

Original article (Orijinal araştırma)

The effect of sublethal doses of flupyradifurone on the life table and esterase enzyme of *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)¹

Flupyradifurone'nun subletal dozlarının *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)'nin yaşam çizelgesi ve esteraz enzimi üzerine etkisi

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Abstract

The aim of this study was to determine the effect of two different sublethal doses (LC₁₀ and LC₃₀) of flupyradifurone on the life table and esterase enzyme of *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae). The experiments were conducted in 2022 in Isparta University of Applied Sciences laboratory and climate rooms in 2022 as 1 control + 2 sublethal doses. For the life table, control, LC₁₀ and LC₃₀ doses were established as 30, 25 and 30 replications, respectively. Female and total lifespan of *M. persicae* adults exposed to LC₁₀ concentrations of flupyradifurone were significantly shortened. Daily and total numbers of the offsprings decreased at both LC₁₀ and LC₃₀ concentrations. Furthermore, these negative effects on the aphid were revealed as a lower intrinsic rate of increase (r), net reproductive rate (R_0), finite rate of increase (λ) and fecundity (F). Based on the obtained data, flupyradifurone seems to suppress the population growth of *M. persicae*. It was determined that esterase enzyme activity involved in pesticide detoxification did not change in populations exposed to two different sublethal doses of flupyradifurone and unexposed (control). It is thought that this study facilitates the understanding of the lethal and sublethal effects of flupyradifurone on aphid performance.

Keywords: Aphid, detoxification, insecticide, life table, sublethal effect

Öz

Bu çalışmanın amacı, flupyradifurone etken maddesinin iki farklı subletal dozunun (LC₁₀ ve LC₃₀) *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)'nin yaşam çizelgesi ve esteraz enzimi üzerine etkisini belirlemektir. Denemeler, 2022 yılında Isparta Uygulamalı Bilimler Üniversitesi laboratuvar ve iklim odalarında 1 kontrol+ 2 subletal doz olacak şekilde yürütülmüştür. Yaşam çizelgesi için kontrol, LC₁₀ ve LC₃₀ dozları sırasıyla 30, 25 ve 30 tekerrür olarak kurulmuştur. Flupyradifurone'nun LC₁₀ konsantrasyonuna maruz kalan *M. persicae* erginlerinin dişi ömrü ve toplam yaşam süreleri önemli ölçüde kısalmıştır. Günlük ve toplam yavru sayıları hem LC₁₀ hem de LC₃₀ konsantrasyonlarında azalmıştır. Ayrıca yaprakbiti üzerindeki bu olumsuz etkiler daha düşük bir kalıtsal üreme yeteneği (r), net üreme gücü (R_0), içsel artış oranı (λ) ve üreme oranları (F) olarak ortaya çıkmıştır. Elde edilen verilere göre, flupyradifurone'nun *M. persicae*'nin popülasyon büyümesini baskıladığı görülmektedir. Pestisit detoksifikasyonunda rol alan esteraz enzim aktivitesinin flupyradifurone'nun iki farklı subletal dozları uygulanmış ve uygulanmamış (kontrol) popülasyonlarında değişmediği belirlenmiştir. Çalışmanın, flupyradifurone'nun yaprakbiti performansı üzerindeki letal ve subletal etkilerinin anlaşılmasını kolaylaştırdığı düşünülmektedir.

Anahtar sözcükler: Yaprakbiti, detoksifikasyon, insektisit, yaşam çizelgesi, subletal etki

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Introduction

Aphids belong to the superfamily Aphidoidea and have a very high number of species (Erol et al., 2018). It is known that there are approximately 5000 species belonging to 510 genera in the world (Blackman & Eastop, 2023). In Turkey, 532 species belonging to approximately 142 genera have been identified (Şenol et al., 2015). The green peach aphid, *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae), is the most economically important aphid pest in the world due to its high host diversity, the damage mechanism it causes to the plant, its life cycle, its ability to spread rapidly, its vectoring of virus diseases and its ability to easily develop resistance to insecticides (Foster et al., 2000; van Emden, 2007; Boukhris-Bouhachem et al., 2017). *Myzus persicae* causes damage by sucking the sap of the plant during the whole development period of the plant. This pest, which has a very wide distribution area in the world, causes damage on a variety of plants in Türkiye. Under ideal conditions, it can continue its activity and reproduction in every month of the year (Lodos, 1986).

