Sublethal effects of diflovidazin on life table parameters of two-spotted spider mite Tetranychus urticae (Acari: Tetranychidae)

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To link to this article: https://doi.org/10.1080/01647954.2017.1417328

Published online: 02 Jan 2018.
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*Tetranychus urticae* (Acari: Tetranychidae)

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**ABSTRACT**

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is a serious pest of many agricultural crops. Using chemical pesticides is one of the main methods for its management. In this study, sublethal effects of diflovidazin including LC$_{25}$, LC$_{10}$, LC$_{20}$ and distilled water were evaluated on biological parameters (such as $r$, $\lambda$, gross reproductive rate, $R_0$, and $T$) of *T. urticae*. The experiments were done under laboratory condition at 25 ± 2°C, 60 ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D) hours. The crude data were analysed based on age-stage, two-sex life table analysis. The results indicated significant reduction in female’s duration of maturation, oviposition period, and total fecundity by increasing examined dose. The highest and lowest values of the net reproductive rate ($R_0$) were obtained 48.88 and 31.14 offspring/individual in control and LC$_{20}$ respectively. The maximum value of intrinsic rate of increase ($r$) was 0.234 day$^{-1}$ that obtained in control treatment, while the minimum value was 0.216 day$^{-1}$ obtained in LC$_{20}$ concentration. Finite rate of increase ($\lambda$) had not significantly descended with concentration enhancing from LC$_{5}$ to LC$_{20}$ compared with the control. The results demonstrated that diflovidazin could be incorporated in integrated pest management programmes of *T. urticae*.

**Introduction**

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is a destructive pest that causes serious damage on a wide range of crops such as cotton, peach, kidney bean, cucumber, soybean, eggplant, etc. (Pritchard and Baker 1955; Hossain et al. 2006; Sedarati et al. 2009, 2011; Khannamani et al. 2013; Maleknia et al. 2016; Mollaloo et al. 2017). This mite attacks to plant species and feeds of plant sap that was barrier vital actions and leaves lose their quality and joy, and in case of severe damage, there is also falling out of leaves (Gorman et al. 2001; El-Kady et al. 2007). In severe infection, a large amount of thin webbing is produced that may completely cover infested areas of leaves (Zhang 2003). Control of this mite species is obviously difficult and mostly relies on the use of acaricides.

The wide use of pesticides leads to affect non-target organisms (Croft 1990), human safety (García-Marí and Enrique González-Zamora 1999), the emergence of secondary pests (Elzen 2001), and development of resistance (Brattsten et al. 1986). One of the commonly used methods to manage resistance creation and the biological agent conservation is reduction of applied concentration by using sublethal doses (He et al. 2013; Song et al. 2013). Sublethal effects can be very delicate and affect populations at concentrations lower than the traditional concentration-response curve (Stark and Banks 2003). Sublethal effect studies have been used to assess the selectivity of pesticides to beneficial arthropods (Teodore et al. 2005; Poletti et al. 2007). Furthermore, application of selective pesticides is considered to be preferable over a broader range of pesticides as they are thought to be safer for non-target species.

Among the common selective miticides in order to cope with the two-spotted spider mite, diflovidazin, SC20% with trade name of Flumite®, is a selective acaricide that belongs to synthetic insecticide and acaricide group of tetrazine that has contact, ovicide, selective with translaminar activity. It is generally accepted that this acaricide leads to growth inhibition Insecticide Resistance Action Committee (IRAC) and used for controlling the wide range of mites from different families such as Tetranychidae, Eriophyidae, Tarsonemidae, and Tenuipalpidae on different crops.

Consequently, taking the activity of this acaricide, in this experiment, the effects of different sublethal concentrations of diflovidazin on *T. urticae* were investigated using demographic toxicological analysis. The probability of incorporating this compound in the management of the two-spotted spider mite was discussed.

**Materials and methods**

**Host plant**

Host plant (*Phaseolus vulgaris* L. var Khomein (Fabaceae)) was grown in plastic pots (15 cm diameter) under controlled greenhouse condition at 25 ± 5°C, 60 ± 10% Relative humidity (RH), and a photoperiod of 16: 8 (L:D) hours.

