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# Sublethal effects of emamectin benzoate on life table parameters of the cotton leafworm, *Spodoptera littoralis* (Boisd.)

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## Abstract

**Background:** The cotton leafworm, *Spodoptera littoralis* (Boisd.), is a serious economic pest in Egypt. Pest control depends mainly on chemical control with several pesticides include conventional and modern insecticides. Comprehensive analysis of pesticides impacts needs to investigate sublethal effects in addition to lethal effect.

**Results:** In the current study, the leaf-dip bioassay method was used to evaluate emamectin benzoate (EMB) sublethal concentrations. Results showed that EMB proved high toxicity against *S. littoralis* with  $LC_{50}$  value of  $0.019 \text{ mg liter}^{-1}$ . Life table analysis showed that treatments with  $LC_5$  and  $LC_{15}$  prolonged larval period, mean longevity of males and females, mean generation time (T), doubling time (DT), adult preovipositional period (APOP), and total preovipositional period (TPOP) compared with control. On the contrary, net reproduction rates ( $R_0$ ), intrinsic rates of increase ( $r$ ), finite rate ( $\lambda$ ), fecundity, gross reproductive rate (GRR), and relative fitness were decreased compared to control.

**Conclusions:** The current study clarified that sublethal concentrations of EMB induce adverse effects and suppress the population growth of *S. littoralis*. Our results would be useful to assess the overall effects of EMB on *S. littoralis* and can contribute effectively in pest management.

**Keywords:** Cotton leafworm, Emamectin benzoate, Sublethal effects, Life table parameters

## Background

The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is a key destructive polyphagous insect pest (Hosny et al. 1986). This pest attacks several plant species belong to about 40 plant families (Pineda et al. 2007). Hosts overlapping contribute to high population densities of the pest and consequently economic losses throughout attacking various plant vegetative parts (Osman and Mahmoud 2009). So, several insecticides include emamectin benzoate were recommended to combat the pest (Egyptian Agricultural Pesticides Committee 2019).

Emamectin benzoate (EMB) is a member of the avermectins group, acts as gamma-aminobutyric acid

(GABA)-gated chloride channels agonist. EMB increases chloride ion flux in neuromuscular junction, causing irreversible paralysis and finally death (Ishaaya et al. 2002). EMB proved high efficiency against *S. littoralis* larvae (Mokbel et al. 2017, 2019). Assessment pesticide sublethal effects are necessary to assess the overall impact of this pesticide against the target pest (Stark et al. 1997). Sublethal effects draw upon insecticide class and pest species (Desneux et al. 2007). Sublethal amounts of pesticides can affect population growth parameters, feeding behavior, and insect bodies nutrient content (Rehan and Freed 2015; Xu et al. 2016). Barrania (2019) clarified that treating 2nd instar larvae of *S. littoralis* with  $LC_5$  and  $LC_{10}$  of emamectin benzoate affected larval weights, larval duration, pupation, pupal mean weight, and adult emergence rates.

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Life table parameters indicate population growth rates and can clarify the insecticide sublethal effects on insect population (Rahmani and Bandani 2013; Chi and Getz 1988). These parameters can be used to delay resistance development, combat pest resurgence, and use pesticides in optimal ways (Kareiva et al. 1996; Forbes and Calow 1999; Zanzuncio et al. 2005; Lashkari and Sahragard 2007; Rezaei et al. 2007; Desneux et al. 2007). Age-stage, two-sex life table analysis can overcome certain drawbacks in other life table methods analysis (Chi and Su 2006). Age-stage, two-sex life table procedure was used to investigate these parameters in several insect pest species including mirid bug, *Apolygus lucorum* (Meyer-Dür) (Zhen et al. 2018), peach fruit moth, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae) (Quan et al. 2016), and chive gnat, *Bradysia odoriphaga* (Zhang et al. 2014a, 2014b).

The present study aims to investigate sublethal effects, to project pest population growth, and to evaluate emamectin benzoate use against *S. littoralis*. These findings can contribute in developing effective pest management measures.

