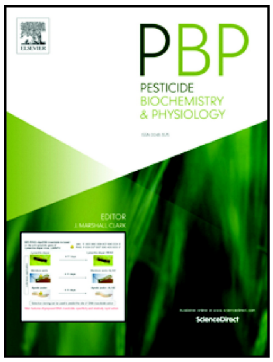

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PII: S0048-3575(18)30334-1
DOI: https://doi.org/10.1016/j.pestbp.2019.01.012
Reference: YPEST 4324

To appear in: *Pesticide Biochemistry and Physiology*

Received date: 20 July 2018
Revised date: 15 January 2019
Accepted date: 27 January 2019

Please cite this article as: M. Hafeez, S. Liu, S. Jan, et al., Enhanced effects of dietary tannic acid with chlorantraniliprole on life table parameters and nutritional physiology of Spodoptera exigua (Hübner), Pesticide Biochemistry and Physiology, https://doi.org/10.1016/j.pestbp.2019.01.012

Enhanced effects of dietary tannic acid with chlorantraniliprole on life table parameters and nutritional physiology of *Spodoptera exigua* (Hübner)

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Abstract

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) is a highly polyphagous pest which causes considerable economic losses to cotton and many vegetable crops. Tannins are among the most important secondary metabolites in cotton plants. We show that tannic acid enhances the toxic effect of chlorantraniliprole on *S. exigua* when presented in combination. Bioassays using third-instar *S. exigua* larvae on an artificial diet showed that consumption of tannic acid with chlorantraniliprole at the concentration of (2 mg/g and LC_{50} 0.018 mg/L) had higher toxicity when compared to either chlorantraniliprole or tannic acid alone (LC_{50} 0.027 mg/L). The diet containing tannic acid with chlorantraniliprole significantly prolonged larval and pupal developmental time and extended mean generation time and total pre-oviposition period compared to either chemical alone. Moreover, fecundity, survival rate, reproductive value, intrinsic rate of increase, finite rate of increase and net reproduction rate declined significantly when exposed to the combined treatment. No difference was observed between tannic acid and the control. Meanwhile, tannic acid with chlorantraniliprole had markedly antifeedant effects; causing significant decline in the relative growth rate (RGR), the relative consumption rate (RCR), the efficiency of conversion of ingested food (ECI), the efficiency of conversion of digested food and an increase in the approximate digestibility (AD) compared to either chemical alone. Tannic acid with chlorantraniliprole also decreased the insect's carbohydrate, lipid and protein contents significantly. The results showed that the interaction between tannic acid and chlorantraniliprole on the growth inhibition of larvae was additive and tannic acid increased the toxicity of chlorantraniliprole to insects. The results of this study provide information useful in integrated pest management programs for *S. exigua* and

show that tannic acid combined with chlorantraniliprole may be a route to reducing the use of synthetic pesticides.

Keywords: Beet armyworm; tannic acid; chlorantraniliprole; nutrient contents; growth; reproduction; nutritional physiology.

1. Introduction

Beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) is an extensively distributed and destructive pest, often causes severe economic losses to important crops like corn, soybeans, cotton, beets, corn, cabbage tomatoes, and alfalfa. Its polyphagous nature makes it a pest to plant species representing more than 30 different families [1,2]. *S. exigua* is a notable problem due to its insecticide resistance, which has been widely documented [3,4]. Although, there are many novel methods for insect control, chemical control using insecticides has long been considered dependable method, as alternative control measures themselves are often insufficient at preventing economic damage [5]. *Spodoptera exigua* has developed a resistance towards different groups of pesticides as a consequence of its polyphagous behavior, overlapping development and longtime exposure to most insecticides [6–8]. Due to the failure of synthetic pesticides to control *S. exigua*, it causes a tremendous economic loss globally to a wide range of crops [7,9]. It has been reported that the selection of pesticides, methods, and frequencies of application play a crucial function in contribution insecticide resistance [10,11]. Almost all type of pesticides have been widely used against the immature and adult phases of insects in agriculture, forestry, and public health fields [12–15]. This, in turn, has harmful effects on non-target organisms and human beings as well as in food chains [16]. In humans and other organisms, the ingestion of pesticides can cause serious health problems that can have multi-generational impact [17].

Plant secondary metabolites exert important defensive roles by interfering with essential metabolic, biochemical, physiological functions and pathways of herbivorous insects [18]. Various important plant secondary metabolites have been shown to provide plants with resistant against many herbivorous insects by acting as feeding deterrents, growth inhibitors or toxins for

several insect orders including Lepidoptera, Coleoptera and Hemiptera [19–21]. It is also reported that insecticide resistance mechanisms show a discrepancy across insect pest populations feeding on different host plant species [10]. Tannic acid and flavone are the important plant secondary metabolites with an insecticidal effect which may have application in integrated pest management (IPM) systems [22]. Tannic acid is one of the most important plant secondary metabolites, involved in the defense system of different plants against phytophagous insects. Exactly how tannic acid affects the herbivore population is still open to debate, however, they are broadly considered as anti-digestive protein binding agents [23]. Some studies have reported that increasing levels of carbon dioxide enhanced the condensed tannic acid and gossypol content in cotton plants, and reduced the growth and development capacity of infesting *B. tabaci*, *A. gossypii* and *S. exigua* indirectly [24–26]. Polyphagous insects can feed on many different host plants across their geographic distribution, however, inside their environments, they commonly feed on a small number of plant species [27]. Variation in the use of different host plants displays the patterns of local adaptation of insect populations and also shows responses to past selection [28]. However, *S. exigua* have acquired a high level of resistance against different groups of insecticides due to high-frequency applications and massive doses of these traditional pesticides exposed [4,29]. The development of insecticide resistance, however, is a major problem in the control *S. exigua*[30]. Therefore, the development of a sustainable, integrated management program against *S. exigua* requires the availability of a wide range of environmentally safe insecticidal compounds with different modes of action.

Chlorantraniliprole belongs to the Ryanoid class of insecticide, which has received extensive attention because of their novel mode of action with high insecticidal activity to target pests, whilst remaining acutely safe for all natural enemies [31,32]. Since its registration in

China, chlorantraniliprole has been widely used on field crops to control several lepidopteran pests including *S. exigua*[33]. Within the body, chlorantraniliprole binds to ryanodine receptor modulators site leading to uncontrolled release of Ca ions from the sarcoplasmic reticulum. The mode of action causes impaired regulation of muscular contraction, which results in termination of feeding, lethargy, paralysis, and ultimately death [34,35]. It has been reported that chlorantraniliprole is highly effective against lepidopteran insect pests, and no cross-resistance has yet been reported with existing pesticides or incidence of low toxic impact on non-target organism [36].

Overall, more attention is being paid to insect pest resistance to insecticides because of the extensive application of chemical control methods. The mechanism of interaction between tannic acid combined with chlorantraniliprole on *S. exigua* has not previously been studied. In this research we determined the effects of tannic acid combined with chlorantraniliprole on larval survival, developmental growth, and reproductive parameters *S. exigua* using the computer program 'age-stage, two-sex life table'. In addition, we determined the antifeedant properties and the addition of energy constituents in the third instar larvae of *S. exigua*.

2. Materials and Methods

2.1. Insects breeding technique

The populations of *S. exigua* used in this study were collected from the field in Jingzhou, Hubei province, China and have been reared in laboratory culture since 2003. All the rearing populations of *S. exigua* were kept at 25 ± 2°C, 70 ± 5% relative humidity (RH) and a photoperiod of 16:8 (L:D). Neonate larvae were shifted are raised in small transparent plastic cups (3.5 diameter, × 3 height) containing an artificial diet following an established methodology [37]. From the third growth stage, larvae were reared in the groups and transferred into transparent 12-well cell culture plates. All adults were supplied with 10% honey solution as food in accordance with previous studies [38].

2.2. Chemicals

Chlorantraniliprole (20% SC, Rynaxypyr™), was purchased from DuPont Crop Protection (Shanghai, China) and 98% Tannic acid (powder) was purchased from China Pharmaceutical Grade Chemicals (www.graderchem.com China).

2.3. Preparation of insecticide plus tannic acid-supplemented diets

Concentrations and preparation methods for treatments was established in a preliminary study. To prepare the insecticide plus tannic acid-supplemented diets, the stock solution of chloranraniliprole (2.5 mg/L in distilled water) was diluted by using 0.1% (w/v) 'Triton X-100' aqueous solution. Tannic acid was first dissolved in 1% dimethyl sulfoxide (DMSO). The same concentration of 1% DMSO was dissolved in all diets contained treatments including control. The different concentrations (0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625 mg/L of insecticide containing 2 mg/g tannic acid in each insecticide concentration) were thoroughly mixed with the artificial diet and then transferred into small transparent plastic cups (3 cm in diameter by 3.5 cm

in height) before the solidification of agar (when it reached 40-45°C). The control group (neither tannic acid nor insecticide) diet was prepared using an equal volume of 1% DMSO and 0.1% (w/v) 'Triton X-100' aqueous solution using the same method.

2.4. Enhanced effects of tannic acid with of chlorantraniliprole against *S. exigua*

The enhanced effect of tannic acid on the toxicity of chlorantraniliprole against third-stage instar was evaluated using diet incorporation method for the bioassay [38]. This developmental stage was selected because second and third instars have been identified as the target stage for optimal insect pest management [39]). The stock solution of chloranraniliprole (2.5 mg/L in distilled water) was diluted using 0.1% (w/v) 'Triton X-100' aqueous solution and the final concentration of 2 mg/g tannic acid mixed into the semi-synthetic diet. After mixing, the diet was cut into 3 cm cubes before being placed into transparent petri dishes (5 cm in diameter). Three small plastic cubes were used for each concentration. Approximately 630 one-day-old third instar larvae were used for each toxicity bioassay with each selected group. All toxicity bioassays were accomplished under same environmental circumstances as the insect rearing. All bioassays were conducted using 6 insecticide concentrations (0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625 mg/L of insecticide containing 2 mg/g tannic acid in each insecticide concentration) with three replications per concentration (90 larvae for each treatment for three replicates, including a control group). An equal volume of DMSO and 0.1% (w/v) 'Triton X-100' aqueous solution was used for control with the same methods as above. Mortalities of the exposed population were assessed for 72 h after exposure, and larvae were noted as dead if they did not show any movement when touched with a fine brush.

2.5. Effect of dietary tannic acid with chlorantraniliprole on feeding index of third-stage larvae of *S. exigua*

Evaluation was conducted on the impact of dietary tannic acid, chlorantraniliprole and both in combination on the feeding indices of *S. exigua*. Semi-synthetic diets containing tannic acid (2 mg/g), chlorantraniliprole (0.027 mg/L) alone and tannic acid plus chlorantraniliprole at dose levels of (2 mg/g plus 0.018 mg/L) were mixed into the semi synthetic diet and, after cooling, were cut into 1.5 g cubes. The control treatment contained an equal volume of DMSO and 0.1% (w/v) 'Triton X-100' aqueous solution. In this experiment, newly molted active third-stage larvae were selected (90 larvae treatment with three replications). Prior to commencing the experiment, all the larvae were starved for four hours and then fed on treated or untreated (control) artificial diet. Furthermore, an additional thirty newly molted active third-stage larvae were first weighed then dried in an oven (at 45 °C) for 72 h, and again reweighed. The initial dry weight of each larva in the experiment was converted to a fresh-to-dry (fresh:dry) weight ratio. The same methodology was used to calculate the initial dry weight of the diet for all treatment groups. All remaining diets material after 72 hours and feces produced by larvae were removed and dried in an oven at 45°c for 24 h. Thereby, the dry weight of food eaten by larva (E), the dry weight of feces produced by larvae (F) and also the dry weight gain of the larvae (P) were calculated. A balance (Sartorius BSA 124 S, sensitive to 0.1 mg) was used to measure all weights. The following formulae were used to estimate the feeding indices from all treatments [40].

Relative consumption rate (RCR) = $\frac{E}{TA}$; Relative growth rate (RGR) = $\frac{P}{TA}$; Approximate digestibility (AD) =100 × $\frac{E-F}{E}$; Efficiency of conversion of ingested food (ECI) = $\frac{100P}{E}$; Efficiency of conversion of digested food (ECD) = $\frac{100P}{E-F}$

In the preceding formulae;

A = mean of the dry weight of larvae during T

E = dry weight of food eaten

F = dry weight of feces produced

P = dry weight gain of insect

T = duration of the experimental period

2.6. Measurement of nutrient contents

Levels of macronutrient content such as total protein, lipid, and carbohydrate were measured at 24, 48 and 74 h intervals. The four treatments used were; 1) tannic acid 2) chlorantraniliprole 3) tannic acid plus chlorantraniliprole 4) untreated control group. For each treatment 150 third-instar *S. exigua* larvae were used. After bioassay work was complete, all samples, including the control, were frozen in liquid nitrogen and stored in a freezer (-80°C) for further analysis. Fifteen larvae were used for each treatment for each biochemical index experiment, and five larvae for each individual replicate (three replicates for each treatment) were used.

