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Full Length Article

Age-Stage, Two-Sex Life Table Study of the Effects of Sub-Lethal Concentrations of Novaluron on *Earias vittella* (Lepidoptera: Noctuidae)

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Abstract

Earias vittella (F) is an important insect pest of cotton (*Gossypium hirsutum*) and okra (*Abelmoschus esculentus*) in Pakistan. The current study was carried out to explore the effects of sub-lethal concentrations of novaluron on the life table parameters of the pest. Bioassays were performed to assess the sub-lethal concentrations (LC_{20} and LC_{50}) of the novaluron and its effects on the demographic parameters of the *E. vittella*. Age-stage, two-sex life table theory was applied to interpret the data for population parameters of *E. vitella*. In the current study, the LC_{20} and LC_{50} were calculated as 2.224 ppm and 9.837 ppm, respectively. The results showed that in novaluron treated samples rates of all biological parameters decreased whereas the larval, pupal period and mean generation time were increased. The intrinsic rate of increase remained high in control as 0.166 d^{-1} in comparison with LC_{50} as 0.128 d^{-1}. The net reproductive rate ranged from 94.542 offsprings per individual (control) to 61.228 offsprings per individual (LC_{50}). Fecundity was dropped in insects treated with sub-lethal concentrations from 330.9 eggs per female (control) to 238.11 eggs per female (LC_{50}). This study revealed that the sub-lethal concentrations of novaluron significantly decreased the biological rate of *E. vitella* under laboratory conditions and suggests that such doses should be practiced in the fields for proper integrated pest management strategies.

Keywords: *Eairas vittella*; Sub-lethal concentration; Novaluron; Insect growth regulator; Two-sex life table

Introduction

Earais vittella (F.) (Noctuidae: Lepidoptera), also known as spotted bollworm, is a notorious polyphagous pest of many malvaceous crops (Aziz *et al.* 2012). Some of its major host crops include *Gossypium* spp. (cotton), *Abelmoschus esculentus* (okra), *Abutilon indicum, Hibiscus cannabinus, Althaearosea* (hollyhock) and *Malwa parviflora* (sonchal) (Rasool *et al.* 2002; Jan *et al.* 2015; Rahman *et al.* 2016). The spotted bollworm is active throughout the year and have 6 to 8 generations during each year. Several buds and bolls are damaged by a single larva in its life span. In cotton, it pupates in bolls and reduces the boll growth (Aziz *et al.* 2011; Jan *et al.* 2015) commonly the buds, flowers, and fruits are attacked by second instar larvae and results in the reduction of quality and quantity. It may reduce the yield up to 50% in cotton and about 69% in okra (Aziz *et al.* 2011).

The use of chemicals plays a key role in the management of pests in fields, resulting in a low risk of yield loss (Popp and Hantos 2011). *E. vitella* infestation has been managed using different insecticides including, pyrethroids and organophosphates (Praveen *et al.* 2007; Umrao *et al.* 2013). Irregular and massive application of such chemicals has resulted in resistance development in field pests (Abbas *et al.* 2014; Gulzar and Wright 2014; Abbas and Shad 2015; Ahmad *et al.* 2019). Novaluron is one of the recent insect growth regulators (IGR) that belong to the insecticidal group, benzoylphenyl urea. It is a chitin synthesis inhibitor that acts through ingestion and contact. It targets the larval stage of insects which synthesize chitin actively (Lohmeyer and Pound 2012). The residual activity of novaluron in field conditions depends upon the environmental conditions and ranges from 10 to 30 days (Ishaaya *et al.* 2003). It has a very low toxic effect on mammals, birds, earthworms (Ishaaya *et al.* 2007) and adults of non-targeted beneficial insect species (Cutler *et al.* 2005).

The application of pesticides in fields does not kill all the pest populations with immediate effects, so over time the pesticide decreases and, as a result, the sublethal effects including behavioral and physiological changes in pests can occur (Rehan and Freed 2015). These sublethal concentrations of pesticides can significantly alter the adult development, adult insect weight, larval and pupa periods and reproduction parameters of the insect (Han *et al.* 2012).

