Article

Sublethal effects of some botanical and chemical insecticides on the cotton whitefly, *Bemisia tabaci* (Hem: Aleyrodidae)

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Abstract
In addition to direct mortalities caused by acute concentrations of insecticides, some biological traits of target pests may be also affected by sublethal doses. The cotton whitefly, *Bemisia tabaci* (Hem: Aleyrodidae) is an important pest of a wide variety of agricultural crops across the world. The control of *B. tabaci* largely relies on wide application of chemical insecticides. In this study, we analyzed the life table parameters to evaluate the sublethal effect of three plant-derived insecticides (*Fumaria parviflora* (Fumariaceae), *Teucrium polium* (Lamiaceae), and *Thymus vulgaris* (Lamiaceae)) and two chemical insecticides (pymetrozin and neemarin) *on B. tabaci*. The whiteflies were allowed to oviposit on plants infected with each of the five insecticides using leaf-dip method. The data were analyzed using the age-stage two-sex life table. We found significant differences in the gross reproductive rate (GRR), the net reproductive rat ($R_{0)}$, the intrinsic rate of increase (r) and the finite rate of increase (λ) of treated whiteflies compared to control. Our results showed that some biological traits of *B. tabaci* are affected by sub-lethal doses of the plant-derived extracts and that these effects are comparable to those of chemical insecticides. Given the detrimental effects of chemical insecticides on human, environment and non-target organisms, plant-derived insecticides may provide valuable environmentally friendly tools for pest management programs.

Keywords *Bemisia tabaci*; life table; population parameters; sublethal effects; plant extract; insecticide.

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1 Introduction

The cotton whitefly, *Bemisia tabaci* (Hem: Aleyrodidae), is one of the most serious pests of agriculture attacking a wide range of plant species across the world (Oliveira et al., 2001; DAFGWA, 2008; De Barro et al., 2011). Economic damages occur only by direct feeding on plant phloem, (Malumphy, 2003) but also by producing high amounts of honeydew (Perumal and Marimutha, 2009) as well as transmission of more than 111 plant pathogenic viruses (Jones, 2003; Antony et al., 2009). At least 24 different biotypes of *B. tabaci* have been identified worldwide with the biotype B (also known as *Bemisia argentifolii* Bellows and Perring) is the most serious and widely distributed biotype (Perring, 2001).

During the two recent decades, *B. tabaci* has spread rapidly around the world to become a major pest of fruits, vegetables, fiber, and ornamental crops in tropical and subtropical regions (Brown et al., 1995; Oliveira et al., 2001). The control of *B. tabaci* has largely depended on wide application of chemical insecticides, which has caused many environmental and health problems (Ren et al., 2001; Lu et al., 2012). In addition to these problems, the satisfactory control of *B. tabaci* has proven to be difficult because of its wide range of hosts, rapid rate of development and reproduction and, more importantly, its high resistance to many commercially available insecticides (De Barro and Driver, 1997). Resistance to different classes of insecticides including organophosphates, carbamates, pyrethroids, neonicotinoids, and insect growth regulators has been frequently reported for populations collected from different geographic areas of the world (Nauen and Rauch, 2003). Therefore, it is necessary to search for alternative methods for effective control of this pest besides reducing the detrimental effects of chemical insecticides on human, environment and non-target organisms. Recently, the use of plant-derived insecticides (known also as botanicals) as potential safe weapons alternative to synthetic pesticides has been received increasing attention (Pascual-Villalobos and Robledo, 1998; Dimetry, 2012).