2.7. Total protein content

To determine the total protein contents, a methodology developed by Bradford was used [41]. First, five larvae (whole body samples) were homogenized in 100 µL extraction buffer containing 50 mM 'Tris-HCl' (pH = 7.1), 0.5% 'Triton X-100' and 20% sucrose all solutions were kept on the ice, then centrifuged at 12,000 rpm (4 °C) for 10 min. Thirty microlitres of the upper layer (supernatant) was moved into the 150 µL 0.01% of Coomassie Brilliant Blue G-250 (Beyotime China) for 5 min. The protein standard curve was drawn using bovine serum albumin (Beyotime China). BIO-RAD xMark Microplate Spectrophotometer was used to measure the absorbance at 595 nm.

2.8. Measurement of lipid content

The analysis technique used for total lipid content was modified from existing methodologies [42,43]. Five larvae (whole bodies) were homogenized in 200 µL solution containing 2% Na_2SO_4. Total lipid contents were extracted in a 750 µL mixture of chloroform: methanol (2:1). The reaction mixture was centrifuged at 12,000 rpm at 4 °C for 10min. Once removed from the centrifuge, 600 µL of the supernatant was collected and dried at 40 °C for 10 hours. A 500 µL solution containing 98% H_2SO_4 was transferred into each tube, followed by incubation in the water bath at 90 °C for 10min. Finally, 30 µL of solution from each sample was mixed into 270 µL of vanillin reagent solution (60 mg vanillin dissolved in 10 mL distilled water and 40 mL solution of 85% H_3PO_4). The absorbance was measured at 530 nm after 30min using (BIO-RAD xMark Microplate Spectrophotometer. Cholesterol standard curve was used to calculate the total lipid content.

2.9. Carbohydrate content

Total carbohydrate content was determined following a previously described methodology [44]. For each replicate, fifteen larvae (whole body) for each treatment were homogenized in the total solution of 160 µL containing 10% trichloroacetic acid (TCA) the solution was chilled on ice before being centrifuged at 12,000 rpm (4°C) for 10min. From this solution, 30 µL of supernatant was mixed with 70-µL solution of 10% of TCA. Then 600-µL solution containing 0.2% anthrone (200mg anthrone substance in 100mL H_2SO_4 98%) was added to the mixture. All the samples were incubated in a water bath for 10 min, and after 10 min, the mixtures were cooled immediately to room temperature. The absorbance was read at 630 nm to draw the glucose standard curve using BIO-RAD xMark Microplate Spectrophotometer.

2.10. Effect of dietary tannic acid alone and combination with chlorantraniliprole on the developmental growth and population parameters of *S. exigua*

Approximately 90 larvae of same-aged third-instar were selected for the growth and life table study. Based on the toxicity of tannic acid and chlorantraniliprole on *S. exigua*, the 120 newly molted (within 24 h) third-instar larvae from each group were taken and fed on semi-synthetic artificial diet treated with tannic acid (2 mg/g), chlorantraniliprole (0.027 mg/L) or tannic acid plus chlorantraniliprole (2 mg/g plus 0.018 mg/L) concentrations. The control population was fed on semi-synthetic artificial diet treated with (DMSO) and the aqueous solution containing 0.1% (w/v) Triton X-100. The number was assigned to all treated individuals and named for the parental generation. After 72 hours of treatment, all live larvae were shifted to small plastic cups. (3 cm in diameter by 3.5 cm in height) and fed on new semi-synthetic artificial diet without any additional supplements. The total developmental stage of each larva in all treatments was recorded every day until they changed into the pupal phase. The old artificial diet was replaced with a fresh one every second day. Twenty, 2-day-old pupae were randomly selected and weighed using AL104 electronic balance (China). Male and female pupae of all treatments were identified using a stereomicroscope before being transferred individually into small transparent plastic cups (3 cm in diameter by 3.5 cm in height) and reared in a breeding room (25 ± 2°C, 70 ± 5% relative humidity) until the adults emerged. One-day-old adults from each treatment were sexed, paired, and transferred into an ovipositional transparent plastic box (9.3 cm in diameter by 6.5 cm in height) with 10% honey solution provided. Nappy liner and white paper were lined inside the plastic box to facilitate oviposition. Nappy liner and paper with eggs were collected replaced every day. The developmental time of larvae from third to fifth instar, pupal duration, total adult longevity, the pre-ovipositional period (POP) and the total number of eggs laid by

each female adult was recorded. All the measurements related to growth and development were recorded every day. Approximately 300 eggs laid by a female (within 24 h) from each treatment were randomly selected, to calculate the hatching rate of eggs as a percentage.

Raw data in the life table for all individuals were analyzed based on the age-stage two-sex life table theory. The age-stage specific survival rate (S_{xj}) represents that every egg will survive to age x and stage j. The age-stage specific fecundity (f_{xj}) demonstrates the number of offspring of age x and stage j produced by females [45]. Age-stage specific reproductive values represent by (v_{xj}). The lifespan (e_{xj}) means that the age (x) and stage (j) that every individual is predicted to measure when age (x).

2.11. Statistical analysis

The data of insecticide concentration-response were calculated by using probit analysis [46]. The control mortality was negligible. The data sets of the pupal weight, percentage hatchability and feeding index were tested for normality and homogeneity of variance using the Kolmogorov-Smirnov D test and Cochran's test, respectively. The effects of tannic acid, chlorantraniliprole alone and tannic acid with chlorantraniliprole on the pupal weight, percentage hatchability and feeding index of *S. exigua* were analyzed using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test. Estimate of the standard errors (SE) associated with these parameters were computed by applying the Jackknife formula [47]. All statistical analyses were performed using SPSS® software, version 19.0 (IBM, SPSS Statistics).

2.12. Life Table Analysis

In this study, the data of all treatments for the life table parameters of *S. exigua* including larval instar and pupal developmental time, survivorship of adults and total fecundity of female adults were analyzed using the age-stage two sex life table theory [48]. All the standard errors for life

table data were calculated by the bootstrap test method [49,50]. Based on the confidence interval of difference, the results of all the different treatments were compared using the paired bootstrap test using TWOSEX-MSChart [48]. All the graphs were constructed using Sigma plot.12.5.

3. Results

3.1. Toxicity of tannic acid with chlorantraniliprole and chlorantraniliprole alone to third-stage larvae of *S. exigua*

Toxicity of diet containing tannic acid (2 mg/g, as established in preliminary assays) with chlorantraniliprole and chlorantraniliprole alone against third-stage larvae of *S. exigua* is shown in **Table 1.**The toxic effect of diet containing tannic acid with chlorantraniliprole was as high as LC_{50} = 0.018 mg/L and LC_{40} = 0.012 mg/L giving enhancement factors of 1.5 as compared to the toxicity of chlorantraniliprole alone against *S. exigua* third-stage larvae (LC_{50} = 0.027 mg/L and LC_{40} = 0.021 mg/L). The tannic acid alone showed a lower toxic effect at concentrations lower than 2 mg/g. These results indicate that tannic acid at the dose of 2 mg/g significantly increased the toxicity of chlorantraniliprole. However, no synergistic effect was found when tannic acid concentration was below 2 mg/g.

3.2. Toxic effects of tannic acid alone and combined with chlorantraniliprole on the development and growth of *S. exigua*

In the parental generation, the toxic effects of diet containing tannic acid with chlorantraniliprole and chlorantraniliprole or tannic acid alone on the growth time, pupal duration and the mean number of total eggs laid by female adults of *S. exigua* were identified (**Table 2).** The toxic effects of diet containing tannic acid with chlorantraniliprole and chlorantraniliprole significantly prolonged the third, fourth and fifth larval period of S. *exigua* compared with the tannic acid

alone or the control treatment. No statistical difference was observed between the tannic acid alone and the control treatment. The pupal duration in diet containing tannic acid with chlorantraniliprole (9.28 d) and chlorantraniliprole (8.55 d) were both significantly longer than in tannic acid alone (7.91 d) and the control (7.76 d). There was no significant difference between the tannic acid alone and control treatments. The mean longevity of female adults was significantly shorter in the tannic acid and chlorantraniliprole alone (10.57 d and 10.33 d, respectively) and tannic acid plus chlorantraniliprole groups (8.5 d) than it was for the control group (12.53 d). There were no significant differences between the tannic acid and chlorantraniliprole. Meanwhile, the mean adult longevity and adult pre-oviposition period (APOP) in all treated groups were showed no difference when compared to the control treatment. However, the total pre-oviposition period (TPOP) of adults and entire female ovipositional days in the diet containing tannic acid with chlorantraniliprole and tannic acid or chlorantraniliprole alone treatments were significantly greater than the control group. While no significant difference was found in total female oviposition days between tannic acid and chlorantraniliprole alone. All treatment diets led to a significant decline in the mean numbers of total eggs laid when compared to the control group. The lower total number of eggs laid by a female adult was found in the combined effect of tannic acid plus chlorantraniliprole group (**Table 2**). Meanwhile, the pupal weights of female and male were significantly greater in the chlorantraniliprole alone compared to the control treatment, while the pupal weights of both female and male were decreased considerably in tannic acid plus chlorantraniliprole treated groups. Tannic acid alone had no significant effect on the male or female pupal weight (**Fig. 1A).** Egg hatchability was also found to decline significantly in the F1 generation when the third-stage larvae of the parent generation were treated with chlorantraniliprole alone. This decline in egg hatchability was

further enhanced in the treatment combining tannic acid and chlorantraniliprole, while no difference was observed between tannic acid alone and control (**Fig. 1B**)

3.3. Toxic effects of tannic acid alone and combined with chlorantraniliprole on the population growth parameters of *S. exigua*

Intrinsic rate of increase (r) was markedly decreased in the combined tannic acid with chlorantraniliprole treatment (0.134 d^{-1}) compared to the control treatment (0.182 d−1). No significant difference to the control was observed when tannic acid or chlorantraniliprole were applied separately. A similar trend was found in the finite rate of increase (λ) and the net reproductive rate (R_0) after the treatment of tannic acid or chlorantraniliprole alone and tannic acid with chlorantraniliprole. However, the mean generation time (T) of *S. exigua* individuals was significantly prolonged in the chlorantraniliprole alone and tannic acid with chlorantraniliprole treatment groups as compared with the tannic acid alone or control groups (**Table 3**).

All curves related to the 'age-stage specific survival rate' (S_{xj}) were demonstrated (**Fig. 2)**. Different life stage overlapping may be because of the development rate varied among individuals. Thus, (S_{xj}) values for female adults were adversely affected by all treated populations when compared to the control which showed the maximum curve value (i.e. 0.48 for females), while this value continually decreased in tannic acid, chlorantraniliprole and tannic acid with chlorantraniliprole treatment groups (0.40, 0.25 and 0.22, respectively). S_{xj} curve shows that the male adults were negatively affected in tannic acid with chlorantraniliprole treatment group (showing a lower rate of 0.21). The 'age-specific survival rate' (l_x) is the probability of newly laid eggs surviving to age 'x,' and it is a basic form of the 'age-stage survival rate' (S_{xj}). The lowest peak value of 'age-specific fecundity' (m_x) can be seen at 32.5 d

in tannic acid with chlorantraniliprole groups (89.6 eggs). The chlorantraniliprole (116.49 eggs), tannic acid (112.69 eggs) and control groups (112.76 eggs), peaks at 31.5, 26 and 24.5 days, respectively. The 'age-specific fecundity' (*fx)* curve signifies the aggregate number of eggs laid by female adults **(Fig. 2)**. The peak values of *fx* under the treatment of chlorantraniliprole alone and tannic acid with chlorantraniliprole were higher whiles under tannic acid alone fecundity was not different to the control **(Fig. 2)**. The curves of 'age-stage specific reproductive values' (V_{xj}) indicates the individual at age '*x'* and stage '*j*' for the next generation **(Fig. 3).** However, this value for male offspring cannot be explained because V_{xj} curve was not drawn for male adults. The results demonstrated that the maximum peak value of 'V_{xj}' declined sharply at the pupal stage, while the time required to attain the peak value was increased in the tannic acid, chlorantraniliprole and tannic acid with chlorantraniliprole groups compared to the control group. **Figure 3** shows that the peak values of V_{xj} for female adults were 260, 320, 350 and 352 for the control, tannic acid with chlorantraniliprole, tannic acid alone and chlorantraniliprole respectively. The age-stage life expectancy curve (e_{xj}) was identified (**Fig. 4**). The (e_{xj}) curves for the third and fourth instar larval stage in the three treated groups sharply dropped; however, this curve significantly increased for the fifth instar larval stage, compared to the control group **(Fig. 4).**

3.4. Enhancement effects of tannic acid combined with chlorantraniliprole on feeding indices of *S. exigua*

After being treated with an artificial diet containing tannic acid or chlorantraniliprole alone and tannic acid with chlorantraniliprole, the feeding indices of *S. exigua* third-instar larvae were significantly decreased compared to the control population. The effect of chlorantraniliprole alone or tannic acid with chlorantraniliprole significantly affected both *S. exigua* feeding

behavior and growth. The approximate digestibility (AD) for the chlorantraniliprole alone and tannic acid with chlorantraniliprole was significantly higher compared with the tannic acid alone and the control group (**Table 4**). Though the efficiency of food ingestion, efficiency of food digestion (ECD), relative growth rate (RGR) and relative consumption rate (RCR) were significantly lower in chlorantraniliprole alone and tannic acid with chlorantraniliprole compared with the tannic acid alone or the control group. No difference was found between the tannic acid and chlorantraniliprole groups (**Table 4**).

