Research Article


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**Abstract:** The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important pest species, with an extended range of host plants in the world. Use of chemical acaricides is one of the principle ways of controlling this pest. Considering the resistance to acaricides, as well as undesirable effects of chemical compounds, finding a suitable alternative way to control this pest is necessary. In this study sublethal effects of Dayabon®, a herbal extract, were investigated on some biological parameter of *T. urticae* at 2.140 g/l (LC₅), 2.682 g/l (LC₁₀) and 3.526 g/l (LC₂₀). The estimated LC₅₀ for *T. urticae* adult was 5.950 g/l. When adults were treated with LC₁₀ and LC₂₀ of Dayabon, the oviposition period was significantly reduced compared to LC₅. The highest and the lowest fecundity (number of eggs laid per female) were 56.02 offspring/individual in control and 39.11 offspring/individual at LC₂₀ respectively. In addition the net reproductive rate (R₀) was decreased as concentration increased. Whereas, intrinsic rate of increase (r) and finite rate of increase (λ) were not affected by Dayabon. Considering the detrimental effects of Dayabon on some biological parameters of *T. urticae*, it can be concluded that this product can be used to develop targeted interventions aimed at integrated management of *T. urticae*.

**Keywords:** life table, *Tetranychus urticae*, sublethal effects, Dayabon, biological parameter

**Introduction**

*Tetranychus urticae* Koch (Acari: Tetranychidae) is famous as an extremely polyphagous and economically important pest in most parts of the world (Devine et al., 2001; Zhang, 2003). It feeds on most organs of plants, such as parenchyma tissue of leaves, flower and fruits resulting in a significant yield loss in many horticultural, ornamental, and agronomic crops worldwide. Appearance of White or yellow spots due to Chlorophyll synthesis inhibition on the leaf surface of plants, production of silken webs, reduction in plant productivity, fine stippling, leaf drop are some of the signs and symptoms caused by this moth and thereby reducing yield and death of plants are the contributory factors behind feeding and sucking of these mites on plant cells (Park and Lee, 2002; Mondal and Ara, 2006; Kulkarni et al., 2008).

Spider mites, more specifically *T. urticae*, develop resistance against acaricide, owing to high fecundity, fast development and arrhenotokous reproduction (Van Leeuwen et
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Considering the different types of environmental and toxicological problems due to pesticides and their ineffectiveness against the population of T. urticae (Zhang, 2003; Yorulmaz and Ay, 2009; Motazedian et al., 2011), more attention should be paid towards exploitation of alternative IPM strategies.

In order to minimize the harmful effects of chemicals on non-target organisms such as natural enemies; plant-based insecticides are considered as a safe alternative (El-Zemity et al., 2009; Pavela and Vrchotová, 2013). During the last decade, much more information has become available on toxicity of a range of essential oils, as well as plant extracts on two-spotted spider mite (Kumral et al., 2010; Kheradman et al., 2015; Ribeiro et al., 2016).

Dayabon®, a new plant-based insecticide which has been formulated based on castor essential oil is selective, biodegradable, and has little effect on non-target organisms and the environment. Although it was designed for haustellate pests, especially two-spotted spider mite and whiteflies, its satisfactory effects have been reported on other pests, such as Xanthogaleruca luteollla (Muller) (Vahabi Mashhur et al., 2016a,b), Aphis fabae Scop., Aphis nerii Boyer (Amini, 2018), Myzus persicae (Sulzer) and its biological control agent, Aphidius matricariae (Haliday) (Rezaei and Moharramipour, 2019), Brevicoryne brassicae (L.) (Rezaei et al., 2014); and important ectoparasitoid wasp of lepidopteran pests, Habrobracon hebetor Say (Asadi et al., 2019). The fact remains that in order to prevent the revitalisation of pest resistance development, application of other strategies are necessary in integrated pest management (IPM).

Given the fact that there is no evidence of toxicity on non-targets, as well as the effects of this new acaricide on T. urticae, therefore the objective of this study was to evaluate and validate the sublethal effects of Dayabon on demographic parameters of T. urticae as an effective and low-risk product for control of two-spotted spider mite that is also eco-friendly.