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For a comprehensive pesticide evaluation, only acute toxicity is not enough, but the sublethal effects may also be included (Zhang *et al.* 2015). Previously, Rahmani and Bandani (2013) found that the sub-lethal doses of thiamethoxam chemical significantly altered the different *Hippodamia variegate* population parameters in adverse modus. This study was planned to elucidate the sublethal effects of the novaluron on population parameters of E. vittella by application of Age-stage, two-sex life table theory. The finding of this study may be helpful to monitor the insecticide resistance in *E. vittella* to insect growth regulators (IGRs) in fields and develop improved integrated pest management strategies.

Materials and Methods

Insect culture and insecticides

Laboratory culture of spotted bollworm (*E. vittella*) was established from the larvae collected from okra fields in the surrounding of Rawalpindi, Taxila and Attock. The infested pods from the fields were transferred into transparent plastic jars (20 cm length and 10 cm wide) and were kept under control conditions (27 ± 2°C, 60 ± 5% R.H. photoperiod of 16 L: 8 D). Insect culture was maintained on okra fruits as described by Al-Mehmmady (2000). Fresh okra fruits were washed thoroughly by tap water and air-dried before feeding to the neonates. The okra fruits were cut into 0.5–1.0 cm pieces, 4–5 larvae were released per piece and placed in the plastic container. The food was replaced daily till pupation. The larvae were carefully removed from the okra pods and the excreta were cleaned. The pupae were shifted to another plastic jar (10 cm length and 5 cm width) until adult emergence. The emerged adults were shifted to adult cages and fed with 10% sugar solution. Nappy strips were hanged in the adult cages as oviposition sites, these strips were replaced regularly when eggs were observed.

Novaluron, insect growth regulator, (Corvus®) FMC Pvt. Ltd. was used at the recommended dose along with different concentrations against the 1st instar larvae of spotted bollworm (*E. vittella*) to investigate the larvicidal effects.

Bioassays

Bioassays were performed by using diet emersion method and toxicity of novaluron was checked against 1st larval instar of spotted bollworm (*E. vitella*). Different concentrations of novaluron by serial dilutions (mg L^{-1}) of stock solution were prepared using distilled water. Fresh okra fruits were dipped separately in each concentration for 10 sec and then dried for 10 min at room temperature. Five okra fruits were used in one replication. Two 1st instar larvae were released on each okra pods in all replications treated with different concentrations. Each treatment was replicated four times. Distilled water was used as a control treatment. All the treated larvae were kept under controlled conditions (Temperature of 25 ± 2°C; R. H. of 65 ± 5%). Mortality data were assessed after 72 h.

Sublethal effects on demographic parameters of *E. vittella*

In life table study, 210 eggs were used, which were collected after 24 h of deposition by females of the laboratory population. Three treatments (control, LC_{20}, and LC_{50}) were prepared for this experiment. Seventy eggs were treated with each treatment. Each egg is an individual petri dish that was considered as one replicate (Huang and Chi 2013; Zhang *et al.* 2015). All the Petri dishes were kept under controlled conditions. The egg hatching data were recorded daily. The neonates from control, LC_{20} and LC_{50} were shifted on the okra pods treated with control, LC_{20} and LC_{50}, respectively. The larval development was observed daily and fresh okra pods were provided after each instar. Pupae were removed and placed in new Petri dishes until emergence. After the adult emergence, they were paired (one male and one female) and transferred to individual plastic containers for oviposition. The adults were checked daily for oviposition and transferred to new containers for egg-laying. The fecundity and survival rate of the adults have assessed until the death of the adults.

Data analysis

The LC values were calculated based on mortality data by using R Statistical Software version 2.9.0 (R Development Core Team 2009). The data regarding different stage development periods, survival rate and fecundity along with oviposition periods were analyzed using Age-stage, two-sex life table theory (Chi and Liu 1985; Chi 1988) with TWO SEX-MS Chart software (Chi 2017). Means of the biological parameters were compared by using 100,000 bootstrap techniques to achieve stable SE estimates (Huang and Chi 2013). The curves for age-specific survival rate, fecundity, life expectancy and reproductive values were generated bu using Sigma Plot 14.0. The net reproductive rate was calculated as:

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

The intrinsic rate of increase (r) is calculated by using the iterative bisection method from:

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

With age indexed from zero (Goodman 1982). The mean generation time (T) is calculated as follow:

$$T = \frac{\ln R_0}{r}$$

The Gross reproductive rate (GRR) is calculated by the

formula as follow:

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

The age-specific survival rate (l_x) and age-specific fecundity (m_x) were given as:

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

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

Results

Toxicity bioassays

The toxicity of novaluron against the 1st instar of *E. vittella* after 72 h is given in Table 1. The LC_{20} and LC_{50} were calculated as 2.224 mg L^{-1} and 9.837 mg L^{-1}, respectively.

