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Sublethal Effects of *Atropa belladonna* Herbal Extract on Biological Performance of the Two-Spotted Spider Mite *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Abstract

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important pests of agricultural products. Using chemical pesticides is one of the main methods for its management. Due to undesirable effects of synthetic pesticides, this research was conducted to achieve alternative safe compounds. In this study sublethal effect of *Atropa belladonna* herbal extract including LC_{10}, LC_{20}, LC_{30} and distilled water were evaluated on biological parameters (such as r, λ, *GRR*, R_0 and T) of *T. urticae*. The experiments were done under laboratory condition at 25±2 °C, 60±5% RH, and a photoperiod of 16:8 (L: D) hours. The crude data were analyzed 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 38.41 and 18.08 offspring/individual in control and LC_{30}, respectively. The maximum values of intrinsic rate of increase (r) was 0.2201 day^{-1} that occurred on control treatment while the minimum values was obtained 0.1937 day^{-1} in LC_{30} concentration. Finite rate of increase (λ) had significantly descended with concentration enhancing from LC_{20} to LC_{30}, compared with the control. The results demonstrated that *Atropa belladonna* herbal extract could be incorporated in integrated pest management (IPM) programs of *T. urticae*.

Keywords: *Atropa belladonna*, *Tetranychus urticae*, life table parameters, sublethal effects

1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is an serious pest of many greenhouse plants and field crops (Cote et al., 2002; Sedaratian et al., 2009; Khanamani et al., 2013; Maleknia et al., 2016). The phytophagous mite *T. urticae* damage may include webbing, fine stippling, leaf yellowing, leaf crop, and even plant death (Helle & Sabelis, 1985). This pest with a short life span, high reproductive ability, multiple births and plant nutrition can stop plant growth and reduce the quality and quantity of the crop (Saeedi & Arbabi, 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 pesticides (Zhao, 2000).

The wide use of pesticides leads to affect non-target organisms (Croft, 1990), human safety (Garcia-Marı & Gonzalez-Zamora, 1999), the emergence of secondary pests (Elzen, 2001) and development of resistance (Brattsten et al., 1986). Also because of the adverse effect of pesticide use, alternative control methods are being researched for *T. urticae* (Yorulmaz et al., 2014). Plant compounds such as extracts were used as insecticides (Ofuya & Okuku, 1994; Kim et al., 2003), acaricidals (Rim & Jee, 2006; Fernandes & Freitas, 2007). The *Atropa belladonna* L. (Solanaceae) is a perennial herb that is cultivated throughout the world. This plant belongs to the Caspian region and grows on the edge of forests and sub-trees. The geographical distribution of this species is in Europe, Turkey and northern Iran (Rita & Animesh, 2011). The scientific name *A. belladonna* is one of the most important medicinal plants of the Solanaceae family (Berdai et al., 2012). It has natural habitats in the northern regions of Iran. The berries are sweet and are consumed by animals that disperse the seeds in their droppings, even though they contain toxic alkaloids (Kay, 1992). A number of studies have been conducted for evaluating the lethal and sublethal effects of various herbal extracts, on two-spotted spider mite (Choi et al., 2004; Wang et

al., 2007; Kumral et al., 2010; Pavela, 2015), but no reports have been made about the effects of this plant extract on two-spotted spider mite. The effect of pesticides on insects usually is estimated in experimental conditions based on LC_{50}. Sublethal effect studies have been used to assess the selectivity of pesticides to beneficial arthropods (Teodoro et al., 2005; Poletti et al., 2007).

