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Multigenerational Insecticide Hormesis Enhances Fitness Traits in a Key Egg Parasitoid, *Trichogramma chilonis* Ishii

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Abstract: Hormesis for the intractable pests can be dreadful, but for natural enemies of pests, it is a puissant strategy in optimizing their mass rearing. We report multigenerational stimulatory effects of widely used insecticide, imidacloprid, on the demographic traits of an important egg parasitoid *Trichogramma chilonis* Ishii. The study investigated the consequences of sublethal (LC_5), low lethal (LC_{30}), and median lethal (LC_{50}) concentrations, as well as a control, for five continuous generations (F_1 to F_5). The initial bioassay experiments revealed imidacloprid exhibiting the highest toxicity for the parasitoid with a LC_{50} of 2 $\mu g \cdot L^{-1}$, whereas LC_5 and LC_{30} were 0.07 $\mu g \cdot L^{-1}$ and 0.6 $\mu g \cdot L^{-1}$, respectively. Among biological traits, compared to the F_1 individuals, a substantial increase in the fecundity of *T. chilonis* was observed in the F_5 individuals by 54.92% and 46.81% when exposed to LC_5 and LC_{30}, respectively ($p < 0.00001$). Further, there was a significant enhancement in the adult longevity as well as oviposition days of the F_5 individuals at both these concentrations. Considering the population traits, along with gross reproductive rate (*GRR*), net reproductive rate (R_0) was also enhanced by both LC_5 and LC_{30} in F_5 individuals than F_1; whereas the intrinsic rate of increase (r) and finite rate of increase (λ) were enhanced only at LC_{30} upon comparing with control. On the other hand, LC_{50} exposure to *T. chilonis* did not result in notable differences in biological or population traits when compared across generations (F_1 and F_5). Low and sublethal concentrations of imidacloprid did not have a major influence on demographic traits of *T. chilonis* at initial generations of exposure but can induce hormetic effects in the subsequent generations. Overall, imidacloprid-induced hormesis stimulating the development of *T. chilonis* might be helpful under circumstances of mild exposure of imidacloprid in fields and could be leveraged for its mass rearing.

Keywords: biological control; demographic parameters; hormesis; imidacloprid; sublethal effects

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1. Introduction

Crops are generally prone to stress throughout the growth period due to the incursion from various pests such as insects, nematodes, diseases, and weeds [1]. Even though there is an appeal for integrated pest management (IPM) systems, chemical management remains a priority [2]. However, the consequences of non-selective pesticides may have ecological fallout such as ruination of predators and parasitoids, pest resurgence and resistance, as well as secondary pest outbreak [3–6]. There is a proclivity for the natural enemies to be at the risk of pesticides as compared to their herbivore host [7]. Although

pesticides are applied at adequate concentrations against the targeted insects, certain spatio-temporal conversions due to the abiotic and biotic factors steer alteration of the targeted concentrations [8]. Thus, besides the lethal consequences, pesticides can have sublethal outcomes on the arthropods exposed [5,9–13]. These sublethal concentrations often have an impact on the demographic and biological traits, thus affecting the growth of the organisms [14–21] and the phenomenon is categorized as 'hormesis' (meaning stimulation due to low concentration and inhibition due to high concentration) [22–24]. The primary focus remains on pesticide effects on pest species, but attention is currently being paid to biocontrol agents of pests that are potentially relevant for agroecosystem services [24,25].

Trichogramma chilonis Ishii is an idiobiont and oligophagous egg parasitoid species known to have a good pest suppression potential [26–30]. As *Trichogramma* is used extensively against key Lepidopteran pests [31–33], there is a need to minimize the repercussion of pesticides on them, demanding the study of the potential effects of commonly used pesticides [34]. Imidacloprid, a widely used neonicotinoid, has been commercially applied in the early nineties [35,36] due to the fact that it possesses systemic insecticidal activity [37,38]. It severely affects the non-targeted insects [5,39–43]. Apart from its lethal effects, sublethal effects are also reported in various insects. For instance, sublethal (LC_5) and low lethal (LC_{15}) concentrations of imidacloprid induced a stimulatory effect on the fecundity and adult longevity of the progeny generations of the *Aphis gossypii* Glover along with a substantial increase in the intrinsic and finite rate of increase [12].

Studies on pesticide-induced hormesis are crucial for insect pest management, however a new arena of recent attention is sublethal (hormetic) stress to optimize mass production and to enhance the quality of bio-agents. Moreover, due to the value of bio-agents in IPM, sublethal effect studies have garnered more importance. Despite the advances in insecticide toxicology, the information on low and sublethal concentrations of imidacloprid on the demographic traits is not well understood in important parasitoids such as *T. chilonis*, a parasitoid of global distribution. Furthermore, studies stipulating the stimulatory effects of other insecticides on the parasitoids are restricted to a single generation. Hence, for the current study, the multigenerational stimulatory effects of imidacloprid on the demographic traits of *T. chilonis* were investigated for five continuous generations (F_1 to F_5). The outcomes of the study would be helpful in providing comprehensive knowledge regarding the possible enhancement of the mass rearing efficiency of *T. chilonis*.

2. Materials and Methods

2.1. Study Environment

The present research was conducted in the Biocontrol Laboratory of Crop Protection Division, National Rice Research Institute, Cuttack (20°27′14.0″ N 85°56′06.0″ E) under standard conditions (temperature 25 ± 1 °C; relative humidity 70 ± 5%; and 14 h light: 10 h dark).

2.2. Insect Colonies

Initial colonies of the factitious host, *Corcyra cephalonica* (Stainton) and parasitoid, *T. chilonis* unexposed to pesticides were obtained from the mass-rearing facility of National Rice Research Institute, Cuttack, Odisha, India. Before utilizing test insects in subsequent experiments, they were reared over 10 generations to nullify any previous effects.

