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Article

## Sublethal effects of bifenazate on life history and population parameters of *Tetranychus urticae* (Acari: Tetranychidae)

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### Abstract

Traditional estimating only by measuring the lethal effect of acaricides may underestimate the total effects of acaricides on the pest mites. In order to investigate the sublethal effect of bifenazate on life history and population parameters of the two-spotted spider mite, *Tetranychus urticae* Koch, the newly emerged females were treated with two lethal concentrations of bifenazate: LC<sub>10</sub> (4.92 µg/mL) and LC<sub>20</sub> (8.77 µg/mL). Subsequently, the development and fecundity of the progeny generations were observed. Compared to the control, exposure to the 10% lethal concentrations (LC<sub>10</sub>) and LC<sub>20</sub> 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%). Besides, the population parameters of the progeny generation from the treated females were also investigated. The results showed that the progeny generation had lower intrinsic rate of increase ( $r_m$ ) and finite rate of increase ( $\lambda$ ), longer mean generation time ( $T_c$ ) compared to the control. The results suggested that the sublethal effects of bifenazate on population growth of *T. urticae* were significant, and the results of this study could be used as a guide for the rational use of bifenazate in the field for better managing pest mites.

**Key words:** two-spotted spider mite, bifenazate, survival, development, fecundity

### Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch, which infests more than 1100 species of host plants, is the most important polyphagous spider mite and a vital pest in temperate regions for many crops (Hamed *et al.* 2011; Amini *et al.* 2016; Migeon & Dorkeld 2016). Although biological control of *T. urticae* has been proven to be successful in many crops growing in greenhouses, acaricides have always played the central role in its control in field crops, owing to their lower cost and strong effects (Sanderson & Zhang 1995; Van Leeuwen *et al.* 2010; Jafari *et al.* 2016). When applied to the field, pesticides not only bring direct acute lethal effect on insects and mites, but also affect the life history traits of individuals surviving through the pesticide treatments. It has been reported that exposure to the sublethal dose of spiromesifen reduced the fecundity or fertility of *T. urticae* (Marcic *et al.* 2010), and exposure to the sublethal concentration of clofentezine decreased the hatchability of eggs produced by the surviving females of *T. viennensis* (Li *et al.* 2006). In addition, exposure to the sublethal concentration of acaricides also affected the population parameters of tetranychid mites and their phytoseiid predators (Hamed *et al.* 2009; He *et al.* 2011; Pakyari & Enkegaard 2015).

Bifenazate belongs to the group of hydrazine derivatives (Van Leeuwen *et al.* 2010), and is being used worldwide for control of spider mites on several crop systems (Dekeyser 2005; Van Leeuwen *et al.*, 2015). Recent studies reported that the resistance of *T. urticae* to bifenazate is tightly linked to the mutations in the mitochondrial cytochrome b (cytb) and the Q<sub>0</sub> 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 showed that the resistance was only inherited maternally (non-Mendelian), supporting a hypothesis called mitochondrial control (Van Leeuwen *et al.* 2006).

Life table, in ecology, is a table for simply and intuitively reflecting the population survival and death process (Chi 1988). 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). The current study was focused on the sublethal effects of bifenazate on the females of pre-ovipositional stage of the two-spotted spider mite, 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 two-spotted spider mite in the field.

## Materials and methods

### *Mite cultures*

The two-spotted spider mite was introduced from the Key Laboratory of Grassland Ecosystem of Education Ministry, College of Grassland Science, Gansu Agricultural University in 2011. After introduction to our laboratory, the mites were reared on cowpea *Vigna unguiculata* in a climate chamber under  $25 \pm 1$  °C,  $75 \pm 5$  % RH, and a photoperiod of L:D = 14:10 h. Before this experiment, the mites had never contacted with bifenazate or any other acaricides.

