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#### Article

## Lethal and sublethal effects of bifenazate on the biological parameters of *Tetranychus truncatus* Ehara (Acari: Tetranychidae)

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#### Abstract

Tetranychus truncatus Ehara (Acari: Tetranychidae) is one of the serious pests that infests different agricultural crops in field and greenhouse crops, and its distribution is limited to mostly Asian countries. The experiments were conducted to know the effectiveness of different concentrations of bifenazate against the T. truncatus, and to evaluate and compare the demographic parameters of T. truncatus on host plant Lablab purpureus (L.) Sweet (Fabaceae), which were obtained from females treated with bifenazate. The  $LC_{15}$ ,  $LC_{30}$ ,  $LC_{50}$  and  $LC_{90}$  values are determined through the bioassay from the results of the first application of bifenazate on adult females of T. truncatus. The  $LC_{15}$ ,  $LC_{30}$ ,  $LC_{50}$  and  $LC_{90}$  concentrations of bifenazate were 0.417, 1.028, 2.591 and 24.792 ml/ l, respectively. The response of adult females against the two lethal concentrations (LC<sub>15</sub> and LC<sub>30</sub>) was mostly alike but had a little bit difference. The differences in life table parameters were observed between control and treated spider mites. The results demonstrated that  $LC_{15}$  and  $LC_{30}$  of bifenazate could reduce the survival rate, oviposition period, fecundity and longevity of females of T. truncatus and it significantly affected the developmental times, especially larval duration and fecundity of T. truncatus. Life-table parameters of T. truncatus were much reduced in  $LC_{15}$  and  $LC_{30}$  compared to the control and their growth and other factors showed significant differences. The present study showed that the lower lethal concentration ( $LC_{15}$  and  $LC_{20}$ ) of the tested acaricide showed negative effects on survivorship and life-table parameters of the subsequent generation of T. truncatus.

Key words: spider mites, bifenazate, sublethal effect, survivorship, life table parameters

#### Introduction

Spider mites have become a serious threat to the many agricultural and horticultural crops production. So far, more than 1300 species of spider mites were recorded, among them about 100 are considered as pests, and about 10 are major pests (Migeon & Dorkland 2021). *Tetranychus truncatus* Ehara (Acari: Tetranychidae) is a serious pest that infests various crops in Bangladesh and was also reported in many far eastern countries, such as Guam, Marianas, China, Indonesia, Philippines, Taiwan, Thailand, Vietnam, Iran, Japan and Korea (Ullah *et al.* 2014, 2021; Migeon & Dorkland 2021).

Acaricides play a major role in the management of spider mite populations but high reproductive potentials, tremendously short life cycle of spider mites along with repeated applications of acaricides boosts resistance development that may result in control failures (Nauen et al. 2001). The collective incidence of pesticide resistance of spider mites has directed to a renewed interest in developing pesticides with alternative modes of action, lower environment impact, greater compatibility in integrated pest management programs and reduced health hazard to humans as well as wild-life. Integrated pest management programs use chemical control as a component giving priority to the use of selective pesticides that kill target pests but have relatively little or no effect on beneficial organisms and other non-target organisms. In order to provide sustainable control of spider mite populations, *i.e.*, keeping the number of individuals below an economic threshold, it is becoming more important to design specific resistance management strategies, e.g., the resistance management guidelines published by the Insecticide Resistance Action Committee (IRAC), especially for spider mite control. Proper attention is required in selecting acaricides for managing the spider mites as they have a remarkable intrinsic potential for rapid evolution of resistance (Croft & Van de Baan 1988; Van Leeuwen et al. 2009). The spider mite populations have often developed a high degree of resistance to a newly introduced compound after only a few years of use, with crossresistance to other compounds having the same mode of action. The Arthropod Pesticide Resistance Database (APRD) reports nearly 800 cases of acaricide resistance in phytophagous mites, 93% of which refer to spider mite resistance (Whalon et al. 2021). Therefore, there is an endless effort to develop new acaricides with novel biochemical modes of action, and to optimize their use in order to prevent or delay the development of resistance and prolong their life span (Dekeyser 2005). In addition, exposure to the sublethal concentration of acaricides also affected the population parameters of tetranychid mites and their phytoseiid predators (Hamedi et al. 2010; He et al. 2011; Pakyari & Enkegaard 2015).