Recent studies have revealed that the acute mortality of insect pests is not the only determinant of insecticide efficiency. On the other word, in addition to direct mortality caused by insecticides, some biological traits of insect pests may be also affected by sublethal doses. Here, a question arises: whether or not the biological properties of resistant populations are still affected by sublethal doses of insecticides even when they do not suffer any direct mortality. To explore this question, in the current study, we used the life table parameters to evaluate the sublethal effects of some chemical and plant-derived insecticides on *B. tabaci*. The life table demonstrates a perfect description of survival, development, stage differentiation, and reproduction of a population (Hu et al., 2010). The traditional age-specific life table (Lewis, 1942; Leslie, 1945) considers only the survival and fecundity of females, and ignores male population as well as the stage differences and overlapping. As the economic damages of many important pests, including *B. tabaci*, occurs by both sexes, ignoring the male population may lead to miscalculation of survival and fecundity curves (Chi, 1988; Chi and Yang, 2003; Chi and Su, 2006). Therefore, in this study, we used the age- stage, two- sex life table, developed by Chi and Liu (1985), to take into account the male populations as well as the variable developmental rate occurring among individuals.

2 Material and Methods

2.1 Host plants

Seeds of cotton, *Gossypium hirsutum* (Var. Varamin) and tomato, *Lycopersicon esculentum* (Var. Bakker brothers) were planted in transplant trays in greenhouse conditions (25 - 27 ºC, 50 - 65% RH, and 16:8 L:D). Cotton plants were used as the main host for mass rearing of *B. tabaci*, while tomato plants were used as experimental host plant. The tomato stalks were transferred into plastic pots which had been filled with a commercial sterile plant growth media (BAGA, Bastare Amade Giah Arganic, manufactured by Dashte Sabz Atie Co., Iran). All glasses were kept in wooden cages (60 × 50 × 80 cm) covered by fine cloth mesh to avoid the entrance of wild *B. tabaci*. Old glasses were monthly replaced by new one to prevent the over-crowding.

2.2 Study insects

Adults of *B. tabaci* were collected from cotton fields (Rafsanjan, Kerman province, Southeastern Iran) by aspirator and released on cotton plants grown in greenhouses of College of Agriculture (Vali-e-Asr University of Rafsanjan, Iran). Several puparia belonging to *B. tabaci* (biotype A) were selected from these colonies and used to establish a stock colony in greenhouse conditions.

Young fully-grown tomato leaves, cut from end of shoots, were put in small plastic glasses (10 cm diameter, height: 15 cm) which were filled by distilled water. Each glass was covered by a similar transparent one to make a glass cages. Adult whiteflies were released into the cage via a small pore provisioned on middle parts of the upper glass. The rearing method was taken from Biondi et al. (2010) with slight modifications. To provide coetaneous 24-h old adults, the eye-red puparia on tomato leaves were checked daily and the newborn adults were collected and released in to prepared glass cages.

2.3 Chemical insecticides

Commercial formulations of the two synthetic insecticides, pymetrozin (Chess®25% WP, Singenata Company), and azadirachtin (Neemarin EC1500), were used. These formulations are among the most widely used insecticides for *B. tabaci* control in Iran.

2.4 Preparation of plant extract

Four medicinally important plants belonging to different families were used as botanical insecticides (Table 1). The aerial parts of these plants (leaves and flowers) were collected in their flowering stage from their natural habitats located at different parts of the Kerman province during May and June 2010. Plant materials were air-dried for 4-5 days and ground by an auto-mixer. Twenty grams of dried materials were placed on filter paper and steeped in ethanol (90 ml) and water (210 ml) for 12 hours. The plant extracts were prepared according to the Soxhlet extraction method (Pascual-Villalobos and Robledo, 1998). After extract preparation, rotation was used to reach an extract amount of one third.

Table 1 Plant species extracts evaluated on adult *Bemisia tabaci*.

Scientific name	Common name	Family name	The used parts of plant
Fumaria parviflora	Fumitory	Fumariaceae	Leaves and stem
Teucrium polium	Germander	Lamiaceae	Leaves
Thymus vulgaris	Thyme	Lamiaceae	Leaves