3.5. Effects of tannic acid alone and combined with chlorantraniliprole on the total nutrient contents of *S. exigua*

The results revealed that chlorantraniliprole and tannic acid with chlorantraniliprole had a significant effect on the content of carbohydrate, lipids, protein and other primary energy substances in the third-stage larvae of *S. exigua*. The total amount of carbohydrate content in third-instar larvae of *S. exigua* was significantly reduced under the chlorantraniliprole and tannic acid with chlorantraniliprole treatments when compared to the control treatment counterparts after 24, 48 or 72h (**Fig. 6A**). The amount of total lipid content in larvae for chlorantraniliprole and tannic acid with chlorantraniliprole treatment groups was significantly lower than that observed in the tannic acid or control group. Additionally, the total content of protein was significantly reduced under the chlorantraniliprole and tannic acid with chlorantraniliprole treatments compared with the control; similarly, no significant difference was observed between the tannic acid and the control treatment after 48 h or 72h (**Fig. 6C**).

4. Discussion

There is a conflict between the necessity of pesticidal application and its potential adverse effects on the environment. Therefore, reducing the extensive use of pesticides and enhancing the unit activity and property could provide a route to overcoming pesticide resistance development in insect pests. Thus, the application of synergists is an effective strategy for increased pesticide's effectiveness and reduced amount of pesticides usage quantity [51]. In the present study, treatment with diet containing tannic acid with chlorantraniliprole and chlorantraniliprole alone showed significant effects on the growth and reproductive traits of *S. exigua*. Our results are aligned with previous reports, in which the toxicity of 5% dietary tannic acid and the effects of various concentrations of different pesticides on the larval growth and development demonstrated adverse impacts through nutritive stress and perturbations within the development of neural tissues [52,53]. Our research, combined with that of previous studies, demonstrates that efficacy of chlorantraniliprole activity on *S. exigua* is enhanced by tannic acid. Previous studies have shown that tannic acid plus Cry1Ac toxin, and different concentrations of the chlorantraniliprole increased the mean larval and pupal developmental period and mortality of lepidopterous insect pests [33,54–56], a result which supports our finding of increased larval and pupal period for *S. exigua* following exposure to a diet of tannic acid with chlorantraniliprole or chlorantraniliprole alone. It is speculated that the *S. exigua* treated larvae on diet containing tannic acid with chlorantraniliprole commit more resources and energy to overcome the nutritive stress and on detoxification rather than growth and development. As a result, larval development takes significantly longer than the control group [52,57]. In the present research, the mean longevity of female adult, mean number of eggs produced by female adults and hatching percentage of eggs of *S. exigua* declined after feeding on the diet containing tannic

acid with chlorantraniliprole or either chemical alone. Furthermore, the reduction in egg laying capacity could result from the fact that the adult female's ovaries were affected by tannic acid and chlorantraniliprole. Similarly, it has been shown that the size of different components of the ovaries, the number of mature ova, the size of basal oocytes and thickness of their follicular epithelial tissue of *S. litura* could reduce by the exposure to sub-lethal (LC_{30}) concentration of chlorfluazuron insecticide which may be the main reason for the decline of total fecundity and hatchability of eggs [58].

Life table parameters reflect the total effect of an insecticide on an insect population. Fecundity, the intrinsic rate of increase (r), the finite rate of increase (λ), and the net reproductive rate (R_0) are the most useful characteristics indicating reproductive expectations for an insect population [43,59,60]. In this study, fecundity, the 'intrinsic rate of increase' (r), the 'finite rate of increase' (λ), and therefore the 'net reproductive rate' (R_0) had a tendency to be lowered in the diet containing tannic acid with chlorantraniliprole when compared to chlorantraniliprole alone, tannic acid alone or the control group. Similar phenomenon in a number of previous studies have shown the toxic effects of other insecticides including chlorantraniliprole on the fecundity and life table parameters of different insect pests [38,55,60]. Tannic acid used in combination with chlorantraniliprole showed enhanced efficacy, as demonstrated by the life table parameters and may be effective in reducing overall insecticide requirement in integrated pest management (IPM) of *S. exigua*.

The developmental time of the larvae and pupae was significantly increased in the treated population. From assessing previous studies, we speculate that this may be as a result of tannic acid and chlorantraniliprole decreasing nutritional intake through inhibition of feeding and thus inhibiting the insect growth [43,59,61]. We also investigated the effect of diet containing tannic

acid with chlorantraniliprole or either tannic acid and chlorantraniliprole alone on the feeding indices *S. exigua* larvae. Larval assays demonstrated that the approximate digestibility (AD) in diet containing tannic acid with chlorantraniliprole and chlorantraniliprole alone treated group was significantly increased. A similar phenomenon was proposed by Nathan [62], who reported that the longer duration of larvae and the availability of food in the digestive tract leads to an increase in AD. Stoyenoff et al. [63], reported that enhanced digestibility was a consequence of reduced consumption, leading the food to pass more slowly through the digestive tract. Results from the present work reveal that relative growth rate (RGR) of the *S. exigua* larvae significantly decreased when treated with diet containing tannic acid with chlorantraniliprole or chlorantraniliprole alone. This result consistent with previous studies which suggested that the RCI, RGR, ECI and ECD of *Cnaphalocrocis medinalis* and *Agrotis ipsilon* fourth instar larvae were significantly reduced after treatment with Melia azedarach [62], biopesticides [64] and cyantraniliprole [65] **.** Jansen and De Groot [66] reported that the decreased RGR of treated larvae might be caused by permanent damage to the cellular surface of the midgut cells. In conclusion, this may be due to the treated population of S. *exigua* requiring greater energetic resources allocated for detoxification and other metabolic processes, therefore limiting the energy available for normal growth and development. This reduced energetic budget may also explain the growth and developmental delay, low survival rate, and reduced reproduction observed. Carbohydrate, lipid and protein contents are essential nutrients that provide the vital energy to sustain the body function of insect life activities. The amount of these macronutrients change between different growth stages and feeding conditions [67]. A previous studies also suggested that different pesticides and phytotoxins, like tannic acid, lowered feeding efficiency, which in turn reduced the biochemical components in the insect's body [43,65,68,69]. In this

study we have shown that total carbohydrates, lipids, and protein content was reduced when third-stage larvae of *S. exigua* were treated with a diet containing tannic acid with chlorantraniliprole or either chlorantraniliprole and tannic acid alone. These results are consistent with previous studies, in which carbohydrate, lipid and protein contents were reduced in *Anastrangalia dissimilis* and *Bradysia odoriphaga* larvae after the treatment of cyantraniliprole, while the total protein content was increased after chlorfenapyr treatment in *B. odoriphaga* compared with the control [43,68]. The outcome may be that tannic acid with chlorantraniliprole causes the protein to be degraded into amino acids to participate in the TCA cycle of acetic acid and modifies the function of carbohydrate metabolism to make up for lower energy under pesticide stress in the insect body [70,71]. In addition, after tannic acid and chlorantraniliprole treatment, the detoxification of larvae required a significant portion of consumed substances to be transform into energy, which may also be another reason for the reduction in the content of carbohydrate, lipid, and protein in larvae of *S. exigua*.

5. Conclusion

The results of this study strongly suggested that toxicity can be enhanced even through a combination of the phytotoxin and pesticides. These results support the idea that the use of phytotoxin with chlorantraniliprole cause growth inhibition, antifeedant effects and inhibit the reproduction of the insect pest, *S. exigua*. Further investigations should explore novel combinations of natural phytotoxins with different groups of insecticides. Additionally, the results of this study suggest that phytotoxic substances like tannic acid enhance the activity of chlorantraniliprole and will be an efficient alternative to conventional synthetic insecticides for the management of *S. exigua*. The utilization of natural phytotoxins as an additive to

chlorantraniliprole may play a prominent role in integrated pest management programs in the future.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant 31171874) [M.W]), Grant 31701791 (S.L.), Hubei Provincial Natural Science Foundation of China (Grant 2017CFB233 [S.L.]), Fundamental Research funds for the Central Universities (Grant 52902-0900202797 [S.L.]), and the China Scholarship Council to Muhammad Hafeez. We are grateful to Dr. Hsin Chi (Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Omer Halisdemir University, Turkey), for their technical guidance and statistical assistance with the two-sex life table theory used in this work.

References

[1] B.N. Dingha, W.J. Moar, A.G. Appel, Effects of Bacillus thuringiensis Cry1C toxin on the metabolic rate of Cry1C resistant and susceptible *Spodoptera exigua* (Lepidoptera: Noctuidae), Physiol. Entomol. 29 (2004) 409–418. doi:10.1111/j.0307-6962.2004.00409.x.

[2] M. Rizwan-Ul-Haq, Q.B. Hu, M.Y. Hu, Q.S. Lin, W.L. Zhang, Biological impact of harmaline, ricinine and their combined effects with Bacillus thuringiensis on *Spodoptera exigua* (Lepidoptera: Noctuidae), J. Pest Sci. (2004). 82 (2009) 327–334. doi:10.1007/s10340-009-0257-x.

[3] W. Che, T. Shi, Y. Wu, Y. Yang, Insecticide Resistance Status of Field Populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) From China, J. Econ. Entomol. (2013). doi:10.1603/EC13128.

[4] M. Ahmad, I.M. Arif, Resistance of beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) to endosulfan, organophosphorus and pyrethroid insecticides in Pakistan, Crop Prot. 29 (2010) 1428–1433. doi:10.1016/j.cropro.2010.07.025.

[5] C.C. Lee, Sublethal effects of insecticides on longevity, fecundity and behaviour of insect pests: a review, J. Biosci. (2000). doi:10.1016/j.ibmb.2010.09.001.

[6] M. Ahmad, A. Farid, M. Saeed, Resistance to new insecticides and their synergism in *Spodoptera exigua* (Lepidoptera: Noctuidae) from Pakistan, Crop Prot. 107 (2018) 79–86. doi:10.1016/j.cropro.2017.12.028.

[7] W. Che, T. Shi, Y. Wu, Y. Yang, Insecticide resistance status of field populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) from China. TL - 106, J. Econ. Entomol. 106 VN- (2013) 1855–1862. doi:10.1603/EC13128.

[8] A. Osorio, A.M. Martínez, M.I. Schneider, O. Díaz, J.L. Corrales, M.C. Avilés, G. Smagghe, S. Pineda, Monitoring of beet armyworm resistance to spinosad and methoxyfenozide in Mexico, Pest Manag. Sci. 64 (2008) 1001–1007. doi:10.1002/ps.1594.

[9] S.D. Stewart, M.B. Layton, M.R. Williams, Occurrence and control of beet armyworm outbreaks in the cotton belt, Proceedings, Beltwide Cott. Conf. 2 (1996) 846–848.

[10] W. Xie, S. Wang, Q. Wu, Y. Feng, H. Pan, X. Jiao, L. Zhou, X. Yang, W. Fu, H. Teng, B. Xu, Y. Zhang, Induction effects of host plants on insecticide susceptibility and detoxification enzymes of *Bemisia tabaci* (Hemiptera: Aleyrodidae), Pest Manag. Sci. 67 (2011) 87–93. doi:10.1002/ps.2037.

[11] M.D. Feliciangeli, J. Rabinovich, Vital statistics of triatominae (Hemiptera : reduviidae) under laboratory conditions II. *Triatoma maculata*, J. Med. Entomol. 22 (1985) 43–48. doi:10.1093/jmedent/22.1.43.

[12] M. Ahmad, M.I. Arif, Z. Ahmad, Susceptibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to new chemistries in Pakistan, Crop Prot. (2003). doi:10.1016/S0261-2194(02)00219-3.

[13] L.T. Wang K, Zang Y, Wang H, Xia X, Influence of three larval diets on susceptibility of selected insecticides and activities of detoxification esterases of *Helicoverpa assulta* (Lepidoptera: Noctuidae)., Pestic. Biochem. Physiol. Biochem Physiol. 96 (2010) 51–55.

