Research Article

SUBLETHAL EFFECTS OF FLONICAMID ON LIFE TABLE OF Myzus persicae (Sulzer)

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ABSTRACT

The green peach aphid, Myzus persicae (Sulzer) (Aphididae: Hemiptera) is a cosmopolitan polyphagous pest of more than five hundred plant species belonging to at least forty different plant families including several economically important agricultural crops. The sublethal effect of LC25 concentration of flonicamid on *M. persicae* was assessed by using an age-stage two-sex life table approach for integrated management of this pest. A leaf-dip bioassay showed that flonicamid was very toxic to the adults of *M. persicae* at 48 h with the calculated LC₂₅ concentration value of 0.0115 g a.i./L. The adults (parental generation) of M. persicae exposed to the LC₂₅ concentration (sublethal dose) of flonicamid showed inhibitory effects on the life history traits across its generations such as reduction in adult longevity and fecundity of parental generation (F_0); shortening the durations of fifth instar nymphs, adult female longevity, oviposition period, total longevity and fecundity of F_1 & F_2 generations. On the other hand, stimulatory effects were observed on the duration of first, second, third, and fourth instar nymphs; and pre-adults in F_2 generation in the flonicamid-treated aphids. Consistently with the effects on individual traits, a higher mean generation time (T) and shorter intrinsic rate of increase (r), finite rate of increase (λ), and net reproductive rate (R₀) of F₂ generation were observed in the flonicamid treated aphids, although r, λ , R₀, T, and gross reproductive rate (GRR) of F_1 generation were not significantly affected. The findings revealed the exposure of parental adults of M. persicae to sublethal concentration of flonicamid could induce hormetic (both inhibitory and stimulatory) effects on their succeeding generations. This study is useful for assessing the overall effects of flonicamid on M. persicae. The hormetic effects should be considered when it used flonicamid in the integrated management of M. persicae in crop fields.

Key words : Flonicamid, hormetic, life table, Myzus persicae, sublethal.

INTRODUCTION

The use of chemical insecticides becomes a frequent means to manage aphids in crops. Frequent use of chemical insecticides to control the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) has resulted in the development of resistance to various classes of insecticides including organophosphates, carbamates, pyrethroids, and neonicotinoids (Bass *et al.*, 2014; Tang *et al.*, 2017).

The indiscriminate and repeated use of insecticides results in multifaceted effects such as immediate death of insects, development of resistance and cross-resistance in insects (Herron *et al.*, 2001; Koo *et al.*, 2014; Cui *et al.*, 2016; Chen *et al.*, 2017b) or bring changes in physiology and behavior of an insect (Rehan and Freed, 2015). Insecticidal effects on insects can generally be divided into direct lethal effects that cause mortality, and sublethal effect that causes no apparent mortality (Rehan and Freed, 2015). However, exposure of an insect to a sublethal dose of an insecticide induces biological and behavioral changes in surviving insects through changes in multiple biological traits of insects such as survivorship, longevity, and fecundity (Boina *et al.*, 2009), feeding and oviposition (Tan *et al.*, 2012; He *et al.*, 2013), and the genetic constitution of successive generations (Lee, 2000; Desneux *et al.*, 2007). Consequently, stage-specific survival rate, reproduction rate, and population parameters are also impacted (Liang *et al.*, 2018). Thus, it is essential to identify sub-lethal effects on demographic traits of arthropod pests for giving a more accurate assessment of insecticide efficiency to optimize the application (Stark and Banks, 2003). For a comprehensive understanding of the sublethal effects of an insecticide on an insect, it is necessary to consider its effect on the survival rate and fecundity throughout the whole life span.

M. persicae is a cosmopolitan polyphagous pest of more than five hundred plant species belonging to at least forty different plant families including several economically important agricultural crops (van Emden and Harrington, 2007) and greenhouse crops (Sanchez *et al.*, 2010; Mehta, 2012). The pest is characterized by its rapid development due to its high reproductive capacity. It causes direct damage to its host plants by sucking sap leading to stunting or death. Moreover, it serves as an efficient vector to transfer more than 100 plant viruses in different hosts, particularly in solanaceous vegetables (Cloyd and Sadof, 1998; Ellis *et al.*, 1998; Blackman and Eastop, 1984; van Emden *et al.*, 1969; Hughes, 1963; Blackman and Eastop, 2000).