Sub-lethal effects of novaluron on biological parameters of *E. vittella*

The developmental periods, fecundity and adult longevity of both males and females of *E. vittella* treated with sub-lethal concentrations (LC_{20} and LC_{50}) of novaluron are given in Table 2. Egg duration was significantly ($P \leq 0.005$, df=2, F=87.61) prolonged when treated with sub-lethal concentrations (4.26 days and 4.07 days for LC_{50} and LC_{20} respectively of novaluron as compared with the control (3.66 days). Total larval time was also increased by treating with sub-lethal concentrations (LC_{50} andLC_{20}) as 12.54 days and11.23 days respectively ($P \leq 0.005$, df=2, F=117.24) as compared with control (11.03 days). No significant variation was noted between LC_{20} (11.35 days) and LC_{50} (11.47 days) in terms of the pupal period but differed significantly ($P \leq 0.005$, df=2, F=122.82) when compared with the untreated larvae (Table 2). Adult male longevity was not significantly different (p=0.104, df=2, F=11.82), while female longevity was significantly ($P \leq 0.005$, df=2, F=92.87) different ranging from highest (12.7 days) on control to the lowest (7.62 days) on LC_{50}. The differences in total pre-oviposition period (TPOP) between sub-lethal concentrations were statistically non-significant ($P \leq 0.01$, df=2, F=7.45), while a significant difference was found between the control and treated larvae. The number of eggs per female varied significantly ($P \leq 0.005$, df=2, F=648.22) between sub-lethal concentrations (238.11 and 268.5 eggs per female in LC_{50} and LC_{20} respectively). The highest fecundity (330.9 eggs per female) was noted for untreated larvae.

Sub-lethal effects of novaluron on population parameters of *E. vittella*

Novaluron significantly altered the population parameters of the *E. vittella* (Table 3). To estimate the population parameters, the bootstrap method with 100,000 replicate sample method was used. The intrinsic rate of increase was decreased by treating with both the concentrations of novaluron (0.128 and 0.140 d^{-1} for LC_{50} and LC_{20}, respectively) as compared with the untreated larvae (0.166 d^{-1}). A similar trend was found in the finite rate of increase (λ), as the highest value for λ was found in control larvae (1.181 d^{-1}) which gradually decreases with an increase in concentration from LC_{20} to LC_{50} as 1.150 and 1.137 d^{-1} respectively. A significant decrease was also observed in net reproductive rate after novaluron treatment from being highest on control larvae (94.542 offsprings per individual) to 76.714 and 61.228 offsprings per individual for LC_{20} and LC_{50} treated larvae respectively. Moreover, the gross reproductive rate (GRR) of LC_{20} treated larvae (146.45, offsprings per individual) was significantly similar to that of LC_{50} and control. The highest GRR was recorded for control larvae (183.42 offsprings per individual), while the lowest recorded for LC_{50} treated larvae (136.77 offsprings per individual) which were both statistically significant to each other. The mean generation time was prolonged in the treated larvae compared to the control larvae. Minimum mean generation time was taken by the control larvae (27.255 d), followed by LC_{20} treated larvae (30.967 d). The maximum days were recorded on LC_{50} treated larvae (31.917 d). The curves of developmental rates of the individuals showed an overlapped nature showing the differences in their development rates (Fig. 1). Females were emerged late in the population than males, while their survival was longer than males. The LC_{20} and LC_{50} treatment were observed with less number of larvae as compared with untreated. The longest time for development was noted for LC_{50} and LC_{20} treated larvae when compared with untreated larvae (Fig. 1).