2.5 Dose-mortality response

The effects of five concentrations of the abovementioned botanical and synthetic insecticides on adult *B. tabaci* were assayed using the leaf-dip method. The concentrations include 106, 150, 210, 298, 420, and 593 µg/ml for pymetrozin, 0.060, 0.135, 0.330, 0.780, and 1.860 mg/ml for neemarin, 44, 58, 76, 100, 132, 173, and 228 mg/ml for *Th. vulgaris*, 58, 76, 100, 132, 173, 223, 300, and 395 mg/ml for *T. polium*, and 100, 153, 234, 359, 550, and 842 mg/ml for *F. parviflora*. Briefly, two-leaves tomatoes stalks were dipped in the prepared dilutions for 5 s (Heydari et al., 2003), then put separately in glass cages. After drying the treated leaflets, 50 coetaneous adults were released into each cage. Cages were maintained under controlled environmental conditions (27±2 °C, 50±5% RH, and a photoperiod of 16:8 h L:D). The total numbers of dead adults were counted after 24 h. Adults were considered dead when they were not able to move properly when stimulated with a fine brush. A solution of 3% ethanol/water was used as control. The mortality percentage was corrected using Abbott (1925) method and the lethal dose of 25% (LD_{25}) was calculated for each insecticide. Three replicates were considered for each of the studied insecticides and concentrations.

2.6 Sublethal effects

The sublethal effects of the selected insecticides were evaluated by analysis of life table parameters of *B. tabaci* eggs treated with the lethal dose of 25% (LD_{25}). Tomato leaflets were dipped in 25% lethal concentration of each insecticide for 5 s (Heydari et al., 2003) and transferred to the glass cages. Thirty new emerged adults were randomly captured from the stock colony and released into cages. After 24 h, all adults were removed from the cages and their laid eggs were incubated for life table studies. The plants containing eggs were checked daily and any change in population parameters were recorded. After adult emergence, the whiteflies were individually transferred to new cages daily. The longevity of adult female and male whiteflies was determined and the number of eggs laid by each female was recorded daily until all females died. All experiments were carried out in controlled conditions (27±2℃, 50%±5 relative humidity (RH) and 16: 8 h L:D).

2.7 Data analysis

Probit analysis was used to estimate the LC50 and LC25 by the Polo-Plus 2.00 software. The population parameters data were analyzed using SPSS software (version 16) followed by one-way ANOVA and Duncan's multiple range tests. The raw life table data were analyzed based on the age- stage, two- sex life table theory (Chi and Liu, 1985; Chi, 1988). The means and standard errors of the life table parameters were estimated using Jackknife method (Sokal and Rohlf 1995). Processing of raw data was facilitated through TWOSEX-MSChart computer program (Chi, 2005) available at http://140.120.197.173/Ecology/ prod02.htm (Chung Hsing University, Taichung, Taiwan) and http://nhsbig.inhs.uiuc.edu.tw/www/chi.html (Illinois Natural History Survey, Champaign, IL). To format age- stage, two- sex life table, daily history of all individuals from birth to death, including daily female's fecundity, was inscribed. The developmental stages were listed as egg, larva, pupa, and adult.

According to Chi and Liu (1985), the total population dimensions (N_t) and the total number of individuals in stage j (N_j) are calculated using the following folmula. So in simulation based on the age-stage, two-sex life table, the curves for every stages and for the total population can be produced (Chi, 1988).

$$N_t = \sum_{i=1}^{n}\sum_{j=1}^{m} n_{ij}$$

$$N_j = \sum_{i=1}^{n} n_{ij}$$

Other life table parameters including the age- stage specific survival rate (S_{xj}), the age- specific survival rate (l_x), the age- stage specific fecundity (f_{xj}), the age- specific fecundity (m_x), the age-specific maternity ($l_x m_x$), the age- stage reproductive value (v_{xj}), the mean fecundity (F), the intrinsic rate of increase (r), the finite rate of increase (λ), the net reproductive rate (R_0), and the mean generation time (T) were calculated using the following formula (Chi and Liu, 1985; Chi, 1988):

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

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

where β is the number of stages.

$$e_{xy} = \sum_{i=x}^{n}\sum_{j=y}^{m} s'_{ij}$$

where n is the number of age, m is the number of stage and $\acute{s}_{ij}$ is the probability of individual survival from age x and stage y to age i and stage j (Chi, 1988). Difference in developmental rate and overlapping of stage growth are common phenomenon frequently reported in many populations of insects. Therefore, individuals with the same age but different stage growth may possess different life expectancies. The two-sex life table method, however, accounts for individual life expectancy with age x and stage y (Istok, 1981; Chi and Su, 2006; Yang and Chi, 2006).