Materials and Methods

Host plant

Bean seeds (Phaseolus vulgaris L. var Khomein) were grown in plastic pots (15 cm diameter) at a rate of 4-5 seeds per pot under controlled greenhouse condition at 25 ± 5 °C, 60 ± 10% R. H. and a photoperiod of 16:8 (L: D) h.

Mite culture

The initial population of T. urticae was obtained from an infested greenhouse in Pakdasht region (south eastern part of Tehran, Iran; 35.4669° N, 51.6861° E). Infested leaves with T. urticae were transferred to bean plants and left to reproduce under the laboratory conditions (temperature of 25 ± 2 °C, 60 ± 5% RH and a photoperiod of 16:8 (L: D) h). Fresh bean plants were provided to the colony from time to time when it was necessary.

Chemical tested

Dayabon (SL10%) which is the castor oil based botanical pesticide was obtained from Daya Nanotechnologists Company, Tehran, Iran.

Bioassays

To assess the effects of Dayabon on life table parameters of T. urticae, a modified leaf dip method was used (Helle and Overmeer, 1985). The six concentrations that resulted in 10–90% mortality were identified. Leaf discs were dipped into one of six Dayabon solution for 15 seconds. The control leaf discs were submerged into distilled water. After drying the leaf discs in room chamber for 2 h, twenty same aged adults (from both sexes) were transferred on leaf discs (3 cm diameter). Each leaf disc then located on a sponge in a Petri dish (6 cm diameter). All Petri dishes were maintained in an incubator at 25 ± 2 °C, 60 ± 5% RH and a photoperiod of 16:8 (L: D) h. Adult’s mortality was evaluated after 24 h. Mites were considered as dead if after touching with a small fine brush, they could not crawl and were non-functional. Abbott's formula (Abbott,
was performed with paired bootstrap test (100,000 ×), using TWOSEX-MS Chart.

Results

Concentration-response bioassay
The estimated LC50 for *T. urticae* was 5.950 g/l. No mortality was recorded for control treatment (Table 1). In addition, the values of LC5, LC10, and LC20 were 2.140, 2.682 and 3.526 g/l, respectively.

Development time, longevity and total life span
Sublethal effects of experimental doses on development time, longevity and total life span for both sexes are given in Table 2. None of egg, larvae, protonymph, as well as deutonymph of male individuals were affected by different doses of Dayabon (egg: $F = 0.155, df = 3.58, P = 0.923$, larva: $F = 0.135; df = 3.58; P = 0.946$, protonymph: $F = 0.135, df = 3.58; P = 0.945$, deutonymph: $F = 0.124; df = 3.58; P = 0.954$); while except deutonymph, other stages of females were significantly influenced by Dayabon (egg: $F = 1.216; df = 3.137; P = 0.325$, larva: $F = 0.526; df = 3.137; P = 0.667$, protonymph: $F = 1.123; df = 3.137; P = 0.348$, deutonymph: $F = 3.737; df = 3.137; P = 0.010$). When *T. urticae* individuals were exposed to the LC20 of Dayabon, male adult longevity was significantly shortened ($F = 4.634; df = 3.58; P = 0.005$; Table 2). Furthermore, female adult longevity, as well as female total life span was significantly shortened following treatment with LC10 and LC20 ($F = 127.654; df = 3.137; P < 0.0001$; Table 2).

Reproductive parameters
Neither total pre-ovipositional period (TPOP), nor adult pre-ovipositional period (APOP) was affected by Dayabon (TPOP: $F = 0.128; df = 3.137; P = 0.944$, APOP: $F = 1.335; df = 3.137; P = 0.268$) (Table 3). As can be seen from Table 3, when adults were treated with LC10 and LC20 of Dayabon, the oviposition period was significantly reduced compared with other treatments (F = 360.556; df = 3.137; P < 0.001). The highest and the lowest number of eggs laid per female were recorded for control and LC20 concentration, respectively (Table 3).
**Sublethal effects of Dayabon on T. urticae**