However, the common method for estimating the effect of pesticides on insects only addresses the effects of lethality and mortality data, so demographic toxicological studies or toxicological evaluation tests on insect life table that provide more accurate information can be recommended. In these studies, the effects of pesticides on a (homogeneous) same age group of survivors from the impact of the pesticide are investigated and the survival, fecundity and mortality of this group are studied until the end of lifespan. It also provides a measure of population growth rates. The use of the inherent rate of population increase as a biometric parameter has been recommended in toxicological studies (Boykin & Campbell, 1982). Therefore, in predicting the effect of pesticide on the insect, life-history tests are more reliable than their lethal dose estimation. Only essential oils derived from some plants can be good alternative methods to controlling this pest. Essences, secondary metabolites of plants with aromatic compounds, have low molecular weight compounds and volatile compounds with proven pesticide properties (Bakkali et al., 2008). A number of essential oils act selectively and do not affect non- target organisms. So far the properties of several herbal essential oils are studied for controlling two-spotted spider mite (Cavalccanti et al., 2010; Han et al., 2010). Also, many studies have been done on the sublethal effects of herbal essential oils, such as repulsion effects, nutritional inhibition, breeding prevention and breeding (Choi et al., 2004). Many of these plant compounds are able to affect the growth and reproduction parameters of pests and their various growth stages (Tsolakis & Ragusa, 2008). According to the researches done so far the effect of *A. belladonna* herbal extract has not been reported on two-spotted spider mite.

Consequently, taking the activity of this herbal extract, in this experiment, the effects of sublethal concentrations of *Atropa belladonna* herbal extract on *T. urticae* were investigated using demographic toxicological analysis. Being a part of systematic evaluation of the acaricidal potential of the plants, the objective of this study was to evaluate and validate the effects of sublethal concentrations of *A. belladonna* herbal extract on demographic parameters of *T. urticae*, to effective and low-risk control of two-spotted spider mite that is eco-friendly.

2. Materials and Method

2.1 Host Plant Rearing

Beans plants (*phaseolus vulgaris* L. var. Khomein) were planted as host plant for *T. urticae* rearing. The plants were reared in pots with 15 cm diameter under laboratory conditions at 25±2 °C, 60±5% R.H. and a photoperiod of 16:8 (L:D) hours.

2.2 Mite Colonies

Primary population of *T. urticae* was collected from infested greenhouses of rose with scientific name *Rosa hybrida* from greenhouse around Pakdasht (South eastern of Tehran, Iran) and then grown on beans planted in the greenhouse. The colony of *T. uricae* was placed in the growth chamber with a temperature of 25±2 °C, a relative humidity of 60±5% and a photoperiod period of 16:8 h (light:dark).

2.3 Preparation of Herbal Extract

The *Atropa belladonna* plants were collected from the forests of Golestan province. The leaves were first crushed and then 40 grams of leaves were extracted with ethanol (300 milliliters 70 percent) for 3 hours at each turn using Soxhlet device. The solvent was isolated using a rotary machine at 40 degrees Celsius at 120 rpm.

2.4 Concentration-Response Bioassay

To determine the effective dose of *A. belladonna*, were used five concentrations . The experiments were assessed according to leaf dip bioassay method (Helle & Overmeer, 1985). The mortality covering the range of 10-90%. The leaf discs were dipped for 15 seconds into solution of herbal extract. The control leaf discs were dipped in distilled water only. Then, leaf discs dried in room condition for about 3 hours. Twenty same age (24-h-old) adults mite (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 touching with a small fine brush, they could not crawl and were non-functional. All experimentas were conducted in the laboratory condition at 25±2 °C, 60±5% RH and a photoperiod period of 16:8 h (L:D). In this study four replicates were used for each concentration. There were five concentrations and four replicates per concentration.

2.5 Effect of Sublethal Concentrations on Biological Parameters of Offspring From Treated Females

In order to evaluate the sublethal effects of *A. belladonna* herbal extract on *T. uricae*, bean leaf discs were treated with sublethal concentrations including LC_{10}, LC_{20}, LC_{30} and allowed to dry for 2 h. One hundred same-aged (24-h-old) gravid females were used for each treatment. After 24 hours, the surviving females were separately transferred to leaf disc with 3 cm diameter on wet filter paper in Petri dishes. After 24 hours, one egg as the basis was placed in each Petri dish and other eggs laid by female and mite were removed from Petri dish.

The newly-emerged females were coupled with males for mating after the adult emergence. Finally, the fecundity of females was daily recorded and population parameters were calculated in both male and females until the death of the last sample. In order to avoid creating tension in mites (due to aging) leaves of beans were replaced by fresh leaves every 48 h. The Petri dishes were checked every 24 hours and changes such as developmental time and survival of them were recorded until the death of the last female.