### *Concentration response bioassay*

Bifenazate, commercial formulation Acramite® (suspension concentrate, 43%, Chemtura, USA) used in this study was purchased from Beijing NewGreen Environ-Tech. Co., Ltd. The toxicity of bifenazate against females of *T. urticae* was determined using leaf-residues method (Hamedi *et al.* 2010; Mahmoudvand *et al.* 2011). Based on the recommended concentration in the field by its manufacturer, bifenazate were diluted to six concentration gradients (430, 215, 107.5, 53.75, 26.88, and 13.44 µg·a.i./mL) with distilled water. The cowpea leaf discs (3 cm in diameter) were placed into the diluted liquid for 5 s and then allowed them to be naturally dried. The leaf discs treated by distilled water only served as control. Then the leaf discs were placed in Petri dish (8.5 cm in diameter × 2 cm in height, with the sponge and a layer of absorbent cotton inside). Thirty mated female mites of pre-oviposition period (each newly emerged female was paired with a male adult for 12 h) were transferred on the surface of each leaf disc. A bioassay with six concentrations of bifenazate and a control was replicated five times. After 24 h, the mortality of the tested mites was recorded under binoculars. Mites that failed to move after a gentle touch by a camel hair brush were considered as dead.

### *Sublethal effects of bifenazate on the survival and reproduction of T. urticae females*

Bifenazate was diluted with distilled water to 10% lethal concentration (4.92 µg/mL) and 20% lethal concentration (8.77 µg/mL) based on above bioassay. The cowpea leaf discs (3 cm in diameter) were put into the diluted liquid for 5 s and then allowed them to be dried, and the leaf discs were placed into Petri dishes. One newly emerged mated female was transferred to the surface of each leaf disc. In total 100 females were treated for each concentration of bifenazate. After 24 h, the survived

female was transferred to new clean leaf discs without bifenthrin, where a male mite was added for mating (the males were re-added if the previous male mite died). The numbers of females survived and eggs were recorded every 24 h, until all females died naturally. The leaf disc treated with distilled water only served as the control.

#### *Sublethal effects of bifenthrin on development and population parameters of the progeny generations*

To evaluate further effects of bifenthrin on the treated females, 100 eggs of each treatment (LC<sub>10</sub>, LC<sub>20</sub> and control) were collected randomly (Hamed *et al.* 2011), and placed on the surface of the leaf disc individually. The survivorship, growth and development were checked every 24 h. After eclosion, males and females were paired. In case there were not enough males to pair with females, males from the stock colony were also used (these males were not included in life table analysis). If the male died or escaped, another male was introduced. The survival of the mites and the number of eggs laid were checked daily until they died. The females that died because of improper handling or escape from the leaf disc were excluded from the data analysis.

#### *Data analysis*

Estimation of the LC<sub>50</sub>, the lethal concentrations and the regression equation for the concentration mortality line were obtained using a probit program of SPSS 16.0 for Windows (SPSS, Chicago, IL, USA). The life history raw data of all individuals were analyzed based on the age-stage, two-sex life table theories (Chi & Liu 1985), which can analyze the growth process of mite population of both females and males (Chi 1988). The means and standard errors of the population parameters were estimated by using the bootstrap technology (Huang & Chi 2013). The computer program TWOSEX-MSChart (Chi 2015) was used for life table analysis. The developmental duration for immature stages and adult longevity, the reproductive period and the total female fecundity, the age-specific survival rate ( $l_x$ ), the age-specific fecundity ( $m_x$ ) and the population parameters: the intrinsic rate of increase from the Euler-Lotka equation:  $\sum e^{-rm(x+1)} l_x m_x = 1$ ; finite rate of increase  $\lambda = e^{rm}$ ; net reproduction rate  $R_0 = \sum l_x m_x$ ; the mean generation time  $T_c = (\ln R_0)/r_m$ , were calculated accordingly. Means, variances and standard errors of longevity, fecundity, and duration of immature stage among different treatments were estimated with the bootstrap technique using 10,000 replications to generate less-variable results.