2.2.1. Rearing of the Factitious Host, *Corcyra cephalonica*

The host insect (*C. cephalonica*) was reared according to the procedure of Gowda et al. [44]. For mass rearing of *C. cephalonica* 0.25 cc (approx. 5000 eggs) of eggs were charged on a medium which consists of sterilized and insecticide free broken maize kernels (2.5 kg), roasted groundnut powder (50 g), yeast (2 g), multivitamin powder (5 g), streptomycin sulphate (0.2 g), and formalin (0.1%, 10 mL). After the moth emergence, they were collected in a moth collection unit (50 L) having an inner oviposition chamber (10 L) fitted with a vacuum suction system (motor power of 120 W). The base of the unit was covered with

wire mesh (15 mesh size) for egg deposition. On the inner wall of the oviposition chamber, a cotton swab soaked in honey–water solution (1:1 ratio) was affixed. The eggs laid were collected from the bottom of the oviposition cage and sieved 3–4 times to obtain cleaner eggs for further use.

2.2.2. Rearing of the Egg Parasitoid, *Trichogramma chilonis*

Trichogramma chilonis was reared according to the method of Gowda et al. [45]. *Corcyra cephalonica* eggs were sterilized in a UV chamber with the help of UV-C lamp (HNS 15W G13; Osram Puritec, Russia) for 45 min to prevent the embryonic development and subsequent cannibalism by hatched larvae on unhatched parasitized eggs. One cc (18,000–20,000) eggs were then glued on paper cards (15 × 10 cm). The eggs were then exposed in a 40:1 ratio to female parasitoid wasps until adult mortality. The parasitoid population was maintained in glass tubes (Borosil®; height × diameter, 19.0 cm × 3.6 cm) plugged with muslin fabric to allow air movement and were kept in insect growth chambers (model: JSPC-420C; JS Research Inc., Gongju, Republic of Korea).

2.3. Insecticide

Technical grade imidacloprid with 98% purity (Sigma Aldrich, St. Louis, MI, USA) was used for the experiment. The required concentrations for contact toxicity studies were obtained by diluting the insecticide with analytical-grade acetone. Only acetone served as a control for each experiment. The insecticide solution used was fresh for all the bioassay experiments to circumvent insecticide decomposition.

2.4. Toxicity Assay

Concentration–mortality bioassay was carried out to evaluate the toxicity of imidacloprid to *T. chilonis*. The dry film deposition assay was performed by exposing *T. chilonis* adults to insecticide residues on glass tubes [3,46]. The concentration range inducing (10–90) percent adult mortality was determined in preliminary experiments. Concentration–mortality association was developed by subjecting *T. chilonis* adults to six different concentrations, i.e., 0.01, 0.1, 1, 10, 100, and 1000 $\mu g \cdot L^{-1}$ of imidacloprid. Insecticides solutions were made and introduced to the inner walls of glass tubes (Borosil®; height × diameter, 19.0 cm × 3.6 cm). In order to ensure homogeneous deposition, one ml solution of insecticide was applied, completely covering the inner surface of the tube. The tubes were rotated until there were no visible droplets on the glass tube's wall. Before introducing the *T. chilonis* adults, the tubes were kept at room temperature for about 2 h to ensure evaporation. Each tube contained a streak of honey and adult wasps of equal age (<24 h old) (n = 300). Tubes were plugged with a muslin fabric affixed with rubber to allow air movement and were kept in an insect growth chamber (model: JSPC-420C; JS Research Inc., Gongju, Republic of Korea) set at 25 °C, 70% RH, 14L:10D. For each concentration of insecticide, three replications (corresponding to three tubes) were used. After 8 h of exposure, adult parasitoids were shifted to another tube free from insecticide and a streak of honey. After 24 h, bioassay results were recorded by counting the number of dead parasitoids and the percentage mortality was calculated. Wasps that did not move when probed were assumed to be dead.

2.5. Multigenerational Sublethal Effects of Imidacloprid on the Demographic Traits of T. chilonis

Sublethal (LC_5), low lethal (LC_{30}), and median lethal (LC_{50}) concentrations of imidacloprid including control (acetone) were applied to investigate the effects on the demographic traits of *T. chilonis* adults for five continuous generations (F_1 to F_5). The demographic traits of *T. chilonis* were assessed by the method suggested by Del Pino et al. [47] under standard laboratory conditions (temperature 25 ± 1 °C; relative humidity 70 ± 5%; and 14 h light: 10 h dark)

2.5.1. Egg to Adult Developmental Time

The development time of *T. chilonis* was carried out at sublethal (LC_5), low lethal (LC_{30}), and median lethal (LC_{50}) concentrations and control (acetone). The prepared insecticide solutions were applied in the glass tubes as described in Section 2.4. After 8 h of exposure, approximately 20 surviving pairs (males and females) were selected from the treated tubes and were exposed to 200 fresh UV-sterilized eggs of *C. cephalonica* for 24 h. All parasitoids were taken out after 24 h. Sixty parasitized eggs (<24 h old) were placed singly in glass vials (Borosil®; height × diameter, 7.5 × 0.8 cm) using a wet fine brush (Camlin No. 0). Each egg isolated individually corresponded to a replicate. The parasitized eggs were monitored daily till adult emergence and their sex was recorded. As the parasitoid completes its larval development inside the egg of its host, the immature stages, namely egg, larva, and pupa, were considered as pre-adult stages. The multigenerational sublethal effects were recorded for five continuous generations (F_1 to F_5) with 20 pairs of surviving genitors (n = 40) in each generation. Each generation was carried forward by genitors treated with designated concentrations of imidacloprid and offered with UV-sterilized *C. cephalonica* eggs.

2.5.2. Adult Fecundity and Longevity

The fecundity and longevity of *T. chilonis* adults were performed at similar environmental conditions mentioned in Section 2.4. Sixty pairs of wasps (<24 h old) acquired from the studies of development (refer Section 2.5.1) were isolated in separate plastic vials (height × diameter; 5.3 × 3.3 cm) and plugged with cotton and a strip of honey solution. Sentinel cards (length × width; 3.5 × 3 cm) consisting of 100 fresh *C. cephalonica* eggs were provided daily to adult parasitoids ad libitum. The cards (parasitized) were taken out daily and were incubated until the emergence of offspring. The longevity and fecundity of the adult, along with emergence rate and sex ratio of the offspring, were recorded [47].