Flonicamid, a pyridine organic compound, has been categorized as a neonicotinoid though the mode of action is different from that of other neonicotinoids (Chen *et al.*, 2014). So it has been recommended against a wide range of homopterous insect pests including *M. persicae*. However, the potential sublethal (inhibitory or stimulatory) effects of flonicamid on *M. persicae* are still feebly assessed. This paper presents the assessment of sublethal effects of the LC_{25} concentration of flonicamid on *M. persicae* using the age-stage two-sex life table approach for integrated management of this pest.

MATERIALS AND METHODS

Cabbage (*Brassica oleracea* L. var. *capitata*) is an important commercial vegetable grown in Nepal for a long time, and is one of the most preferred hosts of *M. persicae*. Thus, this crop was selected as a host plant for *M. persicae* in this study. Cabbage (Green coronet variety) seedlings were grown in Vegetable Development Centre, Khumaltar, Lalitpur under a net house to obtain a regular supply of insect free cabbage seedlings. Such seedlings were grown in a 24-well plastic tray and in a coco pit-compost nursery mixed in a 3:1 ratio in batches at 15 to 20 days intervals to maintain tender leaves for insect rearing.

The colony of *M. persicae* was established inside a nylon cage in the premises of the Central Agricultural Laboratory, Pulchowk, Lalitpur with apterous adults collected from non-pesticide contaminated cabbage fields and was periodically supplemented with nymphs collected from the same fields to maintain its genetic heterogeneity. Before starting the experiment, parent insect stock was reared for 2 to 3 generations in the laboratory in an incubator maintaining 25 ± 1 °C temperature,

 $65 \pm 5\%$ relative humidity (RH), and with a photoperiod of 16: 8 (L: D) h. The experiment was set up in a two-chambered seed germinator available in the seed laboratory under Central Agriculture Laboratory maintaining temperature, humidity, and light as per the objective. All the experiments were performed in controlled conditions keeping the constant relative humidity (RH %) at 65 ± 5 , temperature at 25 ± 1 °C, and photoperiod at 16:8 hours light and dark. Flonicamid (Ulala) 50 % WG, a commercial formulation of flonicamid was used in all experiments. Serial dilutions were made using distilled water, and the desired concentration of the solution was used immediately after preparation to minimize any chemical decomposition.

Lethal toxicity bioassays (Experiment 1) were carried out by preparing five different concentrations of flonicamid 50% WG by diluting in distilled water i.e. 0.05, 0.025, 0.0125, 0.00625, and 0.003125 g a.i/L. Cabbage leaves were dipped in each insecticide solution for 30 seconds separately and were air dried by placing them upside down for 1 h. Leaves were then transferred into insect-rearing cups (0.5-liter capacity with a diameter of 10 cm base, 11.5 cm top, and 6.5 cm height). Six-day-old 10 individuals of *M. persicae* were placed in each rearing cup with the aid of a fine camel hairbrush. The insect-rearing cups were maintained in a growth chamber at 25 ± 1 °C and $65 \pm 5\%$ RH. Four replicates were conducted for each concentration. These bioassays were repeated four times in each concentration at different dates. Insect mortality was assessed after 48 h of continuous exposure to insecticide, and the insects that cannot move or only one leg vibrates slightly (after probing gently with a soft hairbrush) were recorded as dead insects (methodology used in Liang *et al.*, 2018). Based on lethal toxicity bioassays data, LC₂₅ value was calculated by using total mortality data from up to 96 h of experimentation with the help of the linear regression method after making corrections of data wherever necessary by using the following correction factor,

Corrected mortality % of treatment = $\frac{\text{(Mortality\% in treatment - Mortality\% in control)}}{(100 - Mortality\% in control)} \times 100$

To assess the sublethal effects of flonicamid on F_0 generation Experiment 2 was set after completion of the lethal toxicity bioassay. Six days old apterous aphids were treated with LC₂₅ concentration of flonicamid. After 48 h, the survived aphids were transferred individually to untreated fresh leaves in the insect-rearing cup. Adults were observed daily for recording survival and numbers of newborn nymphs which were then removed. The total number of adults used in this study were 100 in each flonicamid exposed and unexposed (control) treatment. The leaf was replaced every alternate day to maintain leaf freshness until all adult aphids in the cohort died.