Acute toxicity testing of pesticides to insects is largely done by lethal dose or concentration determination studies. Median lethal dose (LD₅₀) or median lethal concentration (LC₅₀) is used to determine the effects of pesticides on both pests and natural enemies. These values are parameters used to compare the effects of different active substances or formulations on the test organism. In addition to the direct lethal effects of pesticides, it is also important to determine the effects of low pesticide concentrations on the physiology and behavior of insects (Desneux et al., 2007). Sublethal effects can be defined as physiological, demographic or behavioral effects on individuals or populations that survive exposure to lethal or sublethal doses or concentrations of a toxicant (De França et al., 2017). Sublethal dose means non-lethal; below lethal dose. Sublethal effects in insects can occur in the form of changes in lifespan, development time, population growth, egg production, sex ratios and behavior, deformations, search for food and reproductive sites, shortening of feeding and reproductive time (Lee, 2000). Therefore, the effects of sublethal doses and concentrations on insect physiology, behavior, demographic parameters and natural enemies are crucial in the selection of insecticides for use in integrated pest control programs (De França et al., 2017). It has been reported that low doses (sublethal) of pesticides have stimulatory effects on pests, while higher doses have inhibitory or toxic effects on pests (Calabrese & Baldwin, 2003). Luckey used the term "hormoligosis", which comes from the Greek words "hormo" (excite) and "oligo" (in small quantities), to describe the mild stimulating effects of toxic or non-toxic stress effects on an organism under suitable conditions, such as pesticides, temperature, light, etc. (Luckey, 1968; Cohen, 2006). In entomology, the term hormoligosis is known as sublethal doses of a pesticide on pest or natural enemy species to stimulate fertility or egg production.

Flupyradifurone is the first member of the new class of butanolide insecticides grouped as 4D according to the IRAC classification (Colares et al., 2017). Flupyradifurone can provide rapid and systemic protection with xylem mobility (Barbosa et al., 2017). By reversibly binding to post-synaptic nicotinic acetylcholine receptors (nAChRs), it mimics acetylcholine in the nervous system of insects by keeping them open and eventually causing uncontrolled axonal excitation (Nauen et al., 2015; Colares et al., 2017). Although flupyradifurone targets the nAChR, it differs from other nAChR agonists based on structure-activity relationships (Jeschke et al., 2015). nAChR has been an insecticide molecular target site of increasing importance for many years, playing a central role in mediating fast excitatory synaptic transmission in the insect central nervous system (CNS). The active ingredient, flupyradifurone is a newly licensed product for the control against whitefly in Türkiye. Although this active ingredient is not licensed against *M. persicae*, it is thought to have an effect on aphids somehow in the same environment due to its extensive use in whitefly control, especially in greenhouse production.

Esterases are a large and heterogeneous group of enzymes that metabolize internal and external substrates with ester bonds. Also, esterases; It also plays a role in processes such as insect development,

behavior (by breaking down odors, etc.), reproduction, digestion and pesticide detoxification (Montella et al., 2012). Many groups of insecticides, such as organic phosphorus, benzoylphenyl ureases, organic chlorinates, carbamates, pyrethroids and juvenile hormone analogues, are susceptible to esterase hydrolysis. Although some esterases involved in insecticide resistance have limited catalytic effect, they can be produced in large numbers and bind to the insecticide before reaching their target, reducing availability (Field et al., 1988). This process is known as “sequestration” (Bass & Field 2011).

In this study, the effect of two different sublethal doses (LC₁₀ and LC₃₀) of flupyradifurone on *M. persicae* was investigated. The effects of these doses on average lifespan, total number of offsprings, pre-reproductive, reproductive, and post-reproductive periods were calculated for female *M. persicae* individuals using life tables. Additionally, the effects of two different sublethal doses of flupyradifurone on the esterase enzyme, which plays an important role in pesticide detoxification, were also examined.

Materials and Methods

Aphid culture

Myzus persicae population used in the study was obtained from Ankara Pest Control Research Institute in 2018. To date, the aphid population is produced in the climate rooms without exposure to any pesticide application. Radish, *Raphanus sativus* L. (Brassicales: Brassicaceae) was used as the host plant because it is easy to grow in climate rooms. *Myzus persicae* population was grown on clean radish plants in water-filled tubs covered with tulle and in climate rooms with 26±1°C temperature, 60-65% humidity and 16:8 (L/D) hour photoperiod conditions.

Insecticide

It is the first member of the new class of butenolide insecticides classified by IRAC as flupyradifurone 4D. Sivanto SL 200 (Bayer), a commercial preparation with the active ingredient flupyradifurone, was used in the study.