Bifenazate belongs to the group of hydrazine carbazate acaricide (Van Leeuwen *et al.* 2010), and is one the most frequently used acaricides to manage all growth stages of spider mites (Van Nieuwenhuyse *et al.* 2012). The resistance of *T. urticae* to bifenazate is associated with the mutations in the mitochondrial cytochrome *b* (cytb) and the  $Q_0$  site of cytb complex III of the electron transport chain (Van Leeuwen *et al.* 2008; Van Nieuwenhuyse *et al.* 2009). Reciprocal crosses between the susceptible and resistant mites of *T. urticae* showed that the resistance was only inherited maternally (non-Mendelian), reassuring a hypothesis called mitochondrial control (Van Leeuwen *et al.* 2006).

Acaricides may affect life-history traits (longevity, fecundity, fertility, developmental time, sex ratio etc.) and population growth rates of mites that survived exposure to pesticides (Stark & Banks 2003). The acute toxicity tests have mostly been utilized for toxicity studies, while sublethal effects include any negative effect other than mortality, such as reduced feeding; lower fecundity or egg viability; reduced longevity or increased developmental time; and altered sex ratios (Biondi *et al.* 2013; Beers & Schmidt 2014) and are important for understanding the total effects of pesticides (Parsaeyan *et al.* 2020). Bioassays that address all potential effects of pesticides provide needed information to use in IPM programs (Beers & Schmidt 2014). It is suggested that life-table analysis is one of the best methods to evaluate the lethal and sublethal effects of an acaricide (Kim *et al.* 2006; Li *et al.* 2017).

Life table, in ecology, is a table for simply and intuitively reflecting the population survival and death process (Chi 1988; Ullah *et al.* 2020; Rismayani *et al.* 2021). It is suggested that life table analysis is the best method to evaluate the lethal and sublethal effects of an acaricide (Kim *et al.* 2006; Mohammadi *et al.* 2016). Higher reproductive potential and rapid development are the main causes of spider mite population size increase (Ullah *et al.* 2011, 2012). Several studies reported on the efficacy of different chemical insecticides to control the *T. truncatus*. However, reports on the sublethal effect of acaricides against the populations of *T. truncatus* were rarely found. The current

study was focused on the sublethal effects of bifenazate on the females of pre-ovipositional stage of the spider mite (T. truncatus) as well as the population parameters of their progeny. Our results can serve as a reference to determine the rational use of bifenazate as an effective acaricide to manage the spider mite T. truncatus in a field.

#### Materials and methods

#### Collection and rearing of spider mites

*Tetranychus truncatus* population was collected from jute plant (*Corchorus capsularis* L.; Malvaceae) in field laboratory of Bangladesh Agricultural University, Mymensingh, Bangladesh on August 2019. The population was reared on leaf discs of bean, *Lablab purpureus*. The leaves were placed on a water-saturated polyurethane mats in plastic Petri dishes and kept at 25°C, 60–80% RH and a photoperiod of 16L:8 D hours. The leaves were replaced approximately every 7 days as they became dried or overexploited due mite damage (Islam *et al.* 2017).

#### Toxicity to adults

To test the toxicity of bifenazate (Mito-kohne®, 20% SC, Nissan Chemical Industries, Tokyo, Japan) on adult females, 15 three- to five-day-old mated females of *T. truncatus* were placed on a new bean leaf disc (20-mm diameter). After 24 h, dead or injured individuals were removed and suspensions of the bifenazate (1, 2, 4, 8, 16 and 32 ml/L) were sprayed onto the leaf disc with adult female spider mites (60 females for each concentration, total 360 females) at a rate of 1 ml/cm<sup>2</sup>, using a hand sprayer. The sprayed samples were dried in the shade and then maintained at  $25 \pm 1^{\circ}$ C with a 16L: 8D photoperiod for 72 h. Mites that did not move their appendages when touched with a fine brush were scored as "dead".

### Determination of $LC_{15}$ , $LC_{30}$ , $LC_{50}$ and $LC_{90}$ concentrations of bifenazate based on toxicity level in adults

After application of bifenazate in different concentrations, different toxic effects were observed. Based on the toxicity of the first appliance in adult mites,  $LC_{15}$ ,  $LC_{30}$ ,  $LC_{50}$  and  $LC_{90}$  concentrations were measured. Again,  $LC_{15}$  and  $LC_{30}$  concentrations of bifenazate were applied on adult female of *T. truncatus* with host plant.