[14] F.M. Fishel, Pesticide Toxicity Profile: Synthetic Pyrethroid Pesticides, Univ. Florida IFAS Ext. (2014) 2–4. http://edis.ifas.ufl.edu.

[15] R. Busi, A. Porri, T.A. Gaines, S.B. Powles, Pyroxasulfone resistance in Lolium rigidum is metabolism-based, Pestic. Biochem. Physiol. (2018). doi:10.1016/j.pestbp.2018.03.017.

[16] P.C. Abhilash, N. Singh, Pesticide use and application: An Indian scenario, J. Hazard. Mater. 165 (2009) 1–12. doi:10.1016/j.jhazmat.2008.10.061.

[17] W. Aktar, D. Sengupta, A. Chowdhury, Impact of pesticides use in agriculture: Their benefits and hazards, Interdiscip. Toxicol. 2 (2009) 1–12. doi:10.2478/v10102-009-0001-7.

[18] M. Alon, M. Elbaz, M.M. Ben-Zvi, E. Feldmesser, A. Vainstein, S. Morin, Insights into the transcriptomics of polyphagy: *Bemisia tabaci* adaptability to phenylpropanoids involves coordinated expression of defense and metabolic genes, Insect Biochem. Mol. Biol. 42 (2012) 251–263. doi:10.1016/j.ibmb.2011.12.007.

[19] B.K. Salunke, H.M. Kotkar, P.S. Mendki, S.M. Upasani, V.L. Maheshwari, Efficacy of flavonoids in controlling *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae), a post-harvest pest of grain legumes, Crop Prot. 24 (2005) 888–893. doi:10.1016/j.cropro.2005.01.013.

[20] T. Nyman, R. Julkunen-Tiitto, Manipulation of the phenolic chemistry of willows by gall-inducing sawflies., Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 13184–13187. doi:10.1073/pnas.230294097.

[21] C.A. Elliger, Y. Wong, B.G. Chan, A.C. Waiss, Growth inhibitors in tomato (Lycopersicon) to tomato fruitworm (Heliothis zea), J. Chem. Ecol. 7 (1981) 753–758. doi:10.1007/BF00990307.

[22] Z. Luo, H. Dong, W. Li, Z. Ming, Y. Zhu, Individual and combined effects of salinity and waterlogging on Cry1Ac expression and insecticidal efficacy of Bt cotton, Crop Prot. 27 (2008) 1485–1490. doi:10.1016/j.cropro.2008.06.006.

[23] M. Cardinal-Aucoin, É. Bauce, P.J. Albert, Preingestive detection of tannins by *Choristoneura*

fumiferana (Lepidoptera: Tortricidae), Ann. Entomol. Soc. Am. 102 (2009) 717–726. doi:10.1603/008.102.0417.

[24] G. Wu, J.-Y. Guo, F.-H. Wan, N.-W. Xiao, Responses of three successive generations of beet armyworm, *Spodoptera exigua*, fed exclusively on different levels of gossypol in cotton leaves., J. Insect Sci. 10 (2010) 165. doi:10.1673/031.010.14125.

[25] F. Gao, S.-R. Zhu, Y.-C. Sun, L. Du, M. Parajulee, L. Kang, F. Ge, Interactive Effects of Elevated CO2 and Cotton Cultivar on Tri-Trophic Interaction of *Gossypium hirsutum*, *Aphis gossyppii*, and *Propylaea japonica*, Environ. Entomol. 37 (2008) 29–37. doi:10.1603/0046-225X(2008)37[29:IEOECA]2.0.CO;2.

[26] J.-Y. Guo, G. Wu, F.-H. Wan, Effects of High-Gossypol Cotton on the Development and Reproduction of *Bemisia tabac*i (Hemiptera: Aleyrodidae) MEAM1 Cryptic Species, J. Econ. Entomol. 106 (2013) 1379–1385. doi:10.1603/EC12401.

[27] L. Ruiz-Montoya, J. Núñez-Farfán, J. Vargas, Host-associated genetic structure of Mexican populations of the cabbage aphid *Brevicoryne brassicae L*. (Homoptera: Aphididae), Heredity (Edinb). 91 (2003) 415–421. doi:10.1038/sj.hdy.6800338.

[28] H. Ueno, Y. Hasegawa, N. Fujiyama, H. Katakura, Population differentiation in host-plant use in a herbivorous ladybird beetle, *Epilachna vigintioctomaculata*, Entomol. Exp. Appl. 99 (2001) 263–265. doi:10.1023/A:1018960529858.

[29] M.O. Mello, M.C. Silva-Filho, Plant-insect interactions: An evolutionary arms race between two distinct defense mechanisms, Brazilian J. Plant Physiol. 14 (2002) 71–81. doi:10.1590/S1677-04202002000200001.

[30] M. Hafeez, S. Liu, S. Jan, B. Ali, M. Shahid, G.M. Fernández-Grandon, M. Nawaz, A. Ahmad, M. Wang, Gossypol-induced fitness gain and increased resistance to deltamethrin in beet armyworm, *Spodoptera exigua (Hübner)*, Pest Manag. Sci. (2018). doi:10.1002/ps.5165.

[31] J. Su, Z. Zhang, M. Wu, C. Gao, Geographic susceptibility of Chilo suppressalis Walker (Lepidoptera: Crambidae), to chlorantraniliprole in China, Pest Manag. Sci. 70 (2014) 989–995. doi:10.1002/ps.3640.

[32] A. Dinter, K.E. Brugger, A. Bassi, N.-M. Frost, M.D. Woodward, Chlorantraniliprole (DPX-E2Y45, DuPont™ Rynaxypyr®, Coragen® and Altacor® insecticide) - a novel anthranilic diamide insecticide - demonstrating low toxicity and low risk for beneficial insects and predatory mites, in: Work. Gr. Pestic. Benef. Org. Proc. Meet. Berlin, Ger. 10-12 Oct. 2007, 2007.

[33] T. Lai, J. Su, Effects of chlorantraniliprole on development and reproduction of beet armyworm, *Spodoptera exigua* (Hübner), J. Pest Sci. (2004). 84 (2011) 381–386. doi:10.1007/s10340-011-0366-1.

[34] A.A. Sial, J.F. Brunner, S.F. Garczynski, Biochemical characterization of chlorantraniliprole and spinetoram resistance in laboratory-selected *obliquebanded leafroller*, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), Pestic. Biochem. Physiol. 99 (2011) 274–279. doi:10.1016/j.pestbp.2011.01.006.

[35] M.E. Whalon, D. Mota-Sanchez, R. Hollingworth, Analysis of global pesticide resistance in arthropods, in: Glob. Pestic. Resist. Arthropods, 2008: pp. 5–31. doi:10.1007/s13398-014-0173-7.2.

[36] J. Huang, S.F. Wu, G.Y. Ye, Evaluation of lethal effects of chlorantraniliprole on *Chilo suppressalis* and its larval parasitoid, *Cotesia chilonis*, Agric. Sci. China. 10 (2011) 1134–1138.

doi:10.1016/S1671-2927(11)60103-X.

[37] W.K. Zhang YJ, Lu Q, Gu SH, Lu YH, A kind of artificial diet preparation method for black cutworm larvae and application, n.d.

[38] J. Dong, K. Wang, Y. Li, S. Wang, Lethal and sublethal effects of cyantraniliprole on *Helicoverpa assulta* (Lepidoptera: Noctuidae), Pestic. Biochem. Physiol. (2017). doi:10.1016/j.pestbp.2016.08.003.

[39] G. Ali, J.M. Vlak, W. van der Werf, Biological activity of Pakistani isolate SpltNPV-Pak-BNG in second, third and fourth instar larvae of the leafworm *Spodoptera litura*, Biocontrol Sci. Technol. (2018). doi:10.1080/09583157.2018.1461197.

[40] G.P. Waldbauer, The Consumption and Utilization of Food by Insects, Adv. In Insect Phys. 5 (1968) 229–288. doi:10.1016/S0065-2806(08)60230-1.

[41] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. (1976). doi:10.1016/0003-2697(76)90527-3.

[42] B. Yuval, R. Kaspi, S. Shloush, M.S. Warburg, Nutritional reserves regulate male participation in Mediterranean fruit fly leks, Ecol. Entomol. (1998). doi:10.1046/j.1365-2311.1998.00118.x.

[43] Y. Zhao, Q. Wang, J. Ding, Y. Wang, Z. Zhang, F. Liu, W. Mu, Sublethal effects of chlorfenapyr on the life table parameters, nutritional physiology and enzymatic properties of *Bradysia odoriphaga* (Diptera: Sciaridae), Pestic. Biochem. Physiol. (2018). doi:10.1016/j.pestbp.2018.04.003.

[44] J.H. ROE, The determination of sugar in blood and spinal fluid with anthrone reagent., J. Biol. Chem. 212 (1955) 335–343.

[45] C.H. Huang YB, Life tables of *Bactrocera cucurbitae* (Diptera: Tephritidae): with an invalidation of the jackknife technique, J. Appl. Entomol. 137 (2013) 327–339.

[46] D.J. Finney, Probit Analysis, 1971. doi:10.1002/jps.2600600940.

[47] J.S. Meyer, C.G. Ingersoll, L.L. McDonald, M.S. Boyce, Estimating uncertainty in population growth rates: jackknife vs. bootstrap techniques., Ecology. (1986). doi:10.2307/1938671.

[48] H. Chi, TWOSEX-MSChart: a computer program for the age stage, two-sex life table analysis, Natl. Chung Hsing Univ. Taichung, Taiwan. 2015b. (2015) Available: http://140.120.197.173/Ecology/ (access.

[49] S.C. and C.H. Akca, I, Ayvaz T, Yazici E, Demography and population projection of *Aphis fabae* (Hemiptera: Aphididae): with additional comments on life table research criteria, J. Econ. Entomol. (2015) 108:1466-1478.

[50] E.P. Akköprü, R. Atlihan, H. Okut, H. Chi, Demographic assessment of plant cultivar resistance to insect pests: A case study of the dusky-veined walnut aphid (Hemiptera: Callaphididae) on five walnut cultivars, J. Econ. Entomol. 108 (2015) 378–387. doi:10.1093/jee/tov011.

[51] H. Bao, X. Shao, Y. Zhang, Y. Deng, X. Xu, Z. Liu, Z. Li, Specific Synergist for Neonicotinoid Insecticides: IPPA08, a cis-Neonicotinoid Compound with a Unique Oxabridged Substructure, J. Agric. Food Chem. 64 (2016) 5148–5155. doi:10.1021/acs.jafc.6b01512.

[52] M. Mrdaković, V.P. Mataruga, L. Ilijin, M. Vlahović, D. Todorović, V. Nenadović, J. Lazarević, The effects of tannic acid on the fitness-related traits of *Lymantria dispar* L. larvae, Arch. Biol.

Sci. 63 (2011) 1037–1046. doi:10.2298/ABS1104037M.

[53] N. Desneux, A. Decourtye, J.-M. Delpuech, The Sublethal Effects of Pesticides on Beneficial Arthropods, Annu. Rev. Entomol. 52 (2007) 81–106. doi:10.1146/annurev.ento.52.110405.091440.

[54] Y. Ma, Y. Zhang, R.R. Chen, X.L. Ren, P.J. Wan, L.L. Mu, G.Q. Li, Combined effects of three crystalline toxins from Bacillus thuringiensis with seven proteinase inhibitors on beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), Pestic. Biochem. Physiol. (2013). doi:10.1016/j.pestbp.2013.01.007.

[55] W. Han, S. Zhang, F. Shen, M. Liu, C. Ren, X. Gao, Residual toxicity and sublethal effects of chlorantraniliprole on *Plutella xylostella* (Lepidoptera: Plutellidae), Pest Manag. Sci. 68 (2012) 1184–1190. doi:10.1002/ps.3282.

[56] X. Guan, X. Liu, Z. Lu, Z. Zhao, Q. Zhang, J. Xia, Influence of tannic acid and Cry1Ac toxin of Bacillus thuringiensis on larval survival, growth, and development of *Helicoverpa armigera*, Entomol. Exp. Appl. 132 (2009) 50–58. doi:10.1111/j.1570-7458.2009.00866.x.

[57] G.T. Hannig, M. Ziegler, G.M. Paula, Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups, Pest Manag. Sci. 65 (2009) 969–974. doi:10.1002/ps.1781.

[58] F. Perveen, T. Miyata, Effects of Sublethal Dose of Chlorfluazuron on Ovarian Development and Oogenesis in the Common Cutworm *Spodoptera litura* (Lepidoptera: Noctuidae), Ann. Entomol. Soc. Am. 93 (2000) 1131–1137. doi:10.1603/0013-8746(2000)093[1131:EOSDOC]2.0.CO;2.