Determination of LC values

The study was conducted in Isparta University of Applied Sciences, Faculty of Agriculture, Acarology Laboratory between 2022-2023. The leaf dipping method was used to determine LC values for the flupyradifurone. To determine the LC against flupyradifurone in the aphid population, 1 control + 6 doses (100, 50, 25, 12.5, 6.25, 3.125 µl/100ml) were used, with each dose consisting of 3 replicates. In each replicate, 25±5 adult aphid individuals were used. Flupyradifurone doses were prepared using the 50% dilution method. Only pure water was applied to the control group. First of all, 1% agar powder was mixed with distilled water, boiled and allowed to cool. After cooling, the agar medium was poured into a 9 cm petri dish at a height of approximately 4 mm and the medium was waited for it to freeze. The main purpose of using agar medium in the study is to ensure that the trial leaf meets its moisture need from the environment. After the radish leaves were cut into 3 cm disk shapes, they were dipped into the doses for 10 seconds and the leaves were placed in petri dishes and *M. persicae* adults were transferred onto them with the help of a binocular. Petri dishes were placed in climate rooms with 26±1°C temperature, 60-65% humidity and 16:8 h (L/R) photoperiodic conditions. Dead and alive counts were made at the end of the 72nd hour. The results obtained from the dead alive counts were analyzed and evaluated with the POLO computer package program (LeOra Software, 1994). As a result of the study, in addition to the LC₅₀ value for flupyradifurone, LC₁₀ and LC₃₀ values used as sublethal doses were also determined.

Sublethal dose applications and determination of biological parameters

Generally, pests are exposed to low concentrations of pesticides due to degradation, etc. in the field (Desneux et al., 2007, Biondi et al., 2012). This leads to various physiological and behavioral sublethal effects

in individuals (He et al., 2013, Chen et al., 2016, Zeng et al., 2016). Therefore, in this study, LC₁₀ and LC₃₀ sublethal doses were used to determine the effects of flupyradifurone on biological parameters of *M. persicae*. The experiments were established as 1 control + 2 sublethal doses (LC₁₀ and LC₃₀). In the life table, 30 repetitions were established in the control and LC₃₀ groups and 25 repetitions in the LC₁₀ group. For each replicate, one *M. persicae* female was transferred to the radish leaf. It was checked after 1 day and the mother and other aphids were removed so that 1 newborn aphid was left in each replication. Thus, individuals of the same age were used for each dose and control group throughout the entire experiment. After the mother and other aphids were removed flupyradifurone sublethal doses were prepared and 2 mL insecticide concentration was applied into the petri dish under 1 atm pressure with the help of spray tower (Burkard Manufacturing Co Ltd). Only pure water was applied to the control group. All replicates were checked daily and the reproductive periods of aphid individuals that reached the adult stage and total number of offspring daily were observed. The observations in the experiment continued until the repetitions in all applications died.

Life table studies

In order to determine the effects of flupyradifurone sublethal doses on the life cycle of *M. persicae*, parameters were calculated according to Age-stage, two-sex life table (Chi et al., 2020, 2023). The parameters and formulas for the calculated life tables are as follows.

Survival rate depending on age and period: s_{xj}

Age-specific survival rate: l_x

Age-specific fecundity: m_x (female/female/day)

Net reproductive rate, R_0 (nymphs /individual): $\sum_{x=0}^{\infty} l_x m_x$

Intrinsic rate of increase: r (day⁻¹): $\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$

Fecundity: F (nymphs/female): $\frac{\sum_{x=1}^{N_f} E_x}{N_f}$

Finite rate of increase (λ , (day⁻¹)): $\lambda = e^r$

Mean generation time (T , days): $T = \frac{\ln R_0}{r}$

Population-doubling time (T_2 , day): $T_2 = \frac{\ln 2}{r}$

To compute the differences and SEs, 100,000 bootstrap replicates were performed (Efron & Tibshirani, 1993; Huang & Chi, 2012; Akca et al., 2015; Akköprü et al., 2015). At a 5% significant level, the paired bootstrap test was used to evaluate the differences in demographic parameters between the flupyradifurone sublethal doses - exposed groups and the control group based on the confidence interval of the difference (Wei et al., 2020).

