingestion of DMSO in *S. litura*. To detoxify and lower their susceptibility to plant allelochemicals or insecticides, *S. litura* and other insect pests have evolved detoxifying plant allelochemical or pesticide resistance mechanisms (Feyereisen, 2006; Wang et al., 2015a; Shi et al., 2019; Hou et al., 2021; Shi et al., 2022). Similar to our outcomes, numerous investigations have indicated that esterase, GST, and P450 play a significant part in the detoxification mechanisms of insect pests (Tao et al., 2012; Zhang et al., 2017; War et al., 2018; Hafeez et al., 2019; Shi et al., 2022). In the biochemical analysis using synergistic (PBO), the current study indicates that increased P450 activity is most likely the primary detoxification mechanism causing indoxacarb resistance. Meanwhile, increased P450s, GST, and CarEs activities are most likely the cause of DIMBOA detoxification. This study displays that *S. litura* can develop resistance to indoxacarb and that this resistance is mostly caused by increased detoxification enzymes.

Compared to the DMSO control, *H. armigera* larvae consumption of diets containing flavone, coumarin, DIMBOA, and visnagin significantly increased the activity of carboxylesterase in the fat body by 3.78-, 2.97-, 1.42- and 4.72-fold, respectively (Chen et al., 2019). At the same time, flavone, coumarin, DIMBOA, and visnagin significantly improved the activity of cytochrome P450 in both the midgut and fat body of *H. armigera*. *H. armigera* larvae fed on diets containing flavone and coumarin exhibited the highest P450 activity in the midgut by 2.36- and 2.12-fold, respectively, whereas those fed on diets containing DIMBOA demonstrated the highest activity (4.96-fold) in the fat body of *H. armigera* (Chen et al., 2019). However, over 12 generations of selection (RR = 18.37), the GST activity of the fourth-instar larvae of *S. litura* of the Lab-Sus and Ind-Sel strains was not significantly changed (Hou et al., 2021).

Numerous studies indicate that the metabolic resistance of insects to insecticide is mainly caused by an increased rate of insecticide detoxification, which is due to the overexpression, amplification or mutation of detoxification enzymes and related genes (such as P450s, CarEs, and GSTs) (Li et al., 2007; Luo et al., 2022; Shi et al., 2022). Previous studies have exhibited that seven CarE genes (*SlituCOE009*, *SlituCOE090*, *SlituCOE073*, *SlituCOE050*, *SlituCOE093*, *SlituCOE074,* and *SlituCOE111*) were upregulated in the indoxacarb-resistant strains of *S. litura* (Shi et al., 2019). The transcripts of *SlituCOE009*, *SlituCOE090*, *SlituCOE073*, *SlituCOE050*, *SlituCOE093,* and *SlituCOE074* were significantly upregulated, by 2.53-, 4.01-, 25.49-, 3.27-, 4.69- and 14.21-fold in the indoxacarb-resistant strain and by 2.35-, 6.24-, 9.63-, 11.03-, 2.42- and 5.27-fold in the field indoxacarb-resistant strain than the susceptible strain of *S. litura*, respectively (Shi et al., 2022). Similarly, after feeding on diets supplemented with flavone, coumarin, DIMBOA, or visnagin-induced expression of the *CYP6B2*, *CYP6B6,* and *CYP6B7* genes in both midgut and fat body of *H. armigera* (Chen et al., 2019). Compared to DMSO control, feeding on diets containing DIMBOA for 24 h, the transcription level of *CYP6B6* and *CYP6B7* were significantly induced by 2.9- and 4.1-fold in the midgut of *H. armigera*, respectively (Chen et al., 2019). While, *CYP6B2*, *CYP6B6,* and *CYP6B7* were significantly induced by 3.5-, 7.8- and 6.0-fold in the fat body of *H. armigera* after treatments with DIMBOA for 24 h, respectively (Chen et al., 2019). In this study,