## **Results**

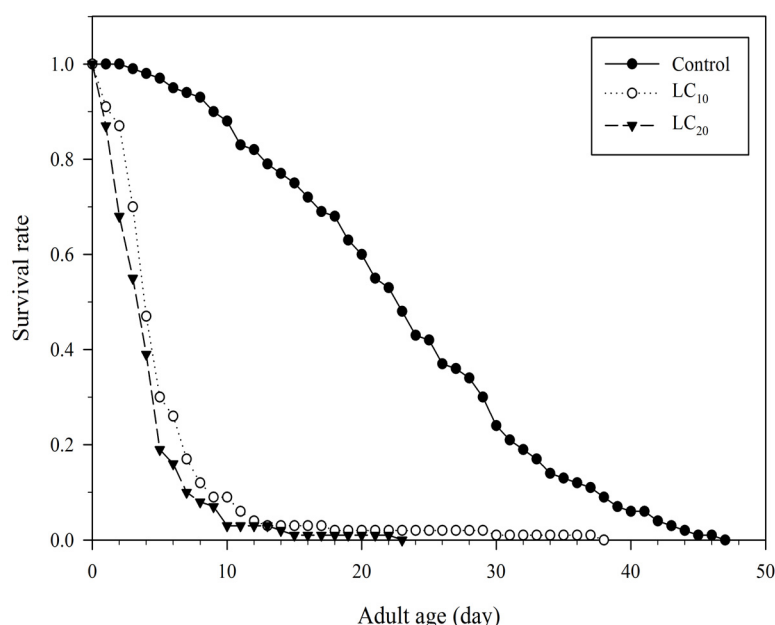
#### *Concentration response bioassay*

The regression equation between concentration and mortality of bifenthrin against females of *T. urticae* was  $y = -2.49 + 1.75 x$ ; [ $y$  = mortality (probit),  $x$  = logarithm of concentration (ppm)], with the correlation coefficient value of 0.9890]. The LC<sub>50</sub>, LC<sub>20</sub> and LC<sub>10</sub> of bifenthrin against *T. urticae* were 26.54, 8.77 and 4.92 µg/mL, respectively. No mortality was observed in the controls.

#### *Sublethal effects of bifenthrin on the survival and reproductions of T. urticae females*

The results showed the 10% lethal concentration (LC<sub>10</sub>) and LC<sub>20</sub> of bifenthrin significantly affected the female oviposition periods, average oviposition, and longevity (Table 1). The number of females that laid eggs (ovipositing individual) and the survival rate were distinctly reduced after exposure to bifenthrin at LC<sub>10</sub> and LC<sub>20</sub>. After a 24 h exposure to bifenthrin, the proportion of females that survived from treatment was 0.91 (LC<sub>10</sub>) and 0.87 (LC<sub>20</sub>), while there were no dead females in control; after 10 days the survival rate in both LC<sub>10</sub> and LC<sub>20</sub> treatments were lower than 0.1, far

below the control (Figure 1). The fecundity of the treated female was obviously less than the control, and fecundity of female treated with LC<sub>20</sub> of bifentazate was higher than that with LC<sub>10</sub> during 5 to 10 d (Figure 2).



**FIGURE 1.** Survival rate of *T. urticae* females survived from the exposure with LC<sub>10</sub> and LC<sub>20</sub> of bifentazate.

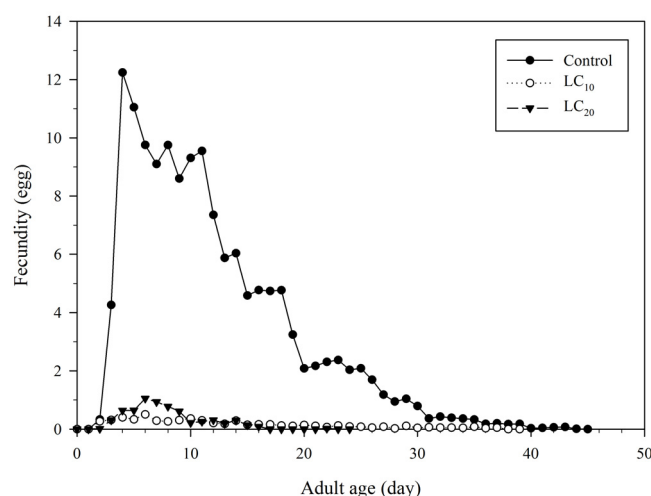
**TABLE 1.** Oviposition per female, oviposition period, female longevity and survival of *T. urticae* females treated with LC<sub>10</sub> and LC<sub>20</sub> of bifentazate, estimated using all individuals and the bootstrap technique.