Esterase activity

This study was conducted to determine whether sublethal doses of flupyradifurone caused changes in the esterase enzyme activity of *M. persicae*. First of all, LC₁₀ and LC₃₀ doses of flupyradifurone were applied to *M. persicae* individuals and two different populations were created. Esterase enzyme activities were determined in three different populations of *M. persicae*. The method developed by (Devonshire, 1975) to determine total esterase activity was rearranged by (Devonshire et al., 1992) by adapting it to a 96-well microplate. 50 µL of 20 mM phosphate buffer (pH: 7.0) containing 0.1% Triton X-100 (Boehringer Mannheim, especially purified) was placed in each well of the microplate with a multichannel micropipette. Adult aphids belonging to the populations to be tested were transferred to each well using a brush. Aphids were homogenized using a multiple homogenizer and 15 minutes were waited for the tissues to dissolve thoroughly.

30 mg of Fast Blue RR Salt was weighed and completed with phosphate buffer (pH: 6.0) to 50 mL, and after filtering through Whatman filter 1, 500 L of 100 mM 1-naphthyl acetate solution was added. 200 µL of the prepared dye-substrate solution was taken and placed into all wells with a multi-channel micropipette. "Optical density" (O.D.) values were obtained by making "kinetic" readings on a Molecular Devices brand microplate reader at 450 nm wavelength with 10-second intervals for a total of 5 minutes.

Data analysis

The logarithmic-probit model was used to calculate the LC₁₀, LC₃₀, LC₅₀ values, slopes and their 95% confidence limits of flupyradifurone against *M. persicae* using POLO computer program (LeOra Software Inc., Berkely, CA). Non-overlapping 95% confidence limits were used to determine statistical differences between populations. The esterase enzyme values in *M. persicae* individuals exposed to LC₁₀ and LC₃₀ sublethal doses of flupyradifurone and individuals in the control group were statistically analyzed by one-way analysis of variance with Tukey's post hoc test with significance set at $p < 0.05$ (IBM, SPSS Statistics, version 22).

Results and Discussion

LC₁₀, LC₃₀ and LC₅₀ values against flupyradifurone in *M. persicae* are given in Table 1.

Table 1. LC values against flupyradifurone in *Myzus persicae*

| Insecticide | n ^a | χ^2 /df/ P ^b | Slope±SE | LC ₁₀ (mga.i. L ⁻¹) (95% CL ^c) | LC ₃₀ (mga.i. L ⁻¹) (95% CL ^c) | LC ₅₀ (mga.i. L ⁻¹) (95% CL ^c) |
|-----------------|----------------|------------------------------|-------------|--|--|--|
| Flupyradifurone | 578 | 0.535/4/0.179 | 1.497±0.142 | 1.219 (0.661-1.882) | 3.908 (2.678-5.186) | 8.756 (6.810-10.851) |

a: number of individuals used in the experiment; b: chi-square/degrees of freedom /p-value; c: confidence limits.

Developmental stages and life span of *M. persicae* individuals exposed to LC₁₀ and LC₃₀ doses of flupyradifurone are given in Table 2.

Table 2. Development stages and life span of *Myzus persicae* individuals exposed to LC₁₀ and LC₃₀ sublethal doses of Flupyradifurone (Days)*

| Biological Period | Type | n | Mean | |
|--|------------------|----|------------|---|
| I. Nymph Stage | Control | 30 | 1.80±0.07 | a |
| | LC ₁₀ | 25 | 1.68±0.16 | a |
| | LC ₃₀ | 30 | 1.73±0.10 | a |
| II. Nymph Stage | Control | 30 | 1.66±0.10 | a |
| | LC ₁₀ | 25 | 1.36±0.12 | a |
| | LC ₃₀ | 30 | 1.56±0.14 | a |
| III. Nymph Stage | Control | 30 | 1.50±0.09 | a |
| | LC ₁₀ | 25 | 1.44±0.11 | a |
| | LC ₃₀ | 30 | 1.76±0.11 | a |
| IV. Nymph Stage | Control | 30 | 1.63±0.11 | a |
| | LC ₁₀ | 25 | 2.04±0.15 | a |
| | LC ₃₀ | 30 | 1.76±0.13 | a |
| Development time (born to from adult) | Control | 30 | 6.60±0.09 | a |
| | LC ₁₀ | 25 | 6.52±0.10 | a |
| | LC ₃₀ | 30 | 6.83±0.12 | a |
| Life span of Adult Female (to adult to died) | Control | 30 | 17.26±0.26 | a |
| | LC ₁₀ | 25 | 14.80±0.42 | b |
| | LC ₃₀ | 30 | 16.46±0.42 | a |
| Total Life Time (to born from died) | Control | 30 | 23.86±0.26 | a |
| | LC ₁₀ | 25 | 21.32±0.47 | b |
| | LC ₃₀ | 30 | 23.30±0.42 | a |

* The difference between the means (± standard errors) marked with the same letter for each parameter is statistically insignificant. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared using the paired bootstrap test ($p < 0.05$).