among the thirty-eight detoxification-related genes (seven CarEs, eight GSTs, and twenty-three P450s), the transcript level of four CarEs, four GSTs, and fifteen P450s was significantly upregulated in the Dim-Sel strain feeding on diets containing LC_{10} indoxacarb after 24 h when compared to the Lab-Sus strain of *S. litura* (Fig. 2). Treatment with LC_{10} indoxacarb for 24 h, the transcript level of six CarEs, four GSTs, and ten P450s were significantly increased in the Ind-Sel strain than those in the Lab-Sus strain of *S. litura* (Fig. 2). Three CarEs (*SlituCOE009*, *SlituCOE073*, and *SlituCOE111*), two GSTs (*SlGSTe1*, and *SlGSTs5*), and six P450s (*CYP321A9*, *CYP321B1*, *CYP6AB12*, *CYP6AE68*, *CYP9A39,* and *CYP9A40*) were significantly increased in both Dim-Sel and Ind-Sel strains than those of in the Lab-Sus strain of *S. litura* (Fig. 2), which indicated that these genes might involve in detoxification of plant allelochemical or insecticides.

5. Conclusions

This study revealed that after selection with plant allelochemical DIMBOA for 18 generations highly induced P450s, GSTs, and CarEs genes transcript and increased the activity of detoxification enzymes in the larvae of *S. litura* which inducement led to reduced larval susceptibility to beta-cypermethrin, chlorpyrifos, phoxim, chlorantraniliprole, and emamectin benzoate. Plant allelochemicals in host plants might through induction enhanced detoxification mechanisms and reduce caterpillar susceptibility to insecticides. In addition, this study indicated that insecticide, whether alone or in combination with DIMBOA, has a negative impact on the fitness cost of *S. litura.* The study provided important information on plant allelochemicals on resistance to *S. litura* and indoxacarb resistance characteristics that will be useful for the integrated pest management of *S. litura*.

CRediT authorship contribution statement

Xi Yang: Methodology, Data curation, Software. **Muhammad Hafeez:** Visualization, Writing – original draft, Data curation, Software, Review & editing. **Chen Hongyu:** Visualization, Data curation. **Wan-Ting Li:** Visualization, Data curation. **Rong-Jie Ren:** Visualization, Data curation. **Yu-Sen Luo:** Data curation, Software. **Yousif Abdelrahman Yousif Abdellah:** Writing – original draft, Project administration, Funding acquisition, Review & editing. **Rui-Long Wang:** Supervision, Project administration, Funding acquisition, 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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115669.

References

Agrawal, A.A., Weber, M.G., 2015. On the study of plant defence and herbivory using comparative approaches: how important are secondary plant compounds. Ecol. Lett. 18 (10), 985–991.

Ahmad, M., Sayyed, A.H., Saleem, M.A., Ahmad, M., 2008. Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. Crop Prot. 27 (10), 1367–1372.

Beran, F., Petschenka, G., 2022. Sequestration of plant defense compounds by insects: from mechanisms to insect-plant coevolution. Annu. Rev. Entomol. 67, 163–180.

Chen, Q.J., Li, G.H., Pang, Y., 2000. A simple artificial diet for mass rearing of some noctuid species. Entomol. Knowl. 37, 325–327.

Chen, S., Elzaki, M.E.A., Ding, C.H., Li, Z.F., Wang, J., Zeng, R.S., et al., 2019. Plant allelochemicals affect tolerance of polyphagous lepidopteran pest *Helicoverpa armigera* (Hübner) against insecticides. Pestic. Biochem. Physiol. 154, 32–38.

Chi, H., 1988. Life-table analysis incorporating both sexes and variable development rates among individuals. Environ. Entomol. 17, 26–34.

Chi, H., 2023. TWOSEX-MSChart: A computer program for the age-stage, two-sex life table analysis. National Chung Hsing University: Taichung, Taiwan, 2023; Available online: ⟨http://140.120.197.173/Ecology/Download/Twosex-MsChart.rar⟩ (accessed on 26 May 2023).