2.6. Statistical Analysis

Mortality data were used for probit analysis by operating PoloPlus version 2.0 (LeOra Software Inc., Berkeley, CA, USA) software and the sublethal (LC_5), low lethal (LC_{30}), median lethal (LC_{50}), and LC_{90} concentrations were calculated with associated 95% fiducial limits [5,12,48]. The raw data on *T. chilonis* life history were evaluated with the help of age-stage, two-sex life table theory [49,50]. The parameters involving finite rate of increase (λ), intrinsic rate of increase (r), gross reproductive rate (GRR), net reproductive rate (R_0) and mean generation time (T), age-stage specific survival rates (s_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), oviposition days, adult pre-oviposition period (APOP), total pre-oviposition period (TPOP), age-specific maternity ($l_x m_x$), age-stage specific life expectancy (e_{xj}), and age-stage reproductive value (v_{xj}), were computed with the aid of TWOSEX-MSChart computer program [18,51]. The mean and standard errors of the traits were computed using 100,000 bootstrap re-samples [52]. Difference of the parameters between the imidacloprid treated and untreated (acetone) groups and between generations within each treatment group were analyzed with the help of the paired bootstrap test.

3. Results

3.1. Toxicity Assay of Imidacloprid on T. chilonis Adults

Concentration–mortality assay of imidacloprid on *T. chilonis* adults showed a linear regression, thereby validating the fitting of the observed data and thus providing a LC_{50} (2 $\mu g \cdot L^{-1}$) and LC_{90} (20 $\mu g \cdot L^{-1}$) with a 95% fiducial limit of 0.9–3 $\mu g \cdot L^{-1}$ and 9–60 $\mu g \cdot L^{-1}$, respectively (Table 1). Furthermore, the sublethal concentration (LC_5) (0.07 $\mu g \cdot L^{-1}$) and low lethal concentration (LC_{30}) (0.6 $\mu g \cdot L^{-1}$) were estimated with a fiducial limit of 0.01–0.2 $\mu g \cdot L^{-1}$ and 0.3–1 $\mu g \cdot L^{-1}$, respectively (Table 1). The LC_5, LC_{30}, and LC_{50} along with the control (acetone) were thus chosen to assess the population and biological traits of imidacloprid on *T. chilonis* adults.

Table 1. Toxicity of imidacloprid on *T. chilonis* adults determined by using dry film deposition assay.

Insecticide	*n*	Slope ± SE	LC_5 µg·L^{-1} (95% CL)	LC_{30} µg·L^{-1} (95% CL)	LC_{50} µg·L^{-1} (95% CL)	LC_{90} µg·L^{1} (95% CL)	χ^2 (df)
Imidacloprid	300	1.189 ± 0.055	0.07 (0.01–0.2)	0.6 (0.3–1)	2 (0.9–3)	20 (9–60)	12.196 (3)

SE—standard error; LC—lethal concentration; CL—confidence limits; and df—degree of freedom.

3.2. Multigenerational Sublethal Effects of Imidacloprid on the Biological Traits of T. chilonis

Multigenerational effects of imidacloprid on the biological traits of F_1 and F_5 generation of *T. chilonis* are provided in Table 2. Results revealed that LC_5 had no notable differences in the developmental time, fecundity, and longevity of the F_1 generation of *T. chilonis*, but a significant effect was noticed on the adult longevity, total longevity, oviposition days, and fecundity of the F_5 generation of *T. chilonis* in comparison to the control. It was reported that the duration of adult longevity ($p < 0.00001$), total longevity ($p = 0.03659$), and reproductive days ($p = 0.00034$) of F_5 individuals were significantly enhanced by the exposure of LC_5 with stimulation in the fecundity from 169.26 ± 4.95 to 241.51 ± 5.39 offspring/individual ($p < 0.00001$) (Table 2). However, no such significant differences were recorded for the immature period, pre-adult duration, and total pre-oviposition period (TPOP) (Table 2). Furthermore, the sublethal concentration LC_5 resulted in the increased fecundity from 155.89 ± 9.24 to 241.51 ± 5.39 offspring/individuals) ($p < 0.00001$) during F_5 than that of the F_1. Additionally, there was a prolongment in the adult duration ($p = 0.00007$) and total longevity ($p = 0.01368$) as well as for the oviposition days ($p = 0.00027$) in the F_5 compared to F_1 generation (Table 2). Concerning $LC_{30,}$ a significant enhancement was noticed in the adult longevity, fecundity, and oviposition days in F_5 generations as compared to control. However, non-significant differences in the pre-adult duration and TPOP of both generations were noticed in comparison to control. When compared between the generations, a significant enhancement in the total longevity by 9.2% ($p = 0.03375$) and fecundity by 46.8% ($p < 0.00001$) was observed in *T. chilonis* individuals of the F_5 generation (Table 2). Similarly, a substantial increase in the duration of adult longevity and oviposition days were reported in the F_5 individuals than that of the F_1 individuals of the imidacloprid LC_{30} treatment ($p < 0.00001$) (Table 2). LC_{50} exposure to *T. chilonis* did not result in any notable differences in biological parameters except for the immature period ($p = 0.03175$) and TPOP ($p = 0.01994$) upon comparing with control during F_5 generation (Table 2).

Table 2. Multigenerational effects of imidacloprid on the biological parameters of F_1 and F_5 generations of *T. chilonis*.