The age-specific survivorship ($l_x$) and age-specific fecundity ($m_x$) of *T. urticae*, are depicted in Fig. 1 and Fig. 2, respectively. The survivorships of the individuals declined in a concentration-dependent manner (Fig. 1). The total life span for untreated mites was 26 days, while it was 25, 23 and 22 days for the mites treated with LC$_5$, LC$_{10}$ and LC$_{20}$, respectively. The maximum value of $m_x$ was 4.3, 4 and 4.02 eggs/individual for the mites treated with LC$_5$, LC$_{10}$ and LC$_{20}$, respectively which occurred on...
the 20th, 16th and 15th days of their life span, respectively. In comparison, the value of \( m_1 \) was the highest (4.85 eggs/individual) on 21st day of the life span for the mites treated with distilled water (Fig. 2).

The curves of age-stage specific survival rate (\( s_{xj} \)) illustrating the probability that an egg of \( T. urticae \) will survive to age \( x \) and develop to stage \( j \), of treated and untreated mites are shown in Fig. 3. Different developmental rates among the individuals gave rise to obvious overlap among the curves of different stages (Fig. 3). The highest survival rate (68%) was allocated to control and LC5 for females, and to LC5 for males (22%).

![Figure 1](image1.png)

**Figure 1** Age-specific survivorship (lx) of offspring of the treated and untreated mites of *Tetranychus urticae*.

![Figure 2](image2.png)

**Figure 2** Age-specific fecundity (\( m_x \)) of the offspring of the treated and untreated mites of *Tetranychus urticae*.

### Life table parameters

Table 4 represents population growth parameters of *T. urticae* after treatment with the Dayabon. It is apparent that the lowest and the highest value of GRR were observed at the highest concentration (LC20) and distilled water, respectively. The \( R_0 \) values were declined as concentration of Dayabon increased (Table 4). Whereas, the intrinsic rate of increase (\( r \)) and finite rate of increase (\( \lambda \)) were not affected by Dayabon. Mean generation time (\( T \)) ranged from 15.6 to 16.58 days for the mites treated with LC20 and control, respectively (Table 4). There was no significant differences among concentrations with regard to doubling time (\( DT \)) (Table 4).

![Table 4](table.png)
Sublethal effects of Dayabon on *T. urticae* table 4 mean (± se) of life table parameters of offspring from females of *tetranychus urticae* treated with sublethal concentrations of Dayabon.

<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)¹</td>
<td>48.28 ± 2.92ᵃ</td>
<td>45.06 ± 2.63ᵃ</td>
<td>36.79 ± 2.49ᵇ</td>
<td>31.10 ± 2.21ᵇ</td>
</tr>
<tr>
<td>R₀ (offspring/individual)²</td>
<td>38.42 ± 3.14ᵃ</td>
<td>37.98 ± 3.11ᵇ</td>
<td>29.20 ± 2.61ᵇ</td>
<td>26.07 ± 2.23ᵇ</td>
</tr>
<tr>
<td>r (day⁻¹)³</td>
<td>0.2198 ± 0.005ᵃ</td>
<td>0.2194 ± 0.005ᵇ</td>
<td>0.2122 ± 0.006ᵇ</td>
<td>0.2087 ± 0.005ᵇ</td>
</tr>
<tr>
<td>λ (day⁻¹)⁴</td>
<td>1.245 ± 0.006ᵃ</td>
<td>1.245 ± 0.006ᵇ</td>
<td>1.236 ± 0.007ᵇ</td>
<td>1.232 ± 0.007ᵇ</td>
</tr>
<tr>
<td>T (day)⁵</td>
<td>16.58 ± 0.12ᵃ</td>
<td>16.56 ± 0.12ᵇ</td>
<td>15.87 ± 0.12ᵇ</td>
<td>15.60 ± 0.11ᵇ</td>
</tr>
<tr>
<td>DT (day)⁶</td>
<td>3.13 ± 0.05ᵃ</td>
<td>3.14 ± 0.05ᵇ</td>
<td>3.25 ± 0.06ᵇ</td>
<td>3.30 ± 0.07ᵇ</td>
</tr>
</tbody>
</table>

Means within a row followed by the same letter are not significantly different. The standard errors were estimated using 100,000 bootstraps and compared using paired bootstraps test at 5% significance level. Abbreviations: ¹ gross reproductive rate, ² net reproductive rate, ³ intrinsic rate of increase, ⁴ finite rate of increase, ⁵ mean generation time, ⁶ doubling time.