The first nymphal stage of *M. persicae* individuals exposed to sublethal doses and individuals in the control group varied between 1.68 days and 1.80 days, and statistically they were all in the same group. Similarly, in the second, third, fourth nymphal stages and development stages, all of them were statistically in the same group. When the adult female life span data were analyzed, it was seen that the LC₁₀ dose was in a different statistical group compared to the control and LC₃₀ values with 14.80 days. Also, total life span was found to be in a different statistical group compared to the control and LC₃₀ values with 21.32 days at LC₁₀ sublethal dose and the difference between them was found to be significant.

Prereproductive, reproductive, postreproductive period (days), daily and total offspring numbers of *M. persicae* individuals exposed to LC₁₀ and LC₃₀ sublethal doses of flupyradifurone and in the control group are given in Table 3. The prereproductive period of *M. persicae* individuals varied between 1.20 days and 3.06 days and each of them were statistically in separate groups. Reproductive periods were determined as 13.66, 12.08 and 14.00 days for control, LC₁₀ and LC₃₀, respectively. In postreproductive periods, all groups were statistically in the same group. The daily and total number of offsprings of individuals exposed to LC₁₀ and LC₃₀ doses were in the same statistical group, while the control group was in a different class in both cases. Daily and total offspring numbers were highest in the control groups and the difference was statistically significant compared to LC₁₀ and LC₃₀ doses of flupyradifurone (Table 3).

Table 3. Prereproductive, reproductive, postreproductive periods (Days), daily and total offspring numbers of *Myzus persicae* individuals exposed to LC₁₀ and LC₃₀ sublethal doses of flupyradifurone*

| Parameter | Type | n | Mean | |
|-----------------------------------|------------------|----|------------|----|
| Prereproductive Period | Control | 30 | 3.06±0.16 | a |
| | LC ₁₀ | 25 | 1.72±0.14 | b |
| | LC ₃₀ | 30 | 1.20±0.13 | c |
| Reproductive Period | Control | 30 | 13.66±0.35 | ab |
| | LC ₁₀ | 25 | 12.08±0.73 | b |
| | LC ₃₀ | 30 | 14.00±0.56 | a |
| Postreproductive Period | Control | 30 | 0.53±0.15 | a |
| | LC ₁₀ | 25 | 1.00±0.33 | a |
| | LC ₃₀ | 30 | 1.26±0.29 | a |
| Daily number of offspring per Day | Control | 30 | 2.45±0.12 | a |
| | LC ₁₀ | 25 | 1.18±0.10 | b |
| | LC ₃₀ | 30 | 1.34±0.11 | b |
| Total number of offspring | Control | 30 | 42.40±2.02 | a |
| | LC ₁₀ | 25 | 17.84±1.65 | b |
| | LC ₃₀ | 30 | 22.53±2.07 | b |

* The difference between the means (± standard errors) marked with the same letter for each parameter is statistically insignificant. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared using the paired bootstrap test ($p < 0.05$).

Life table parameters of *M. persicae* exposed to LC₁₀ and LC₃₀ doses of flupyradifurone and control *M. persicae* individuals are given in Table 4. The differences between the intrinsic rate of increase (r), net reproductive rate (R_0) and finite rate of increase λ (day^{-1}) values of both sublethal doses-exposed and control *M. persicae* individuals separately were statistically significant. The longest mean generation time (T) was 15.51 days in *M. persicae* individuals in the control group and the shortest was 13.89 days in individuals exposed to LC₃₀ sublethal dose (Table 4). The highest fecundity was again observed in the control group. The shortest population doubling time was observed in the control group with 2.87 days and the longest with 3.42 days in individuals exposed to sublethal dose of LC₃₀ (Table 4).

Table 4. Life table parameters of *Myzus persicae* individuals exposed to LC₁₀ and LC₃₀ sublethal doses of flupyradifurone*