Age-specific survival rates (*lx*), age-specific fecundity (*fx*, female), age-specific fecundity for total population and age-specific maternity (*lxmx*) are presented in Fig. 2. A significant decline in the curve of lx was noted in LC_{20} and LC_{50} treated larvae after 32 days of the treatment. The untreated group has the highest top peaks of f*x* and m*x* than compared with the LC_{20} and LC_{50} treated group. A significant variation was shown in the life expectancy (e_{xj}) among the treated (LC_{20} and LC_{50}) and untreated larvae (Fig. 3). The maximum life expectancy of new eggs was recorded in LC_{50} (39.0 days), followed by LC_{20} (38.0 days) while the minimum life expectancy of eggs was recorded in the untreated group (36.0 days). Reproductive rate (v_{xj}) is defined as the measure of dedication to newly coming offspring in the future from age *x* to stage *j* (Fig. 4). The contribution of males in the population to the next generation was not well defined, therefore the curve for males was not included. A decline was observed in the reproductive values when larvae treated with LC_{50} and LC_{20} as compared to the untreated larvae. Maximum v_{xj} was recorded on the untreated group, while the minimum was

Table 1: Acute toxicity of novaluron on the 1st instar of *E. vittella* after 72 h of treatment

Chemical	n	Concentration mg liter^{-1} (95% CL)			Slope ± SE	χ^2 (df)
		LC_{10}	LC_{20}	LC_{50}		
Novaluron	240	1.022 (0.534-2.156)	2.224 (1.162-4.256)	9.837 (5.140-18.827)	1.304 ± 0.144	0.997 (5)

LC (lethal concentration), n (number of samples), CL (confidence level), SE (standard error), χ^2 (Chi square), *df* (degree of freedom)

Table 2: Life table parameters of *E. vittella* treated with sub-lethal concentrations of novaluron

Treatment	Control		LC_{20}		LC_{50}	
	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE
Egg (days)	70	3.66 ± 0.1^{b}	70	4.07 ± 0.094^{a}	70	4.26 ± 0.125^{a}
1st instar (days)	64	2.28 ± 0.08^{c}	60	2.33 ± 0.088^{b}	58	2.52 ± 0.094^{a}
2nd instar (days)	54	2.19 ± 0.076^{b}	50	2.20 ± 0.082^{b}	56	2.54 ± 0.096^{a}
3rd instar (days)	48	2.17 ± 0.078^{b}	44	2.36 ± 0.105^{a}	46	2.48 ± 0.106^{a}
4th instar (days)	46	2.13 ± 0.072^{b}	42	2.19 ± 0.088^{b}	40	2.53 ± 0.125^{a}
5th instar (days)	46	2.26 ± 0.094^{b}	40	2.15 ± 0.082^{b}	38	2.47 ± 0.125^{a}
Larva (days)	46	11.03 ± 0.028^{c}	40	11.23 ± 0.041^{b}	38	12.54 ± 0.013^{a}
Pupa(days)	41	8.74 ± 0.201^{b}	34	11.35 ± 0.15^{a}	31	11.47 ± 0.151^{a}
Male longevity(days)	18	8.52 ± 2.066^{a}	19	7.72 ± 1.56^{a}	16	7.12 ± 0.331^{a}
Female longevity(days)	23	12.7 ± 2.13^{a}	15	9.04 ± 1.21^{b}	14	7.62 ± 0.242^{c}
APOP	23	1.2 ± 0.133^{a}	15	1.00 ± 0.00^{a}	14	1.00 ± 0.00^{a}
TPOP	23	24.5 ± 0.619^{b}	15	28.3 ± 0.26^{a}	14	29.22 ± 0.464^{a}
Fecundity (eggs/f)	23	330.9 ± 26.41^{a}	15	268.5 ± 19.67^{b}	14	238.11 ± 14.24^{c}

LC (lethal concentration), SE (standard error), n (number of insects exposed. Adult pre oviposition period (APOP), total pre oviposition period (TPOP), means sharing similar letters in a row are not different statistical at 5% probability

Table 3: Effect of sub-lethal concentration of novaluron on the biological parameters of *E. vittella*

Population parameters	Control		LC_{20}		LC_{50}	
	Mean	SE	Mean	SE	Mean	SE
Intrinsic rate of increase (*r*)	0.166^{a}	0.011	0.140^{b}	0.009	0.128^{c}	0.010
Finite rate of increase (λ)	1.181^{a}	0.013	1.150^{b}	0.010	1.137^{b}	0.011
Net reproductive rate (R_o)	94.542^{a}	25.23	76.714^{ab}	20.679	61.228^{b}	17.610
Mean generation time (*T*)	27.255^{c}	0.573	30.967^{b}	0.272	31.917^{a}	0.301
Gross reproductive rate (*GRR*)	183.42^{a}	8.055	146.45^{ab}	32.83	136.77^{b}	30.553

LC (lethal concentration), SE (standard error), *df* (degree of freedom), means sharing similar letters in a row are not different statistical at 5% probability

observed in LC_{50} treated group.