The intrinsic rate of increase (*r*) was calculated through the iterative bisection method using the Euler-Lotka formula with age indexed from 0 (Goodman, 1982):

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

The finite rate of increase (λ), the mean generation time (*T*), the gross reproductive rate (*GRR*) and the net reproduction rate (R_0) were calculated as:

$$\lambda = e^{r}$$

$$T = (LnR_0)/r$$

$$GRR = \sum m_x$$

$$R_0 = \sum_{x=\alpha}^{\beta} l_x m_x$$

In the two-sex life table which starts with N eggs, the survival number of each age-stage is $N.S_{ij}$, thus the total number of eggs laid by adults emerged from this original N eggs would be:

$$\sum_{i=1}^{n}\sum_{j=1}^{m} N\, s_{ij} f_{ij}$$

The mean fecundity (*F*) per females was calculated as:

$$F = \left(\sum_{i=1}^{n}\sum_{j=1}^{m} N s_{ij} f_{ij}\right)/N_f$$

where N_f is the number of females emerged from N eggs.

3 Results and Discussion

In this study, we used the age-stage two-sex life table parameters to evaluate the sublethal effects of three plant-derived insecticides and two synthetic chemical insecticides on *B. tabaci*, a key devastating pest of agriculture across the world. The rationale behind selection of these insecticides was that their efficiency for control of different species of whiteflies had been repeatedly approved in previous studies (Pascual-Villalobos and Robledo, 1998; Hummelbrunner and Isman, 2001; Bi et al., 2002; El-Shazly and Hussein, 2004; Mahdavi Arab et al., 2008; Wang et al., 2008).

We did a preliminary bioassay test using five different concentrations of each selected insecticide to estimate the lethal dose of 25% (LD_{25}). The LC_{25} values calculated using these assays were 89.95 µg/ml, 0.070 µg/ml, 69 mg/ml, 90.9 mg/ml, and 314 mg/ml for pymetrozin, neemarin, *Th. vulgaris*, *T. polium*, and *F. parviflora*, respectively.

The age- stage survival rate (S_{xj}) of *B. tabaci*, shows the probability that a recently born will survive to age x and stage j. This curve shows the survival, stages difference, stages overlapping and the variable developmental rate between individuals (Chi, 1988; Yang and Chi, 2006; Hu et al., 2010). We found that the S_{xj} values in pymetrozin and *F. parviflora* treatments decreased compared to other insecticides. The survival rate of male whiteflies in *F. parviflora* was more than that of females. Additionally, a significant overlapping was observed among different stages, which is attributable to the differential developmental rates among individuals (Chi and Su, 2006; Yang and Chi, 2006; Hu et al., 2010).

The age- stage survival rate (l_x), the age- specific fecundity (m_x), and the age- specific maternity ($l_x m_x$) of *B. tabaci* treated by the six insecticides have been shown in Fig. 1. The l_x index presents the probability that a newborn whitefly will survive to age x. The survival rate is estimated by combining all surviving individuals of both sexes and those died during the pre-adult stages. The results showed that the survival rates of adult *B. tabaci* (from birth to death) ranged between 31 and 43 days with the highest and the lowest survival rates were observed in whiteflies treated with *Th. vulgaris,* and *T. polium* and control respectively.

The age- stage fecundity (f_{xj}), which shows the mean number of offspring produced by individual of age x and stage j per day, has been shown in Fig. 1. Since, only females produce offspring, there was only a single curve (Yang and Chi, 2006; Hu et al., 2010). The survival of female *B. tabaci* for treatments of control, pymetrozin, neemarin, *T. polium*, *Th. vulgaris* and *F. parviflora,* were 15, 22, 16, 22, 26 and 14 days respectively. The lowest survival rate and the shortest oviposition period were observed in whiteflies treated by *T. polium* extract. In contrast, the whiteflies treated by *Th. vulgaris* extract had the highest survival rate.