To assess the sublethal effects of flonicamid on F_1 generation Experiment 3 was started one week after the setup of Experiment 2. In this experiment, the newborn nymphs that were produced within 24 h by F_0 adults were collected as F_1 generation and transferred to the rearing cup independently. In both LC₂₅ flonicamid and control treatments, the F_1 generation aphids were reared on fresh untreated leaves. The total number of newborn nymphs used in this study was 65 individuals used in both flonicamid and control treatments. Daily records of the survival and development of the insect were kept. During the reproductive period of the F_1 generation, newly born aphids were counted and then removed. Fresh untreated leaf was replaced every alternate day.

To assess the sublethal effects of flonicamid on F_2 generation, Experiment 4 was started two weeks after the setup of Experiment 3. Here, the newborn nymphs that were produced within 24 h by F_1 adults were collected as F_2 generation and transferred to the rearing cup independently. In both LC₂₅ flonicamid and control treatments, the F_2 generation aphids were also reared on fresh untreated leaves

as done in experiment 3. The total numbers of newborn nymphs used in this study were 60 individuals in each flonicamid and control treatment. Daily records of insect survival and development were conducted. During reproductive period of F_2 generation, newly born aphids were counted and then removed. Fresh leaf was replaced every alternate day as above.

Raw data were analyzed based on the theory of the age-stage, two-sex life table (Chi and Liu, 1985; Chi, 1988). The mean of the development periods for each development stage, longevity for all individuals and adult females, and fecundity of M. persicae were calculated. The total prereproduction period (TPRP) was calculated by including the pre-adult age in the total. The age-stage specific survival rate (s_{xi}) (where x is the age and j is the stage), the age-stage specific fecundity (f_{xi}) , the age-specific survival rate (lx), the age-specific fecundity (mx) and age-stage reproductive value (vxi) were calculated from the daily records of the survival and fecundity of all individuals in the cohort. In addition, the study estimated population parameters, including the intrinsic rate of increase (r), finite rate of increase (λ) , net reproductive rate (R_0) , and mean generation time (T) (Huang and Chi, 2012). The life expectancy (exj) was calculated according to Chi and Su (2006), while the reproductive value (vxj) was calculated according to Tuan et al. (2014). These all parameters were calculated using the computer program TWOSEX-MSChart (Chi, 2018; Chi and Liu, 1985). The variances and standard errors of the population parameters were estimated using the bootstrap procedure with 100,000 random resampling and the difference of population parameters between control and insecticide treatment groups and between generations within each treatment group were compared by using the paired bootstrap test based on the confidence intervals of differences implemented in TWOSEX-MS Chart (Huang and Chi, 2013 & Chi, 2018). All graphs were created using Sigma Plot 12.0 (Systat Software Inc., San Jose, CA, USA).

RESULTS AND DISCUSSION

Toxicity of Flonicamid on *M. persicae* Adults and Calculation of LC₂₅ Concentration of Flonicamid to *M. persicae*

The toxicity of flonicamid to six-day-old *M. persicae* was investigated after 48 h insect exposure to insecticide. The mortality rates of *M. persicae* adults were determined after exposure of insects to different concentrations of flonicamid. No insect mortality occurred 24 h after treatment with flonicamid. However, insect mortality increased with increasing concentration of flonicamid after 48 h. The average insect mortality of the control group in all bioassays was 0.25%. A total of 960 6-day-old aphids were used in the bioassay. The different concentrations of flonicamid used to treat the *M. persicae* adults, and the total insect mortality after 48 to 96 h were used to calculate the LC₂₅ concentration of flonicamid. The calculated LC₂₅ concentration of flonicamid was 0.0115 g a.i./L. The LC₂₅ value was used as the sub-lethal concentration for insect life table studies. The high toxicity of flonicamide has been reported for several sucking pests, including *M. persicae*, *Aphis gossypii*, *Bemisia tabaci*, *Frankliniella occidentalis*, and *Nilaparvata lugens* (Shi *et al.*, 2022).

