RESEARCH ARTICLE

Sub-lethal effects of lufenuron exposure on spotted bollworm *Earias vittella* (Fab): Key biological traits and detoxification enzymes activity

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Abstract

Spotted bollworm, *Earias vittella,* is one of the most serious and devastating insect pests of vegetables and cotton. Currently insecticides are necessary for its control in nearly all crop systems. In this paper we evalute the sub-lethal effects of lufenuron on biological traits and activity of detoxification enzymes; cytochrome P450 monooxygenases, esterase and glutathione S-transeferase (GST) in second instar larvae of *E. vittella*. Results showed that sub-lethal concentrations (LC_{15} and LC_{40} of lufenuron, prolonged larval period (at LC_{40} = 13.86±1.22 d, LC_{15} = 13.14±1.15 d, control = 12.28±0.7), pupal duration (LC_{40} = 11.1± d, LC_{15} = 11.8±0.28 d, control = 9.40±0.52) and extended mean generation time (LC_{40} = 27.3±0.43 LC_{15} = 29.0±1.19 d, control = 26.0±0.65). Sub-lethal exposure significantly prolonged the pre-adult stage, decreased pupal weight and reduced adult longevity in the parent (F0) and F1 generation. Moreover, the fecundity and egg viability were significantly lowered in parental and F1 generations at both sub-lethal concentrations compared to the control. While no significant effects were noted on reproductive parameters such as the intrinsic rate of increase (*r*), finite rate of increase (λ), and net reproduction rate (R_0) of F1 generation when compared to the control. Only mean generation time (*T*) in F1 at LC_{15} was significantly longer compared to the LC_{40} and control (LC_{40}= 3.79±0.37, LC_{15} = 32.28±1.55d, control= 29.79±0.55). Comparatively, the activities of cytochrome P450 monooxygenases and esterase were higher than GST in treated populations. The increase in resistance development against insecticides may possibly because of elevated activity of detoxification enzymes. These results provide useful information for monitoring resistance in integrated pest management (IPM) programs for *E. vittella.*

Keywords: Biological parameters; spotted bollworm; IGR; *Earias vittella*, sub-lethal concentrations, biochemical mechanism.

Introduction

Spotted bollworm, *Earias vittella* Fabricius (Noctuidae: Lepidoptera), is a majorpest of several agricultural crops in south Asian countries (Ahmad and Arif 2009a; Rahman et al. 2016a). *E. vittella* causes can have a severe economic impact through yield losses in important crops such as okra and cotton (Jan et al. 2015; Rahman et al. 2016b). The application of pesticides is often seen as a necessary measure to protect economically important agricultural crops from direct and indirect damage caused by insect pests because non-chemical control measures alone often do not adequately prevent economic losses (Rugno et al. 2015; Santos et al. 2015). *E. vittella* is generally managed by the application of synthetic chemical insecticides in the pyrethroid, organochlorine or carbamate groups (Jan et al. 2015; Ahmad et al. 2017). However, due to the extensive use and selection pressures through repeated application of these chemicals, *E. vittella* has developed high resistance against conventional as well as newly formulated insecticides (Ahmad and Arif 2009b; Jan et al. 2015).

Although pesticides minimize the adverse impact of insect pests by reducing the population of arthropods, inappropriate application of pesticides can remain as residues in the food chain; contaminating soil, air, ground and surface water, thus affecting the entire ecosystem (Lakshmi 1993; Rezaei et al. 2017). Their continued use in agriculture has led to significant consequences not only to public health but also to food quality resulting in an impact load on the fatality of natural predators and as well as the development of pest resistance (Cordeiro et al. 2013; Guedes and Cutler 2014; EFSA 2015; Zhan et al. 2015; Hafeez et al. 2018). In humans, and other organisms, the ingestion of pesticides can cause serious health problems that can have an impact across multiple generations (Aktar et al. 2009).