2.6 Data Analysis

The value of LC_{50} and sublethal concentrations, was calculated using the software SPSS ver. 19.0. To compare the significance of the LC_{50} 95% of the confidence interval were used. The raw data obtained from the two-spotted spider mite life cycle for analyzing and determining the parameters of the life table according to the Chi (1988), and Chi and Liu (1988) by using the computer program, TWO-SEX_MsChart (Chi, 2016). The means of the latter parameters were compared by using the paired bootstrap test at the 5% significance level (Riahi et al., 2017; Khanamani et al., 2017). Differences between means were compared with the Turkey's test was carried out using SAS (SAS Institute 2002). The age-specific fecundity (m_x), age-specific survival rate (l_x), age-stage specific survival rate (S_{xj}) (where, x = age in day and j = stage) and the population growth parameters were calculated accordingly:

$$m_x = \frac{\sum_{j=1}^{k} S_{xj} f_{xj}}{\sum_{j=1}^{k} S_{xj}} \quad (1)$$

where, k is the number of stages.

$$l_x = \sum_{j=1}^{k} S_{xj} \quad (2)$$

The intrinsic rate of increase is estimated by using the iterative bisection method from the following equation:

$$\sum_{x=0}^{k} e^{-r(x+1)} \cdot l_x m_x = 1 \quad (3)$$

with age indexed from 0 to ω (Goodman, 1982).

Other parameters (R_0, λ, T, GRR and DT) were calculated by the following equations:

$$R_0 = \sum_{x=0}^{\omega} \sum_{j=1}^{k} S_{xj} f_{xj} \quad (4)$$

$$\lambda = e^{rm} \quad (5)$$

$$T = \frac{\ln R_0}{r_m} \quad (6)$$

The GRR was calucted as (Fathipour & Maleknia, 2016):

$$GRR = \sum_{x=a}^{\beta} m_x \quad (7)$$

3. Results and Discussion

3.1 Concentration-Response Bioassay

The sublethal concentrations of *Atropa belladonna* herbal extract (LC_{10}, LC_{20} and LC_{30}) for *T. urticae* were estimated to be (1650, 2300 and 3000 ppm), respectively. No mortality was observed in control. The value of p is greater than 0.05, indicating the biometric alignment of the lines, and the Chi-squared value also confirms it.

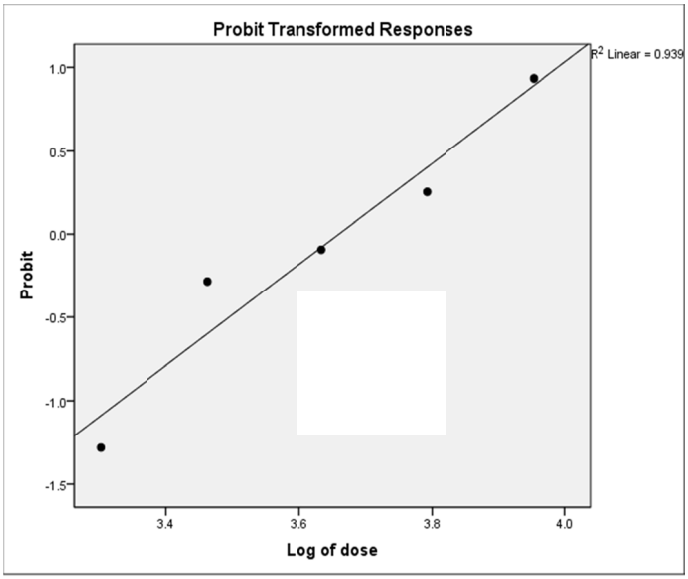

Figure 1. Dose-response curves of *Tetranychus urticae* mites treated with different doses of *Atropa belladonna* herbal extract