| Treatments       | Survival rate<br>(%, 24 h) | Oviposition<br>individual | Oviposition<br>period (day) | Average oviposition<br>per female (egg) | Female<br>longevity (day) |
|------------------|----------------------------|---------------------------|-----------------------------|-----------------------------------------|---------------------------|
| LC <sub>20</sub> | 87                         | 24                        | 3.19±0.37b                  | 34.46±7.29b                             | 3.87±0.34b                |
| LC <sub>10</sub> | 91                         | 44                        | 4.25±0.41b                  | 16.09±5.64c                             | 5.00±0.53b                |
| Control          | 100                        | 100                       | 18.94±0.89a                 | 149.46±6.52a                            | 22.89±1.01a               |

Means followed by a lower case letter were estimated using the bootstrap technique. The same letter within a column indicates no significant difference among treatments.

#### *Sublethal effects of bifentazate on development and population parameters of the progeny generations*

The preadult survival rate of progeny generation in both LC<sub>10</sub> and LC<sub>20</sub> of bifentazate treatment were significantly lower than that of the control. The duration of immature stages of females of the progeny generation were significantly prolonged both in the treatments of LC<sub>10</sub> and LC<sub>20</sub> of bifentazate compared with the control, whereas the oviposition period, female average oviposition and female longevity were not significantly different from the control (Table 2). The duration of immature stages of the male of the progeny generation in treatment of LC<sub>10</sub> was significantly prolonged compared with the control, whereas that of LC<sub>20</sub> had no significant difference with that of the control. On the contrary, the male adult longevity of progeny generation in treatment of LC<sub>20</sub> was significantly shortened compared to the control, whereas that of LC<sub>10</sub> had no significant difference with that of the control (Table 2).



**FIGURE 2.** Fecundity of *T. urticae* females survived from the exposure with LC<sub>10</sub> and LC<sub>20</sub> of bifenthrin.

**TABLE 2.** Developmental durations of immature stages, oviposition periods, longevity of offspring from female *T. urticae* treated with LC<sub>10</sub> and LC<sub>20</sub> of bifenthrin, estimated using all individuals and the bootstrap technique.

| Treatments       | Duration of the immature stages (day) |              | Preadult survival rate | Adult longevity (day) |              | Oviposition period (day) | Average oviposition per female (egg) | Survival rate of female (%) |
|------------------|---------------------------------------|--------------|------------------------|-----------------------|--------------|--------------------------|--------------------------------------|-----------------------------|
|                  | Female                                | male         |                        | Female                | Male         |                          |                                      |                             |
| LC <sub>20</sub> | 12.81±0.16a                           | 11.61±0.23ab | 0.4438±0.0391b         | 21.52±1.54            | 12.65±1.49b  | 19.98±1.34               | 142.29±10.98                         | 43                          |
| LC <sub>10</sub> | 12.56±0.14a                           | 12.21±0.24a  | 0.4735±0.0366b         | 20.95±1.17            | 15.47±2.72ab | 19.10±1.01               | 138.78±8.63                          | 46                          |
| Control          | 12.09±0.14b                           | 11.62±0.16b  | 0.7747±0.0355a         | 20.18±1.18            | 17.38±1.60a  | 17.65±1.08               | 144.06±8.47                          | 52                          |