Over the past four decades, efforts have been made to develop insecticides with selective properties that act specifically on biochemical sites that are present in particular insect groups but with properties that differ from other insecticides (Ishaaya et al. 2005). Lufenuron is a benxoylurea insecticide which inhibits the production of chitin of the insect, causing growth retardation and abnormal moulting from neonate to adult stages. Lufenuron and has been widely used against many economically important agriculture pests including army worm species (Schneider et al. 2008; Gelbic et al. 2011a). Lufenuron has high specificity, low toxicity to non-target organisms, rapid degradation in the environment and is effective on immature stages of insects (Evangelista Jr et al. 2002). These properties make it a strong candidate for use in integrated pest management (IPM) programs when insecticide use is necessary. Many previous studies have been reported that lufenuron showed high insecticidal activity on several insect species in the orders Lepidoptera (Fonseca et al. 2015) and Coleoptera (Arora et al. 2012).

When the target pest is not killed immediately after application of the pesticide in the field, sub-lethal effects, such as physiological and behavioral changes, may occur as the dose of the pesticide decreases over time (Rehan and Freed 2015). Therefore, sub-lethal doses of insecticides can have a great effect on emergence of insects, sex ratios, body weights, adult reproduction, and duration of larval and pupal stages (Han et al. 2012). Pesticides with different modes of action and different application methods show distinct effects against pests when presented at different sub-lethal concentrations (Zhang et al., 2015; Aliabadi and Álvarez-León 2016; Zhao et al. 2018). For example, at a low concentration some insecticides stimulate the reproductive potential of different insect pests. While other studies have shown that sub-lethal doses of insecticides would reduce the survival rates and adult female fecundity of target insect pests (Fonseca et al. 2015; Rehan and Freed 2015).

For the majority of insects, esterase, glutathione S-transeferase (GST) and cytochrome P450-dependent monooxygenase are most important detoxification enzymes, as these are involved in detoxification of OP and pyrethroids. Esterase are the key enzymes involved in hydrolysis and sequestration (Bass and Field 2011), while oxidative metabolism enhanced by monooxygenases is an important mechanism responsible for pyrethroid resistance. Previous study have shown that GST enzymes that catalyze the conjugation of glutathione to insecticides are likely to a contributing factor to organophosphate insecticides (Yu 1984).

However, according to our knowledge, sub-lethal effects of lufenuron on *E. vittella* have not previously been reported. The specific aim of this study was to evaluate the possible sub-lethal effects of lufenuron on *E. vittella* development and biological traits using the age-stage, two-sex life table. We also investigated the detoxification enzyme activities as well as the role of these enzymes in the development of resistance in *E. vittella* in response to sub-lethal concentrations of lufenuron.

Materials and methods

Insect culture

The spotted bollworm was used as the model herbivore species. To establish a laboratory colony, field population of *E. vittella* were collected as eggs and fourth and fifth larval instars from different okra fields for rearing. The experimental insects were reared for two generations. Eggs of *E. vittella* were placed in clear plastic boxes (30×22 cm) with small holes on the lid covered with muslin cloth for aeration as well as the introduction and removal of food. The experimental cultures were maintained at 25 ± 2ºC, 60 ± 5% RH in the laboratory. The neonate larvae hatched from eggs were kept in separate transparent rearing boxes and provided with clean fresh okra fruit. The old okra fruit were replaced with a fresh one on daily basis until the

larvae had developed into pupae. The pupae were transferred into plastic boxes lined with tissue paper at the bottom and covered with muslin cloth. The emerged adults were transferred into plastic rearing cages and provided with 10% sugar solution in distilled water as a food source. Nappy liner strips of different lengths were hung inside the rearing cages in order to facilitate the female moth to lay eggs (Hafeez et al. 2018). Strips with eggs were collected daily and kept in separate plastic boxes. After hatching, the neonate larvae were transferred to fresh okra fruit.

Insecticide

The commercial insecticide product used in the bioassay: Lufenuron MATCH® 5%EC was purchased from Syngenta Pakistan Pvt. Ltd. L-glutathione reduced (GSH), bovine serum albumin (BSA), α-naphthol, α-naphthyl acetate were purchased from Sigma-Aldrich Chemical Hong Kong. 1,4-dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF) 1,2-dichloro-4-nitrobenzene (DCNB), and phenylthiourea (PTU) were purchased from Cheng du Micxy Chemical Co Ltd, China.