#### Immature development duration

A single gravid female of *T. truncatus* obtained from sprayed cultures with specific doses was transferred on a bean leaf disc (2×2cm). Those leaf discs with mites were kept under 25°C, under a long photoperiod (16L: 8D) with 60–80% RH. Newly laid eggs were transferred individually and reared on a fresh leaf disc, and the developmental stages were recorded at 12-h intervals until all individuals reached adulthood. The sex of the spider mites was identified in the teleiochrysalis stage, and each teleiochrysalis female was provided with one adult male from the stock colony to ensure timely mating.

#### Reproduction and female longevity on treated host

When a female of *T. truncatus* reached teleiochrysalis ( $C_3$ ) stage, single male from the stock colony was placed on the female's leaf disc to mate. This male was kept on the disc for total experimental period and replaced with a new one, if it died before females but these males were excluded from the analyses. The females were observed at 12-h intervals to determine the pre-oviposition period.

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Newly emerged females obtained from the above-mentioned experiments at the same environmental conditions were used to calculate reproductive traits and longevity. The number of eggs laid by a female was observed and recorded daily under a stereomicroscope to determine oviposition period, total number of eggs laid per female, eggs laid per female per day, postoviposition period and female longevity until all mites were dead.

#### Life table parameters

The raw data for development, survival, longevity and female daily fecundity of *T. truncatus* were analyzed according to the age-stage, two-sex life table (Chi & Liu 1985; Chi 1988) using the computer program TWOSEX-MSChart as suggested Chi (2021). Any egg batch sampled for the life table study will differentially influence the population parameters by its hatch rate (Mou *et al.* 2015). To calculate survival, development data and daily fecundity and to correctly estimate the life table parameters only the viable, hatched eggs were considered in data analysis by an earlier study (Mou *et al.* 2015). The age-stage-specific survival rate  $(s_{xj}$ , where x = age and j = stage), age-specific survival rate  $(l_x)$ , age-stage-specific fecundity  $(f_{xj})$ , age-specific fecundity  $(m_x)$ , and population parameters, including net reproductive rate  $(R_0)$ , intrinsic rate of increase (r), finite rate of increase  $(\lambda)$  and mean generation time (t), were calculated according to Chi and Liu (1985) by using the following equations:

$$l_x = \sum_{j=1}^k s_{xj}$$

where, k is the number of stages, and

$$m_x = \frac{\sum_{j=1}^k s_{xj} f_{xj}}{\sum_{j=1}^k s_{xj}}.$$

The intrinsic rate of increase (r) was calculated using the Lotka–Euler equation with age indexed from zero (Tuan *et al.* 2016) as follows:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} \, l_x m_x = 1.$$

The net reproductive rate  $(R_0)$  is the total number of offspring that an individual can produce during its lifetime (Tuan *et al.* 2016) and was calculated as follows:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \,.$$

The mean generation time (t) represents the amount of time that a population requires to increase its size to  $R_0$ -fold as time approaches infinity and the population settles to a stable age-stage distribution (Tuan *et al.* 2016). Mean generation time was calculated as follows:

$$t = \frac{\ln R_0}{r}.$$

The finite rate of increase ( $\lambda$ ) is a multiplication factor of the original population at each time period. The finite rate of increase was calculated as follows:

$$\lambda = e^r$$
.

The GRR was calculated as follows: GRR =  $\sum m_x$ 

The age-stage life expectancy  $(e_{xj})$  is the time length that an individual of age x and stage j is expected to survive, and it was calculated as follows:

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{k} S'_{iy'}$$

where  $s_{iy}$  is the probability that an individual of age x and stage j will survive to age i and stage y, and was calculated by assuming  $s_{iy} = 1$ , following the procedure described in Chi (1988).

Oviposition days  $(O_d)$  is the mean number of days on which adult females actually lay eggs and was calculated as follows:

$$O_d = \frac{\sum_{x=1}^{N_{fr}} D_x}{N_{fr}}$$

where  $N_{fr}$  is the number of reproductive females, i.e., females that laid at least one egg, and  $D_x$  is the number of oviposition days of the *x*th reproductive female (Chen *et al.* 2018).

The mean fecundity of reproductive female  $(F_r)$  was calculated as follows:

$$F_r = \frac{\sum_{x=1}^{N_{fr}} E_x}{N_{fr}},$$

where  $E_x$  is the total number of eggs laid by reproductive females.