3.2 Development time, Longevity and Total Life Span

The impact of *A. belladonna* herbal extract at different stages of growth of two-spotted spider mite is mentioned in Table 1. The longest egg duration was recorded 4.21 days that belongs to LC_{10}. However according to El-gengaihi et al. (2011) the length of egg duration of two-spotted spider mite in treatment with alcoholic extract of *Dondonea viscosa* L. was reported 4.3 days which are consistent with the length of egg stage period in treatment with *A.belladonna* herbal extract. The length of larval period in treatment mites does not show significant difference with control treatment. In the immature stages the sublethal concentration of the *Atropa belladonna* herbal extract does not show significant effect with control. Duration of egg, larval, and deutonymph for both sexes demonstrated the no significant difference between control and experimental concentrations. In fact *A. belladonna* herbal extract had nontoxic effect in this period. But protonymph period markedly affected with LC_{30} in compare to other treatments. The longevity of treated male with LC_{20} and LC_{30} of haerbal extract was significant difference from control and LC_{10}. By increasing concentration from LC_{10} to LC_{30} significantly reduced in longevity of treated females. The minimum value of total life span was obtained 18.83 and 19.35 days for male and female treated with highest concentration of haerbal extract.

The length of lifespan period in control, LC_{10}, LC_{20} and LC_{30} concentrations for female mites, were 23.53, 22.44, 21.44, 19.35 ($F = 242.6$, df = 3, $P < 0.0001$) days, respectively. The control caused the most prolonged life time of *T. urticae*.

Table 1. Effects of sublethal concentrations of *Atropa belladonna* herbal extract on developmental times, adult longevity, and total life span (day±SE) of *Tetranychus urticae*

Parameter	Control	LC10	LC20	LC30
Male				
Egg (day)	4.21±0.11a	4.30± 0.01a	4.30±0.1a	4.13±0.07a
Larva(day)	2.14±0.10a	2.22±0.09a	2.22±0.09a	2.00± 0.09
Protonymph (day)	2.14± 0.10a	2.26±0.09a	2.26±0.09a	2.09±0.12a
Deutonymph (day)	2.14±0.10a	2.26±0.09a	2.26±0.09a	2.35±0.1a
Longevity (day)	10.21±0.15a	9.70 ±0.12a	9.13± 0.17b	8.26±0.23c
Total life span (day)	20.86±0.25a	20.74±0.27a	20.17±0.28ab	18.83±0.34b
Female				
Egg (day)	4.19±0.06a	4.21±0.05a	4.18±0.05a	4.11±0.04a
Larva (day)	2.15±0,05a	2.17±0.05a	2.12±0.05a	2.06±0.04a
Protonymph (day)	2.30±0.07a	2.15±0.04a	2.11±0.04ab	2.06±0.04bc
Deutonymph (day)	2.15±0.05a	2.09±0.04a	2.09±0.04a	2.02±0.05a
Longevity (day)	12.74±0.09a	11.82±0.13b	10.94±0.015c	9.11±0.08d
Total life span (day)	23.53±-0.13a	22.44±0.18b	21.44±0.17a	19.35±0.12d

Note. Means within a row followed by the same letter are not significantly different (Turkey-kramer $p < 0.05$).

3.3 Reproduction

According to Table 2 the duration of oviposition period in treated females with different concentrations significantly reduced in compared to control. However, no significant difference was observed for pre-oviposition period between untreated and treated cohort. In this study, an increase in the dosage of leaf extract was followed by a significant decrease in oviposition period. The shortest period of oviposition (7.06 [$F = 542.3$, df = 3, $P < 0.0001$] days) was recorded for mites that treated with LC_{30} concentration and the longest oviposition time (10.74 [$F = 542.3$, df = 3, $P < 0.0001$] days) was recorded for distilled water. Our findings showed that fecundity of female mites significantly decreased under the influence of sublethal concentrations of *A. belladonna* herbal extract.