Toxicity bioassay

Formulated insecticide was used in all bioassays, toxicity bioassays were conducted on newly molted second instar larvae of *E. vittella* under laboratory conditions following the fruit dip method (Thara et al. 2009) with slight modification. Five to six 2-fold serial concentrations of insecticides were diluted in distilled water and only distilled water was used as a control. Toxicity tests of lufenuron were carried out against second instar larvae of 3rd generation laboratory-reared populations. Fresh okra fruits were collected from a research field area, washed with distilled water, and air-dried for 30 minutes. The fruits were dipped in different concentrations of insecticide for 10 seconds and dried at room temperature for 30 minutes to establish the treatment groups. A total of 630 newly molted second instar larvae were used for

each bioassay with each selected group including a control and 90 newly molted second instar larvae for each concentration, including the control group. Thirty individuals were tested per replicate with three replicates completed for each concentration of the toxicity bioassays. The toxicity bioassays were performed under the same environmental conditions as the insect rearing. Mortality was assessed after 72 h exposure to lufenuron. Larvae were recognized as dead if they did not make any coordinated movement after being pushed with a probe.

Treatment of sub-lethal concentrations of lufenuron for F_0

Fresh okra fruits were washed with distilled water and air dried for 30 minutes before being treated with LC_{40} and LC_{15} concentrations of lufenuron (0.626 and 0.193 mg/L). The control was treated with distilled water only. For each replicate, thirty second-instars larvae were placed in individual clear-plastic cups with three treated okra fruits. Five replications per treatment (30 larvae for each replicates, including a control group), providing a total of 150-second-instar for each concentration.

Experimental setup of the life table study for F_1

The survival, growth, and reproduction data of the offspring of lufenuron-treated parent population of *E. vittella* were recorded to evaluate if the parental exposure to lufenuron would also affect the filial generation. Ninety eggs were used for each concentration and control separately. After hatching, each larva was reared separately in the small transparent plastic cups containing one fresh okra fruit. Larvae were checked every day for the occurrence of a molt and for survivorship from first to fifth instars prior to pupation. Individuals that survived to pupation were removed, separated by sex and weighed for comparison between strains. Male and female were identified at the third day of pupation. For this experiment, each larvae provided one replicate (Chi and Yang 2003). Overall survivorship, development time, and pupal weights were

compared. The newly emerged male and female adults were separately paired into a family. We established 15 families, and these were divided into three groups. Each group consisted of five families, and each group served as a replicate for each treatment. Plastic boxes (8 × 11 cm) were used and provided with nappy liner hung vertically to permit oviposition. Fecundity as eggs/female, and adult longevity were recorded. The okra fruits were replaced once after three days to avoid any effects of spoilage throughout the experiment.

Enzymes activity

Esterase activity.

The assay was performed as previously described method (van Asperen 1962; Yang et al. 2004). Twenty microliters of total proteins (80 µg) from each sample and three replicates were added to 300 µl of sodium phosphate buffer (0.1 M pH 7.6) in a 1.5-mL Eppendorf tube. 200 µl substrate solution which contained (5 mL 0.1 M pH 7.6 phosphate buffer, 10 mg Fast Blue RR salt and 0.1 mL 100 mM a-NA) were added, and mixed gently, then the absorbance values were immediately measured on (BIO-RAD xMark Microplate Spectrophotometer) once every 15 s at 450 nm and the recording lasted for 2 minutes. The mixture without protein was used as a blank control. A naphthol standard curve was used to convert absorption into concentration. The activity was expressed as nmol naphthol per min per mg protein.

Assays of glutathione S-transferase activity

GSH-ST detection kit (Nanjing Jiancheng Bioengineering) was used in which GST catalyzes the conjugation of GSH to DCNB through the thiol group of the glutathione according to the method described (Xu et al. 2014). The enzyme solution was prepared with 15 third-instar larvae homogenized in 1 mL of homogenization buffer (0.1 M sodium phosphate buffer with 1 mM

EDTA, pH 6.5). The reaction solutions contained 50 µL of enzyme, 790 µL of the homogenization buffer, 30 µL of 30mM CDNB and 30 µL of 30 mM GSH. An enzyme solution instead of buffer used as control. The OD at 340 nm was recorded at intervals of 1s for 2mins at 25 °C with BIO-RAD xMark Microplate Spectrophotometer.