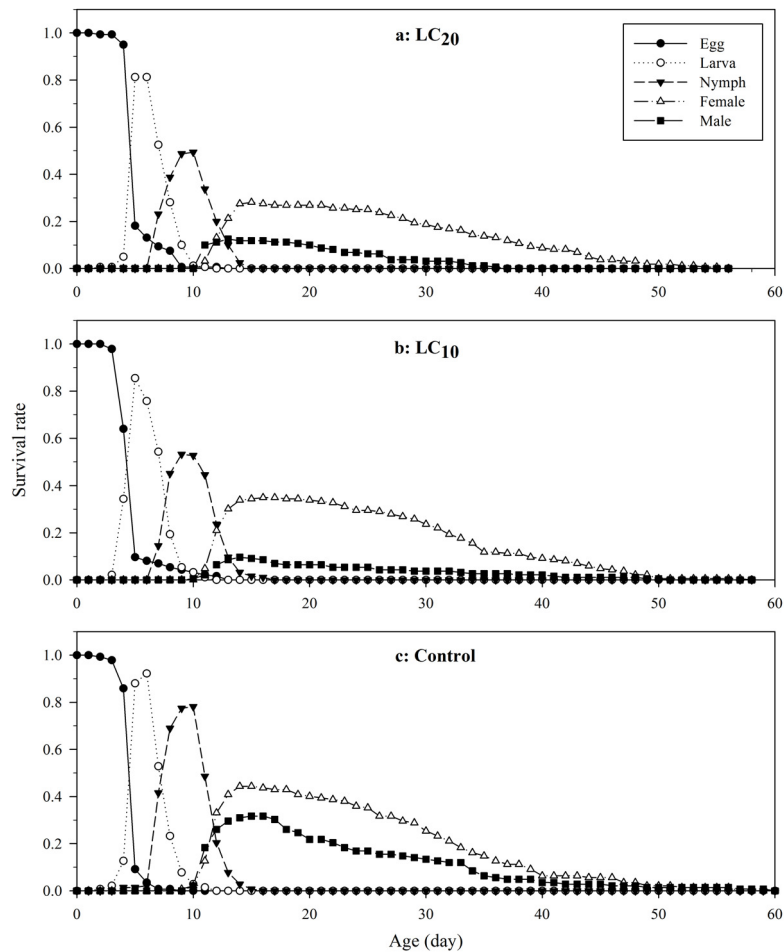
Means followed by a lower case letter were estimated using the bootstrap technique. The same letter within a column indicates no significant difference among treatments.

The  $r_m$  and  $\lambda$  of population treated with the LC<sub>10</sub> and LC<sub>20</sub> of bifenthrin were both significantly decreased, and the progeny generation had a longer  $T_c$ , which these three population parameters of progeny generation treated between LC<sub>10</sub> and LC<sub>20</sub> of bifenthrin were not different significantly (Table 3). The value of  $R_0$  of progeny population treated with the 20% lethal concentration was reduced significantly. Nevertheless, the value of  $R_0$  of population treated with the 10% lethal concentration had no significant difference from the control (Table 3). The  $l_x$  of female offspring decreased with the increasing concentrations of bifenthrin, however, the  $l_x$  of male offspring of LC<sub>20</sub> was higher than that of LC<sub>10</sub>, although both of them were lower than  $l_x$  of male offspring of the control (Figure 3). The  $m_x$ ,  $f_x$  and  $l_x m_x$  of the female offspring showed the synchronization for both two lethal concentrations compared with the control (Figure 4).

**TABLE 3.** Population parameters, mean ± the standard error, of offspring *T. urticae* from female treated with LC<sub>10</sub> and LC<sub>20</sub> of bifenthrin, estimated using all individuals and the bootstrap technique.

| Treatments       | $R_0$        | $r_m$          | $\lambda$      | $T_c$       |
|------------------|--------------|----------------|----------------|-------------|
| LC <sub>20</sub> | 42.72±6.09b  | 0.1773±0.0073b | 1.1940±0.0087b | 21.12±0.29a |
| LC <sub>10</sub> | 51.47±5.93ab | 0.1915±0.0060b | 1.2111±0.0073b | 20.55±0.24a |
| Control          | 65.85±7.07a  | 0.2110±0.0059a | 1.2350±0.0073a | 19.82±0.28b |

$R_0$ , net reproductive rate;  $r_m$ , the intrinsic rate of increase;  $\lambda$ , finite rate of increase;  $T_c$ , mean generation time. Means followed by a lower case letter were estimated using the bootstrap technique. The same letter within a column indicates no significant difference among treatments.