Biological Parameters	Generations	Mean ± SE			
		Control	LC_5	LC_{30}	LC_{50}
Immature	F_1	6.03 ± 7.55 ^{aA}	5.89 ± 0.06 ^{aA}	5.91 ± 0.09 ^{aA}	6.12 ± 6.71 ^{aA}
	F_5	6.07 ± 7.05 ^{aA}	5.89 ± 0.06 ^{aA}	5.84 ± 0.09 ^{aB}	6.28 ± 7.01 ^{aA}
Pre-adult (days)	F_1	0.93 ± 0.03 ^{aA}	0.93 ± 0.03 ^{aA}	0.93 ± 0.03 ^{aA}	0.93 ± 3.22 ^{aA}
	F_5	0.93 ± 0.03 ^{aA}	0.93 ± 0.03 ^{aA}	0.93 ± 0.03 ^{aA}	0.93 ± 3.20 ^{aA}
Adult longevity (days)	F_1	4.76 ± 0.17 ^{aA}	5.11 ± 0.19 ^{bA}	5.30 ± 0.12 ^{bA}	4.96 ± 0.15 ^{aA}
	F_5	5.12 ± 0.15 ^{aB}	6.03 ± 0.12 ^{aA}	6.18 ± 0.12 ^{aA}	5.05 ± 0.12 ^{aA}
Total longevity (days)	F_1	10.48 ±0.23 ^{aA}	10.65 ± 0.26 ^{bA}	10.87 ± 0.22 ^{bA}	10.77 ± 0.22 ^{aA}
	F_5	10.82 ± 0.23 ^{aB}	11.52 ± 0.23 ^{aA}	11.58 ± 0.25 ^{aA}	11.03 ± 0.19 ^{aA}
TPOP (days)	F_1	6.02 ± 0.08 ^{aA}	5.95 ± 0.08 ^{aA}	5.95 ± 0.11 ^{aA}	6.28 ± 0.08 ^{aA}
	F_5	5.99 ± 0.08 ^{aA}	5.95 ± 0.08 ^{aA}	5.95 ± 0.11 ^{aA}	6.28 ± 0.08 ^{aA}
Oviposition days	F_1	4.85 ± 0.21 ^{aA}	5.18± 0.22 ^{bA}	5.39 ± 0.14 ^{bA}	5.10 ± 0.16 ^{aA}
	F_5	5.28 ± 0.18 ^{aB}	6.15 ±0.14 ^{aA}	6.26 ± 0.13 ^{aA}	5.13 ± 0.15 ^{aA}
Fecundity (offspring/individual)	F_1	156.64 ± 5.23 ^{aA}	155.89 ± 9.24 ^{bA}	191.43 ± 5.67 ^{bA}	154.95 ± 8.31 ^{aA}
	F_5	169.26 ± 4.95 ^{aB}	241.51 ±5.39 ^{aA}	281.05 ± 8.49 ^{aA}	163.43 ± 6.65 ^{aA}

Mean and Standard Errors (SE) were determined with the help of bootstrap technique with 100,000 re-samples. Different lower-case letters denote significant differences between F_1 and F_5 generations within each treatment group, whereas upper case letters denote significant differences between control and imidacloprid treatments in each generation ($p < 0.05$, paired bootstrap test).

3.3. Multigenerational Sublethal Effects of Imidacloprid on the Population Traits of T. chilonis

Multigenerational effects of imidacloprid on the population traits of F_1 and F_5 generation of *T. chilonis* were given in Table 3. Compared to control, sublethal (LC_5) treated individuals of *T. chilonis* had no noteworthy differences in the population traits for the initial (F_1) generation. Concerning later (F_5) generation, a significant difference was observed for the net ($p = 0.01182$) and gross ($p = 0.00226$) reproductive rate, whereas non-significant results were noticed for the intrinsic rate of increase (r), finite rate of increase (λ), and mean generation time (T) (Table 3). On the contrary, the low lethal (LC_{30}) treatment showed an increasing trend in the population parameters such as intrinsic rate of increase (r) ($p = 0.04648$), finite rate of increase (λ) ($p = 0.04638$), net reproductive rate (R_0) ($p = 0.00053$), and gross reproductive rate (GRR) ($p = 0.00002$) as compared to control during F_5 generation (Table 3). Compared across the generations, there was an increase in the net reproductive rate (R_0) by 46.8% ($p = 0.00748$) and gross reproductive rate (GRR) by 74.7% ($p = 0.00036$) during F_5 than F_1 generation of LC_{30} treated adults (Table 3). There was no such significant difference in the LC_{50} treated individuals and control in both the generations except for mean generation time (T) ($p = 0.02127$) (Table 3).

Table 3. Multigenerational effects of Imidacloprid on the population parameters of F_1 and F_5 generations of *T. chilonis* adults.

Population Parameters	Generations	Mean ± SE			
		Control	LC_5	LC_{30}	LC_{50}
r (d^{-1})	F_1	0.59 ± 0.01 ^{aA}	0.59 ± 0.01 ^{aA}	0.61 ± 0.02 ^{aA}	0.57 ± 0.01 ^{aA}
	F_5	0.59 ±0.01 ^{aA}	0.62 ± 0.01 ^{aA}	0.63 ± 0.02 ^{aA}	0.56 ± 0.01 ^{aA}
λ (d^{-1})	F_1	1.79 ± 0.03 ^{aA}	1.79 ± 0.03 ^{aA}	1.84 ± 0.03 ^{aA}	1.77 ±0.03 ^{aA}
	F_5	1.80± 0.03 ^{aA}	1.86 ± 0.03 ^{aA}	1.88 ± 0.03 ^{aA}	1.76 ± 0.02 ^{aA}
R_0(offspring/individual)	F_1	101.82 ± 10.21 ^{aA}	101.33 ± 11.32 ^{bA}	124.43 ± 12.36 ^{bA}	100.72 ± 10.94 ^{aA}
	F_5	110.02 ± 10.93 ^{aB}	156.98 ± 15.32 ^{aA}	182.68 ± 18.23 ^{aA}	106.23 ± 10.93 ^{aA}
T (days)	F_1	7.89 ± 0.1 ^{aA}	7.88± 0.11 ^{aA}	7.88 ± 0.12 ^{aA}	8.05 ±0.10 ^{aA}
	F_5	7.96 ± 0.09 ^{aA}	8.15± 0.09 ^{aA}	8.21 ± 0.13 ^{aA}	8.27 ± 0.09 ^{aA}
GRR (offspring/individual)	F_1	125.7 ± 11.07 ^{aA}	130.91 ± 12.83 ^{bA}	155.03 ± 13.70 ^{bA}	127.5 ±11.89 ^{aA}
	F_5	133.94 ± 11.71 ^{aB}	194.4 ± 15.91 ^{aA}	270.91 ± 26.75 ^{aA}	129.59 ±11.99 ^{aA}

r is the intrinsic rate of increase; λ is the finite rate of increase; R_0 is the net reproductive rate; and T is mean generation time and *GRR* is gross reproductive rate. Means and Standard Errors (SE) were determined with the help of bootstrap technique with 100,000 re-samples. The lower-case letters denote significant differences between F_1 and F_5 generations within each treatment group, whereas the upper-case letters denote significant differences between control and imidacloprid treatments in each generation ($p < 0.05$, paired bootstrap test).