Chi, H., Liu, H., 1985. Two new methods for study of insect population ecology. Bull. Inst. Zool. Acad. Sin. 24 (2), 225–240.

Chi, H., Su, H.Y., 2006. Age-stage, two-sex life tables of *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae) and its host *Myzus persicae* (Sulzer) (Homoptera: Aphididae) with mathematical proof of the relationship between female fecundity and the net reproductive rate. Environ. Entomol. 35, 10–21.

Chi, H., You, M., Atlıhan, R., Smith, C.L., Kavousi, A., Özgökçe, M.S., et al., 2020. Age-Stage, two-sex life table: an introduction to theory, data analysis, and application. Entomol. Gen. 40, 103–124.

Cui, L., Wang, Q.Q., Qi, H.L., Wang, Q.Y., Yuan, H.Z., Rui, C.H., 2018. Resistance selection of indoxacarb *Inhelicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae): cross-resistance, biochemical mechanisms and associated fitness costs. Pest Manag. Sci. 74 (11), 2636–2644.

Dawkar, V.V., Chikate, Y.R., Lomate, P.R., Dholakia, B.B., Gupta, V.S., Giri, A.P., 2013. Molecular insights into resistance mechanisms of lepidopteran insect pests against toxicants. J. Proteome Res. 12 (11), 4727–4737.

Feyereisen, R., 2006. Evolution of insect P450. Biochem. Soc. Trans. 34 (Pt 6), 1252–1255.

Glauser, G., Marti, G., Villard, N., Doyen, G.A., Wolfender, J.L., Turlings, T.C.J., et al., 2011. Induction and detoxification of maize 1,4-benzoxazin-3-ones by insect herbivores. Plant J. 68 (5), 901–911.

Gong, C., Wang, Y., Huang, Q., Xu, Z., Zhang, Y., Hasnain, A., Zhan, X., He, Y., Zhang, T., Shen, L., Pu, J., Awais, M., Wang, X., 2022. MAF regulates the overexpression of CYP307A1, which is involved in the fitness advantage of Bistrifluron-resistant *Spodoptera litura* (fab.) (Noctuidae: Lepidoptera). Ecotoxicol. Environ. Safe 234, 113425.

Gul, H., Ullah, F., Hafeez, M., Tariq, K., Desneux, N., Gao, X., et al., 2021. Sublethal concentrations of clothianidin affect fecundity and key demographic parameters of the chive maggot, *Bradysia odoriphaga*. Ecotoxicology 30 (6), 1150–1160.

Guo, J., Liu, S., Jing, D., He, K., Zhang, Y., Li, M., et al., 2022. Genotypic variation in field-grown maize eliminates trade-offs between resistance, tolerance and growth in response to high pressure from the Asian corn borer. Plant, Cell Environ. https://doi.org/10.1111/pce.14458.

Guo, Z., Gai, C., Cai, C., Chen, L., Liu, S., Zeng, Y., Yuan, J., Mei, W., Dai, H., 2017. Metabolites with insecticidal activity from Aspergillus fumigatus JRJ111048 isolated from mangrove plant *Acrostichum specioum* endemic to Hainan Island. Mar. Drugs 15 (12), 381.

Gupta, G.P., Rani, S., Birah, A., Raghuraman, M., 2005. Improved artificial diet for mass rearing of the tobacco caterpillar, *Spodoptera litura* (Lepidoptera: Noctuidae). Int. J. Trop. Insect Sci. 25, 55–58.

Hafeez, M., Liu, S., Jan, S., Ali, B., Shahid, M., Fernández-Grandon, G.M., et al., 2019. Gossypol-induced fitness gain and increased resistance to deltamethrin in beet armyworm, *Spodoptera exigua* (Hübner). Pest Manag. Sci. 75 (3), 683–693.