| Parameter | Type | n | Mean |
|---|------------------|----|-----------------|
| Intrinsic rate of increase, r (day ⁻¹) | Control | 30 | 0.2415±0.0002 a |
| | LC ₁₀ | 25 | 0.2020±0.0001 c |
| | LC ₃₀ | 30 | 0.2230±0.0002 b |
| Net reproductive rate, R_0 (offspring/individual) | Control | 30 | 42.39±0.25 a |
| | LC ₁₀ | 25 | 17.84±0.11 c |
| | LC ₃₀ | 30 | 22.53±0.11 b |
| Finite rate of increase, λ (day ⁻¹) | Control | 30 | 1.2729±0.0009 a |
| | LC ₁₀ | 25 | 1.2242±0.0003 c |
| | LC ₃₀ | 30 | 1.2495±0.0009 b |
| Fecundity, F (nymphs/female) | Control | 30 | 42.39±0.31 a |
| | LC ₁₀ | 25 | 17.84±0.12 c |
| | LC ₃₀ | 30 | 22.53±0.12 b |
| Mean generation time, T (day) | Control | 30 | 15.51±0.20 a |
| | LC ₁₀ | 25 | 14.22±0.15 b |
| | LC ₃₀ | 30 | 13.89±0.19 b |
| Theoretical population-doubling time, DT (day) | Control | 30 | 2.87 |
| | LC ₁₀ | 25 | 3.42 |
| | LC ₃₀ | 30 | 3.09 |

* The difference between the means (± standard errors) marked with the same letter for each parameter is statistically insignificant. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared using the paired bootstrap test ($p < 0.05$).

Age and stage dependent survival rate (s_{xj}), age-specific survival rates (l_x) and fertility rates (m_x) curves of *M. persicae* individuals exposed to LC₁₀ and LC₃₀ sublethal doses of flupyradifurone and individuals in the control group are given in Figure 1-2.

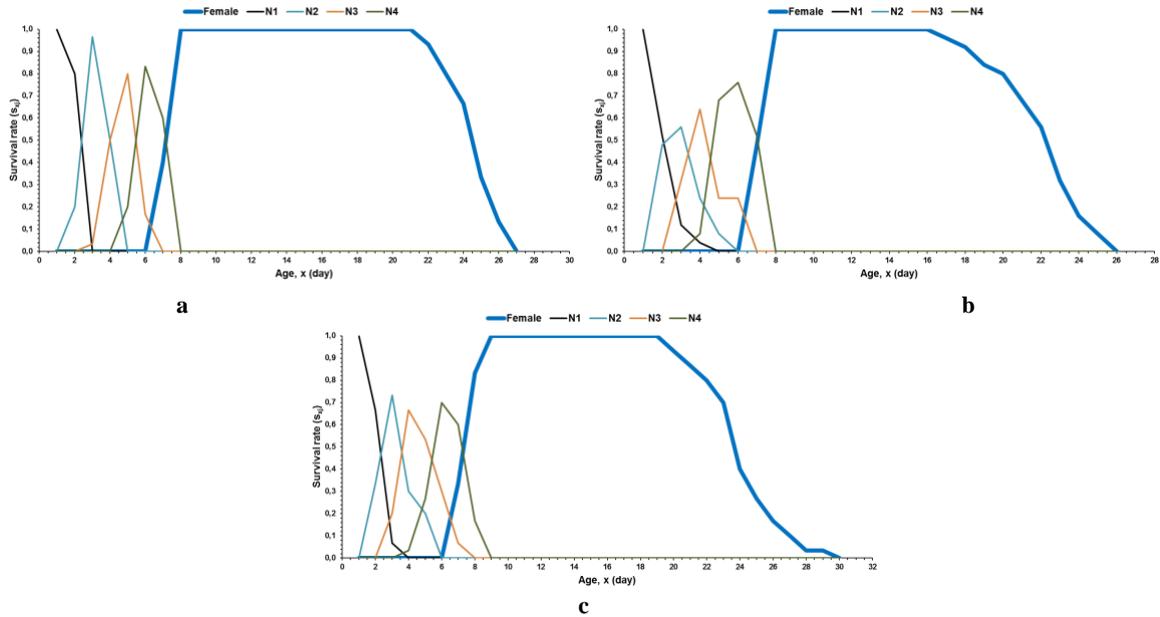


Figure 1. Age- and stage-dependent survival rates (s_{xj}) of *Myzus persicae* individuals (a: control, b: LC₁₀, c: LC₃₀) (Female: female, N1: 1st instar nymph, N2: 2nd instar nymph, N3: 3rd instar nymph, N4: 4th instar nymph).