The lowest value of eggs laid per female was found in the LC_{30} treatment. Therefore, the treated mites with this concentration will produce the least population in their second generation. However according to Binaghi et al (2014) the total fecundity of two-spotted spider mite in control group were 64.44 per female and 32.46 and 31.08 eggs for cumin and clove essential oil treatments respectively. Also the results of present study is consistent that the findings reported by Castagnoli et al. (2005), Numa et al. (2011) and Sivira et al. (2011), who observed that increasing the concentration of the extract applied to *T. urticae* adults, cause to reducing the fercundity of the phytophagous females. In another study, Refaat et al. (2002) investigated two essential oils, *Ocimum basilicum* L. (Lamiaceae) and Lavandula of Ficinalis Chaix (Lam-iaceae) on *T. urticae*. Their results showed that both essential oils have significant reduction in the total number of eggs laid by this mite.

Table 2. Effects of sublethal concentration of *Atropa belladonna* herbal extract on reproductive parameters of *Tetranychus urticae*

Parameter	Control	LC_{10}	LC_{20}	LC_{30}
Pre-oviposition Period (day)	1.00±0.00a	1.00±00.0a	1.00±0.00a	1.02±0.02a
Oviposition Period (day)	10.74±0.09a	9.80±0.13b	8.94±0.15c	7.06±0.08d
TPOP (day)	11.79±0.13a	11.62±0.13a	11.5±0.12a	11.26±0.11b
Total fecundity (offspring/individual)	55.57±0.50a	46.45±0.7b	37.62±0.94c	27.39±0.51d

Note. TPOP: total pre-oviposition period. Means within a row followed by the letter are not significantly different. The ±SE were estimated by using 100,000 bootstraps and compared by using paired bootstraps test at 5% significance level.

3.4 Population Parameters

The demographic parameters of *T. urticae* affected by sublethal treatments of *A. belladonna* herbal extract are depicted in Table 3. *GRR* decreased significantly with increasing sublethal concentrations. Net reproductive rate (R_0) in the LC_{30} dose was significantly inferior to other treatments. Given that the amount of R_0 depends on the number of eggs produced and the survival rate, the data indicated a low survival and oviposition rate in the treated population. The highest and lowest values of R_0 were 18.08 and 38.41 offspring/individual, respectively, for control and LC_{30} treatment. Assessed intrinsic rate of increase for mites influenced by sublethal doses was ranged of 0.2201 to 0.1937 day^{-1}. Also, in mites treated with different doses of *Atropa belladonna* herbal extract, the values of r and λ were significantly less than control.

A. belladonna herbal extract caused a reduction in longevity, survival and fecundity of *T. urticae* and thereby causes a reduction in r_m. The higher r value, population increase is faster and growth period is shorter. The results that obtained from this study is consistent with reported by Gholamzadeh Chitgar et al. (2013) that noted population parameters of *T. urticae* that treated by *Thymus vulgaris* essential oil had a significant difference at r, R_0, T and λ parameters. Therefore, it can be concluded that the doses used from the extract reduce the growth potential of TSSM in comparison with the control. The results are similar to those reported by Binaghi et al. (2014) that cumin and clove oil reduces the growth potential of two spotted spider mite population. The average of a mean generation time needs 16.55 and 14.93 days in control and LC_{30} concentration.

The lowest average generation length was observed for the LC_{30} dose of the treated mites ($P < 0.0001$, $df = 3$). Kumral et al. (2013) reported that leaf extract of D. stramonium had toxic and repellent effect both on *P. ulmi* and its predator *Stethorus gilvifrons* (Muls.) (Coleoptera: Coccinellidae).

Table 3. Life table parameters (mean±SE) of offspring from females of *Tetranychus urticae* treated with sublethal concentrations of *Atropa belladonna* herbal extract

Parameters	**Control**	**LC_{10}**	**LC_{20}**	**LC_{30}**
GRR (offspring/individual)	48.34±2.89a	38.44±2.28b	30.47±1.91c	21.36±1.36d
R_0 (offspring/individual)	38.41±3.14a	30.66±2.24b	24.82±1.89c	18.08±1.34d
r (day^{-1})	0.2201±0.005a	0.2117±0.004a	0.2033±0.005a	0.1937±0.005ab
λ (day^{-1})	1.246±0.006a	1.235±0.006a	1.225±0.006a	1.213±0.006ab
T(days)	16.55±0.12a	16.15±0.11b	15.77±0.10c	14.93±0.09d

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

3.5 Survival and Fecundity

Figure 2, compares the l_x, of *T. urticae* at different concentration of *Atropa belladonna* herbal extract. The total life time for untreated mites was scored 26 days, while it was 25, 24 and 22 days for the mites treated at LC_{10}, LC_{20} and LC_{30} of experimental concentrations.