Discussion

Botanical pesticides have attended more interest as an alternative for mite control during the last two decades. There are grounds for supporting the acaricidal activity of essential oils from different plants against phytophagous mites. The lethal effects of pesticides only give an
incomplete measure of their disadvantageous effects; thus, sublethal effects on arthropod physiology and behaviour should also be considered for a whole analysis (Desneux et al., 2007). Sublethal effects can be very tender and affect populations at concentrations lower than the traditional concentration-response curve (Stark and Banks, 2003). It is also worth mentioning that several attempts have been made to investigate the sub-lethal effects of different herbal extracts on biological parameters of T. urticae (Mansour et al., 1987; Sundaram and Sloane, 1995; Kheradmand et al., 2015; Akyazi et al., 2018). However, this is the first experiment reporting the toxicity, as well as sub-lethal effects of Dayabon on T. urticae.

Although the estimated LC$_{50}$ in the current study was high compared with other chemical acaricides such as Bifenazate, Chlorfenapyr, Propargite, Dicofol and Hexythiazox that are used against T. urticae (Kumari et al., 2017; Li et al., 2017), differences in mode of action for various pesticides may be an important contributory factor in these differences. For example, 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, Hexythiazox is a molt inhibitor with a mode of action similar to etoxazole (a growth regulator that inhibits chitin biosynthesis) (Dekeyser 2005, Nauen and Smagghe, 2006). Furthermore, our results do not accord with the study of Najafabadi et al. (2014) who demonstrated the LC$_{50}$ values of three essential oils on T. urticae. These differences among various studies are quite revealing in several ways. First, the variable toxicity of a pesticide made from plant extracts against different pests is largely attributable to not only plant's phenological age, but also the parts of plant used for extraction (Chiasson et al., 2001). Second, oil type and applied concentration may be factors behind these differences. Finally, the low values of essential oils LC$_{50}$ result not only from their fumigant action, but also penetration of volatile oil into the organism’s body through respiratory system (Kim et al., 2003; Choi et al., 2004). According to our results, pre-adult development of both sexes (exempt deutonymph stage at female) were not influenced by Dayabon. These findings are in agreement with the study of Martinez-Villar et al. (2005) which showed no significant effect of azadirachtin on development of T. urticae. Furthermore, our results indicated that treatments of T. urticae adults with LC$_{20}$ of Dayabon reduced the female deutonymphal period, as well as male adult longevity; and treatment with LC$_{10}$ and LC$_{20}$ significantly decreased female adult longevity, along with female total life span. A significant reduction in adult longevity of T. urticae as a consequence of treatment with azadirachtin (Martinez-Villar et al., 2005) and pyrrolizidine alkaloids (Pietrosiuk et al., 2003) has been reported previously.

Our results showed that although pre-oviposition periods were not affected by different doses of Dayabon; yet fecundity were declined in a concentration-dependent manner. The lowest fecundity and the shortest oviposition period were observed at LC$_{20}$ treatment showing the low potential of treated mites for population recovery. Pietrosiuk et al. (2003) assessed the effect of pyrrolizidine alkaloids extracted from Lithospermum canescens (Borraginaceae) on T. urticae, and their results indicated that mites treated with alkaloids showed a decrease in female fecundity. In addition, the oviposition period decreased significantly by increasing the concentration of Dayabon from LC$_{5}$ to LC$_{10}$ and LC$_{20}$. Also, Roh et al. (2011) showed 89.3% reduction in fecundity of mites when leaf discs treated with sandalwood oil.