## Methods

### Insects and insecticides

Laboratory colony of *S. littoralis* was reared in Central Agricultural Pesticides Laboratory (CAPL), Agricultural Research Center, Dokki, Giza, Egypt. *Spodoptera littoralis* has been reared on fresh castor bean (*Ricinus communis*) leaves, maintained at a constant temperature of  $25 \pm 2$  °C, relative humidity (RH) of  $65\% \pm 10$ , and a photoperiod of 14 h:10 h L: D. Emamectin benzoate formulation used was Biolarve 5% EC (CHEMVET, China).

### Bioassays

The leaf dip bioassay method was used to determine concentrations of mortality response. A stock solution was prepared from insecticide formulation by using tap water. Five concentrations with five replicates in each concentration were used to obtain the toxicity line. Castor bean leaves were dipped into insecticide solution for 30 s and allowed to dry for a half-hour. Leaves dipped into tap water served as control. Each replicate includes ten 1st instar larvae in Petri dishes (5 cm) where the treated leaves were placed. Then, Petri dishes containing treatments were kept in the aforementioned rearing chamber until mortality was scored after 24 h. Larvae were considered dead if they failed to exhibit coordinate movement when probed with a soft camel hairbrush.

### Life table construction

Life table experiment involved three treatments (untreated control,  $LC_5$ , and  $LC_{15}$ ). From each treatment, one hundred 1st instar larvae of *S. littoralis* were taken,

and each larva was considered as one replicate. Larvae were kept in Petri dishes (5 cm) in the aforementioned rearing chamber. The larval stage was reared on fresh castor bean leaf disks which were replaced daily to avoid fungal growth. Larval mortality and duration were recorded individually. When larvae reach the prepupae, it weighted for a final time. Also, pupae weight, duration, adults' emergence, and female ratio were determined. After adults' emergence, a female was paired with a male to be a couple. Each family was kept in a glass jar with a vertical hanging tissue paper for egg laying. Eggs were collected daily by eliminating the tissue paper daily until female death. The fecundity was recorded as the average number of eggs per female.

### Data analysis

Larval mortality was corrected according to Abbott's formula (Abbott 1925) and then subjected to the probit analysis (Finney 1971) to determine EMB lethal concentrations ( $LC_{50}$ ,  $LC_{15}$ , and  $LC_5$ ) using EPA probit analysis version 1.5. Age-stage, two-sex life table analysis was used to derive biological aspects and population growth parameters. Mean values and standard errors were obtained throughout the bootstrap method included in the TWOSEX-MS chart program (Chi 2012). Significant differences between mean values of pre-pupae weight, pupae weight, and pupae period were analyzed throughout the ANOVA method using the SPSS program at ( $P < 0.05$ ) (Cor, IBM SPSS 2016). Survival rate ( $S_{xj}$ ), reproductive value ( $V_{xj}$ ), survival ( $l_x$ ), fecundity ( $m_x$ ), ( $f_{x4}$ ), and ( $l_x m_x$ ) curves were constructed using GraphPad Prism 7. The relative fitness of the treatments was calculated according to Shen et al. (2017) as follows: Relative fitness =  $R_0$  of the treatment/ $R_0$  of the control. Where  $R_0$  represents the net reproductive rate.

## Results

### Toxicity of emamectin benzoate on the 1st instars larvae of *S. littoralis*

Emamectin benzoate exhibited high toxicity against the 1st instar larvae of *S. littoralis* with  $LC_{50}$  value of 0.019 mg liter<sup>-1</sup>. In extension, concentrations that caused 5 and 15 percent mortality were 0.001 mg liter<sup>-1</sup> and 0.003 mg liter<sup>-1</sup>, respectively (Table 1).

### Sublethal effects of EMB on biological characteristics of *S. littoralis*

The effect of EMB sublethal concentrations on biological characteristics were investigated (Table 2). Sublethal treatments significantly increased larval period, pupae period, mean longevity (days), mean longevity of females (days), adult pre-ovipositional period (APOP), and total preovipositional period (TPOP). In contrast, it reduced significantly pupae period, ovi-days, and fecundity.