**Source of *T. urticae***

Stock population of the two-spotted spider mite was obtained from infected greenhouses of Pakdasht (central part of Iran) and were reared on host plants under laboratory conditions of 25 ± 2°C, 60 ± 5% RH, and a photoperiod of 16:8 (L:D) hours.

**Acaricide**

Diflovidazin, IUPAC name 3-(2-chlorophenyl)-6-(2,6-difluorophenyl)-1,2,4,5-tetrazine, commercial formulation Flumite® (suspension concentrate 20%), was used in this experiment.
Effects of sublethal concentrations of diflovidazin on developmental time, adult longevity, and total life span (mean ± SE) of *Tetranychus urticae*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>LC$_{5}$</th>
<th>LC$_{10}$</th>
<th>LC$_{20}$</th>
<th>Slope ± SE</th>
<th>p-Value</th>
<th>X$^2$</th>
<th>n</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg (day)</td>
<td>4.19 ± 0.10$^a$</td>
<td>4.15 ± 0.06$^a$</td>
<td>4.21 ± 0.05$^a$</td>
<td>4.23 ± 0.12$^a$</td>
<td>3.20 ± 0.31</td>
<td>0.92</td>
<td>0.46</td>
<td>480</td>
<td>4</td>
</tr>
<tr>
<td>Larva (day)</td>
<td>2.11 ± 0.09$^a$</td>
<td>2.08 ± 0.06$^a$</td>
<td>2.07 ± 0.07$^a$</td>
<td>2.08 ± 0.08$^a$</td>
<td>2.13 ± 0.10$^a$</td>
<td>0.95</td>
<td>0.46</td>
<td>480</td>
<td>4</td>
</tr>
<tr>
<td>Protonymph (day)</td>
<td>2.12 ± 0.09$^a$</td>
<td>2.08 ± 0.04$^a$</td>
<td>2.14 ± 0.06$^a$</td>
<td>2.09 ± 0.08$^a$</td>
<td>10.9 ± 0.07</td>
<td>0.07</td>
<td>0.46</td>
<td>480</td>
<td>4</td>
</tr>
<tr>
<td>Deutonymph (day)</td>
<td>2.09 ± 0.06$^a$</td>
<td>2.15 ± 0.05$^a$</td>
<td>2.14 ± 0.06$^a$</td>
<td>2.09 ± 0.08$^a$</td>
<td>11.6 ± 0.08</td>
<td>0.11</td>
<td>0.46</td>
<td>480</td>
<td>4</td>
</tr>
<tr>
<td>Longevity (day)</td>
<td>10.56 ± 0.18$^a$</td>
<td>10.46 ± 0.33$^a$</td>
<td>9.93 ± 0.37$^a$</td>
<td>8.23 ± 0.39$^a$</td>
<td>20.66 ± 0.11</td>
<td>0.05</td>
<td>0.46</td>
<td>480</td>
<td>4</td>
</tr>
<tr>
<td>Total life span (day)</td>
<td>21.00 ± 0.25$^a$</td>
<td>20.92 ± 0.46$^a$</td>
<td>20.47 ± 0.42$^a$</td>
<td>18.76 ± 0.47$^a$</td>
<td>60.02 ± 0.24</td>
<td>0.05</td>
<td>0.46</td>
<td>480</td>
<td>4</td>
</tr>
</tbody>
</table>

Means within a row followed by the same letter are not significantly different (Tukey–Kramer $p < 0.05$).

**Laboratory bioassays**

To determine the effective dose of diflovidazin, five concentrations including 1000, 1500, 2200, 3300, and 5000 ppm were used. The experiments were done according to leaf dip method (Helle and Overmeer 1985). The mortality covering the range of 10–90%. The leaf discs were immersed for 15 s at concentrations of diflovidazin. The control leaf discs were dipped in distilled water. Then, leaf discs dried in room condition for 3 h. Twenty same-aged adults (from both sexes) were transferred to leaf discs with a diameter of 3 cm and placed in Petri dishes with diameter of 6 cm. Mortality of adult was evaluated after 24 h. Mites were considered as dead if after excitation by brush did not show any reaction. In this study, four replicates were used for each concentration.