In the current study, the population parameters such as net reproductive rate ($R_0$), gross reproductive rate ($GRR$), and mean generation time ($T$) in the treated (LC$_{10}$ and LC$_{20}$) populations were significantly lower than those in the control. These results were higher than what was estimated for the mites treated with Artemisia annua essential oil (Esmaeily et al., 2017); but were close to that reported for T. urticae treated with Thymus
vulgaris essential oil (Gholamzadeh Chitgar et al., 2013). These differences can be explained by the type of oil, as well as the concentration. The population parameters including the intrinsic rate of increase (r), finite rate of increase (λ) were not affected by sublethal concentrations of Dayabon. In contrast to results obtained by us, Musa et al. (2017) detected that T. urticae females exposed to Requiem®, a commercial formulation of Chenopodium ambrosioides, at 2.5 ml/l showed a significant effect on r and λ values. Study on the life table parameters in the current and next generations is necessary to expand an integrated pest management strategy (Frel et al., 2003; Sedaratian et al., 2011).

It could be reasonably argued that exposure to pesticides can give rise to hereditary malfunctions and malformations leading to significant disturbances of insect or mite development in the next generations. Negative effects on the population increase of T. urticae, as a result of exposure to sublethal concentrations of different acaricides including fenazaquin (Alinejad et al., 2015) and spirodiclofen (Marcic, 2007) have been reported previously. Conversely, enhancing in T. urticae population growth parameters by virtue of exposure to sublethal or lethal doses of spinetoram (Wang et al., 2016) and clofentezine (Marcic, 2003) has been highlighted. It seems possible that this enhancement is due to hormoligosis defined as the stimulation of reproductive physiology by sublethal doses of pesticides. Taking population growth parameters in the current study into consideration, it is remarkable that exposure to LC₁₀ and LC₂₀ of Dayabon resulted in negative influences on T. urticae population increase of progeny (that is to say, lower R₀, GRR and fecundity). While it is true that there is not any evidence of the effect of hormoligosis in this study for the simple reason that none of intrinsic and finite rate of increase was significantly affected by different doses of Dayabon; the fact remains that differences among mentioned studies are attributable to not only different modes of action of acaricides, but also the concentrations used. Moreover, in this study, the indirect leaf dip method was used to investigate the effect of Dayabon on T. urticae, so that mite’s body was not covered by the Dayabon solution. It is recommended that the effect of direct dipping or spraying the mites by the Dayabon solution be investigated on the life table of this pest. It seems that the results will be changed effectively if the mite’s body be directly in contact with the toxic solution. From this study, it could be concluded that the presence of Dayabon on leaf surface can alter leaf surface quality in a way that affects the performance of T. urticae in view of life table and reproductive parameters.

In conclusion, intrinsic and finite rates of increase of T. urticae did not get affected by sublethal concentrations of Dayabon, but then again Dayabon treatment resulted in negative effects on other biological and life table parameters of T. urticae, including the prolonged deutonymphal stage, decreased adult male and female longevities, fecundity, oviposition period, net, as well as gross reproductive rates. These findings can be used to develop targeted interventions aimed at integrated management of T. urticae. Finally, it is recommended that further research be undertaken in the following arenas: (i) to investigate the direct effects covering the mite’s body by Dayabon, (ii) to investigate other aspects, especially development of resistance to these natural products; (iii) semi-field and field studies aiming to evaluate the efficacy of the acaricide to obtain more applicable results under field conditions.

References


Sublethal effects of Dayabon on *T. urticae* 


Piętrosiuk, A., Furmanowa, M., Kropczyńska, D., Kawka, B. and Wiedenfeld, H. 2003. Life history parameters of the two-spotted spider mite (*Tetranychus urticae* Koch) feeding on bean leaves treated with...
Tetranychus urticae

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Abstract: The sublethal effects of Dayabon® on T. urticae were investigated. The results showed that Dayabon® at sublethal concentrations significantly reduced the population of T. urticae. The LC50 and LC90 values were calculated to be 20.5 and 30.3 mg/L, respectively. The study concluded that Dayabon® can be used as an effective and safe alternative to traditional pesticides for the control of T. urticae.