Discussion

In the current study, Age-stage, two-sex life table theory was utilized to calculate the population parameters of *E. vittella* exposed to the sublethal concentrations of novaluron. Age-stage, two-sex life table study is a promising way to estimate the population parameters of the pest by considering both the sexes of the existing population (Rahmani and Bandani 2013; Huang and Chi 2013). The intrinsic rate of increase, reproductive rate and total oviposition period are the most important characteristics of the life table study to predict the insect population effected by insecticides (Papachristos and Milonas 2008).

The sub-lethal concentrations (LC_{20} and LC_{50}) of novaluron for *E. vittella* were calculated as 2.224 and 9.837 mg L^{-1}, respectively in this study. Cutler *et al.* (2005) studied the acute toxicity of novaluron on the second instar of *L. decemlineata* and calculated LC_{50} as 18.7 ppm and categorized it as broad-spectrum insecticides. Population parameters of *E. vittella* studied in this paper showed that the sub-lethal (LC_{20} and LC_{50}) concentrations of novaluron have decreased the intrinsic rate of increase, finite rate of increase, reproductive value, survival rate, and net reproductive rate, however, the larval period, pupal period and TPOP were increased by the sub-lethal concentrations. The above results proved that the population growth of *E. vittella* was significantly reduced by the sub-lethal concentrations of novaluron. These results are according to the sub-lethal effects of thiamethoxam on *Bradysia odoriphaga* (Zhang *et al.* 2015). Significantly no difference was found in the adult pre-oviposition period (APOP) among treatments, while a significant variation was found in the total pre-oviposition period (TPOP), which was positively correlated with the intrinsic rate of increase of the *E. vittella*. Sub-lethal concentrations significantly prolonged the larval duration, this may have occurred because of agitations in nerve tissue development by neurotoxic chemical contact (Desneux *et al.* 2007). Similar results were found when the population of *E. vittella* treated with sublethal doses of lufenuron (Hafeez *et al.* 2019). The reason for the prolonged larval duration would be that the treated larvae were more intense with the detoxification of the sub-lethal effect of novaluron causing the increased larval period as compared to the control (Meng *et al.* 2018). The number of eggs per female also reduced to a significant level by the application of sub-lethal concentrations of

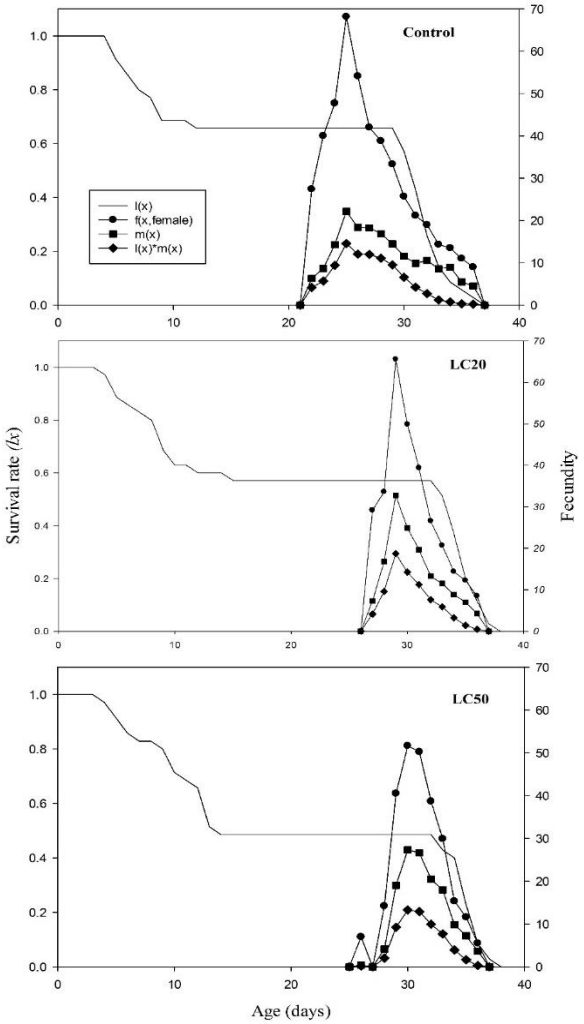

Fig. 2: Survival rate (l_x) and fecundity of *E. vittella* exposed to sublethal concentration of novaluron