The age-stage reproductive value  $(v_{xj})$  is the contribution of *n* individuals of age *x* and stage *j* to the future population. Based on Tuan *et al.* (2016), the reproductive value  $(v_{xj})$  in the age-stage, two-sex life table was calculated as follows:

$$v_{xj} = \frac{e^{r(x+1)}}{S_{xj}} \sum_{i=x}^{\infty} e^{-r(i+1)} \sum_{y=j}^{k} S'_{iy} f_{iy}$$

where  $f_{iy}$  is the probability that an individual of age x and stage j will reproduce to age i and stage y.

#### Statistical analyses

Pooled data for either adult females or eggs were subjected to Probit analysis (transforming the percentage killed into a "probability unit") using POLO Plus program and values of  $LC_{15}$ ,  $LC_{30}$  and  $LC_{90}$  with 95% confidential limits (CL) were estimated (Finney 1971) using Abbott's correction for natural mortality (Abbott 1925):

Corrected mortality (%) =  $[100 \times (\text{Treated -Control}))/((100\text{-Control})]$ 

The age-stage survival rates and fecundity of all three spider mites were calculated using the computer program TWOSEX-MSChart (Chi 2021). The variances and standard errors of the population parameters were estimated by bootstrap analysis. Because bootstrap analysis uses random resampling, a small number of replications will generate variable means and standard errors. To reduce the variability of the results, we used 100000 bootstrap iterations. We then used the paired bootstrap test to examine the differences among the temperature treatments (Efron & Tibshirani 1993).

#### Results

Toxicity of bifenazate on Tetranychus truncatus

Bifenazate was highly toxic to *T. truncatus* adults, the concentration required to kill 50% of the test population ( $LC_{50}$ ) was 2.591 ml/l, while  $LC_{15}$  and  $LC_{30}$  for adult female were 0.417 and 1.028 ml/l, respectively (Table 1). The  $LC_{90}$  value of bifenazate was 24.79 ml/l.

#### Immature development

Immatures of both sexes of *T. truncatus* successfully completed their development on bean leaves at 25°C in all applications that include control and bifenazate treatments with lethal concentrations ( $LC_{15}$  and  $LC_{30}$ ). However, the immature developmental periods of both sexes were significantly affected by the lethal concentrations of bifenazate (Table 2). The larval duration was increased with the increase of lethal concentrations. Egg to adult development time of both males and females were also significantly increased with the increase of concentration. Total development time from eggs to adults of males and females was higher in  $LC_{30}$  than in  $LC_{15}$ . The interactions between lethal concentrations and sex showed no significant influence on developmental durations of different stages in *T. truncatus*.

TABLE 1. Toxicity of bifenazate to adult female of Tetranychus truncatus.

Parameter	Toxicity on adult female		
Slope	$1.306\pm0.158$		
n	300		
$\chi^2$	4.526		
df	4		
LC <sub>15</sub> (95% CL)	0.417 (0.102-0.851)		
LC <sub>30</sub> (95% CL)	1.028 (0.396–1.729)		
LC <sub>50</sub> (95% CL)	2.591 (1.484–3.838)		
LC <sub>90</sub> (95% CL)	24.792 (14.192–71.001)		

Note: LC values expressed in ml/l of commercially formulated bifenazate

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<b>IABLE 2.</b> Development duration (days $\pm$ S.E.) from egg to adult of <i>letranychus truncatus</i> on different
treatments of bifenazate reared on bean plants at 25°C, 16L:8D photoperiod.

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Treatmen	t Sex	$N^{a}$	Egg	Larva	Protonymph	Deutonymph	Egg-to-Adult
Control	Ŷ	51	$4.75\pm0.06Bab$	$3.01\pm0.05Ab$	$2.10\pm0.04 Aa$	$2.42\pm0.05Aab$	$12.28\pm0.08Bbc$
	8	9	$5.00\pm0.08Aa$	$2.94\pm0.13 Ab$	$1.94\pm0.06Bbc$	$2.50\pm0.08 Aab$	$12.39\pm0.16Abc$
LC <sub>15</sub>	9	52	$4.83\pm0.04Aa$	$3.05 \pm 0.05 Ab$	$2.07\pm0.04 Aab$	$2.52\pm0.05 Aab$	$12.46\pm0.07Aab$
	8	8	$4.38\pm0.08Bc$	$3.06\pm0.06Ab$	$2.12\pm0.08Aab$	$2.62\pm0.12 Aa$	$12.19\pm0.19Bc$
LC <sub>30</sub>	9	54	$4.91{\pm}~0.05 Aa$	$3.24\pm0.04Aa$	$2.14\pm0.03Aab$	$2.39\pm0.03Abc$	$12.68\pm0.08Aa$
	8	6	$4.75\pm0.11Bab$	$3.25\pm0.11 Aa$	$2.50\pm0.18Aa$	$2.33\pm0.11 Abc$	$12.83 \pm 0.17 Aa \\$

<sup>a</sup>Number of individuals tested.