**Effect of sublethal concentrations on biological parameters of *T. urticae***

In order to evaluate the sublethal effects of Flumite® on two-spotted spider mite, bean leaf discs were treated with sublethal concentrations including LC$_{5}$, LC$_{10}$, and LC$_{20}$ and allowed to dry for 2 h. One hundred same-aged (24 h old) adult mite were used for each treatment. After 24 h, they were separately transferred to leaf disc with 3 cm diameter on wet filter paper in Petri dishes. After 24 h, one egg as the basis was placed in each Petri dish and other eggs laid by female and mite were removed from Petri dish.

Females were paired with males for study fecundity and population parameters. In order to avoid creating tension in mites (due to aging), leaves of beans were replaced by fresh leaves every 48 h. All Petri dishes were checked every 24 h, and changes were recorded until the death of the last female.

**Statistical analysis**

In order to determine the LC values and sublethal concentrations, we used IBM SPSS Ver. 19.0. All crude data obtained from life cycle for determination of biological parameters were analysed based on life table theory (Chu and Liu 1985) by using the computer programme WOSEX-MS Chart (Chu 2016). The means and standard errors were estimated, using with Bootstrap method (100,000 replications). Differences between means were compared with the Tukey–Kramer procedure by using SAS (SAS Institute 2002).

**Results**

**Concentration–response bioassay**

The LC$_{5}$ values and sublethal concentrations (LC$_{10}$, LC$_{20}$) for *T. urticae* were estimated to be 725.1, 941.2, and 1290.8 ppm, respectively. No mortality was observed in control (Table 1).

**Development time, longevity, and total life span**

There was no significant difference among pre-adult stages such as egg, larva, protonymph, and deutonymph for male in all experimental treatments (egg: $F = 0.09, df = 3, p = 0.96$; larva: $F = 0.11, df = 3, p = 0.95$; protonymph: $F = 0.13, df = 3, p = 0.94$; deutonymph: $F = 0.21, df = 3, p = 0.88$). By the way, there was no significant difference in egg and protonymph stages for tested females (egg: $F = 1.81, df = 3, p = 0.14$; protonymph: $F = 1.97, df = 3, p = 0.11$). The maximum time required to complete larval and protonymph stages was 2.2 and
2.4 days for females treated with LC₅ and LC₂₀ doses, respectively (larval: $F = 2.95$, $df = 3$, $p = 0.03$; protonymph: $F = 1.97$, $df = 3$, $p = 0.11$). The longevity of male and female was ranged between 8.23 to 10.56 and 9.90 to 13.01 days, respectively ($df = 3$, $p < 0.0001$ for both sex). The minimum value of total life span was obtained for LC₂₀ in both sex (Table 2).

**Reproduction**

The pre-oviposition period was not affected by all experimental treatments ($F = 0.99$, $df = 3$, $p = 0.39$) (Table 3). The total pre-oviposition period was ranged between 11.76 and 11.94 ($F = 2.6$, $df = 3$, $p = 0.05$). The minimum number of oviposition day was 7.72 for LC₂₀ treatment ($F = 3.98$, $df = 3$, $p < 0.0001$). Total fecundity ranged from 38.92 to 61.10 (offspring/individual) ($F = 4.88$, $df = 3$, $p < 0.0001$).

**Population parameters**

The demographic parameters of *T. urticae* affected by sublethal treatment of diflovidazin are depicted in Table 4. Gross reproductive rate (GRR) demonstrated a decline trend by increasing experimental concentrations. The lowest value of $R_0$ was obtained for the mites affected by LC₂₀ treatment. Assessed intrinsic rate of increase for mites influenced by sublethal doses was ranged from 0.21 to 0.23 day$^{-1}$. The finite rate of increase ($\lambda$) indicated a non-significant difference with increasing dose from control to LC₂₀. The mean generation time obtained was 15.74 and 15.84 days for LC₁₀ and LC₂₀ which were significantly different from LC₅ and the mites treated by distilled water. In cohort treated with LC₂₀, every 3.18 days the population doubled, which was the highest value between other treatments.