**Table 1** Toxicity of emamectin benzoate to the first larvae of *Spodoptera littoralis*

Insecticide	Concentration (mg (ai) liter <sup>-1</sup> ) (95% CL) <sup>b</sup>			Slope ± SE <sup>a</sup>	χ <sup>2c</sup>	df <sup>d</sup>
	LC <sub>5</sub>	LC <sub>15</sub>	LC <sub>50</sub>			
EMB	0.001 (0.00–0.002)	0.003 (0.001–0.005)	0.019 (0.013–0.027)	1.35 ± 0.19	5.75	3

<sup>a</sup>Standard error<sup>b</sup>95% confidence limits<sup>c</sup>Chi-square value (χ<sup>2</sup>)<sup>d</sup>df, degrees of freedom

Furthermore, pre-pupal and pupal weights were decreased significantly compared with controls. Sublethal treatments prolonged the developmental duration of larval stages from (15.71 days) in control to 16.15, and 16.26 days for LC<sub>5</sub> and LC<sub>15</sub>, respectively. Sublethal effects continued into the adult stage, adult preovipositional period (APOP) showed prolonged periods and recorded 1.77, 2.22, and 3 days with control, LC<sub>5</sub>, and LC<sub>15</sub>, respectively. Similarly, the total preovipositional period (TPOP) showed 29.66, 31.44, and 32.11 days with control, LC<sub>5</sub>, and LC<sub>15</sub>, respectively. On the other hand, EMB sublethal concentrations reduced the ovi-days period from 5.17 days for control to 4.67 days and 3.57 days for LC<sub>5</sub> and LC<sub>15</sub> treatments, respectively.

#### Effect of sublethal concentrations on population growth parameters

Sublethal concentrations of EMB showed pronounce effects on *S. littoralis* population life table parameters (Table 3). Intrinsic rate of increase ( $r$ ), net reproduction rate ( $R_0$ ), finite rate of increase ( $\lambda$ ), gross reproductive rate (GRR), mean generation time ( $T$ ), doubling time (DT), and fecundity were affected by EMB treatment. Intrinsic rate of increase ( $r$ ) decreased for both LC<sub>5</sub> and LC<sub>15</sub> (0.174, and 0.161 day<sup>-1</sup>, respectively), compared with the control (0.208 day<sup>-1</sup>). The finite rate of increase ( $\lambda$ ) showed a similar trend and recorded 1.230, 1.190,

and 1.175 day<sup>-1</sup> for control, LC<sub>5</sub>, and LC<sub>15</sub>, respectively. Gross reproductive rate (GRR) was varied significantly in treatments than control recording 1197.05, 514.13, and 451.34 for control, LC<sub>5</sub>, and LC<sub>15</sub>, respectively. Similarly, the net reproductive rate significantly decreased after emamectin benzoate treatment recording 959.11, 368.23, and 264.90 offspring/individual for control, LC<sub>5</sub>, and LC<sub>15</sub>, respectively. Similarly, the mean fecundities recorded 2038.12, 1043, and 965 per female in control, LC<sub>5</sub> and LC<sub>15</sub>, respectively. In contrast, the mean generation time and the doubling time recorded increased values with EMB treatments. The mean generation time ( $T$ ) of treated individuals was longer than control larvae (32.99, 33.83, and 34.57 days in control, LC<sub>5</sub> and LC<sub>15</sub>, respectively). The doubling time (DT) is the time period required to redouble a certain population. The obtained data showed that doubling time increased with increasing the used sublethal concentration recording 3.33, 3.97, and 4.30 for control, LC<sub>5</sub>, and LC<sub>15</sub> treatments, respectively.

Age-stage specific survival rate ( $S_{xj}$ ) of insects expresses the survival potential of newly hatched larvae to the age  $x$  and stage  $j$ . Stages overlap observed in Fig. 1 interpreted, as a result to individuals variations in developmental rates. Figure 1 declares that LC<sub>5</sub> and LC<sub>15</sub> treatments declined relative numbers of alive larvae compared with controls, while LC<sub>5</sub> and LC<sub>15</sub> treatments increased total development compared with control.