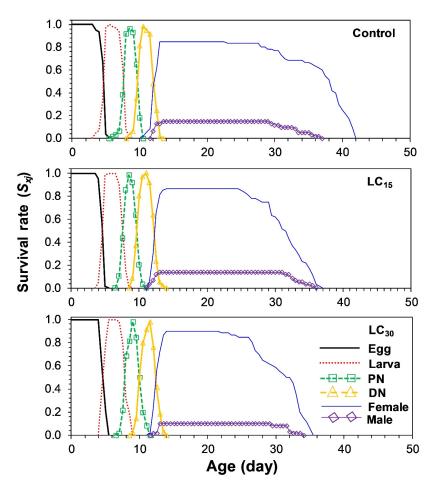
<sup>b</sup>Means in the same column followed by the different small letters denote significant differences in different doses and capital letters denote significant differences in sexes based on the paired bootstrap test at 5% significant level.

**TABLE 3.** Average pre-oviposition period (APOP), total pre-oviposition period (TPOP), oviposition days, female longevity (days  $\pm$  S.E.), and eggs per female (mean  $\pm$  S.E.) of *Tetranychus truncatus* on different treatments of bifenazate reared on bean plants at 25°C, 16L:8D photoperiod.

Treatment	N <sup>a</sup>	APOP	ТРОР	Oviposition days	Female longevity	Eggs per female
Control	51	$1.75\pm0.06a$	$14.04\pm0.07a$	$20.09\pm0.49a$	$37.48 \pm 0.63a$	$128.63\pm2.64a$
LC <sub>15</sub>	52	$1.73\pm0.04a$	$14.19\pm0.08a$	$16.43\pm0.32b$	$32.00 \pm \mathbf{0.43b}$	$98.54\pm2.56b$
$LC_{30}$	54	$1.73\pm0.04a$	$14.41\pm0.08b$	$15.38\pm0.37c$	$30.27\pm0.48\text{c}$	$87.43\pm2.39c$

<sup>a</sup>Number of individuals tested.

<sup>b</sup>Means in the same column followed by the different letters denote significant differences based on the paired bootstrap test at 5% significant level.



**FIGURE 1.** Age-stage specific survival rates  $(s_{xj})$  of *Tetranychus truncatus* on different treatments of bifenazate reared on bean plants at 25°C, 16L:8D photoperiod.

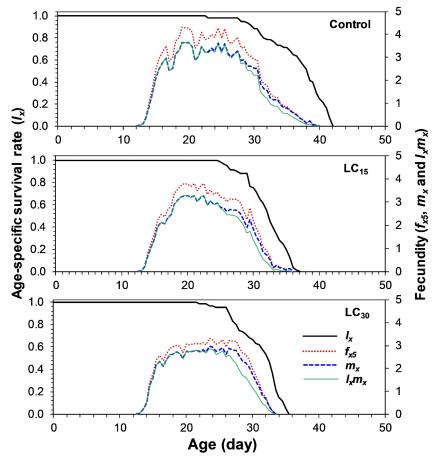
#### Adult longevity and reproduction

The total pre-oviposition period, oviposition days, female longevity and total eggs/female were significantly affected by bifenazate concentrations. In contrast mean pre-oviposition period was not significantly affected by its concentrations, but the total pre-oviposition period increased with the increase of concentrations. The oviposition days and female adult longevity decreased with the increase of bifenazate concentrations (Table 3). The eggs per female was negatively affected by the concentrations of bifenazate. The fecundity reduced significantly with the increase of bifenazate concentrations.

#### Age-stage and two-sex life table

The overlap of the age-stage specific survivorship  $(s_{xj})$  curves of the cohorts of *T. truncatus* reared on host treated with different concentration of bifenazate was presented by an age stage, twosex life table (Fig. 1). The age-stage survival rate curves  $(s_{xj})$  of different treated concentrations of bifenazate on *T. truncatus* illustrate the probability that a newly emerged individuals would survive to age *x* and stage *j*. However, the overlapping among the stage-specific survivorship curves revealed the variation in developmental rates among individuals. The age-stage survival rate  $(l_x)$  of the *T*.