3.4. Multigenerational Sublethal Effects of Imidacloprid on the Age–Stage Specific Survival Rate, Fecundity, and Life Expectancy of T. chilonis

The age–stage specific survivorship curve (s_{xj}) depicts the probability that a newborn is expected to remain alive from age x to stage j (Figure 1). It is clear that due to the difference in the developmental durations of the individuals there was overlap between the different stages (Figure 1). The age–specific survival rate (l_x), age–specific fecundity of total population (m_x), and age–specific maternity ($l_x m_x$) of *T. chilonis* of imidacloprid in F_1 and F_5 generations were mentioned in Figure 2. The l_x shows an outline of the survival rate without the reckoning differentiation of the stage. The l_x curves significantly showed a declining trend for both the F_1 and F_5 generations of LC_5 and LC_{30} at 14 or 15 days as compared to the control and LC_{50} where the trend ended at 13 days (Figure 2). The age–specific fecundity (m_x) and age–specific maternity ($l_x m_x$) were significantly higher in the LC_5 and LC_{30} than in the control (Figure 2). The age–stage–specific life expectancy (e_{xj}) denotes the duration of an individual surviving to age x and stage j (Figure 3). The curve denotes that exposure of the adults to the LC_5 and LC_{30} tends towards individuals to surviving longer as compared to the control and LC_{50} (Figure 3). Age–stage–reproductive value (v_{xj}) typifies the diligence of the population from age x to stage j with regard to the future progenies (Figure 4). A higher trend in the reproductive value was observed for the LC_5 and LC_{30} in comparison to control and LC_{50} of imidacloprid (Figure 4).

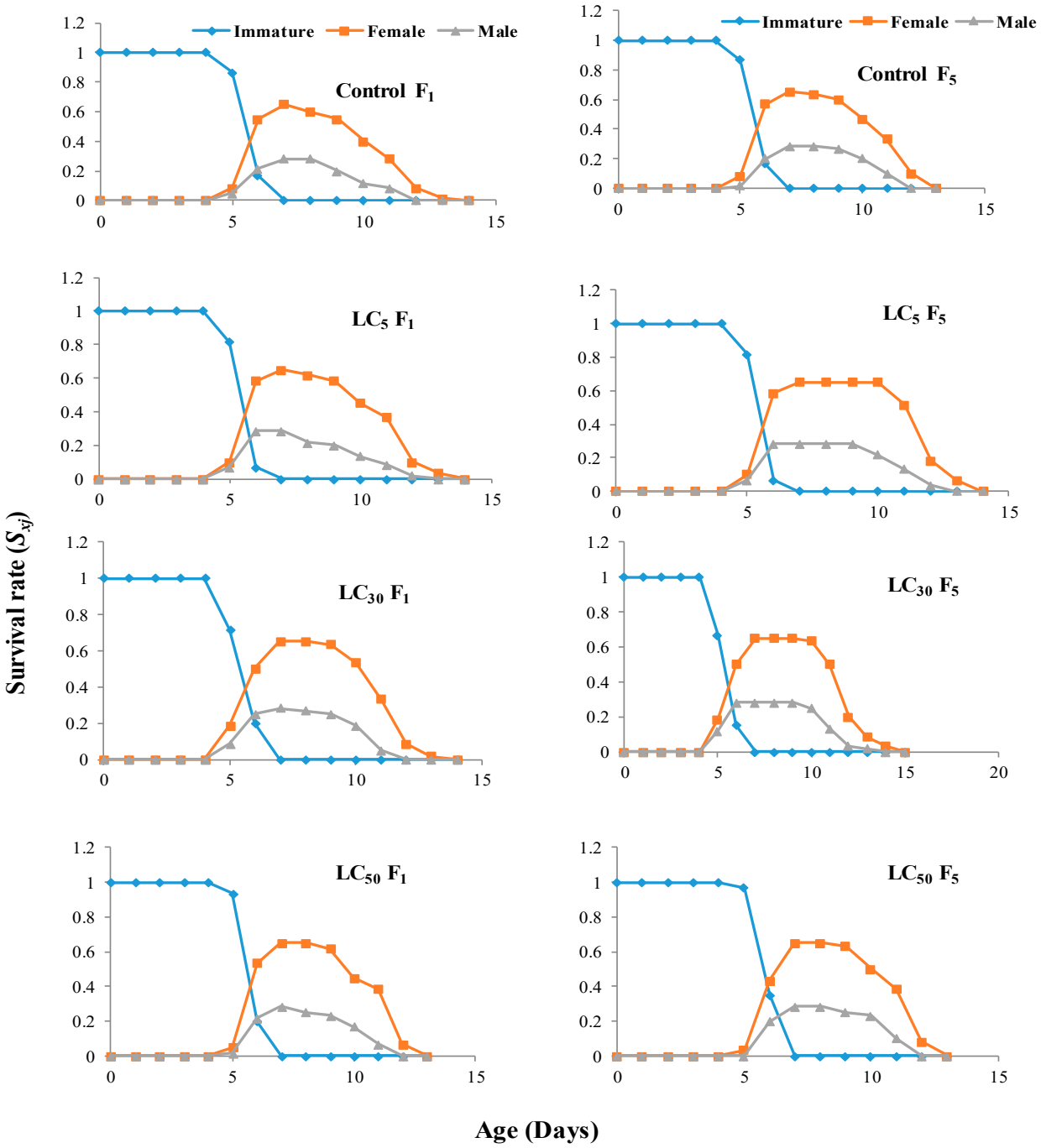

Figure 1. Age–stage–specific survival rates (S_{xj}) of *T. chilonis* adults treated with acetone and LC_5, LC_{30}, and LC_{50} concentrations of imidacloprid in F_1 and F_5 generations.