Assays of P450 PNOD activities

Cytochrome P450 monooxygenase activity was measured by (PNOD) as a substrate according to the method described (Wen et al. 2009) with slight modification. 2 µl of 15 mg /mL p-nitroanisole (P-Na) was added to two hundred microliter of total proteins (200 µg), the reaction mixture containing substrate was incubated for 3 min at 30 C in water bath, and the reactions were initiated by adding of 30 µl of 10 mM NADPH. After 30 min in water bath, ethanol (200 µl) was added in the reaction mixture to precipitate the protein and stop the reaction. For blank control, proteins were added after ethanol to account for the absorbance value of each sample. The tubes were centrifuged at 2500 × g for 10 min, and the supernatant was used to read the absorbance in a spectrophotometer at 405 nm. The change in absorbance was calculated as the difference between the sample absorbance and the absorbance of each blank control. A p-nitrophenol standard curve was used to obtain the molar extinction coefficients to convert the absorbance into concentration. The activity was expressed as nmol p-nitrophenol per min per mg protein.

Age-stage, Two-sex Life Table Analysis

Different life-stage developmental times, survival, adult longevity, and fecundity parameters were statistically analyzed using age-stage two-sex life table theory (Chi and Liu 1985; Chi 1988) and the TWOSEX-MSChart software (Chi H. 2017). Means and standard errors (SE) of long-term table parameters were calculated via 100 000 bootstrap replicates to obtain stable SE

estimates (Huang and Chi 2012; Akca at al., 2015). All treatments were compared using the paired bootstrap test; both bootstrap and paired bootstrap tests were computed in TWOSEX-MSChart (Chi H. 2017), while the software Sigma Plot 12.5 was used to generate curves for all population life table parameters, including survival rate, fecundity, reproductive values, and life expectancy. The age-specific survival rate (*lx)* and age-specific fecundity (*mx)* were calculated as:

$$l_x = \sum_{j=1}^{k} s_{xj} \tag{1}$$

$$m_x = \frac{\sum_{j=1}^{k} s_{xj} f_{xj}}{\sum_{j=1}^{k} s_{xj}} \tag{2}$$

Where s_{xj} is the age-stage specific survival rate, i.e, the probability that an individual will survive to age *x* and in stage *j*. The intrinsic rate of increase (*r*) was then estimated iteratively from the Euler–Lotka equation with age indexed from 0 (Goodman 1982):

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \tag{3}$$

The net reproductive rate R_0 is calculated as:

$$\sum_{x=0}^{\infty} l_x m_x = R_0 \tag{4}$$

Relationship between R_0 and mean female fecundity (*F*), is as follows:

$$R_0 = F \frac{N_f}{N} \tag{5}$$

Where *N* is the total number of individuals used for the life table study and N_f is the number of female adults (Chi 1988). The gross reproduction rate is defined as follows:

$$GRR = \sum_{x=0}^{\infty} m_x \tag{6}$$

The mean generation time is defined as the length of time that a population needs to increase to R_0-fold of its size (i.e., $e^{rT} = R_0$ or $\lambda^T = R_0$ at the stable age-stage distribution. The formula for T is:

$$T = \frac{\ln R_0}{r} \quad (7)$$

Statistical Analysis

Bioassay data were collected and analysed by standard probit analysis after Abbott's correction (Abbott 1925) for control mortality. The LC_{40} and LC_{15} values selected as sub-lethal concentrations were determined by using the statistical program POLO-PC (Robertson et al. 1980). The sub-lethal concentration values were calculated in milligrams of active ingredient per liter and considered significantly different when their 95% confidence limits did not overlap. The mean, variance, and standard error of the biological parameters were estimated with the bootstrap procedure (Efron and Tibshirani 1993; Huang and Chi 2012; Alinejad et al. 2014). The results of pupal weight, s_{xj}, l_x, and m_x were plotted using Software Sigma Plot 12.2.

Pair-wise evaluations of LC_{40} and of LC_{15} principles were important at the 1% stage if their specific 95% confidence intervals did not overlap. Information for larval length, pupal length, pupal bodyweight and mature durability, variety of egg and percent egg stability were determined by ANOVA. The means were compared by Tukey HSD post-hoc test using SPSS v 20.0.