[59] C. Xu, Z. Zhang, K. Cui, Y. Zhao, J. Han, F. Liu, W. Mu, Effects of Sublethal Concentrations of Cyantraniliprole on the Development, Fecundity and Nutritional Physiology of the Black Cutworm (Lepidoptera: Noctuidae), PLoS One. 11 (2017) 11(6): e0156555. doi:10.1371/journal.pone.0156555.

[60] Hafeez M, L Sisi, S Jan, B Ali, M Shahid, M Fernández-Grandon, M Nawaz, A Ahmad, M. Wang, Gossypol-induced fitness gain and increased resistance to deltamethrin in beet armyworm, *Spodoptera exigua* (Hübner), Pest Manag. Sci. (2018) doi: 10.1002/ps.5165.

[61] Feeny PP, Plant apparency and chemical defense, in: Recent Adv. Phytochem., 1976: pp. 1–40.

[62] S.S. Nathan, Effects of Melia azedarach on nutritional physiology and enzyme activities of the rice leaffolder *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae), Pestic. Biochem. Physiol. 84 (2006) 98–108. doi:10.1016/j.pestbp.2005.05.006.

[63] J.L. Stoyenoff, J.A. Witter, M.E. Montgomery, Nutritional indices in the gypsy moth (Lymantria dispar (L.)) under field conditions and host switching situations, Oecologia. 97 (1994) 158–170. doi:10.1007/BF00323145.

[64] S.S. Nathan, P.G. Chung, K. Murugan, Effect of biopesticides applied separately or together on nutritional indices of the rice leaffolder *Cnaphalocrocis medinalis*, Phytoparasitica. 33 (2005) 187–195. doi:10.1007/BF03029978.

[65] C. Xu, Z. Zhang, K. Cui, Y. Zhao, J. Han, F. Liu, W. Mu, Effects of sublethal concentrations of cyantraniliprole on the development, fecundity and nutritional physiology of the black cutworm *agrotis ipsilon* (Lepidoptera: Noctuidae), PLoS One. 11 (2016). doi:10.1371/journal.pone.0156555.

[66] B.J.M. Jansen, A. De Groot, Occurrence, biological activity and synthesis of drimane

sesquiterpenoids, Nat. Prod. Rep. 21 (2004) 449–477. doi:10.1039/b311170a.

[67] R.F. Chapman, The Insects: structure and Function, 4th Edition, Cambridge Univ. Press. (1998) 21–24; 50. doi:10.1098/rsif.2013.0304.

[68] J. Ding, C. Xu, Z. Zhang, Y. Zhao, F. Liu, W. Mu, Effects of cyantraniliprole on development, fecundity and nutritional utilization of *athetis dissimilis*, Sci. Agric. Sin. 50 (2017). doi:10.3864/j.issn.0578-1752.2017.22.007.

[69] D. Raubenheimer, Tannic acid, protein, and digestible carbohydrate: dietary imbalance and nutritional compensation in locusts, Ecology. 73 (1992) 1012–1027. doi:10.2307/1940176.

[70] B. Surendra Nath, Changes in carbohydrate metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* L., exposed to organophosphorus insecticides, Pestic. Biochem. Physiol. 68 (2000) 127–137. doi:10.1006/pest.2000.2509.

[71] B.S. Nath, A. Suresh, B.M. Varma, R.P.S. Kumar, Changes in Protein Metabolism in Hemolymph and Fat Body of the Silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) in Response to Organophosphorus Insecticides Toxicity, Ecotoxicol. Environ. Saf. 36 (1997) 169–173. doi:10.1006/eesa.1996.1504.

Table 1. Enhancement effects of tannic acid to chlorantraniliprole on the third-instar larvae of *S. exigua*

Treatments	Concentrations (mg/L) (95%CL)$^{-1}$		Slope ± SE	X^2	*df*	*P. values*	EF[a]
	LC_{50}	LC_{40}					
Tannic acid 2 mg	----------	---------	----	----	----	-----	----
Chlorantraniliprole	0.027 (0.024 ~ 0.031)	0.021(0.018-0.024)	2.16 ± 0.17	2.317	4	0.67	----
Chlorantraniliprole + Tannic acid	0.018 (0.015 ~ 0.021)	0.012 (0.009-0.015)	1.52 ± 0.15	2.827	4	0.58	1.5

[a]Enhancement Factor (EF) = LC_{50} for chlorantraniliprole alone/LC50 in presence of tannic acid.

Table 2. Effects of tannic acid, chloantraniliprole and tannic acid plus chloantraniliprole on the development and growth of *S. exigua.*

	Control		Tannic acid		Chlorant[a]		T. acid+chlorant[b]	
Stages	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE
L3 (day)	90	3.04 ± 0.03 a	90	3.1 ± 0.07 a	90	3.6 ± 0.08 b	90	4.33 ± 0.11 c
L4(day)	88	2.98 ± 0.02 a	87	3.03 ± 0.05 a	78	3.37 ± 0.08 b	71	3.76 ± 0.07 c
L5(day)	87	3.07 ± 0.04 a	85	3.12 ± 0.06 a	72	3.57 ± 0.08 b	65	3.9 ± 0.09 c
Pupa(day)	86	7.76 ± 0.07 a	84	7.91 ± 0.07 a	68	8.55 ± 0.09 b	61	9.28 ± 0.11 c
Mean longevity of female adult (day)	42	12.53 ± 0.38 a	43	10.57 ± 0.66 b	30	10.33 ± 0.09 b	22	8.5 ± 1.07 c
Mean longevity of male adult (day)	44	10.52 ± 0.39 a	40	10.05 ± 0.66 a	28	9.75 ± 0.72 a	23	9.23 ± 0.98 a
APOP (day)	42	1.53 ± 0.17 a	41	1.46 ± 0.14 a	26	1.77 ± 0.2 a	22	1.8 ± 0.25 a
TPOP (day)	42	24.33 ± 0.3 a	38	25.69 ± 0.29 a	26	27 ± 0.3 b	22	29 ± 0.45 c
Oviposition (days)	41	7.33 ± 0.23 a	36	6.08 ± 0.29 b	26	6.38 ± 0.37 b	22	5.4 ± 0.4 c
Mean number of eggs(per female)	40	595.67 ± 13.4 a	34	508.14 ± 41.2 b	22	483.6 ± 54.39 b	18	331.25 ± 50.0 c

Standard errors were estimated using 100,000 bootstraps. Means marked with different letters in the same row are significantly different as calculated using the paired bootstrap test at the 5% significance level. APOP = adult pre-ovipositional period; TPOP = total pre-ovipositional period.

[a]Chloantraniliprole, [b]Tannic acid+chlorantraniliprole

Table 3 Effects of tannic acid, chloantraniliprole and tannic acid plus chloantraniliprole on *S. exigua* parent population parameters

	Control	Tannic acid	Chlorant[a]	Tannic acid+chlorant
Population parameters	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Intrinsic rate of increase (r) (d^{-1})	0.182 ± 0.008 a	0.167 ± 0.008b a	0.162 ± 0.009 a	0.134 ± 0.019 b
Finite rate of increase (λ) (d^{-1})	1.201 ± 0.010 a	1.182 ± 0.010 a	1.176 ± 0.019 a	1.143 ± 0.011 b
Net reproductive rate (R0) (offspring)	178.7 ± 38.90 a	142.28 ± 34.05 a	145.08 ± 35.13 a	79.5 ± 23.071 b
Mean generation time (T) (d)	28.368 ± 0.28 a	28.71 ± 0.19 a	30.75 ± 0.254 b	32.69 ± 0.298 c

Standard errors were estimated by using 100,000 bootstraps. Means marked with different letters in the same row are significantly different as calculated using the paired bootstrap test at the 5% significance level.

[a]Chloantraniliprole.

Table 4. Feeding indices of third instar larvae of *S. exigua* after treatment with tannic acid, chloantraniliprole and tannic acid plus chloantraniliprole.

Treatment	AD (%)	ECI (%)	ECD (%)	RCR mg/mg/day	RGR mg/mg/day
Control	72.85 ± 0.37 a	11.41 ± 0.124 a	15.66 ± 0.114 a	2.06 ± 0.016 a	0.234 ± 0.006 a
Tannic acid	76.42 ± 0.38 ab	10.84 ± 0.361 a	14.47 ± 0.396 a	1.72 ± 0.004 b	0.222 ± 0.002 a
Chlorantraniliprole	78.22 ± 0.55 b	8.25 ± 0.409 b	10.57 ± 0.442 b	1.39 ± 0.004 b	0.187 ± 0.002 b
Tannic acid+ Chlorantraniliprole	84.00 ± 1.71 c	5.54 ± 0.418 c	5.54 ± 0.418 c	0.64 ± 0.013 c	0.084 ± 0.004 c

Means ± standard deviation followed by the same letter within columns indicate no significant difference (Student-Newman-Keuls test: $P < 0.05$). RGR—relative growth rate, RCR—relative consumption rate, ECI—efficiency of food ingestion, ECD—efficiency of food digestion, AD Approximate digestibility.

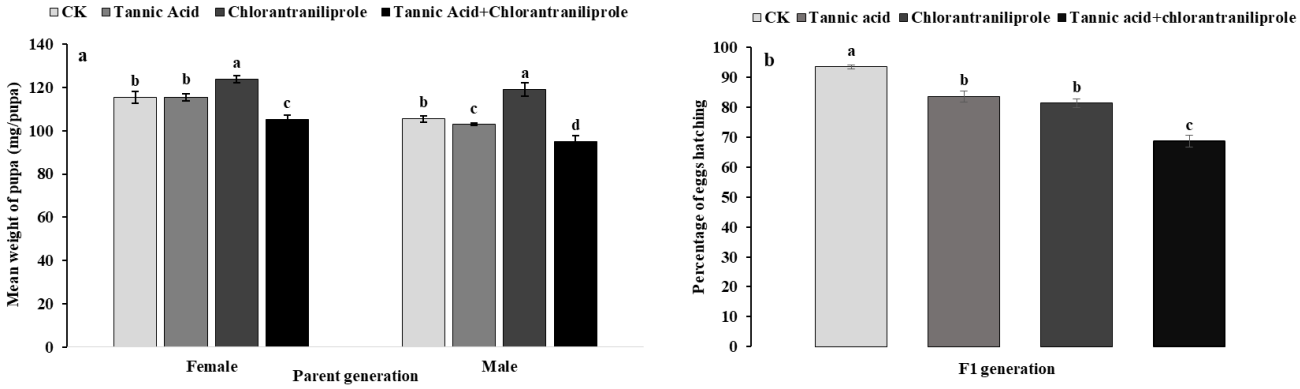
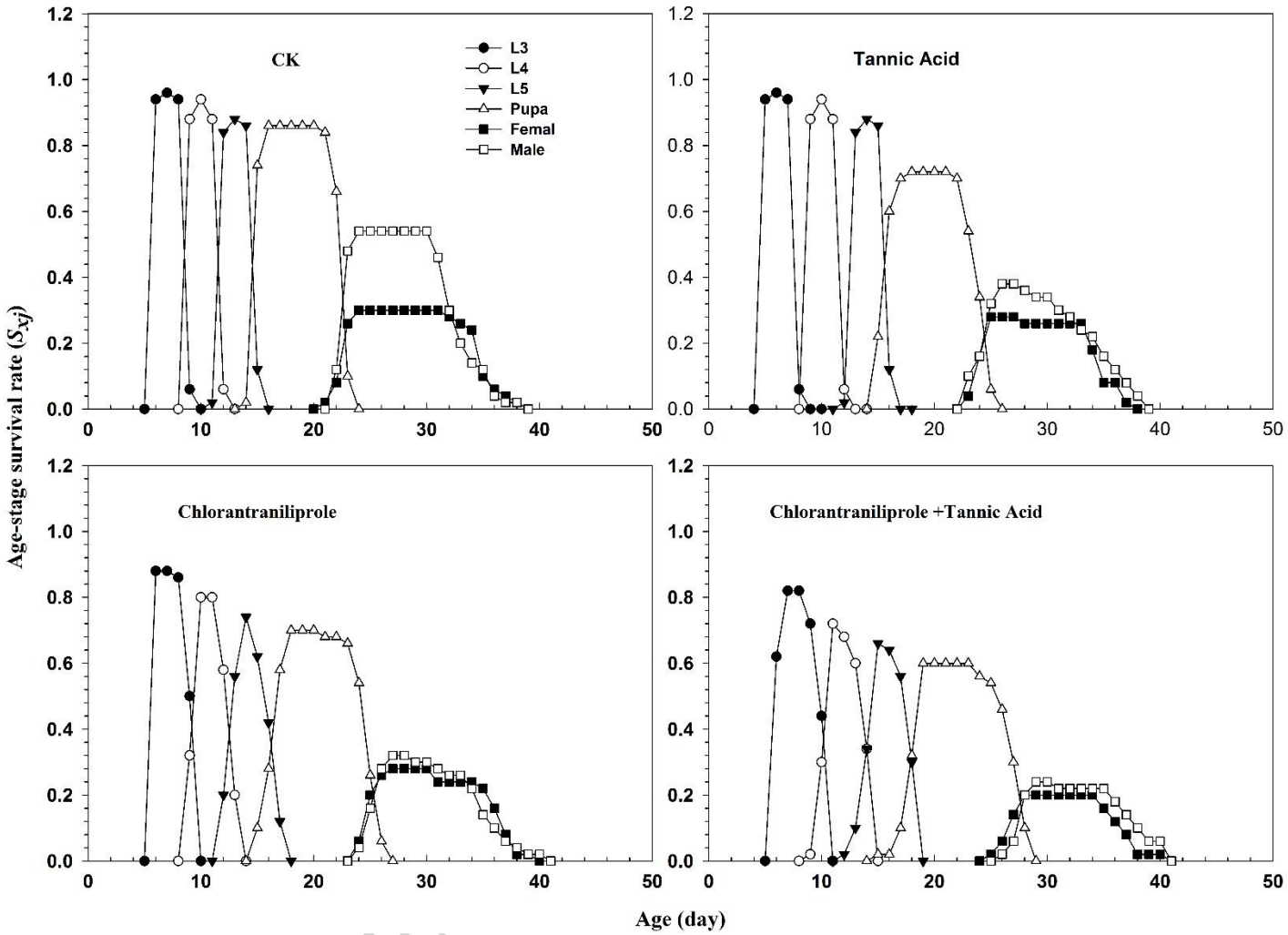