*truncatus* emerged and mites successfully survived to adult (Fig. 1). When the stage differentiation is ignored, a simplified version of the age-specific survival rate  $(l_x)$  is obtained.



**FIGURE 2.** Age-specific survivability  $(l_x)$ , age-stage specific fecundity  $(f_{xs})$ , age-specific fecundity  $(m_x)$  and age-specific maternity  $(l_x m_x)$  of *Tetranychus truncatus* on different treatments of bifenazate reared on bean plants at 25°C, 16L:8D photoperiod.

Female adults started to die on days 23, 24 and 22 in control,  $LC_{15}$  and  $LC_{30}$ , respectively. All females had died on days 42, 37 and 35 in control,  $LC_{15}$  and  $LC_{30}$ , respectively. The age-specific fecundity rate ( $m_x$ ) of *T. truncatus* treated with different concentrations of bifenazate fluctuated throughout the oviposition period, showing an asymmetrical pattern and early aged spider mites showed higher fecundity. Oviposition in females began on days 12, 13 and 13 in control,  $LC_{15}$  and  $LC_{30}$ , respectively. Age-specific fecundity ( $m_x$ ) reached its peak on days 19, 21 and 24 in control,  $LC_{15}$  and  $LC_{30}$ , respectively. The age-specific maternity also showed the similar trend with age-specific fecundity in each concentration of bifenazate (Fig. 2).

#### Population parameters

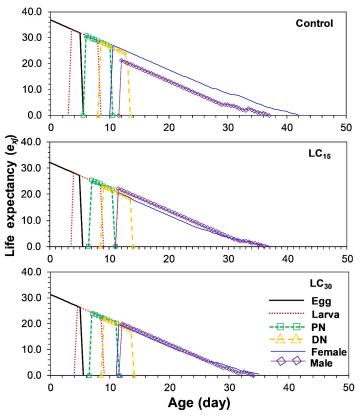
Population parameters of *T. truncatus* were significantly affected by different concentrations of bifenazate on bean plants (Table 4). The net reproduction rate ( $R_0$ ), intrinsic rate of natural increase (r), mean generation time (t), and gross reproduction rate (GRR) were significantly affected by different treatments of bifenazate (P < 0.05). The  $R_0$ -values were significantly higher in control than LC<sub>15</sub> and LC<sub>30</sub>. GRR also showed the similar trend of the net reproductive rate (Table 4). The finite

rate of increase was higher in control than in  $LC_{15}$  and  $LC_{30}$  (Table 4). The mean generation time decreased with the increase of bifenazate doses. The intrinsic rate of natural increase was reduced significantly with the increase of bifenazate from  $LC_{15}$  to  $LC_{30}$ , and it was highest in control and lowest in  $LC_{30}$  (Table 4).

**TABLE 4.** Demographic parameters (mean  $\pm$  S. E.) of *Tetranychus truncatus* on different treatments of bifenazate reared on bean plants at 25°C, 16L:8D photoperiod: net reproductive rate ( $R_0$ ), intrinsic rate of natural increase (r, day<sup>-1</sup>), mean generation time (t, day), finite rate of increase ( $\lambda$ ), and gross reproduction rate (*GRR*).

Treatment	$R_0$	r	t	λ	GRR
Control	$109.33\pm 6.31a$	$0.2210 \pm 0.0032a$	$21.24\pm0.14a$	$1.2474 \pm 0.0039a$	$114.84\pm 6.23a$
LC <sub>15</sub>	$85.40\pm4.83b$	$0.2116 \pm 0.0030 b \\$	$21.02\pm0.12a$	$1.2356 \pm 0.0037 b$	$90.61\pm5.22b$
LC <sub>30</sub>	$78.68 \pm 3.99 b$	$0.2090 \pm 0.0026 \texttt{c}$	$20.88\pm0.02b$	$1.2325\pm0.0032ab$	$85.93 \pm 4.22 b$

<sup>a</sup> Means in the same column followed by the different letters denote significant differences based on the paired bootstrap test at 5% significant level.



**FIGURE 3.** Age-stage life expectancy  $(e_{xj})$  of *Tetranychus truncatus* on different treatments of bifenazate reared on bean plants at 25°C, 16L:8D photoperiod.