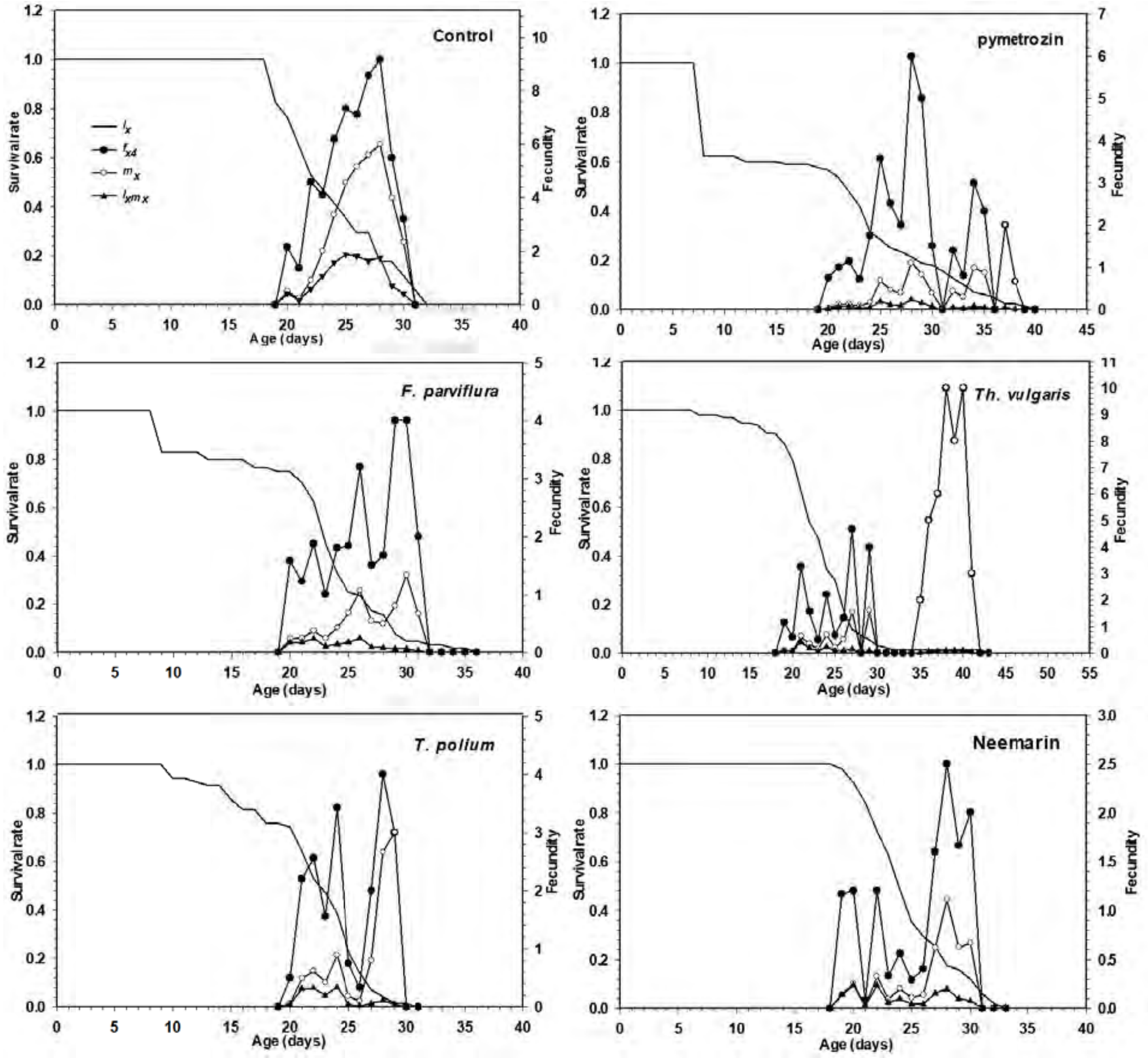

Fig. 1 Effects of botanical and chemical insecticides on age-specific survival rate (l_x), female age-specific fecundity (F_{x4}) (eggs/female), and age-specific maternity ($l_x m_x$) of *Bemisia tabaci*.