Results

Susceptibility to lufenuron

The mortality data of insecticide bioassay by using lufenuron against second instar larvae of *E. vittella* after 72 h. The LC_{50}, LC_{40} and LC_{15} values of lufenuron (0.873, 0.598 and 0.183mgL^{-1} respectively), against second instar larvae of *E. vittella* is presented in **Table 1.**

Low-lethal and sub-lethal effect of Lufenuron on parental F0 and offspring F1 generation

Sub-lethal effects of lufenuron on pupal period, pupal weight, pupation rate and adult emergence of *E. vittella* are presented in (**Table 2).** The average pupal period of *E. vittella* was significantly different across the parent generation ($LC_{40:}$ 11.76±0.29d, LC_{15}: 12.02±0.46d and control: 9.62±0.18d) and offspring generation (LC_{40}: 11.8±0.06d, LC_{15}: 10.5±0.28d and control: 9.40±0.07d) and decreased with increasing insecticide concentration respectively (**Table 2)**. The mean pupal weight was significantly decreased in the both sub-lethal concentrations in parental generation (LC_{40}: 48.24±1.64, LC_{15}: 49.56±1.36 and control: 61.14±2.14) while only in sub-lethal concentration LC_{40} (48.24±1.64) of offspring generation as compared with the control. There was no difference in pupal weight at the sub-lethal LC_{15} concentration between the offspring and control. Furthermore, we found a significant decrease in pupation rate and adult emergence percentage of parental (LC_{40}: 64.94±1.48, LC_{15}: 73.58±1.23 and control: 96.20±3.21) and offspring generation (LC_{40}: 72.20±3.72, LC_{15}: 82.0±4.64 and control: 97.40±2.66) at both sub-lethal concentrations. We found a significant lower adult longevity in parental generation (LC_{40}: Male 7.75±0.24d, Female 7.08±0.04d, LC_{15}: Male 7.91±0.25d, Female, 8.17±0.18d and control: Male 9.78±1.34d, Female 13.62±1.26d) and offspring generation (LC_{40}: Male 8.58±0.26d, Female 9.60±0.52d, LC_{15}: Male 8.93±0.25d, Female, 9.42±1.12d and control: Male 9.96±0.17d, Female 13.10±1.27) at both sub-lethal concentrations as compared to the control, whereas no significant difference between the low-lethal and sub-lethal concentration. Furthermore, we observed that the female longevity of parental and offspring generation was greatly affected when second instars of parental generation was exposed to sub-lethal

concentrations of lufenuron. The mating pair success was significantly impaired in parental and offspring generation in both sub-lethal concentrations of lufenuron. Additionally, data from our study demonstrates that the fecundity of the spotted bollworm female moth of parental generation (LC_{40}: 226.78±13.59, LC_{15}: 231.67±14.03 and control: 326.56±22.63) and offspring (LC_{40}: 234.8±16.32, LC_{15}: 244.3±19.33 and control: 319.5±24.88) remarkably decreased at both concentrations of lufenuron (**Table 3).**

Sub-lethal effects of lufenuron on the individuals (F1) of *E. vittella.*

The developmental periods for eggs, larval instars, larval, oviposition, adult pre-oviposition period (APOP) and total pre-ovipostion period (TPOP) of second instar *E. vittella* F_0 and F_1 generations exposed to sub-lethal concentrations of lufenuron are shown in (**Table 4).** There were no significant differences in egg developmental period of *E. vittella*, when second instars larvae of F_0 generation were treated with sub-lethal concentrations of lufenuron compared to the control. The developmental period of first larval instars development duration was also reduced significantly at both sub-lethal concentrations of lufenuron treated populations compared to the control (LC40: 2.06±0.04 d, LC15: 2.14±0.05 d, control: 2.58±0.02 d), however, no significant difference was observed between both concentrations **(Table 4).** The same outcome was observed in lufenuron groups, which extended developmental period of fifth larval instars (LC_{40}: 2.58±0.1 d, LC_{15}: 3.16±0.16 d, control 2.16±0.06 d). The mean larval developmental period from second instar to pupation of *E. vittella* of F1 generation treated with LC_{15} and LC_{40} concentration (LC_{15}: 11.8±0.28 d, LC_{40}; 11.1 + 0.06) of lufenuron was significantly longer as compared with the control (9.40 ±0.52 d). Similarly, the LC_{15} (11.8±0.28d) and LC_{40} (11.1±0.06d) concentration of lufenuron significantly extended the mean pupal duration of *E. vittella* of offspring generation