The age-specific fecundity (m_x) curves for both treated and untreated mites are depicted in Figure 3. The maximum values of m_x was 4.74 eggs/individual/day for control which was occurred on 21^{th} day of lifespan. This value were estimated to be 3.95, 3.69, 3.39 eggs/individual/day that were happened on 17, 15, 15^{th} day which were recorded for the mites treated at LC_{10}, LC_{20} and LC_{30}, respectively.

The age-stage survival rate (S_{xj}) indicated chance that a *T. uricae* egg will survive to age x and stage j (Figure 4). This curve indicated overlaps due to the varation in different life stages of *T. urticae*. The highest female and male survival rate obtained 69% and 23% in control (female) and LC_{10}, LC_{20} and LC_{30} (male) treatment respectively.

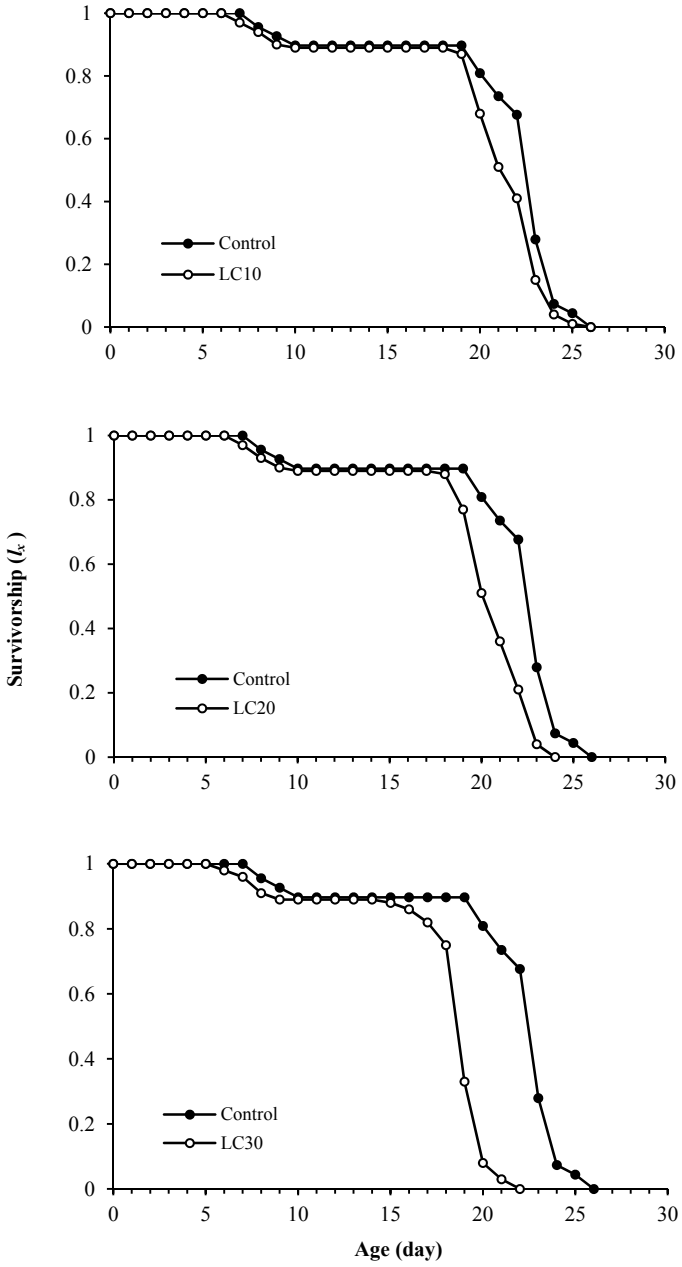

Figure 2. Age-specific survivorship (*lx*) of offspring of the treated and untreated mites of *Tetranychus urticae* by of *Atropa belladonna* extract