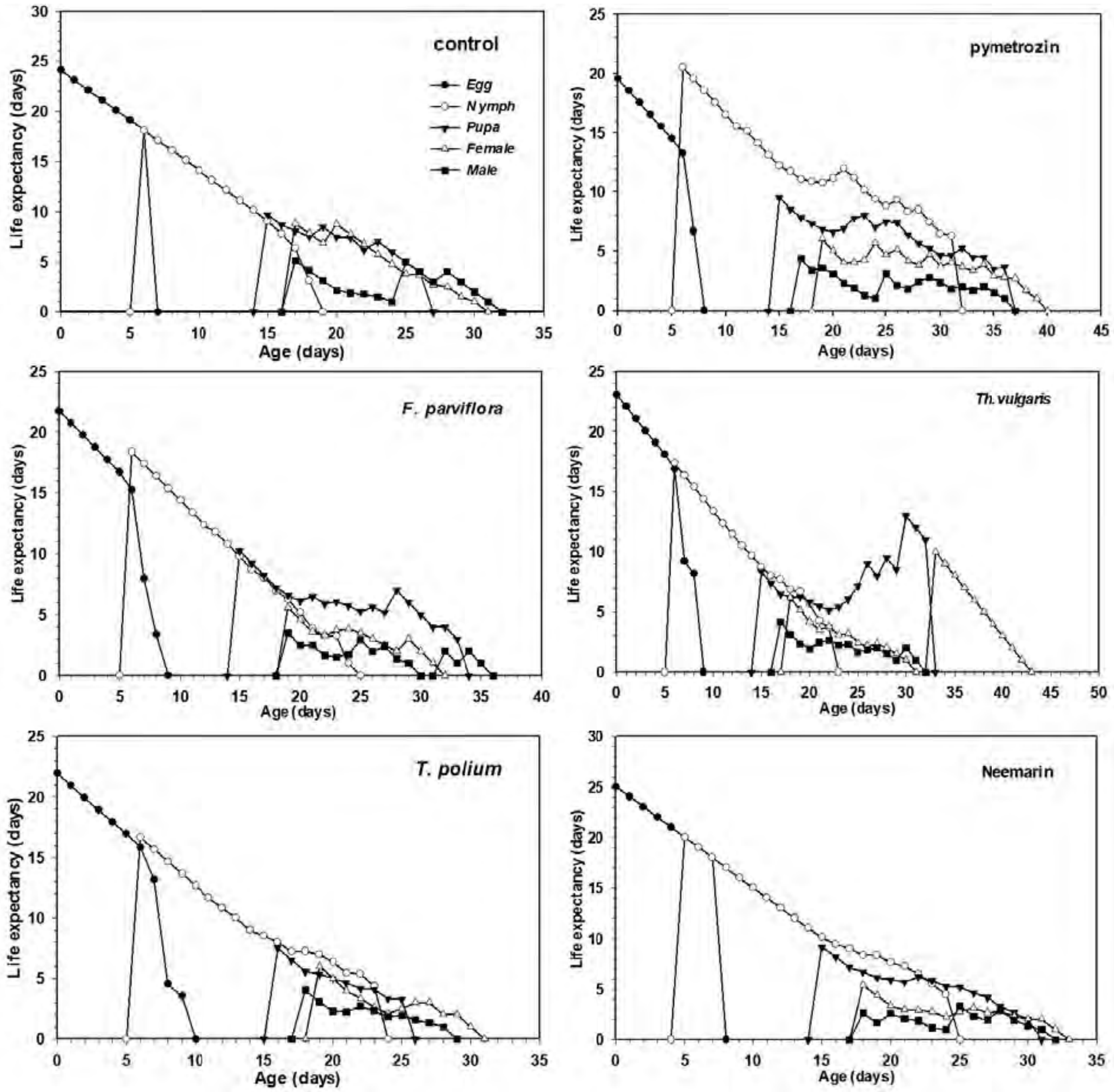

Fig. 2 Effects of botanical and chemical insecticides on age-stage specific life expectancy (e_{xj}) of *Bemisia tabaci*.