Hafeez, M., Li, X., Ullah, F., Zhang, Z., Zhang, J., Huang, J., et al., 2022. Characterization of indoxacarb resistance in the fall armyworm: selection, inheritance, cross-resistance, possible biochemical mechanisms, and fitness costs. Biology 11 (12), 1718.

He, F., Sun, S., Tan, H., Sun, X., Qin, C., Ji, S., et al., 2019. Chlorantraniliprole against the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae): From biochemical/physiological to demographic responses. Sci. Rep. 9 (1), 10328.

Hou, W.T., Staehelin, C., Elzaki, M.E.A., Hafeez, M., Luo, Y.S., Wang, R.L., 2021. Functional analysis of CYP6AE68, a cytochrome P450 gene associated with indoxacarb resistance in *Spodoptera litura* (Lepidoptera: Noctuidae). Pestic. Biochem. Physiol. 178, 104946.

Hua, D., Li, X., Yuan, J., Tao, M., Zhang, K., Zheng, X., et al., 2023. Fitness cost of spinosad resistance related to vitellogenin in *Frankliniella occidentalis* (Pergande). Pest Manag. Sci. 79 (2), 771–780.

Huang, Y.B., Chi, H., 2012. Age-stage, two-sex life tables of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) with a discussion on the problem of applying female age-specific life tables to insect populations. Insect Sci. 19, 263–273.

Iftikhar, A., Hafeez, F., Hafeez, M., Farooq, M., Aziz, M.A., Sohaib, M., et al., 2020. Sublethal effects of a juvenile hormone analog, Pyriproxyfen demographic parameters of non-target predator, *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae). Ecotoxicology 29 (7), 1017–1028.

Jia, B., Liu, Y., Zhu, Y.C., Liu, X., Gao, C., Shen, J., 2009. Inheritance, fitness cost and mechanism of resistance to tebufenozide in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Pest Manag. Sci. 65 (9), 996–1002.

Lai, T., Jia, L., Su, J., 2011. Monitoring of beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) resistance to chlorantraniliprole in China. Pestic. Biochem. Physiol. 101, 198–205.

Li, X., Schuler, M.A., Berenbaum, M.R., 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu. Rev. Entomol. 52, 231–253.

Liu, S.W., Elzaki, M.E.A., Staehelin, C., Ma, Z.H., Qin, Z., Wang, R.L., 2019. Exposure to herbicides reduces larval sensitivity to insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae). Insect Sci. 26, 711–720.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 25, 402–408.

Luo, Y.S., Abdellah, Y.A.Y., Hafeez, M., Yang, X., Hou, W.T., Kong, X.H., et al., 2022. Herbivore-induced tomato plant volatiles lead to the reduction of insecticides susceptibility in *Spodoptera litura*. Pestic. Biochem. Phys. 187, 105215.

Manuwoto, S., Scriber, J.M., 1982. Consumption and utilization of three maize genotypes by the southern armyworm. J. Econ. Entomol. 75, 163–167.

Niemeyer, H.M., 2009. Hydroxamic acids derived from 2-hydroxy-2H-1,4-benzoxazin-3 (4H)-one: key defense chemicals of cereals. J. Agr. Food Chem. 57 (5), 1677–1696.

Okuma, D.M., Bernardi, D., Horikoshi, R.J., Bernardi, O., Silva, A.P., Omoto, C., 2018. Inheritance and fitness costs of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistance to spinosad in Brazil. Pest Manag. Sci. 74 (6), 1441–1448.

Ortego, F., Rui, z, M., Castañera, P., 1998. Effect of DIMBOA on growth and digestive physiology of Sesamia nonagrioides (Lepidoptera: Noctuidae) larvae. J. Insect Physiol. 44 (2), 95–101.

Padovez, F.E.O., Kanno, R.H., Omoto, C., Guidolin, A.S., 2022. Fitness costs associated with chlorantraniliprole resistance in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains with different genetic backgrounds. Pest Manag. Sci. 78 (3), 1279–1286.