as compared with the control (9.40 + 0.52d) (Table 4). There were significant differences in APOP at both concentrations (LC_{15}: 1.00±0.00d, LC_{40}: 1.00±0.00d) of lufenuron as compared to the control (1.2±0.132d), while no significant difference was observed in APOP between the both concentrations respectively. Furthermore, the TPOP values at LC_{15} (29.0 ± 1.19d) and LC_{40} (27.3 ± 0.43d) concentrations in offspring generation were significantly increased as compared with the control (26.0 ± 0. 65 d). (**Table 4).**

Comparison of biological parameters of F1 generation *E. vittella*

Low-lethal and sub-lethal effects of lufenuron on biological parameters of the *E. vittella* were calculated by using bootstrap method. The data regarding to gross reproduction rate (*GRR*), intrinsic rate of increase (*r*) and net reproductive rate R_0 between low-lethal and sub-lethal concentration of lufenuron were not significantly different as compared with the control. While, only LC_{15} concentration had a significant effect on mean generation rate (*T*) as compared with the LC_{40} concentration and control **(Table 5).**

Effect on survival rate, life expectancy, reproduction value and fecundity of F1 generation

The age-stage survival rate (s_{xj}) shows the likelihood that newly laid eggs will survive to age *x* and stage *j* (**Fig. 1).** A difference was exhibited in the developmental rate among individuals (Fig. 1). It was also demonstrated that there was a significant difference in overlapping projected curves among the different developmental stages for LC_{40} and LC_{15} of lufenuron and control. The peak lines in the plotted curves showed a different pattern for every developmental stage for LC_{40} and LC_{15} of lufenuron and the control, with exceptions that curves end earlier for male adults than female. The results show that with LC_{15} concentration of lufenuron, females emerged after 24 days and males at 25 days. For LC_{40}, emergence was seen for both sexes at 24 days while in the control group emergence occurred after 23 days (Fig. 3). Age-stage reproductive

values (v_{xj}) of *E. vittella* are show that at the pupal stage, the reproductive value at low-lethal concentration was lower compared to the sub-lethal concentration and the control group (Fig. 3). The result also showed that when female emerged there was lower plotted curve in both concentrations as compared to the control. Age-stage-specific survival rate (l_x), female age-stage-specific (f_x), age-stage-specific fecundity of the total population (m_x), and age-stage-specific maternity ($l_x m_x$) of *E. vittella* for each concentration were attained (Fig. 2). The curves of l_x declined significantly for the LC_{40} and LC_{15} treated larvae after (29 d), (30 d) compared to the control group (33 d). Lower peaks in the f_x curves were observed for LC_{40} and LC_{15} than to the control. Furthermore, the peak value of the curve in the control group was greater than those of the LC_{40} and LC_{15} groups. The life expectancy (e_{xj}) of *E. vittella* was significantly varied between the low-lethal and sub-lethal concentrations of lufenuron and control group (**Fig. 4).** The life expectancy of newly laid eggs of the F_1 generation at LC_{40} was 34.0 d was lowered as compared to and LC_{15} (38.0 d) and the control group (36.0 d). The peak life expectancy of first instar larvae was (33.4 d, 30.0 d and 36.0 d) in the LC_{40}, LC_{15} and the control treatment, respectively (**Fig.4).**

Metabolic enzyme activity

Earias vittella fitness traits are interpreted in light of the differential activity of three major xenobiotic detoxifying enzymes mechanism, ESTs, P450s and GSTs at LC_{15} and LC_{40} concentrations of lufenuron. In present study, cytochrome P450 monooxygenase activity (µmol/min/mg protein) using p-nitroanisole as substrate feeding on okra fruits exposed to low-lethal and sub-lethal concentrations (LC_{40}; 1.53 ± 0.06 and LC_{15}; 1.28 ± 0.023) of lufenuron

was significantly increased as compared to the control (0.58 ± 0.050) **(Table 6).** Similarly, significantly elevated esterase activity was also observed at both concentrations (LC40; 123.83 ± 3.05 and LC15; 115.73 ± 1.84) of lufenuron as compared to the control (92.15 ± 2.28). While, no significant difference was observed in GSTs activity at LC_{40} and LC_{15} concentrations as compared to the control group respectively (Table 6).