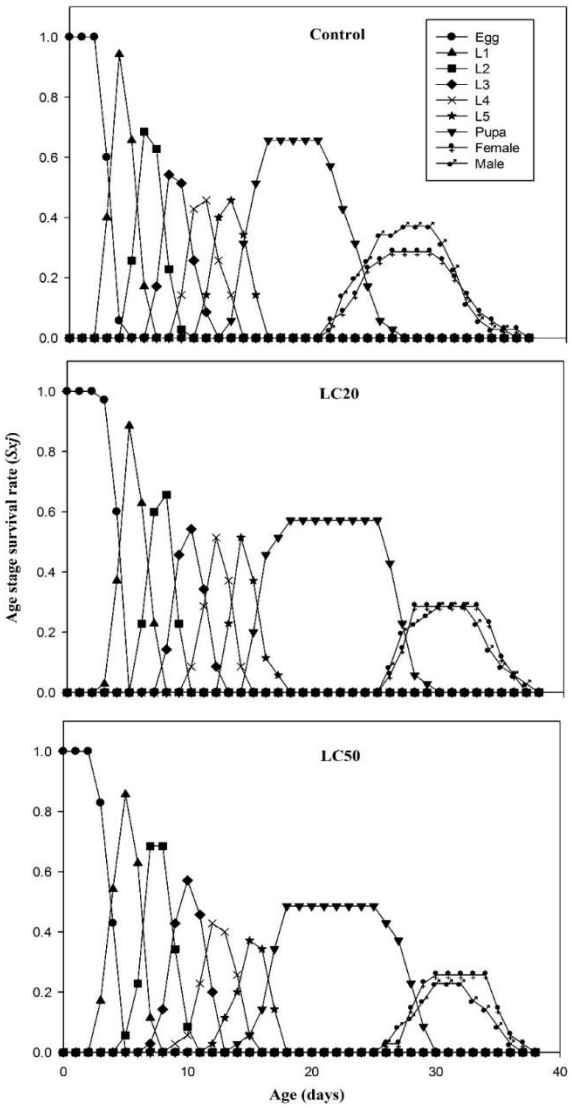

Fig. 1: Age-stage specific survival rate (s_{xj}) of *E. vittella* exposed to sublethal concentration of novaluron

novaluron. This showed that the chemical has produced some effects on the ovaries of the females in treated groups (Seth *et al*. 2004; Qu *et al*. 2017). It has been found that exposure of insects to insecticides results in the reduction of ovarioles size, basal oocytes, and firmness of follicular epithelium of armyworm, which was the main reason for reduced fecundity (Perveen and Miyata 2000). The reduction may also be due to changes in the behavioral and physiological effects of the insecticide. The short life span of adults will result in the short mating periods, which will ultimately result in fewer eggs in the field and thus a decline will be found in the field population over time. In this study, the population parameters *viz*., intrinsic rate of increase, gross reproductive rate, net reproductive rate and finite rate of increase were decreased with the LC_{20} and LC_{50} treatments. These results are in line with the result of previous studies, in which the intrinsic rate of increase, finite rate of increase and net reproductive rate statistically declined in diamondback moth and cabbage aphid by the treatment of spinosad and imidacloprid respectively (Lashkari *et al*. 2007; Yin *et al*. 2008). This study provides a clear knowledge about the population dynamics of *E. vittella* subjected to the sub-lethal effects of the insecticides. These sublethal concentrations of novaluron can be utilized in the field to delay the development rate and reduce the reproduction ability of the females in the pest population. These concentrations will reduce the number of insecticides used in fields, reduce the risk of resistance development in the insect pests and reduce environmental pollution.