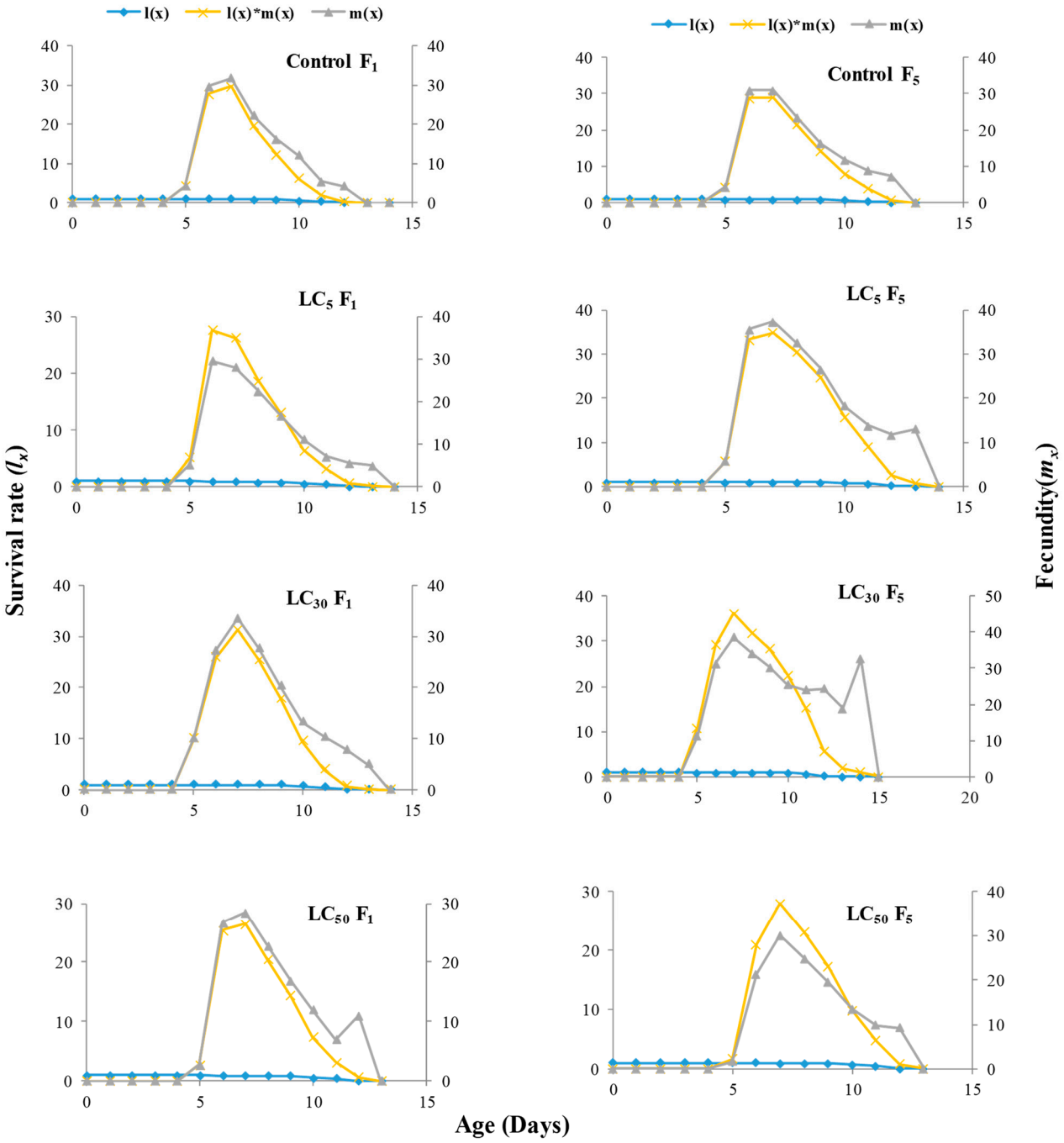

Figure 2. Age–specific survival rate (l_x), age–specific fecundity of total population (m_x), and age–specific maternity ($l_x m_x$) of *T. chilonis* adults of imidacloprid in F_1 and F_5 generations.