Discussion

In the present study, we investigated the sub-lethal effects of lufenuron on biological traits and detoxification enzymes activity on second instar larvae of *E. vittella*. Lufenuron is considered an effective insect growth regulator which greatly impacts reproduction parameters of insects (Medina et al. 2002). The sub-lethal effects on larval growth may be due to the perturbations in the development of neural tissues by the exposure to the neurotoxic substance (Desneux et al. 2007). Thus, study of pesticides with sub-lethal effect is imperative to understanding the greater impact on target and non-target pest organisms (Guedes and Cutler 2014). Prolonged larval duration, pupal stage and reduced pupal weight were observed in parent (F_0) and F1 generations after the exposure to sub-lethal concentrations of lufenuron. Similar results were reported by (Salokhe et al. 2003; Sáenz-de-cabezón et al. 2006; Fonseca et al. 2015) in *Tribolium castaneum* (herbst), *Lobesia botrana* and *Diatraea flavipennella* when exposed to sub-lethal concentrations of lufenuron and flufenoxuron. This is likely to be a consequence of a resource trade-off as lufenuron-treated *E. vittella* larvae spend more resources on detoxification of the substance rather than development causing prolonged larval development in the control group (Meng et al. 2018). Our results demonstrate that pupation rates, adult emergence rates, male and female longevity, fecundity and eggs viability of *E. vittella* were significantly decreased at LC_{15} and LC_{40} concentrations of in parent (F0) and F1 generation **(Table 2 & 3)**. Previous studies

depicted that the mean number of eggs laid by female adults, the percentage of offspring eggs hatching, male and female longevity would decrease after treatment of insect growth regulator insecticides (Schneider et al. 2003; Seth et al. 2004; Mahmoudvand et al. 2011; Qu et al. 2017; Meng et al. 2018). Reductions in fecundity may result from both physiological and behavioural effects of pesticide treatment (Desneux et al. 2007). In the present study prolonged larval and pupal developmental time, decreased adult's longevity and mean number of eggs laid by female adults and percentage of eggs hatching of *E. vittella* were observed following by the exposure of sub-lethal treatments of lufenuron. It is speculated that the ovaries of treated females were affected by lufenuron, which could cause the reduction in egg laying, though they also exhibited reduced time spent for oviposition and the decreased longevity of the adults may contribute to decrease the oviposition opportunity. One factor that may contribute to this is the abruption of prostaglandin E2 biosynthesis, which is identified as one of the most important factors related with oviposition (Moreno and Nakano 2002). Perveen et al., (2000) showed that LC_{30} concentration of chlorfluazuron could decrease the size of different parts of the ovarioles, the number of mature ova, the size of basal oocytes, and thickness of their follicular epithelium of *Spodoptera litura*, which may be the main reason for the reduction in fecundity and hatchability. The present study also demonstrated that lufenuron could affect the female ovaries and reduced the egg-laying tendency of *E. vittella*. Similar observations were made for a number of non-lepidopteran species, in which hatchability significantly decreased when *D. melanogaster* and *Callobruchus maculantus* were exposed to lufenuron (Wilson and Cryan 1997). Shorter longevity of males and females may possibly reduce the coupling period under field conditions and may cause the female to lay fewer eggs, which will result in a decline of field populations

over time. Reducing the number of eggs laid by female adults in the parental generation and reducing the egg hatching percentage are both important factors for success in pest management.