Sublethal Effects of Flonicamid on F0 Generation of M. persicae

Short-term exposure of adult *M. persicae* to LC_{25} concentration of flonicamid for 48 h had a significant effect on the longevity and fecundity of the exposed individuals (F₀ generation) (Fig. 1). As compared to the control group, the adult longevity of the F₀ generation was significantly reduced from 18.9 d to 13.6 d by flonicamid treatment. The fecundity of F₀ adults was also significantly reduced from 33.01 to 14.95 offspring/female after exposure to LC_{25} dose of flonicamid.

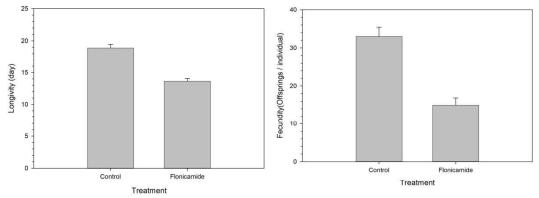


Fig. 1. The longevity and fecundity of initial parental adults (F₀ generation) of *Myzus persicae* treated with LC₂₅ concentration of flonicamid for 48 h.

Effects of Flonicamid on F1 and F2 Generations of M. persicae

The development time, longevity, pre-reproductive period, fecundity and total pre-adult survival rate of the succeeding progenies (F_1 and F_2) were evaluated (Table 1). The duration of the fifth instar nymph (P = 0.0000), the duration of adult female (P = 0.00089), oviposition period (P = 0.0026) and fecundity (P = 0.0000) were significantly decreased in F_2 generation in comparison to F_1 generation within treatment group despite a significant increase observed in the duration of first instar nymph (P = 0.0027), second instar nymph (P = 0.0000), third instar nymph (P = 0.0489), and preadult stage (P = 0.0000). In F₂ generation, the duration of the fifth instar nymph (P = 0.0000), total longevity (P = 0.0000), the duration of adult female (P = 0.0000), oviposition period (P = 0.0000) and fecundity (P = 0.0000) were significantly decreased by flonicamid treatment in comparison to F₂ control despite significant increase found in the duration of second instar nymph (P = 0.0085), third instar nymph (P = 0.0032), fourth instar nymph (P = 0.0081) and preadult stage (P = 0.0000). These results showed the sublethal effects of flonicamide on M. persicae which usually happened with most insect pests after exposure to sublethal concentration of insecticides (Desneux et al., 2007; Tang et al., 2019). There is a reduction in fecundity and longevity of *M. persicae* across generations once the parent aphid is exposed to flonicamide which may decrease the rate of population growth, unlike exposure to sublethal concentration of imidacloprid and cyantraniliprole that would lead to a hormetic (stimulatory) response of M. persicae (Zeng et.al., 2016).

Effect of Flonicamid on Population Parameters of M. persicae

The effects of flonicamid (LC₂₅) on the population parameters of F₁ and F₂ generations of *M. persicae* were evaluated with a bootstrap technique based on the insect life tables (Table 1). Within the treatment group, the mean generation time (*T*) of *M. persicae* was significantly increased from F₁ to F₂ generation (P = 0.0000), and the intrinsic rate of increase (*r*) (P = 0.0000), finite rate of increase (λ) (P = 0.0000), the net reproductive rate (R_0) (P = 0.0000) and gross reproductive rate (*GRR*) (P = 0.0000) were significantly decreased. Moreover, the *r*, λ , R_0 and *GRR* were also significantly decreased in the treatment group in comparison to the control group of F₂ generation. However, there were no significant differences in the *r*, λ , R_0 and GRR in the treatment group in comparison to the control of F₁ generation.