The life expectancy (e_{xj}) of each age- stage group of *B. tabaci* has been shown in Fig. 2. The life expectancy (e_{xj}) was calculated as the life duration of adult female and male whiteflies from emergence to death. The life expectancy of *B. tabaci* in control, pymetrozin, neemarin, *T. polium*, *Th. vulgaris* and *F. parviflora* treatments were calculated as 8.83, 6.03, 5.35, 3.76, 6.16, and 5.58 days for females and 5.12, 4.36, 2.64, 4.64, 4.15, and 3.48 days for males, respectively. The life expectancy based on age- stage two- sex life table shows the difference among individuals of the same age, but of difference stages or different sexes (Yang and Chi, 2006; Hu et al., 2010).

The age-stage reproductive value (v_{xj}) of *B. tabaci*, which shows the contribution of an individual to the next population, has been illustrated in Fig. 3. The main peak in reproductive capability of female whiteflies in control, pymetrozin, neemarin, *T. polium*, *Th. vulgaris* and *F. parviflora*, occurred at the age of 21 d (v_{21}= 36.54), 24 d (v_{24}= 14.91), 28 d (v_{28}= 4.70), 20 d (v_{20}= 8.87), 35 d (v_{35}= 40.64), and 29 d (v_{29}= 9.85), respectively. The reproductive value significantly increased when reproduction was maximized. These findings are in accordance to previous studies, which reported that individuals at the reproduction peak contribute much more than a recently born individuals (Yang and Chi, 2006; Hu et al., 2010) (Fig. 3).

If an adult female produce no offspring from a given age, its reproductive value would be zero from this age onward, although, it may still survive for the following days (Yang and Chi, 2006; Hu et al., 2010). For example, the reproductive capability of whiteflies treated by *F. parviflora* extract ended at 32th day after treatment, although they lived by 36th day (see Fig. 3). Such asynchronies in survival and reproductive rates have been previously reported for whiteflies and other insects (Yang and Chi, 2006; Hu et al., 2010; Huang and Chi, 2012). Our results also showed that the susceptibility to both botanical and chemical insecticides varied among different developmental stages of *B. tabaci*, a phenomenon that has been previously documented by Liu and Stansly (1995).