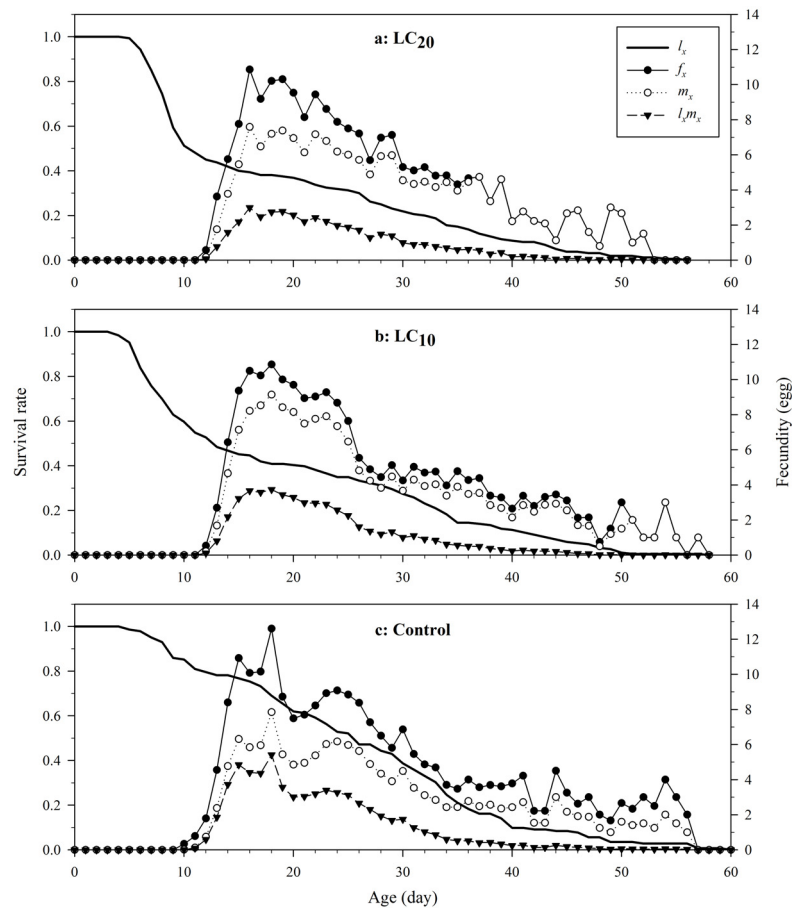


**FIGURE 3.** Age-stage specific survival rate of offspring from *T. urticae* females treated with LC<sub>10</sub> and LC<sub>20</sub> of bifentazate. **a, b** and **c**: Survival rates of offspring from *T. urticae* females treated with LC<sub>20</sub>, LC<sub>10</sub> of bifentazate and distilled water, respectively.

## Discussion

Bifenazate had acaricidal activity against *T. urticae* (Ochiai *et al.* 2007), and the resistance mechanism of *T. urticae* to bifentazate was thoroughly illuminated by several studies (Van Leeuwen *et al.* 2007; Van Leeuwen *et al.* 2008; Van Nieuwenhuysse *et al.* 2009; Van Leeuwen *et al.* 2011). Our study here is the first comprehensive investigation on the sublethal effects of bifentazate on life table parameters of progeny generation of *T. urticae*. Our results demonstrated that bifentazate had a strong adulticidal activity on *T. urticae*, which was in accordance with the previous report (Ochiai *et al.* 2007). Furthermore, our results found that LC<sub>10</sub> and LC<sub>20</sub> of bifentazate could reduce the survival rate, oviposition period, average oviposition and longevity of the female of *T. urticae*. This was also consistent with previous studies. For example, Marcic (2005) concluded that females of *T. urticae* survived from the treatment of tebufenpyrad at protonymphal or deutonymphal stage had the lower fertility, and the females of two-spotted spider mite treated with the sublethal concentration/dose of spirotetramat (Marcic 2007), spirotetramat (Marcic *et al.* 2012), *Beauveria bassiana* (Seyed-Talebi

*et al.* 2012), fenpyroximate and pyridaben (Kim *et al.* 2006) had shorter or lower longevity, reproduction period and fecundity than the controls.



**FIGURE 4.** Age specific survival rate ( $l_x$ ), fecundity ( $m_x$ ), maternity ( $l_x m_x$ ) and age-stage specific fecundity ( $f_x$ ) of offspring from *T. urticae* females treated with LC<sub>10</sub> and LC<sub>20</sub> of bifentazate. **a, b** and **c**:  $l_x$ ,  $m_x$ ,  $l_x m_x$  and  $f_x$  of offspring from *T. urticae* females treated with LC<sub>20</sub>, LC<sub>10</sub> of bifentazate and distilled water, respectively.