Statistics	Stage or sex	Generation	Control		Treated	
			Ν	Mean ± SE ^{a, b}	Ν	Mean ± SE ^{a, b}
Pre-adult duration (d)	N1	F_1	59	$1.32\pm0.07aB$	59	$1.51\pm0.07aB$
		F2	57	$1.72\pm0.08 \text{aA}$	57	$1.88\pm0.1\text{aA}$
	N2	F1	56	$1.61\pm0.07 aA$	53	$1.49\pm0.07aB$
		F2	57	$1.68 \pm 0.06 b A$	53	$1.98\pm0.1\text{aA}$
	N3	F1	52	$1.58\pm0.07 aA$	50	$1.7\pm0.09aB$
		F2	57	$1.6\pm0.08 bA$	52	$1.94 \pm 0.09 \mathrm{aA}$
	N4	F_1	49	$1.84 \pm 0.09 a A$	49	$1.67\pm0.07 aA$
		F2	56	$1.57\pm0.07bB$	43	$1.72 \pm 0.08 \mathrm{aA}$
	N5	F_1	9	$1.22\pm0.15 \text{aA}$	7	1.14 ± 0.14 aA
		F2	7	$1.14\pm0.14aA$	8	$1.12 \pm 0.13 \text{bB}$
	Adult /	F1	49	$16.88\pm0.57aA$	49	$15.59\pm0.54 aA$
	female	F2	56	$18.3\pm0.88 aA$	43	$11.98 \pm 1.3 \text{bB}$
	Preadult	F_1	49	$6.59\pm0.12aA$	49	$6.61\pm0.09aB$
		F2	56	$6.7\pm0.11\text{bA}$	43	$7.7 \pm 0.12 aA$
	Total	F_1	63	$19.11 \pm 1.13 aB$	61	$18.59 \pm 1.04 \mathrm{aA}$
	longevity	F2	57	$24.68\pm0.93 aA$	57	16.7 ± 1.22 bA
Oviposition period (d)	Female	F1	49	$13.49\pm0.51 aA$	49	$11.82 \pm 0.5 bA$
		F ₂	56	$14.32\pm0.73aA$	40	$8.32 \pm 1.03 \text{bB}$
Fecundity (offspring/female)	Female	F1	49	$41.61 \pm 2.23 aA$	49	$38.59 \pm 2.24 aA$
		F ₂	56	$37.38 \pm 2.25 a A$	40	$20.05\pm2.8bB$
APRP (d)	Female	F1	49	$1.29\pm0.08 aA$	49	$1.08\pm0.09 aA$
		F2	56	$1.27\pm0.08 \text{aA}$	40	$1.32 \pm 0.13 \mathrm{aA}$
TPRP (d)	Female	F1	49	$7.88 \pm 0.14 b A$	49	$7.69\pm0.15 aB$
		F2	56	$7.96 \pm 0.14 a A$	40	$8.95\pm0.2aA$
The intrinsic rate of increase, r		F_1	63	$0.2688 \pm 0.01 a A \\$	61	$0.2755\pm0.01 aA$
		F ₂	57	$0.259138 \pm 0.01 aA \\$	57	$0.1778 \pm 0.01 \text{bB}$
The finite rate of increase, λ		F_1	63	$1.3084 \pm 0.01 a A$	61	$1.3172\pm0.01aA$
		F ₂	57	$1.295813 \pm 0.01 aA \\$	57	$1.1946 \pm 0.01 \text{bB}$
The net reproductive rate, R_o		F_1	63	$32.4\pm2.8aB$	61	31 ± 2.6aA
		F ₂	57	$36.7193 \pm 2.3 a A$	57	$14.1 \pm 2.3 \text{bB}$
The mean generation time, T		F1	63	$12.9\pm0aB$	61	$12.5\pm0.2aB$
		F ₂	57	$13.905\pm0.2aA$	57	$14.9\pm0.3\text{bA}$
The gross reproductive rate, <i>GRR</i>		F ₁	63	$47.5\pm2.2aA$	61	$43.4\pm2.6aA$
		F2	57	$52.47 \pm 3.2aA$	57	$34.7 \pm 3.3 \text{bB}$

Table 1. Life history and parameters of the succeeding generations after parental adults of M. persicae exposed to sublethal dose (LC₂₅) of flonicamid

1. Standard errors (SE) were estimated by using the bootstrap technique with 100,000 re-samplings.

2. Significant differences at P<0.05 between two different treatments and generations were compared with paired bootstrap test implemented in TWOSEX MS-Chart.