Fig 1. Pupae weight of male and female of *S. exigua* in parent population (a) and percentage of offspring eggs that hatched in the offspring population (b) after the parent third-instar larval stage was exposed to tannic acid, chlorantraniliprole or tannic acid plus chlorantraniliprole. Bars labeled with the same letters do not differ significantly (a, 100,000 bootstraps; b, Student-Newman-Keuls test, $P < 0.05$)

Fig. 2. Graphs showing Age-stage specific survival rate (S_{xj}) of *S. exigua* larvae exposed to tannic acid, chlorantraniliprole and Tannic acid plus chlorantraniliprole



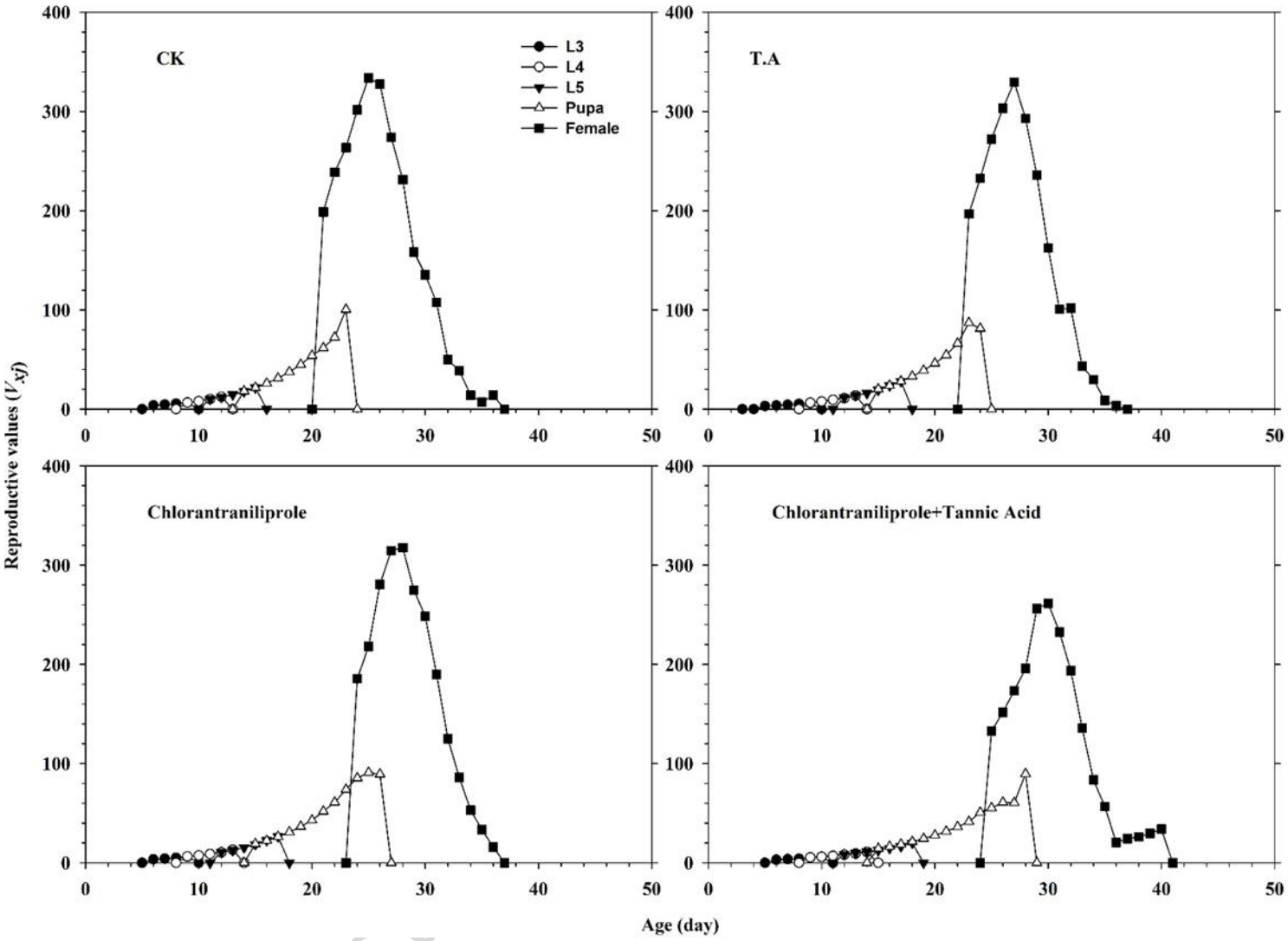


Fig. 3. Graphs showing age-stage specific reproductive values (v_{xj}) of *S. exigua* larvae exposed to tannic acid, chlorantraniliprole and Tannic acid plus chlorantraniliprole

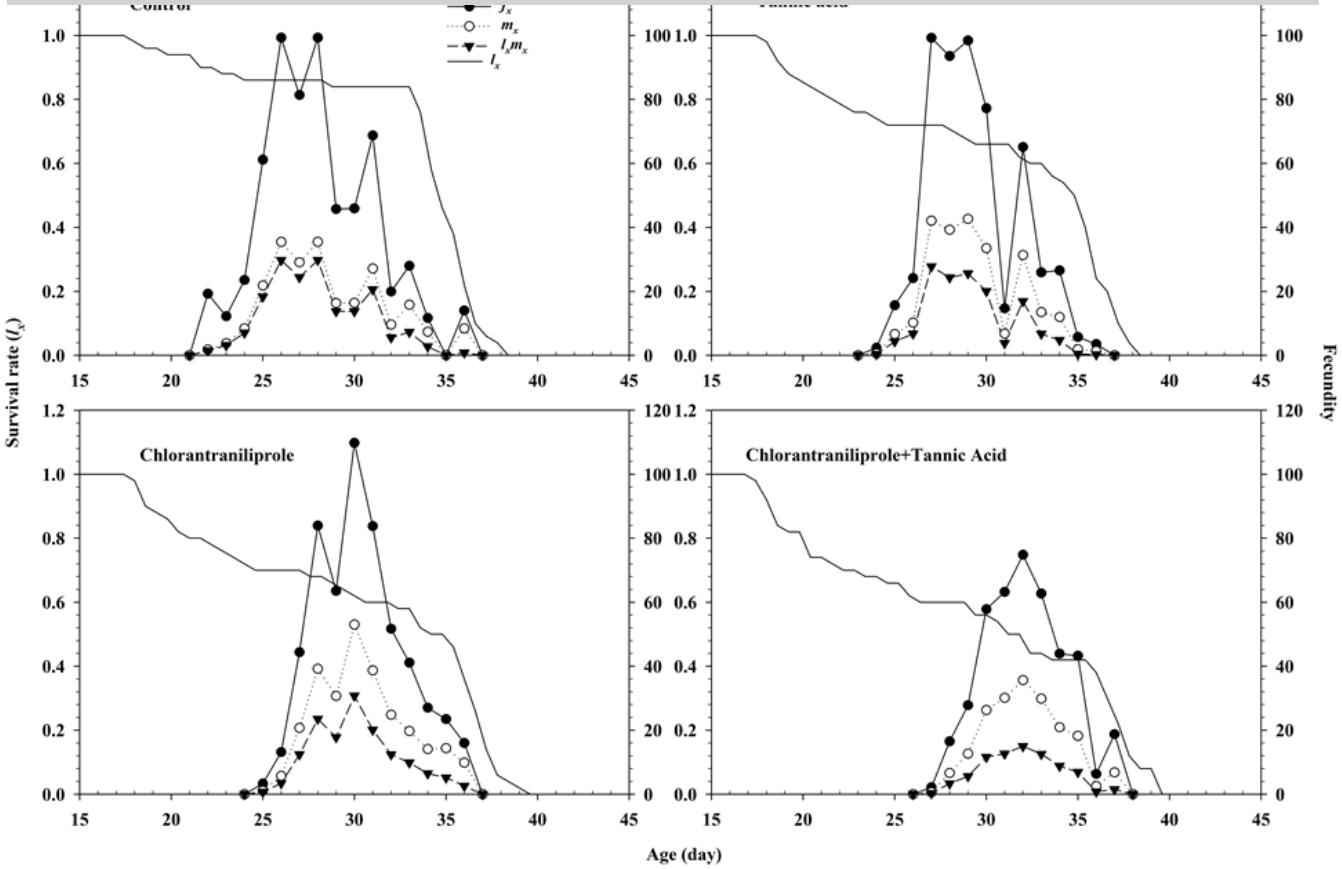


Fig. 4. Graphs showing Age-specific survival rate (l_x), female age-specific fecundity (f_{x9}), age-specific fecundity of the total population (m_x), and age specific maternity ($l_x m_x$) of *S. exigua* larvae exposed to tannic acid, chlorantraniliprole and tannic acid plus chlorantraniliprole.

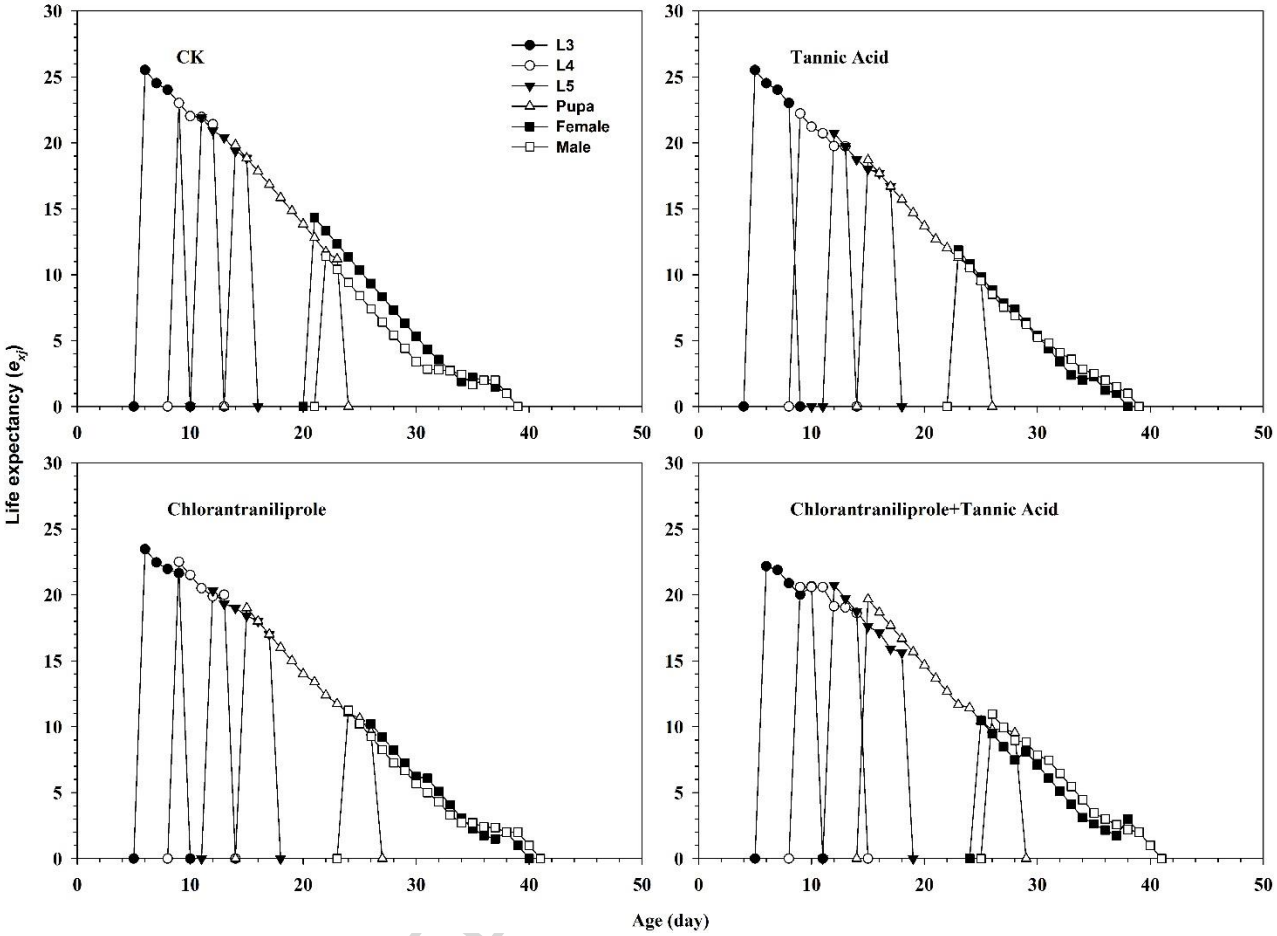


Fig. 5. Graphs showing Life expectancy (e_{xj}) of *S. exigua* larvae exposed to Tannic acid, chlorantraniliprole and tannic acid plus chlorantraniliprole