Conclusion

This study publicized that the novaluron with sub-lethal concentrations significantly decreased the biological rate of *E. vitella* in the laboratory conditions and advocates the

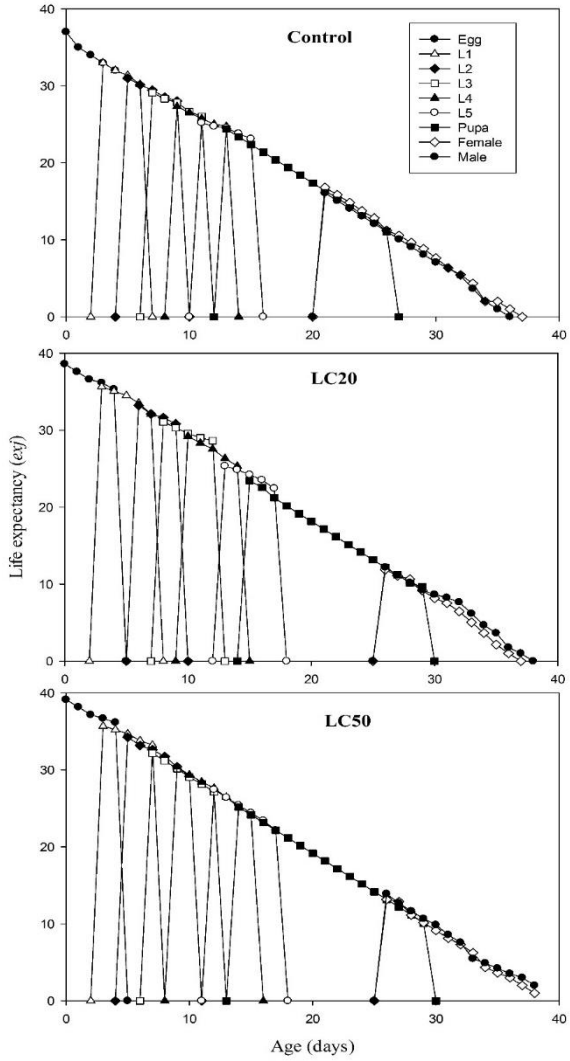

Fig. 3: Life expectancy (e_{xj}) of *E. vittella* exposed to sublethal concentration of novaluron

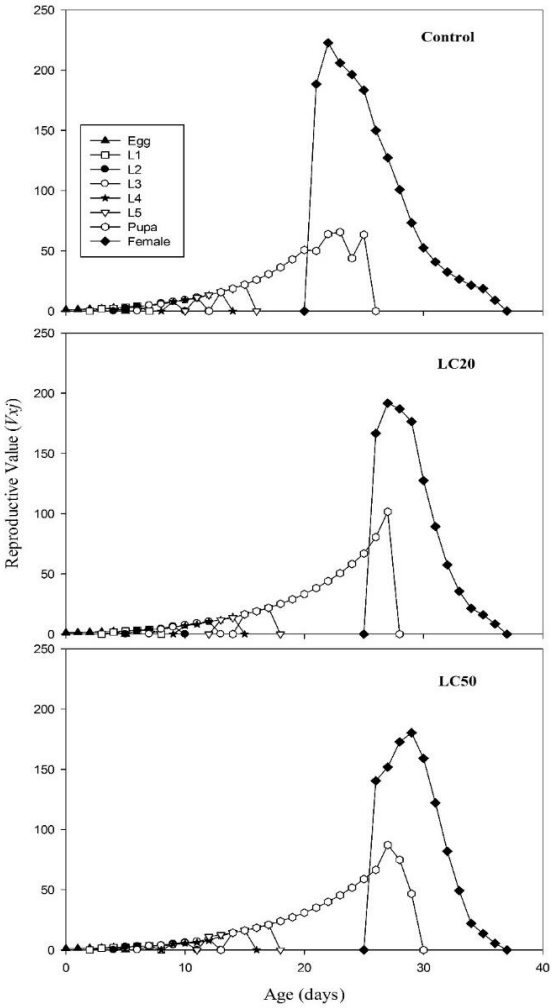

Fig. 4: Reproductive value (V_{xj}) of *E. vittella* exposed to sublethal concentration of novaluron

implementation of such doses in the fields for incorporation in integrated pest management strategies.

Author Contributions

DK, AG, BR and MT designed the research. DK, SK and SA conducted the experiments. BR and DK analyzed the data. DK, BR and IH wrote the manuscript. All the authors read and approved the manuscript.

Conflict of Interest

Authors declare that there is no conflict of interest

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