The small letter shows significant difference between control and flonicamid treatment in each generation, while the capital letter indicates the significant differences between F_1 and F_2 generations within each treatment group (P<0.05)

Effects of Flonicamid on Age-Stage Specific Survival Rate and Fecundity of M. persicae

The age-stage specific survival rate (s_{xj}) of *M. persicae* gives the probability that a newborn will survive to age *x* and stage *j*. Overlapping among the curves of the age-stage specific survival rate (s_{xj}) of *M. persicae* in F₁ and F₂ generations treated with LC₂₅ dose of flonicamid in both treated and the control groups (Fig. 2) are due to the variable developmental rate among individuals.

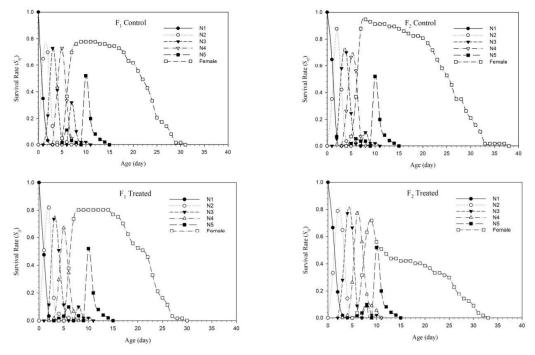


Fig. 2. Age-stage specific survival rate of M. persicae in F_1 and F_2 generations treated with LC_{25} dose of flonicamid.

The age-specific survival rate (lx) demonstrates a simplified overview of the survival rate without accounting for stage differentiation (Fig. 3). Interestingly, a lower l_x of F₁ generation was observed in the flonicamid treated group from day16, whereas a lower lx of F₂ generation in the flonicamid treatment was observed from day 3 day (Fig. 3).



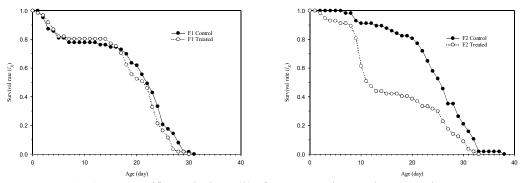


Fig. 3. Age-specific survival rate (l_x) of *M. persicae* in F₁ and F₂ generations treated with LC₂₅ dose of flonicamid.

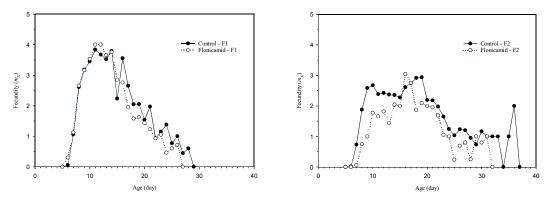


Fig. 4. Age-specific fecundity (m_x) of initial *M. persicae* exposed to LC₂₅ dose of flonicamid in F₁ and F₂ generations.

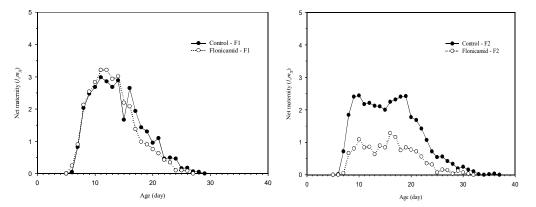


Fig. 5. Age-specific maternity $(l_x m_x)$ of *M. persicae* initially exposed to LC₂₅ dose of flonicamid in F₁ and F₂ generations.

The age-specific fecundity (m_x) of the flonicamid treated *M. persicae* were higher than that of the control group in F₁ generation (Fig. 4), while a similar trend of m_x of F₂ generation *M. persicae* were observed both in the treatment and the control groups (Fig. 4). The age-specific maternity (l_xm_x) (Fig. 5) showed the similar pattern as the age-specific fecundity (m_x) . The highest age-specific maternity (l_xm_x) (Fig. 5) showed the similar pattern as the age-specific fecundity (m_x) . The highest age-specific maternity (l_xm_x) peak observed in F₁ treated group on 12th day and the lowest was in F₂ treated group on 16th day (Fig. 5). The data demonstrate that sublethal concentrations of flonicamid could suppress the growth of *M. persicae* across generations. Shi *et al.* (2022) has also reported suppression of growth of *A. gossypii* after exposure of sublethal and low lethal concentrations of flonicamid.