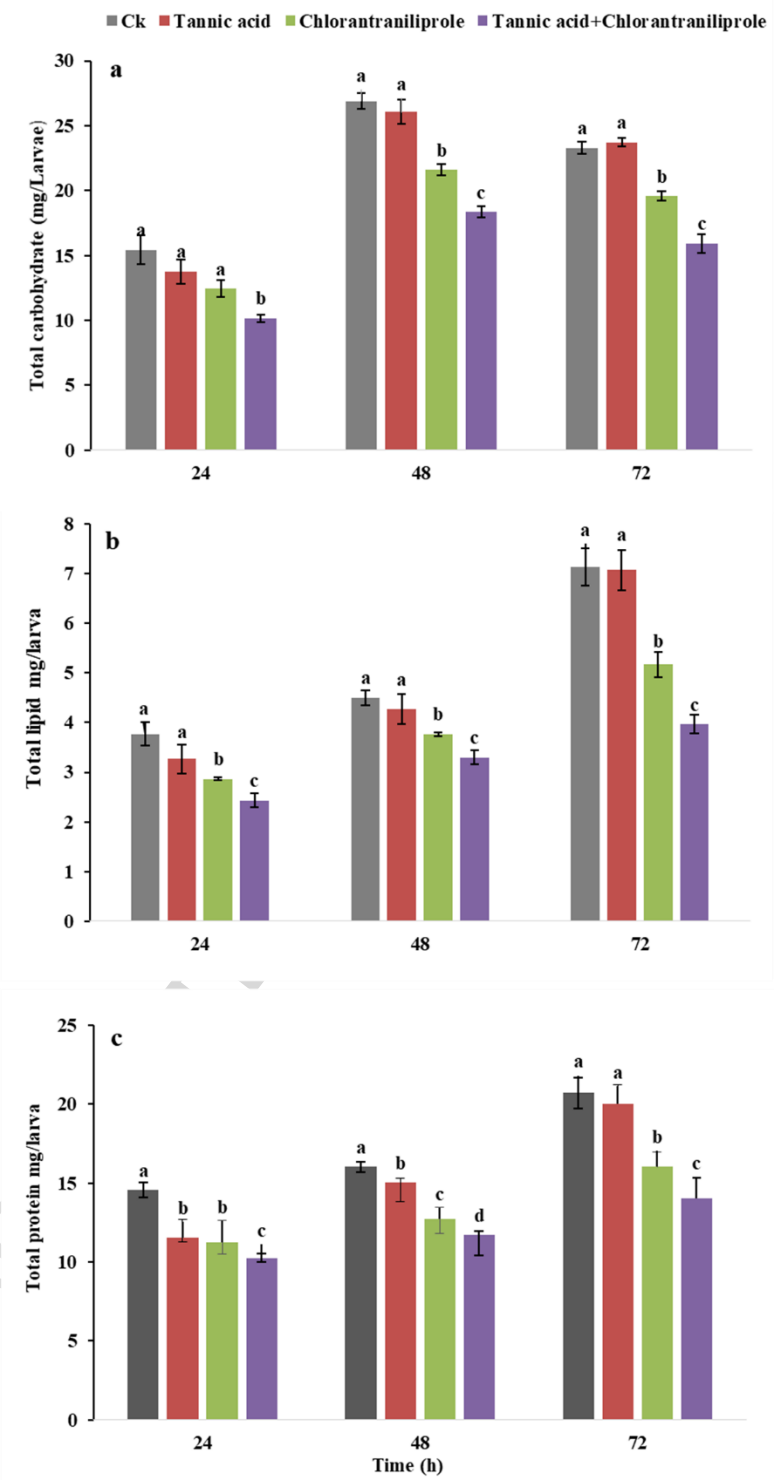
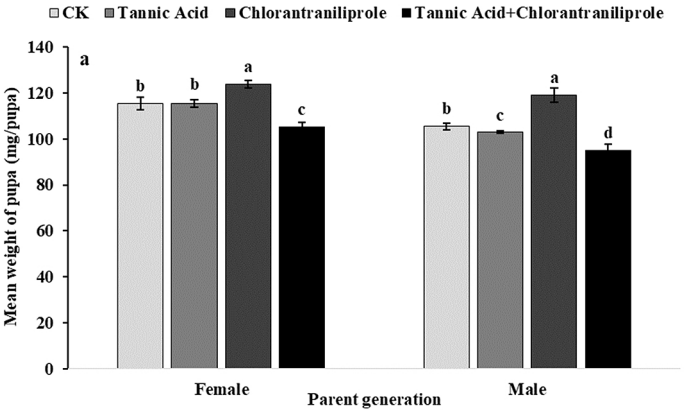


Fig 6. The amount of nutrients (a: carbohydrate, b: lipid, c: total protein) in third-instar larvae of *S. exigua* (Mean ± SE) after treatment with of tannic acid or chlorantraniliprole alone and tannic acid + chlorantraniliprole. Bars labeled with the same letters do not differ significantly LSD test, $P < 0.05$)

Highlights

- Tannic acid enhances toxicity of chlorantraniliprole against *S. exigua*
- Dietary tannic acid & chlorantraniliprole reduces fecundity and fitness of *S exigua*
- Additive effects decreased the carbohydrate, lipid and protein contents of pest
- Enhancement effect observed in combination tannic acid with insecticide


CK
Tannic Acid
Chlorantraniliprole
Tannic Acid+Chlorantraniliprole
a
Mean weight of pupa (mg/pupa)
0
20
40
60
80
100
120
140
b
b
a
c
b
c
a
d
Female
Male
Parent generation

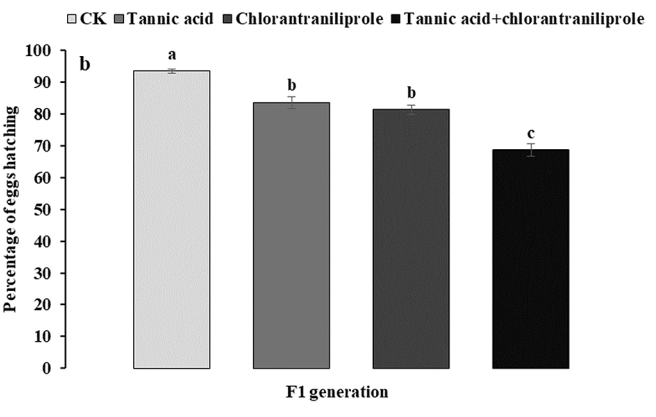

CK
Tannic acid
Chlorantraniliprole
Tannic acid+chlorantraniliprole
b
Percentage of eggs hatching
0
10
20
30
40
50
60
70
80
90
100
a
b
b
c
F1 generation

Figure 1

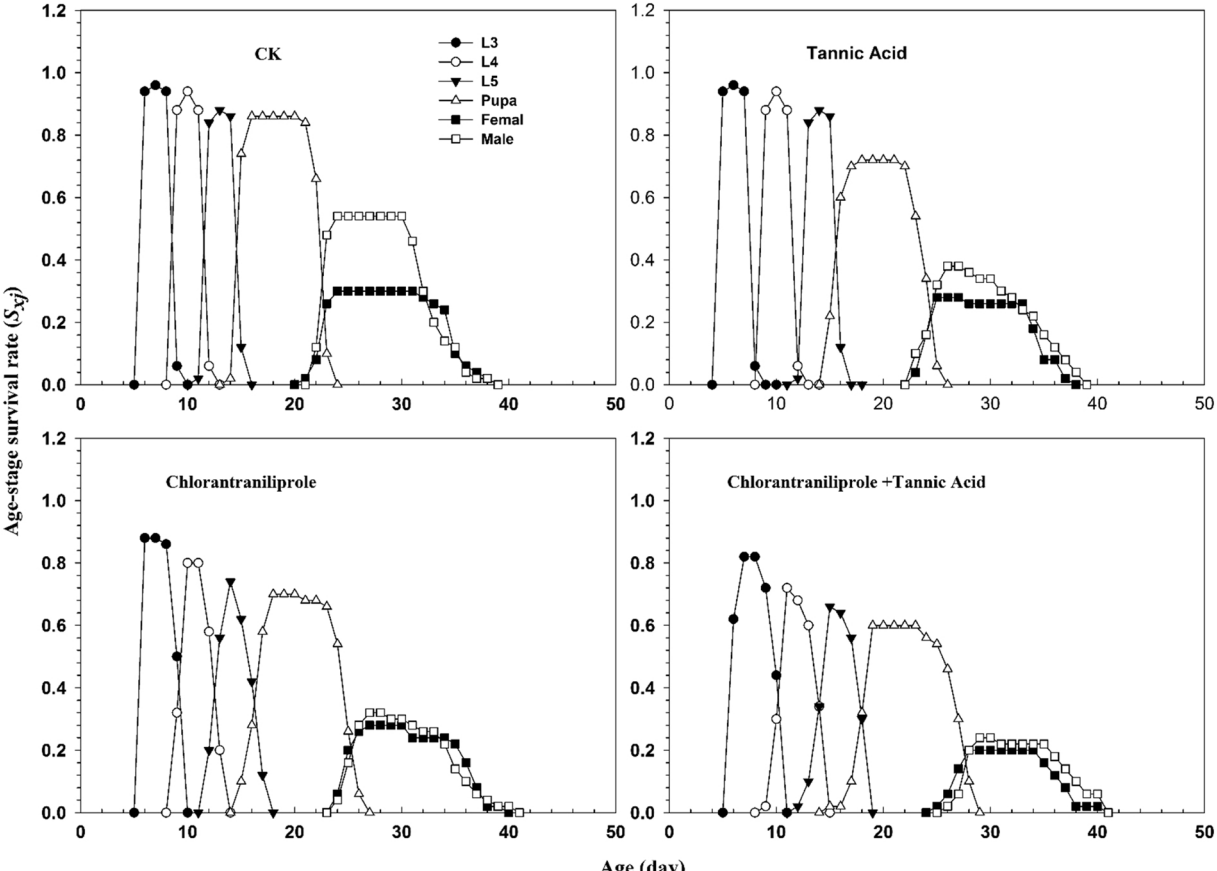

CK
Tannic Acid
Chlorantraniliprole
Chlorantraniliprole +Tannic Acid
L3
L4
L5
Pupa
Femal
Male
Age-stage survival rate (S_{xj})
Age (day)