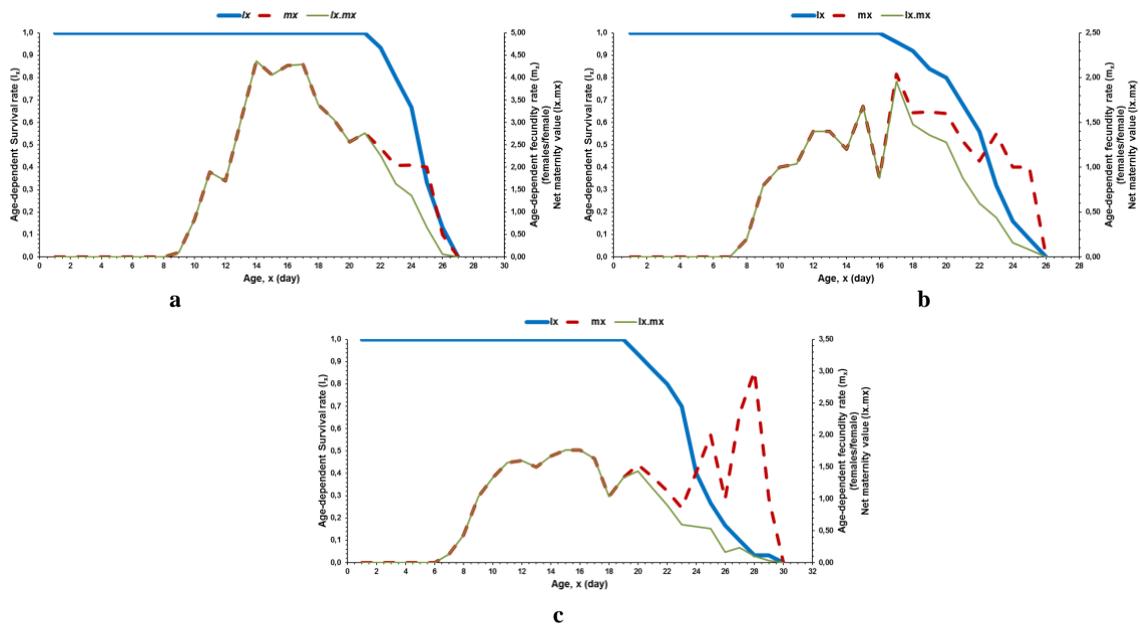


Figure 2. Age-specific survival rates (l_x) and fertility rates (m_x) of *Myzus persicae* individuals (a: control, b: LC₁₀, c: LC₃₀).

Esterase enzyme values in individuals exposed to LC₁₀ and LC₃₀ doses of flupyradifurone and in the control group were found to be 1.80, 2.05 and 1.55 mOD min⁻¹ mg⁻¹ protein, respectively (Table 5). According to these data, it was observed that esterase enzyme activity did not change with control, LC₁₀ and LC₃₀ sublethal doses and all of them were in the same statistical group ($p < 0.05$).

Table 5. Esterase enzyme values in individuals exposed to LC₁₀, LC₃₀ doses of flupyradifurone and in the control group

| Population | n* | Total Esterase mOD/min/mg protein ± SE | R/S** |
|------------------|----|---|-------|
| Control | 4 | 1.55 ± 0.28 a*** | |
| LC ₁₀ | 4 | 1.80 ± 0.65 a | 1.16 |
| LC ₃₀ | 4 | 2.05 ± 0.45 a | 1.32 |

* Number of repetition;

** Enzyme activity of the tested population/ enzyme activity of the control;

*** Letters in each column show statistical differences according to Tukey test ($F(2, 30): 13.07, p < 0.05$) for total esterase.

The extensive use of various insecticides in the control of aphids has led to resistance to many insecticides with different modes of action (Wei et al., 2017; Fouad et al., 2022). The development of new alternative insecticides such as flupyradifurone is a great necessity. The high toxicity of flupyradifurone has been determined for several sap-feeding pests, including *M. persicae*, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) and *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) (Nauen et al., 2015; Tang et al., 2019). In this study, since the active ingredient flupyradifurone is not licensed in Türkiye for *M. persicae*, LC₅₀ determination studies were first carried out against this substance in aphids and it was found to be quite toxic as 8.756 mg/L. Similarly, in the study conducted by (Tang et al., 2019), the LC₅₀ analysis result of *M. persicae* in adult individuals at the end of 48 hours was 8.491 mg/L, indicating that it is very toxic. Sial et al. (2018), *M. persicae* individuals were exposed to deltamethrin and lambda cyhalothrin for 48 hours and as a result, LC₅₀ values were found to be 381 mg L⁻¹ and 1010 mg L⁻¹, respectively.