**Table 2** Effect of emamectin benzoate (EMB) sublethal concentrations on various biological characteristics of *Spodoptera littoralis*

Stages	Untreated		LC <sub>5</sub>		LC <sub>15</sub>		df <sub>1</sub>	df <sub>2</sub>	F	P
	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE				
Larvae period	86	15.41 ± 0.07 <sup>a</sup>	80	16.98 ± 0.08 <sup>b</sup>	70	16.86 ± 0.07 <sup>b</sup>	2	233	280.16	0.00
Pre-pupae weight	84	344.62 ± 6.43 <sup>a</sup>	76	305.13 ± 6.14 <sup>b</sup>	70	294.85 ± 6.28 <sup>b</sup>	2	227	915.89	0.00
Pupae weight	84	324.17 ± 6.46 <sup>a</sup>	74	273.08 ± 6.29 <sup>b</sup>	60	266.40 ± 4.15 <sup>b</sup>	2	215	4365.92	0.00
Pupae period	84	10.16 ± 0.13 <sup>a</sup>	74	9.24 ± 0.11 <sup>b</sup>	60	9.23 ± 0.09 <sup>b</sup>	2	215	43.42	0.00
Mean longevity of males (days)	36	37.79 ± 0.18 <sup>a</sup>	38	39.05 ± 0.36 <sup>b</sup>	32	38.88 ± 0.36 <sup>c</sup>	2	103	9.03	0.00
Mean longevity of females (days)	48	38.5 ± 0.25 <sup>a</sup>	36	40.11 ± 0.35 <sup>b</sup>	28	39.99 ± 0.33 <sup>b</sup>	2	109	20.70	0.00
(APOP)	48	1.67 ± 0.14 <sup>a</sup>	36	2.22 ± 0.15 <sup>b</sup>	28	3.07 ± 0.4 <sup>c</sup>	2	109	20.78	0.00
(TPOP)	48	30.04 ± 0.14 <sup>a</sup>	36	31.44 ± 0.26 <sup>b</sup>	28	32.21 ± 0.37 <sup>b</sup>	2	109	45.12	0.00
Ovi-days	48	5.17 ± 0.37 <sup>a</sup>	36	4.67 ± 0.28 <sup>a</sup>	28	3.57 ± 0.23 <sup>b</sup>	2	109	64.31	0.00

APOP adult preovipositional period, TPOP total preovipositional period (from egg to first oviposition)

Means in the same row followed by the same letter are not significantly different ( $P > 0.05$ )