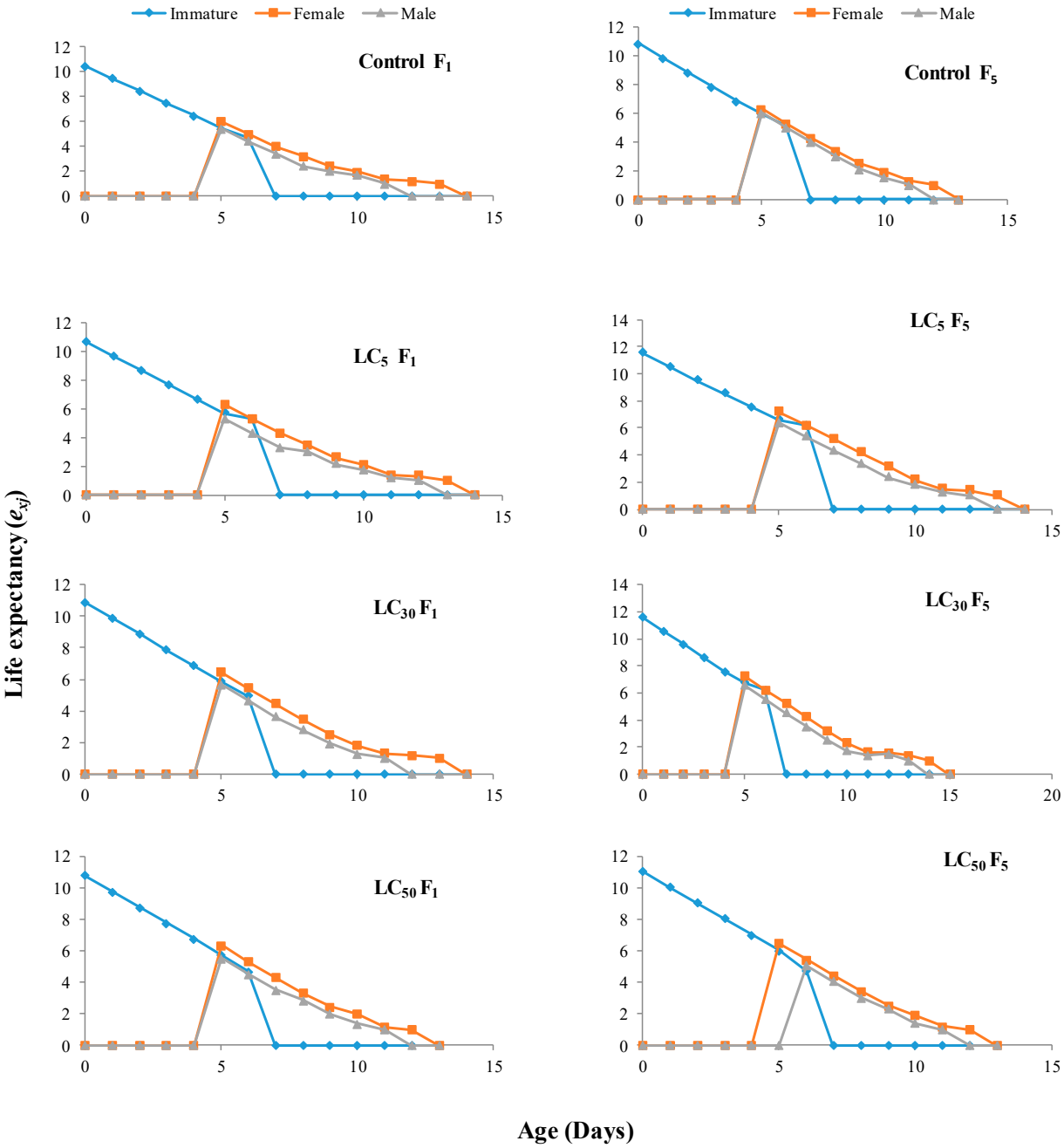

Figure 3. Age–stage–specific life expectancy (e_{xj}) of *T. chilonis* adults of imidacloprid in F_1 and F_5 generations.

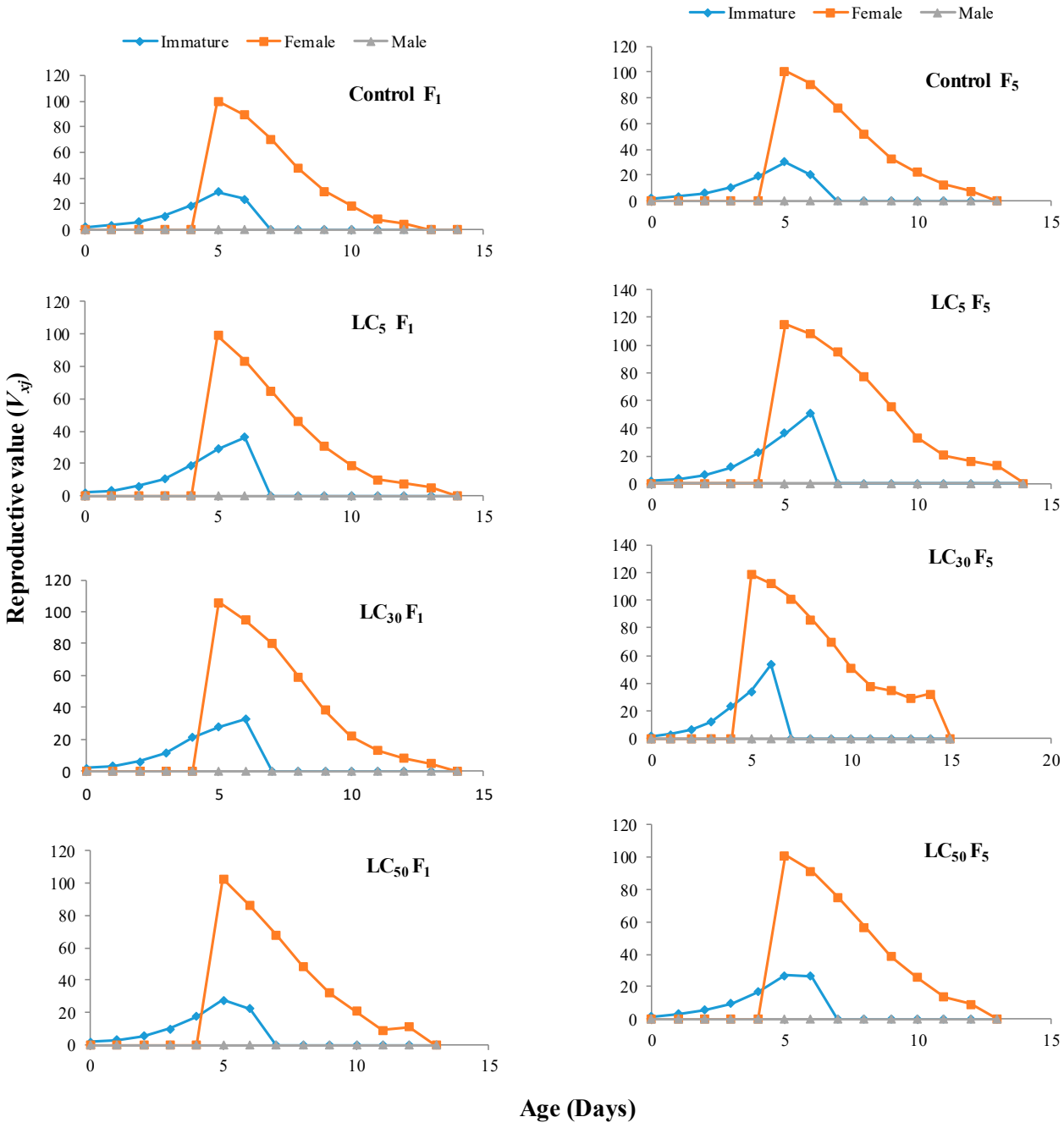

Figure 4. Age–stage–reproductive value (v_{xj}) of *T. chilonis* adults treated with acetone and LC_5, LC_{30}, and LC_{50} concentrations of imidacloprid in F_1 and F_5 generations.