Figure 2

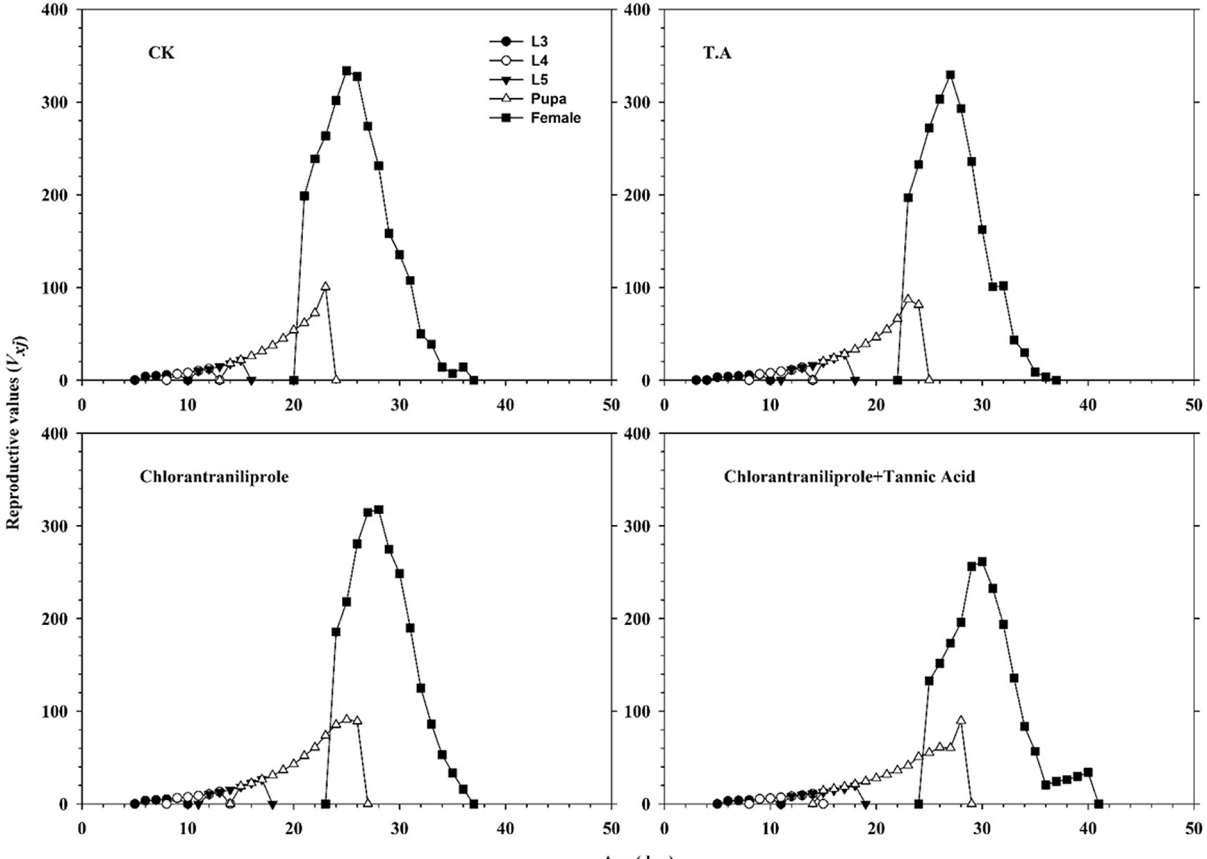

CK
T.A
Chlorantraniliprole
Chlorantraniliprole+Tannic Acid
L3
L4
L5
Pupa
Female
Reproductive values (V_{xj})
Age (day)
0
100
200
300
400
10
20
30
40
50

Figure 3

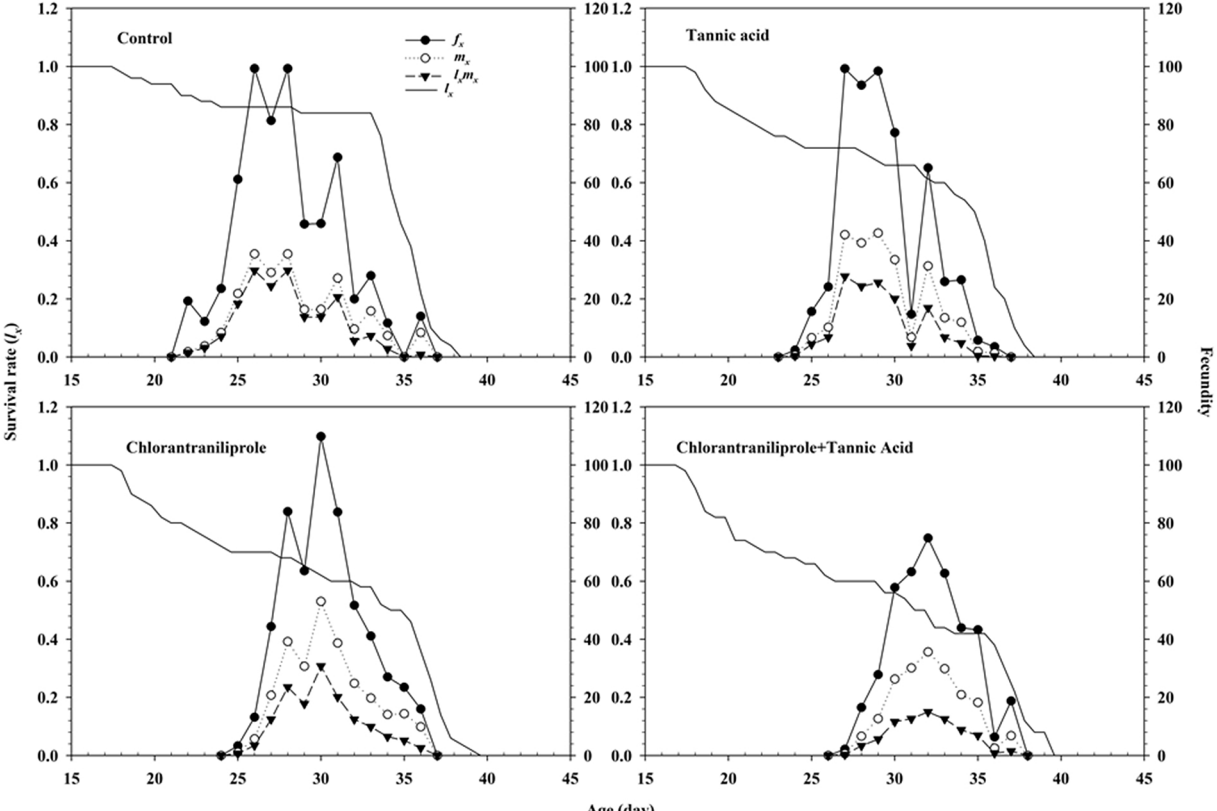

Control
Tannic acid
Chlorantraniliprole
Chlorantraniliprole+Tannic Acid
f_x
m_x
$l_x m_x$
l_x
Survival rate (l_x)
Fecundity
Age (day)

Figure 4

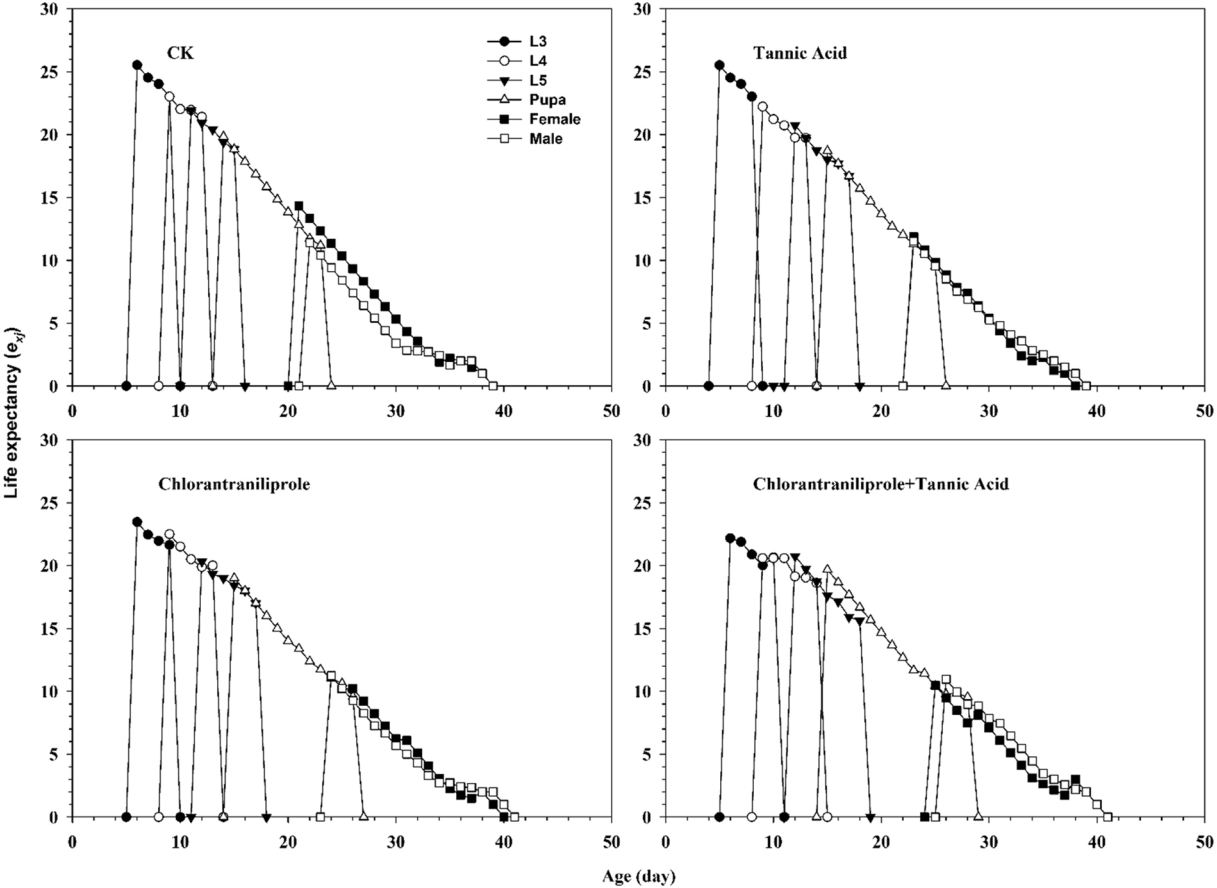

CK
Tannic Acid
Chlorantraniliprole
Chlorantraniliprole+Tannic Acid
L3
L4
L5
Pupa
Female
Male
Life expectancy (e_{xj})
Age (day)

Figure 5

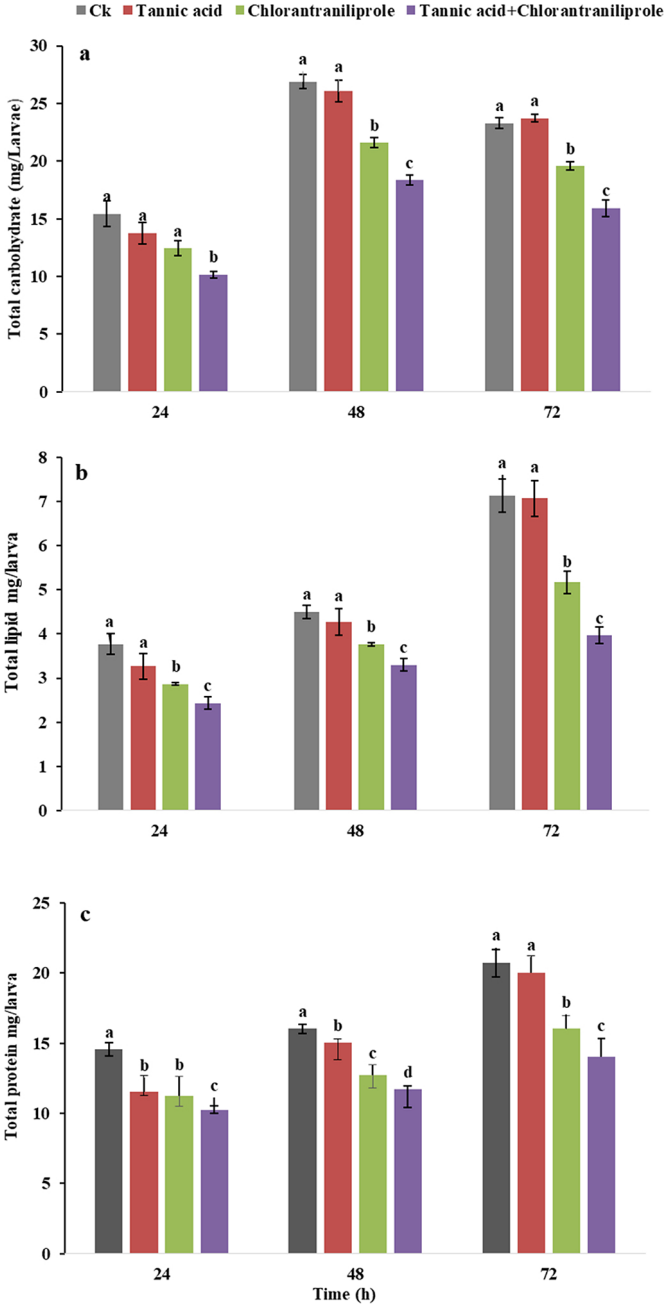

Ck
Tannic acid
Chlorantraniliprole
Tannic acid+Chlorantraniliprole
a
Total carbohydrate (mg/Larvae)
30
25
20
15
10
5
0
a a a b
a a b c
a a b c
24
48
72
b
Total lipid mg/larva
8
7
6
5
4
3
2
1
0
a a b c
a a b c
a a b c
24
48
72
c
Total protein mg/larva
25
20
15
10
5
0
a b b c
a b c d
a a b c
24
48
72
Time (h)

Figure 6