df1, degree of freedom within groups

df2, degree of freedom within groups

**Table 3** The sublethal effects of emamectin benzoate on *S. littoralis* population parameters

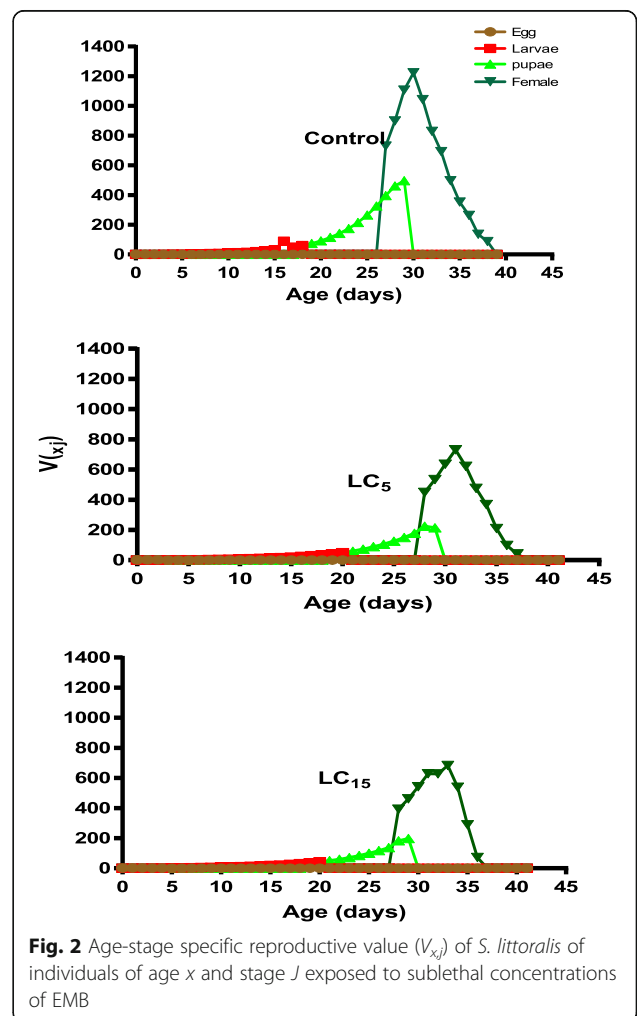
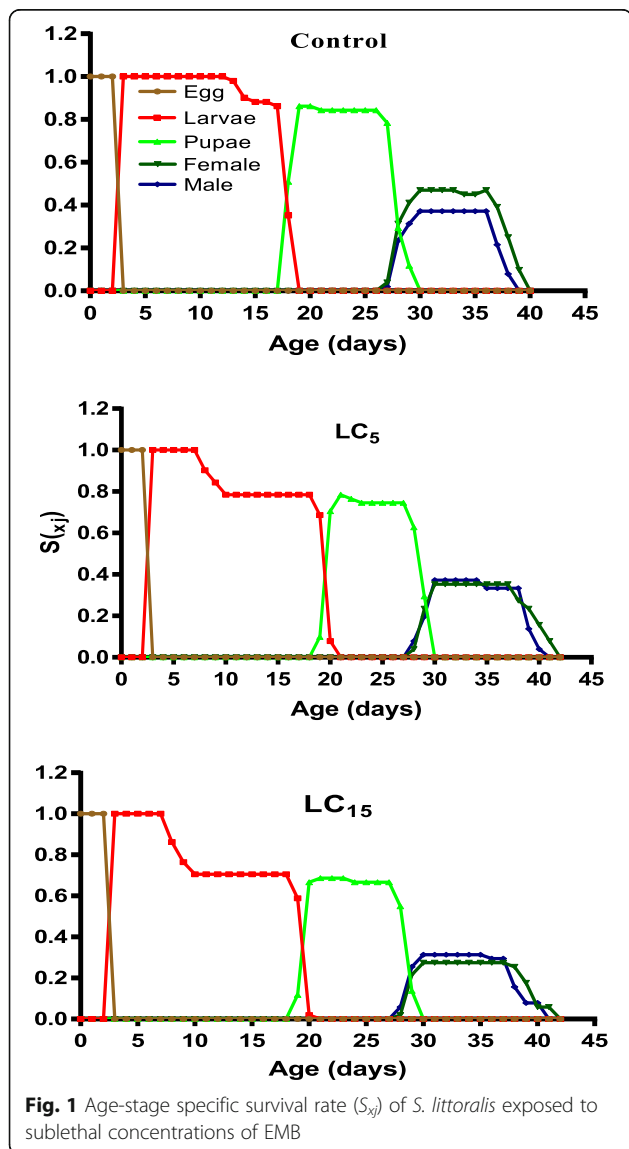
	Untreated	LC <sub>5</sub>	LC <sub>15</sub>
Net reproductive rate ( $R_0$ ) (offspring/individual)	958.74 ± 169.19 <sup>a</sup>	368.235 ± 73.300 <sup>b</sup>	264.90 ± 66.103 <sup>b</sup>
Intrinsic rate of increase ( $r$ ) ( $d^{-1}$ )	0.208 ± 0.006 <sup>a</sup>	0.174 ± 0.006 <sup>b</sup>	0.161 ± 0.007 <sup>b</sup>
Finite rate of increase ( $\lambda$ ) ( $d^{-1}$ )	1.231 ± 0.006 <sup>a</sup>	1.190 ± 0.007 <sup>b</sup>	1.175 ± 0.009 <sup>b</sup>
Mean generation time $T$ ( $d$ )	32.99 ± 0.178 <sup>a</sup>	33.83 ± 0.205 <sup>b</sup>	34.57 ± 0.216 <sup>c</sup>
The doubling time (DT)	3.33	3.97	4.3
Fecundity (eggs/female)	2038.12 ± 194.216 <sup>a</sup>	1043.33 ± 62.977 <sup>b</sup>	965 ± 103.09 <sup>b</sup>
Gross reproductive rate (GRR)	1197.05 ± 193.06 <sup>a</sup>	514.13 ± 91.52 <sup>b</sup>	451.34 ± 100.32 <sup>b</sup>
Relative fitness	1	0.38	0.28