4. Discussion

With an increasing interest of hormesis research in human health, there is also an urge to explore hormesis principles for practical purposes such as improved natural enemy mass production, maintenance, and performance [24]. The plausibility of the mild stress to stimulate natural enemies has garnered recent attention. It is quite evident that natural enemies are also being subjected to low concentration of chemical stress due to pesticide drift or degradation, suggesting that even mild stress could be helpful in boosting the survival instincts of natural enemies along with their behavior or reproduction and may be reducing pest populations [24]. Hence, an attempt in a similar direction has been made in the current study. The present investigation demonstrated that imidacloprid can induce multigenerational hormesis in *T. chilonis*. The results depict the high toxicity of

imidacloprid on *T. chilonis* having LC_{50} value of 2 µg L^{-1} which is in accordance with the previous findings having an LC_{50} value of 2.7 µg L^{-1} [53]. Accompanying the lethal effects, insecticides also evince various sublethal effects owing to the decomposition of the insecticides due to various factors such as temperature, rainfall, radiation, microbes, etc. [5,10,13]. For example, due to high temperature, volatilization of pesticides takes place, reducing the amount of pesticides. The toxicity of an applied insecticide is also affected by microbial or chemical breakdown in or on soil and foliage which varies with moisture, temperature, adsorption, and pH [22]. These outcomes may tend to be positive or negative on the exposed arthropods [54] thereby affecting the fecundity, longevity, and development of the individuals which are critical deciding factors in the pest management programs [14,55,56]. There are many findings with regard to the hormesis effects in various pests such as *Myzus percsicae* Sulzer [57–59], *Aphis gossypii* [12,60], and *Helicoverpa armigera* Hubner [20], but the understanding on the hormesis effects of insecticides on predators and parasitoids is limited [25] and restricted to a single generation. Additionally, in order to improve the productivity and quality during mass rearing, the concept of hormesis could be helpful to predators and parasitoids for augmentative biological control [24]. Moreover, as *Trichogramma* is an extensively used bio-agent against various lepidopteran pests [26,28,30], the knowledge regarding the potential of hormesis on its mass rearing and quality becomes mandatory. Therefore, in the present study, we evaluated the multigenerational hormesis effect of imidacloprid on *T. chilonis*. Our results revealed sublethal concentration (LC_5) of 0.07 µg·L^{-1} and low lethal concentration (LC_{30}) of 0.6 µg·L^{-1} on *T. chilonis* adults.

Sublethal effects have a significant effect on the biological parameters such as longevity, fecundity, oviposition days, etc. For example, the fecundity, longevity, and oviposition days were stimulated in the predatory bug *Podisus distinctus* Stal due to the low doses of permethrin [61], the longevity of the *Podisus nigrispinus* Dallas is greatly increased owing to the low doses of gamma-cyhalothrin [62]. The longevity of the bio-agents, especially parasitoids, greatly relies on the insecticide type, species of bioagent, and application techniques [63]. Reduced longevity mainly occurs in those parasitoids where insecticides have been treated during the developing stages in the host [14,64]. However, certain findings where the effect of sublethal concentrations of insecticides increased longevity of *T. chilonis* (study had single generation observation) was also reported by [46]. In our study, a significant increase in the longevity of the individuals in the F_5 generations of both the sublethal (LC_5) and low lethal (LC_{30}) concentrations of imidacloprid was observed, whereas no such noteworthy observation was depicted for the median lethal concentrations (LC_{50}). Our findings depicted a higher fecundity in the LC_5 and LC_{30} treated individuals. Similar such results were reported by [46], where LC_{30} exposure of chlorflurazuron and tebufenozide increased the female fecundity. However, the fecundity of *T. chilonis* reduced significantly when exposed to the LC_{30} of spinosad, avermectins, fipronil, cartap, and β-cypermethrin, as reported by [46]. However, some findings of sublethal effects of spinosad and abamectin on the biological traits of the other species of *Trichogramma* led to a reduction in fecundity and longevity of the *Trichogramma pretiosum* Riley [65]. In another study on *T. pretiosum* [66], thiodicarb and chlorfenapyr treated individuals saw decreases in adult emergence. Both these studies on *T. pretiosum* owed to the presence of residues of insecticides on host egg chorion. Suh et al. [64] also recorded reduced longevity and emergence of *Trichogramma exiguum* Pinto and Platner owing to the treatment of spinosad. This was explained by the fact that spinosad was unable to penetrate the host eggs (*H. armigera*). Our findings also revealed an increasing trend in the oviposition days of the LC_5 and LC_{30} treated individuals. However, no influence on the oviposition days on the *Trichogramma evanescens* Westwood was reported upon LC_{30} exposure of spirotetramat and flupyradifurone [67].

Our findings with regard to the sublethal (LC_5) effect of imidacloprid on the parasitoid depicted a higher net reproductive rate (R_0) and gross reproductive rate (GRR) without any noteworthy differences in the intrinsic rate of increase (r), finite rate of increase (λ), and mean generation time (T). In contrast, LC_{30} conveyed a significant increase in the intrinsic

rate of increase (r) and finite rate of increase (λ) as well as in the net reproductive rate (R_0) and gross reproductive rate (GRR). Interestingly, the LC_{30} exposure of spirotetramat, flupyradifurone, and deltamethrin on *T. evanescens* significantly lower the intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), and gross reproductive rate (GRR) [67]. The population traits were also reduced when *Trichogramma brassicae* Bezdenko were exposed to the LC_{25} of indoxacarb, spinosad, chlorantraniliprole, and abamectin [30]. Similar results were also reported by [68].

5. Conclusions

Our results demonstrated how effective the sublethal (LC_5) and low lethal (LC_{30}) concentrations of imidacloprid induced hormetic effect over generations, which can be considered important as stimulatory effects of the insecticides are limited to a single generation. Additionally, it has mostly been carried out for the herbivorous insects with a little knowledge about the bioagents. Furthermore, *T. chilonis* is a commonly utilized bioagent against several lepidopteran pests, and in the interest of ecological balance and environmental safety it is high time we use improved bioagents having better fitness characteristics for pest suppression. Hence, such inducement of hormesis in the *T. chilonis* is a great opportunity to stimulate the development of the parasitoid during mild exposure of imidacloprid in the field and as well as in its mass rearing.

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