In addition to acute toxicity, sub-lethal effects, such as reduced egg viability, compromise population growth. The suppression of population growth occurs because intrinsic growth rates depend on developmental stage and viability (eggs, larvae and pupae), plus offspring sex ratio, age-specific fertility and female survival rate (Biondi et al. 2013). In the present study, no significant differences were observed in gross reproduction rate (*GRR*), intrinsic rate of increase (*r*), net reproductive rate (*Ro*) or finite rate of increase (λ) which contrasts with the results of Mahmoudvand et al. (2011) who found that R_0, *r*, *Ro*, and λ were significantly different following IGR exposure. Similarly, only mean generation rate (*T*) was significantly increased in sub-lethal (LC_{40}) concentration compared to the low sub-lethal (LC_{15}) concentration and control groups. The life table parameters assessed in this study reflect a trend already well observed in the published literature; e.g. the effect of lufenuron and methoxyfenozide on *Lobesia botrana* (Lepidoptera: Tortricidae) fall army worm (Sáenz-de-Cabezón et al. 2006; Zarate et al. 2011).

The life table study is recommended as a comprehensive method to evaluate total effect of insecticides on insect populations (Tuan et al. 2016). For gauging the impact of lufenuron on the *E. vittella* populations, an age stage, two-sex life table study provides a valuable tool. We therefore able to evaluate the survival rate, adult longevity, fertility, and population parameters through the use of age-stage, two-sex life tables. Our results showed that Age-specific survival (S_{xj}), fecundity (mx), reproductive value (V_{xj}) and life expectancy (e_{xj}) decreased dramatically in the lufenuron-treated populations. Life expectancy (e_{xj}) of *E. vittella* declined sharply at the stage of second-instar onward in the LC_{40} treated groups as lufenuron kills the most susceptible

individuals. Meanwhile, the e_{xj} of eggs was lower in the treated groups compared to the control group because of the increasing pressure of lufenuron.

Almost all phytophagous insects utilize diverse mechanisms of metabolic detoxification to evade the toxicity of plant secondary metabolites and synthetic insecticides. Elevation 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 (Wu et al. 2011; Tao et al. 2012). The acquisition of resistance to insecticides often carries an associated fitness cost when resources normally directed toward fitness-enhancing traits are redirected instead toward production and maintenance of resistance (Carrie et al. 1994). Our results show that the activities of P450 might play a key role in the development of resistance to *E. vittella*. However, elevated acivities of P450 monooxygenase, and esterase were observed when *E. vittella* larvae exposed to LC_{15} and LC_{40} concentrations of lufenuron, while no significant difference was observed in GST activity compared with LC_{15} and LC_{40} concentrations and control. Our results are in consistent with previous studies (Ahmad et al. 2007; Bilal et al. 2018; Hafeez et al. 2018). Additionally, it has been showed that the esterase's inhibitor dramatically potentiates the toxicity of metaflumizone against the field-evolved resistant populations of *S. exigua* (Tian et al. 2014). Further studies show that different insecticide resistance mechanisms in *S. litura, bemisia tabaci* and *Drosophila melanogaster* were associated with the enhanced activity of cytochrome P450 monooxygenase (MFO) and esterase (Young et al. 2006; Karuppaiah et al., 2017; Wang et al. 2018). Mechanisms involved in P450 monooxygenase and esterase are also observed in butne-fipronil exposed *D. melanogaster* (Arain et al. 2018). To our best knowledge, this is the first study of the sub lethal effects of lufenuron exposure on key biological traits and detoxification enzymes activity.

Establishing the sub-lethal effects of lufernuron on *E. vittella* is critical to assessing the efficacy of applying it to commercially important crops. These results can help us establish a more carefully consideration and rational approach to pest control. Such an approach may allow us to reduce insecticide usage and mitigate against environmental damage.

In conclusion, the results presented here show that lufenuron is toxic to *E. vittella* and increases the different pre-adult developmental period while decreasing the adult's longevity and fecundity. Sub-lethal effects of this insecticide have practical and commercial implications for the pest management strategies. Importantly, the effects of insecticide through the use of methods other than life tables may provide different results and conclusions from when the lethal and sub-lethal effect of pesticide are the primary focus. This approach delivers a more comprehensive answer to the research questions associated to the population biology of a species. Therefore, the use of age-stage, two-sex life tables is needed for further studies on the sub-lethal effect of insecticides on target as well as non-target organisms. In addition, the present study has defined higher activities of different enzymes in *E. vittella* after treated with different concentrations of lufenuron. This study will be a valuable source of information for further understanding the sub lethal effects of lufenuron on biochemical mechanism and for assisting the development of resistance management programs.

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