**Survival and fecundity**

Figure 1 compares the $i_x$, $m_x$, and $f_j$ of *T. urticae* at different concentration of diflovidazin. According to Figure 1, the total life time for untreated mites was 26 days, while it was 26, 23, and 24 days for the mites treated at LC₅, LC₁₀, and LC₂₀ acaricide concentrations.

The highest value of $m_x$ was 5.34 eggs/individual/day for control which was occurred on twenty-first day of life span. This value was estimated to be 5.06, 4.81, and 4.82 eggs/individual/day that was happened on twenty-first, seventeenth, and sixteenth day which was recorded for the mites treated at LC₅, LC₁₀, and LC₂₀, respectively. The age-stage-specific survival rate ($S_{ij}$) curve indicated chance that a spider mite egg will survive to age $x$ and stage $j$ (Figure 2). This curve showed separately different life stages of *T. urticae* (Figure 2). The highest female survival rate obtained 80% and 16% in LC₂₀ and control for female and male, respectively.

**Discussion**

This study provides the population parameters and demographic data related to offspring in treated *T. urticae* with sublethal concentrations of Flumite. The recommended field rate for controlling the two-spotted spider mite is 500 μg ml$^{-1}$, based on the instruction mentioned in the label (Agro-Chemie). A number of studies have been conducted for evaluating the lethal and

### Table 4. Life table parameters (mean ± SE) of offspring from females of *Tetranychus urticae* treated with sublethal concentrations of diflovidazin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>LC₅</th>
<th>LC₁₀</th>
<th>LC₂₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRR (offspring/individual)</td>
<td>54.01 ± 2.14$^a$</td>
<td>54.47 ± 1.96$^a$</td>
<td>39.26 ± 1.65$^b$</td>
<td>36.99 ± 1.81$^b$</td>
</tr>
<tr>
<td>$R_0$ (offspring/individual)</td>
<td>48.88 ± 2.44$^a$</td>
<td>48.50 ± 2.39$^a$</td>
<td>35.48 ± 1.85$^b$</td>
<td>31.14 ± 1.61$^b$</td>
</tr>
<tr>
<td>$\lambda$ (day$^{-1}$)</td>
<td>0.234 ± 0.003$^a$</td>
<td>0.233 ± 0.003$^a$</td>
<td>0.226 ± 0.003$^b$</td>
<td>0.216 ± 0.03$^b$</td>
</tr>
<tr>
<td>$T$ (day)</td>
<td>16.61 ± 0.08$^a$</td>
<td>16.62 ± 0.08$^a$</td>
<td>15.74 ± 0.07$^b$</td>
<td>15.84 ± 0.09$^b$</td>
</tr>
<tr>
<td>DT (day)</td>
<td>2.94 ± 0.05$^a$</td>
<td>2.95 ± 0.05$^a$</td>
<td>3.04 ± 0.06$^a$</td>
<td>3.18 ± 0.07$^a$</td>
</tr>
</tbody>
</table>

Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 100,000 bootstraps and compared by using paired $t$-test at 5% significance level.

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Figure 1. Age-specific survivorship ($i_x$), age-stage fecundity of female ($f_j$) (offspring), and age-specific fecundity ($m_x$) of *Tetranychus urticae* treated with acaricide concentrations.
sublethal effects of various pesticide groups such as tetrazine, tetracnide, pyrazolium, pyrethroid, organophosphate, pyridine azomethines, and neonicotinoid derivatives on two-spotted spider mite, as well as its predatory mites (Marcic 2003; Castagnoli et al. 2005; Li et al. 2006; Çobanoğlu and Alzoubi 2008; Hamedi et al. 2010, 2011; Lima et al. 2013; Alinejad et al. 2016). However, no evidence is available with respect to the sublethal effects of diflovidazin (an acaricide from tetrazine group) on biological parameters of the two spotted-spider mite. Based on the data, the experimental concentrations played a negative role during all pre-adult developmental stages such as egg, larva, protonymph, and deutonymph among males. Regarding females, no significant difference was observed between the various stages of pre-puberty for all the tested concentrations, except in egg and protonymph stages. Contrarily, Li et al. (2017) reported that an increase in the concentration caused a significant difference in both males and females when treated by sublethal concentrations of bifenazate during pre-adult stages of *T. urticae* due to the acaricides mode of action. According to Van Nieuwenhuyse et al. (2012), bifenazate acts as a synergist or the allosteric modulator of functionally expressed Gamma-Aminobutyric acid (GABA) receptor homologues, while diflovidazin acts as the inhibitors of mite growth (Dubey et al. 2016). The results of the present study indicated that the use of different concentrations of the diflovidazin had a significant and negative effect on the longevity and the total lifetime in both males and females. The results are consistent with those of Alinejad et al. (2015), in which a significant decrease happened in longevity and life span after treating with sublethal concentrations of fenazaquin. Similarly, in the study of Martinez-Villar et al. (2005), a significant decrease occurred in the longevity for mites treated with azadirachtin at 64 and 128 ppm.