#### *Life expectancy*

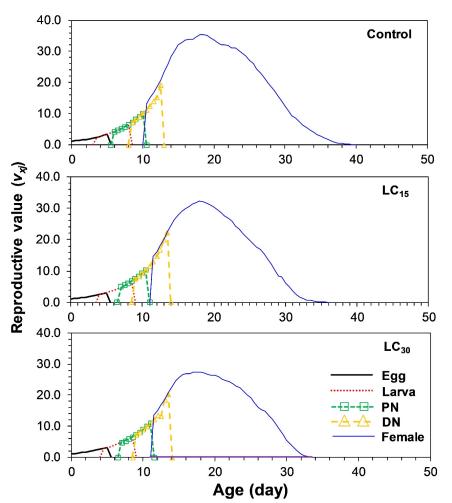
The life expectancy of a newly laid egg reared on different treatments of bifenazate on bean plants was 37, 32 and 30 days, respectively (Fig. 3). The peak life expectancy  $(e_{xj})$  adult females reared on control, LC<sub>15</sub> and LC<sub>30</sub> was 11, 12 and 12 days at 25°C, respectively (Fig. 3).

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#### Reproductive values

The contribution of an individual to future population was defined as reproductive value by Fisher (1930). The reproductive values  $(v_{xj})$  of *T. truncatus* individuals at age and stage are presented in (Fig. 4). The  $v_{xj}$  of a newly-laid egg on control, LC<sub>15</sub> and LC<sub>30</sub> was 1.1 day (Fig. 4). Females near the peak of reproduction, however, contributed considerably more than those at other ages and stages. The peak  $v_{xj}$  of an adult female in control, LC<sub>15</sub> and LC<sub>30</sub> was 18, 17 and 17 days, respectively (Fig. 4).



**FIGURE 4.** Age-stage fecundity  $(v_{xj})$  of *Tetranychus truncatus* on different treatments of bifenazate reared on bean plants at 25°C, 16L:8D photoperiod

#### Discussion

The present study shows that *T. truncatus* successfully survived and completed their development, but their development, fecundity and life table parameters greatly affected at different concentrations of bifenazate. Indeed, the results show that the sublethal concentrations of bifenazate had substantial effects on the intrinsic rate of natural increase (r), the net reproductive rate ( $R_0$ ), female progeny, and the survival of the adult stage.

Demographic toxicology or life-table analysis is one of the best methods for evaluation and combining lethal and sublethal effects of pesticides (Daniels & Allan 1981; Day & Kaushik 1987; Kim *et al.* 2004). LC<sub>15</sub> and LC<sub>30</sub> concentrations of bifenazate influenced the life-table parameters of *T. truncatus*. Our study is the first comprehensive research on the sublethal effects of bifenazate on life-table parameters of *T. truncatus*. The results demonstrated that LC<sub>15</sub> and LC<sub>30</sub> of bifenazate could reduce the survival rate, oviposition period, fecundity and longevity of the female of *T. truncatus*.