Means in the same row followed by the same letter are not significantly different ( $P > 0.05$ )

Finite rate of increase ( $\lambda$ ) ( $d^{-1}$ ), the number of females per female per day

Gross reproductive rate (GRR), defines as total lifetime reproduction in the absence of mortality

Age-stage specific reproductive value ( $V_{x,j}$ ) indicates the status of future offspring from age  $x$  to stage  $j$  (Fig. 2). The males curve was not included in  $V_{x,j}$  graph because males contribution in future population was not defined.  $V_{x,j}$  values declined in LC<sub>5</sub> and LC<sub>15</sub> treated larvae compared with the control group.



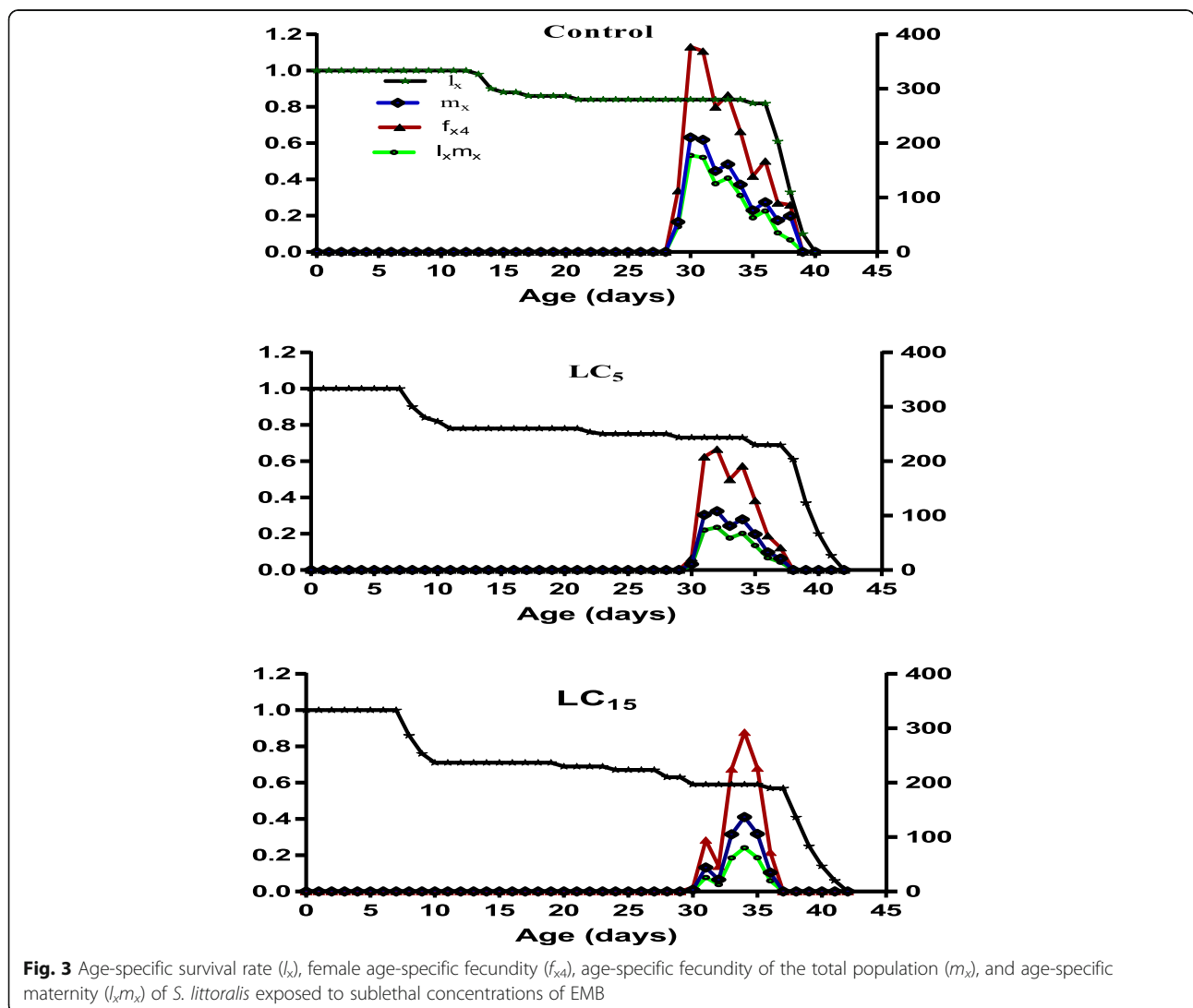
The age-specific survival rate ( $l_x$ ), female age-specific fecundity ( $f_{x4}$ ), age-specific fecundity ( $m_x$ ), and age-specific maternity ( $l_x m_x$ ) are presented in Fig. 3. Age-specific survival rate ( $l_x$ ) is the probability of a newly hatched larva to survive to age  $x$ .  $l_x$  simply expressed the age-stage survival rate ( $S_{xj}$ ). The  $l_x$  avoids the overlapping phenomenon observed in  $S_{xj}$  due to an individual's differences in developmental rates (Fig. 1). The curves of  $l_x$  declined significantly in treated larvae compared with control, and consequently sublethal concentrations of EMB proved a higher impact on larval mortality. Age-specific fecundity of the total population ( $m_x$ ) showed the highest peaks in the control group than the LC<sub>5</sub> and LC<sub>15</sub> groups.