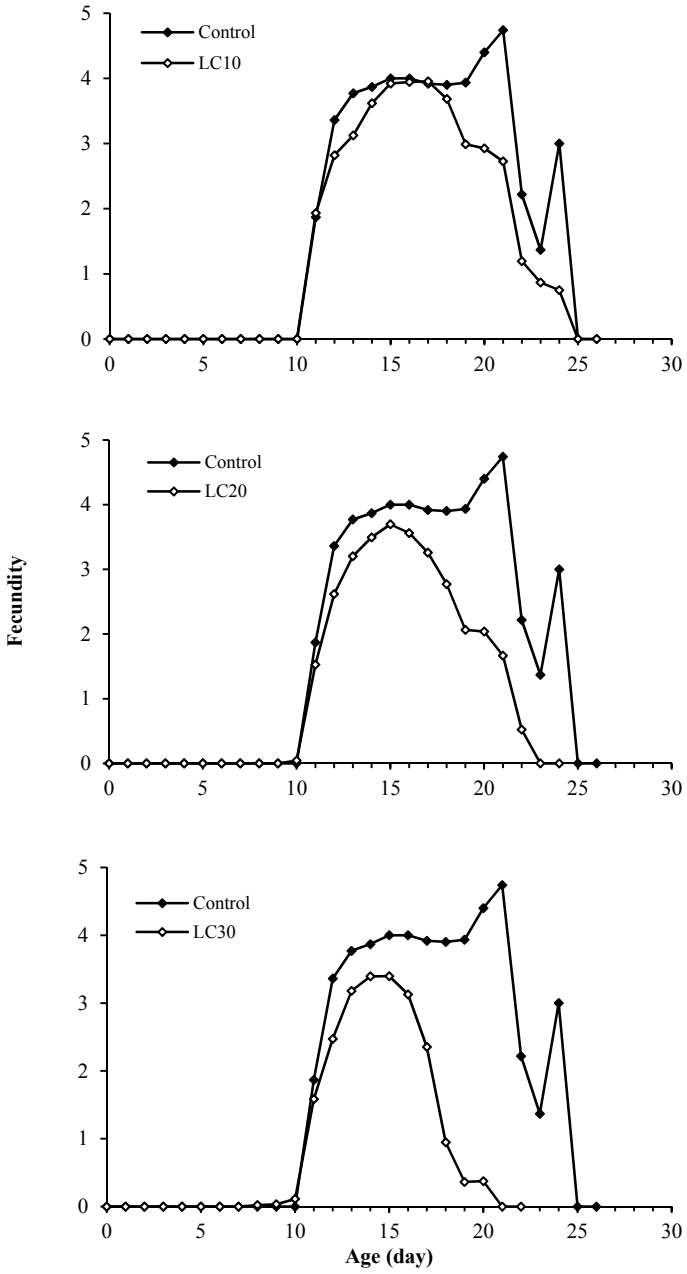

Figure 3. Age-specific fecundity (m_x) of the offspring of the treated and untreated mites of *Tetranychus urticae* by *Atropa belladonna* extract