Rakoczy-Trojanowska, M., Święcicka, M., Bakera, B., Kowalczyk, M., Stochmal, A., Bolibok, L., 2020. Cocultivating rye with berseem clover affects benzoxazinoid production and expression of related genes. Crop Sci. 60, 3228–3246.

Rehan, A., Freed, S., 2014. Selection, mechanism, cross resistance and stability of spinosad resistance in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Crop Prot. 56, 10–15.

Robertson, J.L., Russell, R.M., Preisler, H.K., Savin, N.E., 2007. Pesticide Bioassays with Arthropods, 2nd edition.,. CRC Press,, Boca Raton, FL.

Rose, R.L., Barbhaiya, L., Roe, R.M., Rock, G.C., Hodgson, E., 1995. Cytochrome P450-associated insecticide resistance and the development of biochemical diagnostic assays in *Heliothis virescens*. Pestic. Biochem. Physiol. 51, 178–191.

Rostás, M., 2007. The effects of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one on two species of *Spodoptera* and the growth of *Setosphaeria turcica* in vitro. J. Pest Sci. 80, 35–41.

Saska, P., Skuhrovec, J., Lukáš, J., Chi, H., Tuan, S.J., Honěk, A., 2016. Treatment by glyphosate-based herbicide alters life history parameters of the rose-grain aphid *Metopolophium dirhodum*. Sci. Rep. 6, 27801.

Shad, S.A., Sayyed, A.H., Fazal, S., Saleem, M.A., Zaka, S.M., Ali, M., 2012. Field evolved resistance to carbamates, organophosphates, pyrethroids, and new chemistry insecticides in *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). J. Pest Sci. 85, 153–162.

Shi, L., Shi, Y., Zhang, Y., Liao, X.L., 2019. A systemic study of indoxacarb resistance in *Spodoptera litura* revealed complex expression profiles and regulatory mechanism. Sci. Rep. 9 (1), 14997.

Shi, L., Shi, Y., Liu, M.F., Zhang, Y., Liao, X.L., 2021. Transcription factor CncC potentially regulates the expression of multiple detoxification genes that mediate indoxacarb resistance in *Spodoptera litura*. Insect Sci. 28 (5), 1426–1438.

Shi, Y., Li, W., Zhou, Y., Liao, X., Shi, L., 2022. Contribution of multiple overexpressed carboxylesterase genes to indoxacarb resistance in *Spodoptera litura*. Pest Manag. Sci. 78 (5), 1903–1914.

Silva-Brandão, K.L., Murad, N.F., Peruchi, A., Martins, C.H.Z., Omoto, C., Figueira, A., et al., 2021. Transcriptome differential co-expression reveals distinct molecular response of all-armyworm strains to DIMBOA. Pest Manag. Sci. 77 (1), 518–526.

Sun, R., Jiang, L., Chen, W., Xu, Y., Yi, X., Zhong, G., 2023. Azadirachtin exposure inhibit ovary development of *Spodoptera litura* (Lepidoptera: Noctuidae) by altering lipids metabolism event and inhibiting insulin signaling pathways. Ecotox Environ. Safe 262, 115151.

Tao, X.Y., Xue, X.Y., Huang, Y.P., Chen, X.Y., Mao, Y.B., 2012. Gossypol-enhanced P450 gene pool contributes to cotton bollworm tolerance to a pyrethroid insecticide. Mol. Ecol. 21 (17), 4371–4385.

Tong, H., Su, Q., Zhou, X.M., Bai, L.Y., 2013. Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. J. Pest Sci. 86 (3), 599–609.

Wang, R.L., Christian, S., Xia, Q.Q., Su, Y.J., Zeng, R.S., 2015a. Identification and characterization of CYP9A40 from the tobacco cutworm moth (*Spodoptera litura*), a cytochrome P450 gene induced by plant allelochemicals and insecticides. Int. J. Mol. Sci. 16 (9), 22606–22620.