**Discussion**

Sublethal effects defined as physiological, behavioral, and survival responses after exposure to a sublethal amount

of toxic compound (Tao and Wu 2006; Desneux et al. 2007). These effects resulted due to either attack the nervous system or disrupt hormonal balance (Moustafa et al. 2016). Exposure to sublethal concentrations results from either variable insecticides distribution or continuous degradation (Biondi et al. 2012; Tang et al. 2015).

The present work investigated the sublethal effects of EMB against *S. littoralis*. EMB showed high toxicity against *S. littoralis* consistent with other previous findings of Mokbel et al. (2017). The first larval instar of *S. littoralis* treated with LC<sub>5</sub> and LC<sub>15</sub> of EMB showed a significant increase in the larval period compared with control. Extended larval duration has interpreted as a result of either larval starvation or increasing consumed energy. As many pesticides reduce nutrition efficiency and increase larval consumption of energy in detoxification processes (Lai and Li 2011; Xu et al. 2016). The previous reasons can also interpret the reduction in pre-



**Fig. 3** Age-specific survival rate ( $l_x$ ), female age-specific fecundity ( $f_{x4}$ ), age-specific fecundity of the total population ( $m_x$ ), and age-specific maternity ( $l_x m_x$ ) of *S. littoralis* exposed to sublethal concentrations of EMB



pupae and pupae weight in treated larvae. The sublethal effects were investigated with several insects includes the cotton leafworms, *S. littoralis* (El-Naggar Jehan 2013; Metayi et al. 2015), the diamondback moth, *Plutella xylostella* L. (Plutellidae: Lepidoptera) (Guo et al. 2013), the cabbage aphid, *Brevicoryne brassicae* (Aphididae: Hemiptera) (Lashkari and Sahragard 2007), and whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) (He et al. 2013).