Several studies have been done on the effect of pesticides on spider mites. For example, Marcic (2007) reported that sublethal concentrations/doses of spirodiclofen reduced the survival rate and other life-table parameters of T. urticae. Stark et al. (1997) revealed that reproductive potential of T. urticae can be greatly influenced by their susceptibility to acaricides. They described and compared the effects of  $LC_{25}$  concentrations of tested pesticides by using the age-stage, two-sex life table. The present study shows that bifenazate had sublethal negative effect on life-table parameters of adult T. truncatus. The lethal concentration LC<sub>30</sub> of bifenazate applied to females was enough to affect fecundity and longevity. Similar effects on life-table parameters were recorded by Marcic (2007), who treated T. urticae adult females with several spirodiclofen concentrations. Their results showed that higher concentrations caused greater reduction in r. Our results revealed that the population growth rate of T. truncatus was affected by LC15 and LC30 of bifenazate, as a result of negative effects on immature developmental time, reproduction period, fecundity and finally in the population parameters (i.e., r,  $\lambda$ ,  $R_0$ , and t). Our findings are in agreement with many studies on the effects of pesticides on development, survival, and reproduction of T. urticae (Kim & Seo 2001; Marcic 2003; Ashley et al. 2006; Hardman et al. 2007; Wang et al. 2014). Compared with the control, the reproductive values in mites treated with LC15 and LC30 were reduced, which may cause restriction in reproduction and survivorship. In accordance with Alinejad et al. (2014), the sublethal dose of fenazaquin affected survivorship and fecundity of T. urticae. Intrinsic rate of natural increase (r) is the best factor to describe the effect of pesticides on pests (Hamedi et al. 2010; Stark & Banks 2003), because it demonstrates the overall effects on both survivorship and fecundity (Li et al. 2017). The results of this study showed that r in T. truncatus was reduced by bifenazate compared with the control indicating the adverse effect of the pesticide on this parameter. Li et al. (2017) also reported that the r values of progeny generation of the treated mites with bifenazate decreased significantly. The net reproductive rate  $(R_0)$  of T. truncatus treated with bifenazate is significantly lowered compared to that of the control (Table 4). The mean generation time (t) of T. truncatus treated with bifenazate was also influenced significantly. Moreover, Alinejad et al. (2014) and Tuan et al. (2016) illustrated that because of the variable developmental rates occurring among T. urticae individuals, the survival rate curve showed significant stage overlap. This agrees with our results. In our experimental conditions, the mites treated with bifenazate had reduced the age-specific survival rate  $(l_x)$  (Fig. 2). Furthermore, age-specific maternity  $(l_x m_x)$  was reduced by bifenazate, in agreement with results of Marcic (2007). His results indicated that the tested acaricides would significantly reduce mite growth. Lower age-specific reproductive value and life expectancy were observed in treated mites by acaricides. Mohammadi et al. (2016) reported that life expectancy of T. turkestani Ugarov and Nikolskii was affected differently in individuals of the same age, stages and sexes. Similar results were observed in this study: for example,  $e_{xi}$  of female adults was about 11–12 days, whereas the value was 10.5 days treated with biomite (Mohammadi et al. 2016).

Based on the data, the experimental concentrations played a negative role during all pre-adult developmental stages such as the egg, larva, protonymph, and deutonymph among males and females. 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. Bifenazate acts as a synergist or the allosteric modulator of functionally expressed Gamma-Aminobutyric acid (GABA) receptor

homologues (Van Nieuwenhuyse et al. 2012), and the duration of immature stages of T. urticae was significantly prolonged when treated with bifenazate (Li et al. 2017). Li et al. (2017) observed that exposure to the  $LC_{10}$  and  $LC_{20}$  lethal concentrations of bifenazate severely affected the parental generation of T. urticae, including survival rate (reduced 9% and 13%), oviposition period (reduced 77.6% and 83.1%), fecundity per female (decreased 89.2% and 76.9%) and longevity (decreased 79.2% and 83.1%) compared to the control. In this study we observed that exposure to the  $LC_{15}$  and LC<sub>30</sub> lethal concentrations of bifenazate to *T. truncatus* increased the development duration from egg to adult female (1.4 and 3.2%) and total pre-oviposition period (1.1 and 2.6%), and decreased the oviposition days (18.2 and 23.4%), female longevity (14.6 and 19.2%) and fecundity (23.4 and 32.0%) compared to control. The results of the present study indicated that the use of different concentrations of the bifenazate 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 observed in longevity and life span after treating with sublethal concentrations of fenazaquin. The sublethal effect on biological parameters in different species effect differentially because each individual and species may present a different response to each insecticide (de França et al. 2017).

Finally, studies of sublethal effects on life-table parameters of pests allow us to have the most complete delineation of the population-level responses to pesticides. Quantification of the importance of pesticide impact on life cycle timing at the population level of target pest species will assist the researchers to manage the pest population properly. It was reported that pesticides at lower concentrations may have significant effects on population levels and might affect population dynamics of *T. urticae* (Stark & Banks 2003). According to our results, the lower lethal concentrations (LC<sub>15</sub> and LC<sub>30</sub>) of the tested acaricides showed negative effects on survivorship and life-table parameters of the subsequent generation of *T. truncatus*. Despite the negative effects of LC<sub>30</sub> of bifenazate on life-table parameters of *T. truncatus* at laboratory study, more detailed evaluations are needed on total effects of these acaricides on the mite and its biological control agents under greenhouse and field conditions.

This study showed that LC15 and LC30 of bifenazate significantly affected the developmental times especially larval duration and fecundity of *T. truncatus*. Demographic parameters of *T. truncatus* were much reduced in treatments  $LC_{15}$  and  $LC_{30}$  compared to the control and their growth and other factors were substantially decreased when they were treated with bifenazate. The results of this study could be used as a guide for the rational use of bifenazate in the field for better management of *T. truncatus*.

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#### **Conflict of interest**

The authors have no conflict of interest to declare

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