The net reproduction rate (R$_0$) reduced significantly for spider mites treated with LC$_{10}$ and LC$_{20}$ compared to the control treatment. The results are in line with those of Wang et al. (2014) and Marcic (2007), who examined the sublethal doses of bifenthrin (LC$_{10}$ and LC$_{20}$) and spiromoctfen (6, 12, 24, 48, and 96 mg/l) on the two-spotted spider mite, respectively. Based on the results of the present study, an increase in the intrinsic rate (r) resulted in decreasing the population treated with the LC$_{10}$ and LC$_{20}$ concentrations of diflovidazin compared to the control treatment, which had harmful effects on the offspring produced by each individual, leading to a reduction in population, compared to the untreated cohort. However, the results were inconsistent with those of Wang et al. (2016), in which an increase of LC$_{10}$ to LC$_{20}$ in spinetorom led to the enhancement of r, which may be related to the pesticide group (spinetorom is the fermentation product of *Saccharopolyspora spinosa*, and an analogue of spinosad).

In the present study, a non-significant difference was observed in increasing the finite rate of λ between different concentrations for the treated *T. urticae*, which is consistent with the results of Sænzer-de-Cabezón et al. (2006), who investigated the effect of triflumuron on *T. urticae*. The lowest mean generation time (T) occurred in LC$_{20}$ dose, which indicated a significant difference, compared to the control treatment. The result is congruent with those reported by Alinejad et al. (2015). The doubling time (DT) was higher for the spider mites treated by the LC$_{20}$ concentration in the diflovidazin compared to the control treatment. In other words, more time is needed for spider mites which are exposed to higher concentration in order to make up for the lost people. The result is incongruent with the finding of Mohammadi et al. (2016) regarding the sublethal effects of biomite on the *T. turkestani*. The difference can be related to the pest species and their examined concentration (LC$_{20}$).

Based on the results of the present study, the exposure to sublethal concentrations of diflovidazin during the adult stage played a negative effect on biological parameters of *T. urticae* (i.e. lower $R_0$, r values, and longer DT). Regarding the curves of survival and age-specific fecundity, an increase in the concentration of this acaricide has a downward trend in $l_x$ and $m_x$ values. The similar trend was reported for the survival probability of *T. urticae* and *Phytoseiulus persimilis* Athias-Henriot, as the predator, when the mites were treated by lethal doses of bifenthrin and dimethoate (Alzoubi and Çobanoglu 2010). The results indicated an overlap between the various stages of two-spotted spider mite life regarding the curve of the age-stage-specific survival rate ($s_{xy}$).

The current study showed that exposure of sublethal doses of diflovidazin severely affects various life table attributes of *T. urticae*, i.e. survival, fecundity, GRR, mean generation time (T), and net reproductive rate ($R_0$), whereas effect of diflovidazin is not significant on increase rate of increase (r) and finite rate of increase (λ) at sublethal concentration. Further,
based on the results of another study, no adverse effects of this acaricide was observed on one species of the phytoseiid mites, Neoseiulus californicus (Havasi et al., unpublished data). Consequently, it is recommended that applying difludazin at lower rates could lead to efficient control of *T. urticae* through releasing the appropriate predatory mites.

Acknowledgement

We greatly appreciate the University of Tehran’s support on this project.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the University of Tehran.

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References


Mollaloo MG, Kheradmand K, Sadeghi R, Talebi AA. 2017. Demographic analysis of sublethal effects of spiromesifen on