Life table studies have been used to assess the effects of different variables on insect population dynamics (Rahmani and Bandani 2013). Life table parameters such as the intrinsic rate of increase, net reproductive rate, and total preoviposition period can be used to determine population growth characteristics (Papachristos and Milonas 2008; Rahmani and Bandani 2013). The current study exhibited the ability of sublethal concentrations of EMB to decrease life table parameters such as intrinsic rates of increase ( $r$ ), finite rates of increase ( $\lambda$ ), net reproduction rate ( $R_0$ ), gross reproductive rate (GRR), survival rate, and reproductive value. In contrast, mean generation time ( $T$ ), doubling time (DT), adult preovipositional period (APOP), total preovipositional period (TPOP), and larval durations were prolonged by treatments. Consequently, sublethal concentrations of EMB inhibited *S. littoralis* population growth. Several reports confirmed the inhibitory effect of sublethal concentration of EMB on several insects include *S. littoralis* (El-Naggar Jehan 2013; Metayi et al. 2015). Other insects showed similar trends after treatment with sublethal concentrations of different insecticides including the cabbage moth, *Mamestra brassicae* L. (Lepidoptera: Noctuidae) (Moustafa et al. 2016), the house fly, *Musca domestica* L. following the treatment with Cantharidin (Yasoob et al. 2017) and the chive gnat, *Bradysia odoriphaga* after treatment with thiamethoxam (Zhang et al. 2014b), the seven-spot ladybeetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) (Jiang et al. 2019). Similar findings observed with sulfoxaflor on the mirid bug, *Apolygus lucorum* (Meyer-Dür) (Heteroptera: Miridae) (Zhen et al. 2018).

Survival rate describes survivorship and stage transitions of insects, varies in different conditions and within different insect species. Results indicated that the number of alive larvae was reduced by the  $LC_5$  and further reduction followed the  $LC_{15}$  treatment. Furthermore, the Age-stage specific survival rate ( $s_{xj}$ ) was decreased with EMB sublethal concentrations. These results are in line with that obtained with (Zhang et al. 2014b) for the sublethal effects of thiamethoxam on *Bradysia odoriphaga* (Yang and Zhang). Also, fecundity parameters which include age-specific fecundity ( $f_{x4}$ ), age-specific maternity ( $l_x m_x$ ), and reproduction value ( $V_{xj}$ ) were decreased in treatments compared with control. These results indicate the potential of sublethal concentrations of EMB to

decrease the biological productivity of *S. littoralis*. Low fecundity of the treated groups may interpret a result of either ovaries immaturities (Perveen 2000) or inhibiting the production of nymphs (Devine et al. 1996).

Laboratory experiment of sublethal effect provides a good vision to insect population dynamic and can be used to develop a pest control strategy (Chi and Su 2006). The current study explains the role of sublethal concentrations in suppressing insect population growth. Furthermore, integrate such concentrations in pest control programs to decrease pesticide selection pressure, pesticide amount, control costs, and environmental pollution (Zhang et al. 2014). The present study showed that EMB sublethal concentrations affecting various biological aspects and life table parameters, and consequently, decreased population growth. So, the current results fortified the role of such concentrations in pest management.

## Conclusions

The study proved the pronounced effects of EMB sublethal concentrations on biological aspects and population growth of the cotton leafworm population. These concentrations strongly suppressed *S. littoralis*. These findings can contribute in planning pest management programs, concepting the future use of the pesticide, and using of these sublethal concentrations in pest management.

## Abbreviations

EMB: Emamectin benzoate; T: Mean generation time; DT: Doubling time; APOP: Adult preovipositional period; TPOP: Total preovipositional period;  $R_0$ : Net reproduction rates;  $r$ : Intrinsic rates of increase;  $\lambda$ : Finite rate; GRR: Gross reproductive rate;  $S_{xj}$ : Age-stage specific survival rate;  $V_{xj}$ : Age-stage specific reproductive value;  $l_x$ : The age-specific survival rate;  $m_x$ : Age-specific fecundity;  $f_{x4}$ : Female age-specific fecundity;  $l_x m_x$ : Age-specific maternity

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## Authors' contributions

Dr. Amal contributed in the study design and experimental work. Dr. Sayed was a major contributor in designing the study, conducting the experimental part, and analyzing the obtained data. All authors read and approved the final manuscript.

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## Availability of data and materials

All data generated during this study are included in this published article.

## Ethics approval and consent to participate

The manuscript does not contain any studies involving human participants, human data, or human tissue.

## Consent for publication

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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