It is known that exposure to pesticides can lead to hereditary malfunctions and malformations, and hence can lead to significant disturbances of insect or mite development in the next generation (Adamski *et al.* 2009). Our results demonstrated that exposure to lethal concentrations of bifentazate at LC<sub>10</sub> and LC<sub>20</sub> during the female adult stage had negative effects on the two-spotted mite population increase of progeny generation (i.e. lower  $r_m$  and  $\lambda$  values, but higher  $T_c$ ). According to He *et al.* (2011), exposure to sublethal concentrations of avermectin during the adult stage had negative effects on the population increase (i.e. lower  $r_m$ ,  $R_0$ , and  $\lambda$  values, longer  $D$ ) of the progeny generation of *Panonychus citri* (McGregor). Li *et al.* (2006) also reported that the values of  $r_m$  in offspring of abamectin-treated adult females of *Amphitetranychus viennensis* decreased significantly. However, Landeros *et al.* (2002) reported that there was a significant increase in the  $R_0$  of *T. urticae* after applications of abamectin at LC<sub>10</sub>. In this study, the mites treated with LC<sub>20</sub> of bifentazate had negative effects on  $R_0$  of the mites. LC<sub>20</sub> treatment caused significant changes in the progeny including the prolonged immature stages, decreased preadult survival rate and adult male longevity.



The  $r_m$  is the most important parameter to describe the growth potential of a pest population. The results of this study showed that values of  $r_m$  of progeny generation of the treated mites significantly decreased. Although the preadult survival rate and population growth of progeny generation of treated mites was substantially lower, the female adult longevity, oviposition period and average oviposition per female were not different from those of population of the control. In other words, the surviving females of the progeny generation treated with LC<sub>10</sub> and LC<sub>20</sub> of bifenazate were not affected. This phenomenon may refer to the acaricide resistance of bifenazate. Van Leeuwen *et al.* (2010) reported the progeny of *T. urticae* could absorb small amount of bifenazate from female treated with the sublethal concentration of bifenazate by maternal inheritance referring to the bifenazate resistance. In some other studies, low concentration of bifenazate caused an effect called hormesis, which is referred to biphasic dose response relationship with stimulatory effect at low doses and inhibitory effect at high doses of a stressor or pesticide (Luckey 1968; Guedes & Cutler 2014). There are two hypotheses which provided the potential mechanistic explanation for hormesis: the growth hormesis theory (Stebbing 2000), and the principle of physiological resource allocation (Weltje *et al.* 2005). According to the growth theory, this hypothesis is based on control systems and growth analysis (Stebbing 1998; Stebbing 2000). The control mechanisms that regulate growth counteract the perturbing or inhibitory effects of toxic agents (Guedes & Cutler 2014). This idea remains highly speculative, focused on growth as a life-history trait and lacking a general mechanistic underpinning (Thayer *et al.* 2005; Mushak 2007; Jager *et al.* 2013). The principle of resource allocation is based on physiological energetic models that predict trade-offs in resource allocation among different physiological processes (Forbes 2000; Guedes & Cutler 2014). Hormesis by acquisition is explained by an unlimited increase in energy uptake from the environment, when such a resource is limitless (Jager *et al.* 2013). This increased energy uptake favors growth rate, and possibly maximizes size, which increase reproduction and population growth rate (Guedes & Cutler 2014). The results of our study showed that, the LC<sub>10</sub> and LC<sub>20</sub> of bifenazate had no negative effects on the offspring of the treated female *T. urticae* in fecundity and longevity. This situation may be explained by resource allocation, but further studies are necessary to confirm this conjecture.

Investigations on sublethal effects of an acaricide aim to discover the negative non-lethal impacts of the acaricide on various life table parameters that might affect population dynamics (Stark & Banks 2003), and help us gain more information about the acaricide and put it to better use. Our study is the first step to explore the sublethal effect of bifenazate on progeny generation of *T. urticae*. Based on our results, the survival and the population growth of progeny generation of *T. urticae* was substantially decreased, indicating that low concentration of bifenazate could play an important role when this acaricide was combined with other biological control agents like natural enemies. However, future field investigation will provide more information to guide the rational use of this acaricide.

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