In addition to the lethal effects of insecticides, insect populations are often exposed to low concentrations of insecticides in the field due to the variable distribution and continuous degradation of insecticides (Bonmatin et al., 2005; Desneux et al., 2005). Therefore, sublethal effects of insecticides can increase or decrease insect populations (Desneux et al., 2007). Evaluation of development, survival, reproduction and

behavioral response is important for an overall understanding of the effects of flupyradifurone for IPM. Investigating different toxicity parameters, such as sublethal effects, is essential to delay the development of resistance (Liang et al., 2019). Sublethal effects of flupyradifurone have been reported in several pests such as *B. tabaci*, *A. gossypii*, *M. persicae*, *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Liviidae) and *Lygus hesperus* (Knight, 1917) (Hemiptera: Miridae) (Smith & Giurcanu, 2013; Joseph & Bolda, 2016; Chen et al., 2017; Liang et al., 2019; Tang et al., 2019). In the study conducted for this purpose, the sublethal effects of LC₁₀ and LC₃₀ sublethal doses of flupyradifurone on life table characteristics in *M. persicae* were evaluated. Effects that reduce fecundity, longevity, and alter behavior have been observed in many pests, often after exposure to sublethal insecticide concentrations (Desneux et al., 2007; Han et al., 2012; Guo et al., 2013; Zeng et al., 2016; Tang et al., 2019). For example, sublethal concentrations of endosulfan significantly decreased the fecundity of *Apolygus lucorum* Meyer-Dür, 1843 (Hemiptera: Miridae) (Liu et al., 2008), while sublethal doses of buprofezin shortened the adult life span of *B. tabaci* (Sohrabi et al., 2011). In this study, female longevity and total life span of *M. persicae* adults were significantly shortened when exposed to leaf discs treated with a sublethal LC₁₀ concentration of flupyradifurone. However, no significant effect was found on nymph stage periods and development time. Daily and total offspring numbers decreased at both LC₁₀ and LC₃₀ concentrations. Moreover, these negative effects on the aphid were manifested as a lower intrinsic rate of increase (r), net reproductive ability (R_0), finite rate of increase (λ) and fecundity (F). This suggests that flupyradifurone suppresses population growth of *M. persicae*. Similarly, sublethal effects of insecticides on population growth have been reported in many pests such as *A. gossypii*, *A. lucorum*, *B. tabaci*, *Brevicoryne brassicae* (L., 1758) (Hemiptera: Aphididae), *Bradysia odoriphaga* Yang & Zhang, 1985 (Diptera: Sciaridae), *M. persicae* and *Lipaphis erysimi* (Kaltenbach, 1843) (Hemiptera: Aphididae) (Devine et al., 1996; Lashkari et al., 2007; Wang et al., 2008; Tan et al., 2012; Chen et al., 2016; Liang et al., 2019; Hosseini et al., 2020). Under laboratory conditions, as a result of sublethal doses of rotenone and abamectin application to the green peach aphid, its reproduction decreased by 44.29% and 54.01%, respectively; with fenvalerate application, the average daily reproduction per female decreased significantly compared to the control (Wang et al., 2008). In the study conducted by Wang et al. (2008), it was reported that the sublethal concentration (LC₂₅) of six different insecticides (Imidacloprid, Rotenone, Fenvalerate, Abamectin, Pirimicarb, Azadirachtin) did not have a significant effect on the reproduction of *M. persicae*. Another study showed that exposure to low concentrations of afidopyropen significantly decreased the lifespan and fecundity of *M. persicae*, and that the life parameters of the F1 progeny were also affected (Liu et al., 2022). The findings provide a basis for further investigation of the sublethal effects of afidopyropen and other insecticides on aphids (Liu et al., 2022).

Many studies show that one of the main reasons for insect resistance to pesticides is the increased detoxification capabilities of enzymes associated with pesticide metabolism (Cai et al., 2021). The role of acetylcholinesterase, carboxylesterase or other esterase enzymes in insecticide resistance in aphids has been studied (Gao et al., 1992; Song et al., 1995). Metabolic enzymes reported to provide resistance in *M. persicae* include esterase E4 (or its Mediterranean variant, FE4), which confers broad-spectrum resistance to organophosphates, carbamates, and pyrethroids, and cytochrome P450 CYP6CY3, which imparts resistance to neonicotinoids (Bass et al., 2014). In the study, it was determined that the difference between the control and the populations exposed to LC₁₀ and LC₃₀ sublethal doses was not statistically significant according to the activity of the esterase enzyme.

It is thought that this study facilitates the understanding of the lethal and sublethal effects of flupyradifurone on aphid performance. However, additional studies are needed to fully evaluate the sublethal effects of this new insecticide on *M. persicae* under field conditions. In addition, the effects on natural enemies should be investigated in order to preserve the natural balance.

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