Wang, R.L., Li, J., Staehelin, C., Xin, X.W., Su, Y.J., Zeng, R.S., 2015b. Expression analysis of two P450 monooxygenase genes of the tobacco cutworm moth (*Spodoptera litura*) at different developmental stages and in response to plantallelochemicals. J. Chem. Ecol. 41 (1), 111–119.

Wang, R.L., He, Y.N., Christian, S., Liu, S.W., Su, Y.J., Zhang, J.E., 2017a. Identification of two cytochrome monooxygenase P450 genes, *CYP321A7* and *CYP321A9*, from the tobacco cutworm moth (*Spodoptera litura*) and their expression in response to plant allelochemicals. Int. J. Mol. Sci. 18 (11), 2278.

Wang, R.L., Zhu-Salzman, K., Baerson, S.R., Xin, X.W., Li, J., Su, Y.J., et al., 2017b. Identification of a novel cytochrome P450*CYP321B1* gene from tobacco cutworm (*Spodoptera litura*) and RNA interference to evaluate its role in commonly used insecticides. Insect Sci. 24 (2), 235–247.

Wang, X.G., Huang, Q., Hao, Q., Ran, S., Wu, Y.Q., Cui, P., et al., 2018. Insecticide resistance and enhanced cytochrome P450 monooxygenase activity in field populations of *Spodoptera litura* from Sichuan, china. Crop Prot. 106, 110–116.

War, A.R., Taggar, G.K., Hussain, B., Taggar, M.S., Nair, R., M., Sharma, H.C., 2018. Plant defence against herbivory and insect adaptations. AoB Plants 10, ply37.

Wing, K.D., Sacher, M., Kagaya, Y., Tsurubuchi, Y., Muldering, L., Connair, M., et al., 2000. Bioactivation and mode of action of the oxadiazine indoxacarb in insects. Crop Prot. 19 (8–10), 537–545.

Wouters, F.C., Reichelt, M., Glauser, G., Bauer, E., Erb, M., Gershenzon, J., et al., 2014. Reglucosylation of the benzoxazinoid Dimboa with inversion of stereochemical configuration is a detoxification strategy in lepidopteran herbivores. Angew. Chem. Int. Ed. Engl. 53 (42), 11320–11324.

Xu, L., Mei, Y., Liu, R., Chen, X., Li, D.Z., Wang, C.J., 2020. Transcriptome analysis of *Spodoptera litura* reveals the molecular mechanism to pyrethroids resistance. Pestic. Biochem. Physiol. 169, 104649.

Yang, Y., Wu, Y., Chen, S., Devine, G.J., Denholm, I., Jewess, P., et al., 2004. The involvement of microsomal oxidases in pyrethroid resistance in *Helicoverpa armigera* from Asia. Insect Biochem. Mol. Biol. 34 (8), 763–773.

Zhang, N., Liu, J., Chen, S.N., Huang, L.H., Feng, Q.L., Zheng, S.C., 2016. Expression profiles of glutathione *S*-transferase superfamily in *Spodoptera litura* tolerated to sublethal doses of chlorpyrifos. Insect Sci. 23 (5), 675–687.

Zhang, S.Z., Zhang, X.L., Shen, J., Li, D.Y., Wan, H., You, H., et al., 2017. Cross-resistance and biochemical mechanisms of resistance to indoxacarb in the diamondback moth, *Plutella xylostella*. Pestic Biochem. Phys 140, 85–89.

Zhao, Y.J., Wang, Z.Q., Zhu, J.Y., Liu, N.Y., 2020. Identification and characterization of detoxification genes in two cerambycid beetles, *Rhaphuma horsfieldi* and *Xylotrechus quadripes* (Coleoptera: Cerambycidae: Clytini). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 243–244, 110431.

Zou, X., Xu, Z., Zou, H., Liu, J., Chen, S., Feng, Q., et al., 2016. Glutathione *S*-transferase SlGSTE1 in *Spodoptera litura* may be involved in feeding adaption of host plants. Insect Biochem. Mol. Biol. 70, 32–43.