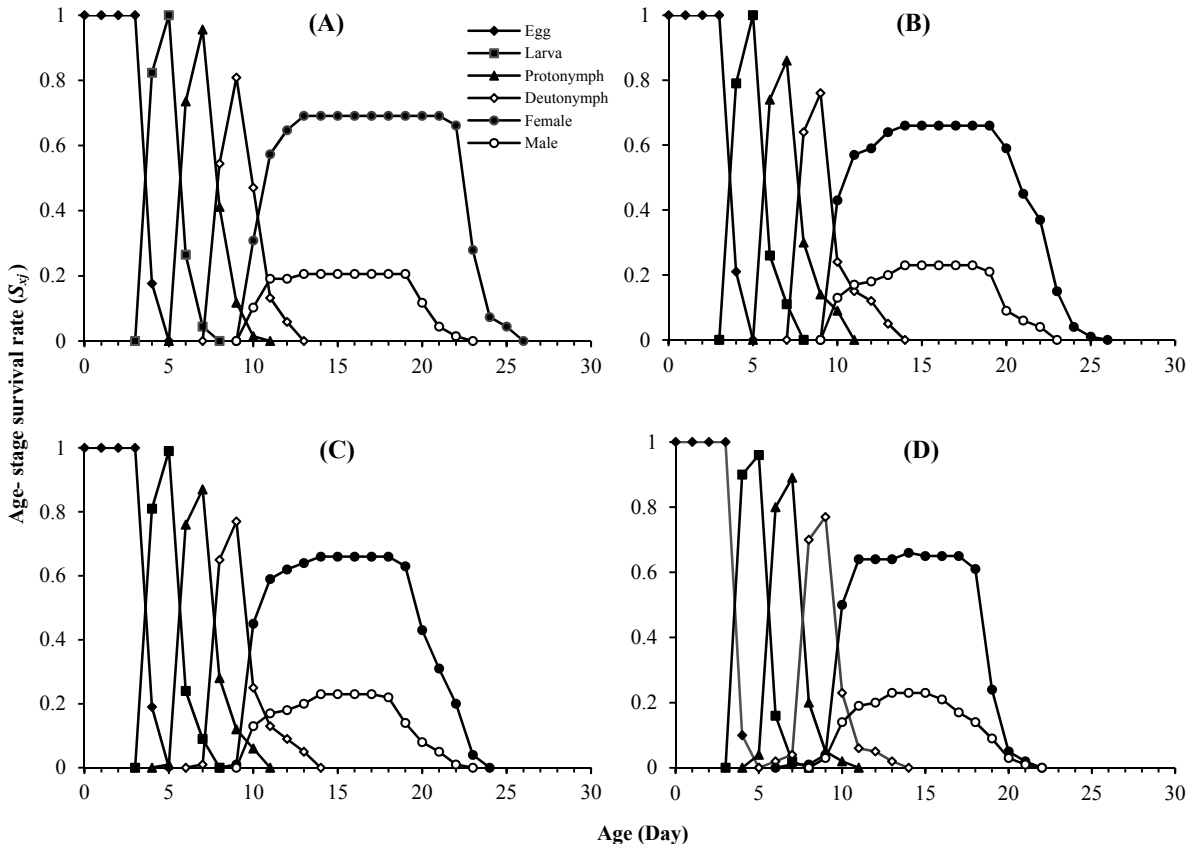

Figure 4. Age-stage specific survival rate (s_{xj}) of *Tetranychus urticae* for control and different concentrations of *Atropa belladonna* herbal extract: Control (A), LC_{10} (B), LC_{20} (C), LC_{30} (D)

According to the OECD (Organisation for Economic Co-operation and Development) guidance biopesticides should only be authorized if they pose minimal or no risk to mammals, other non-target organisms and the environment (Marcic & Medo, 2014). This study provides the population parameters and demographic data related to offspring in treated *T. urticae* with sublethal concentrations of *Atropa belladonna* herbal extract. Our results clearly showed that *Atropa belladonna* herbal extract can be consid-ered as an acaricide against the two spotted spider mite, causing reduction in fecundity and longevity in the laboratory at concentrations. This herbal extract is a promising natural alternative for the control of *T. urticae*. Pesticides based on plant or their constituents have demonstrated efficacy against a range of stored product pests (Gholamzadeh Chitgar et al., 2013).

The results of this study showed that the application of the *Atropa belladonna* herbal extract has effects on the *Tetranychus urticae* population. The LC_{30} concentration caused a higher mortality in mites population than LC_{20} and LC_{10}. It also reduced the parameters such as the immature period. Also the life span both of the sex. This herbal extract has an impact on population growth and reproductive rates of TSSM and can cause a decrease in the population treated in the next generation. Consequently, it is recommended that apply sublethal concentrations of *Atropa belladonna* herbal extract at lower rates could lead to efficient control of *T. urticae*.

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