The age-stage-specific life expectancy (e_{xj}) is the length of time that an individual of age x and stage j is expected to survive after age x (Fig. 6). The life expectancy (e_{xj}) curves indicated that offspring (F₁ and F₂) of adult *M. persicae* with flonicamid exposure could to survive shorter than the control (Fig. 6).

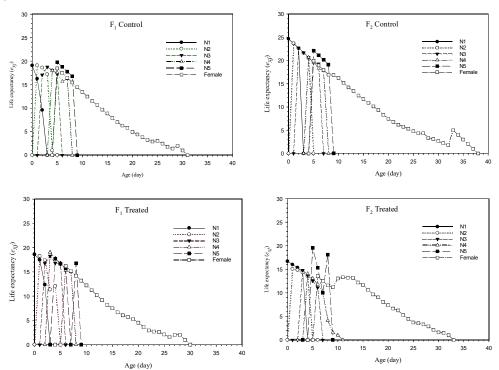


Fig. 6. Age-stage specific life expectancy (e_{xj}) of *M. persicae* initially exposed to LC₂₅ dose of flonicamid in F₁ and F₂ generations.

The age-stage-specific reproductive value (v_{xj}) describes the contribution of an individual of age x and stage j to the future population as defined by Fisher (1930). A higher maximum reproductive value of each stage and a shorter preadult period of F₁ generation of *M. persicae* were observed in the treatment group than those of the control (Fig. 7), while in F₂ generation, except the 1st instar nymph, a lower v_{xj} of each stage in the treatment group was observed than that of the control (Fig. 7).

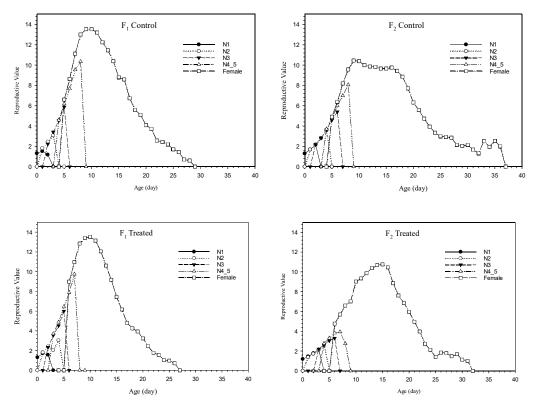


Fig. 7. Age-stage specific reproductive value (v_{xy}) of *M. persicae* initially exposed to LC₂₅ dose of flonicamid in F₁ and F₂ generations.

The approach for ecotoxicological analysis based on life histories and population fitness using demography and measures of population growth rate results in more accurate assessments of the impacts of pesticides and other toxicants because measures of population growth rate combine lethal and sublethal effects (Stark and Banks, 2003).

CONCLUSIONS

The study results indicated that the application of LC₂₅ concentration of flonicamid causes hormesis in *M. persicae* across three generations. The exposure of parental aphids to a sublethal dose of flonicamid resulted in a significant decrease in adult longevity, total longevity, oviposition period, fecundity, and population parameters r, λ , R_0 , and *GRR* and increase *T* in F_2 generation. These results suggested that short-term exposure of *M. persicae* to the sublethal concentration of flonicamid might induce hormesis in the insect across generations. Since life table analysis integrated all these factors into population parameters, it is the most important tool for the overall evaluation of population fitness and hormesis. Nevertheless, given that the genetic variation in field populations is naturally greater than that of laboratory strains, the situation in the field may be more complex, and further investigations on the sublethal effects of this insecticide on *M. persicae* in the field are advisable. Insecticide-induced hormesis, which may potentially occur after application, should be taken into consideration for the integrated management of *M. persicae* in the field.

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