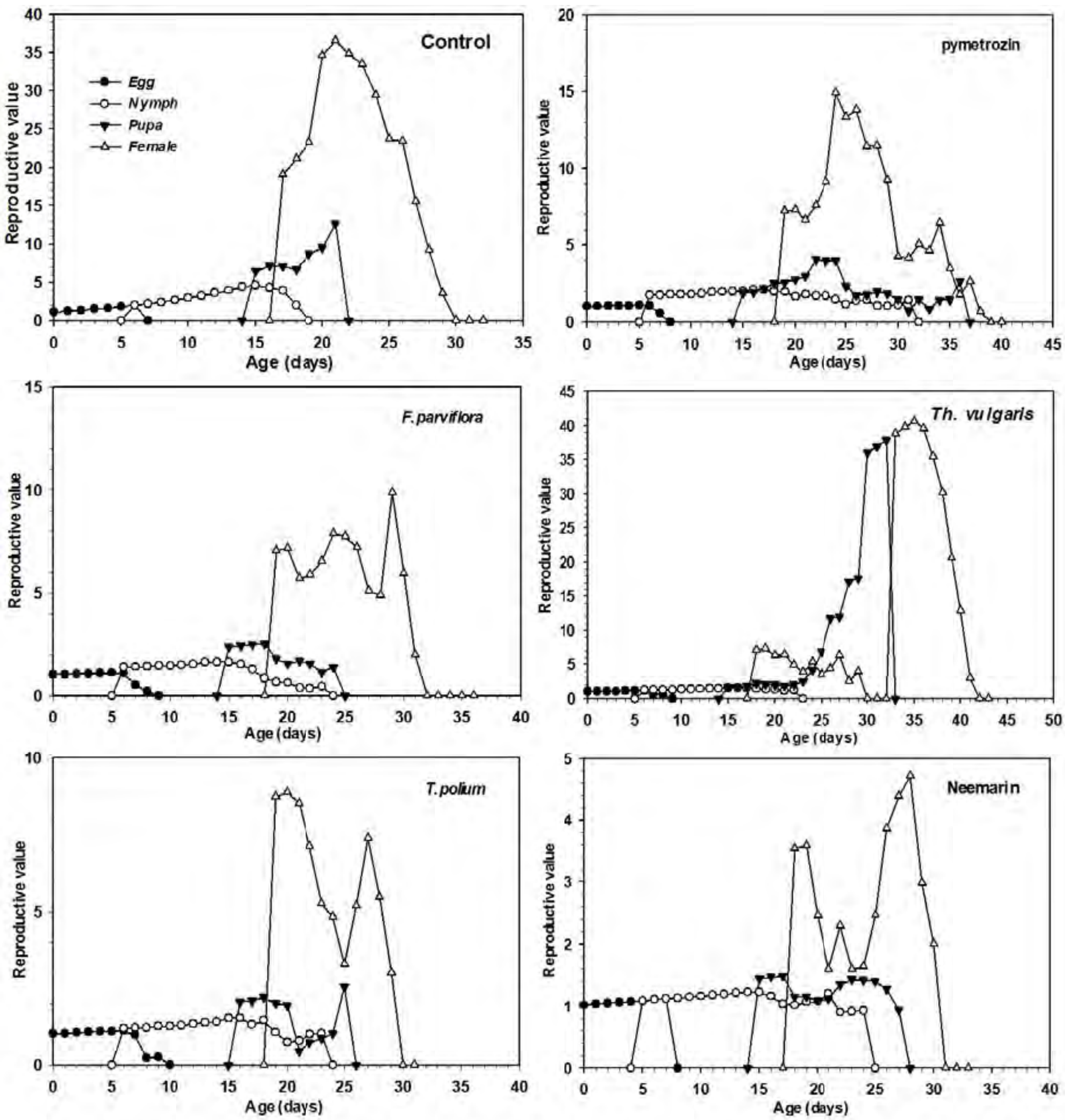

Fig. 3 Effects of botanical and chemical insecticides on age–stage reproductive value (v_{xj}) of *Bemisia tabaci*.

The population parameters including the intrinsic rate of increase (r), the finite rate of increase (λ), the gross reproductive rate (GRR), the net reproductive rate (R_0), and the mean generation time (T) were also calculated using age-stage two-sex life table (Table 2). Analyses of variances revealed significant differences in effects of both botanical and chemical insecticides on the net reproductive rate (One way ANOVA: $F_{6,754}$=12.489 P<0.001), the intrinsic rate of increase (One way ANOVA: $F_{6,754}$=2.946, P<0.01), and the finite rate of increase (One way ANOVA: $F_{6,754}$=3.192 P<0.01) compared to control. However, there was no significant difference in these parameters among whiteflies treated by different insecticides. These findings show the efficiency of the botanical insecticides in control of *B. tabaci* comparable to the commonly used chemical insecticides. The gross reproductive rate (One way ANOVA: $F_{6,754}$=1.044, P=0.28) and the mean generation time (One way ANOVA: $F_{6,754}$=1.098 P=0.362), in contrast, were not statistically different among whiteflies treated by different insecticides.

Table 2 Comparison of means related to the effect of different herbal compounds in comparison with pesticides on population parameters with using the age-stage two-sex life table of eggs exposed to infected plant of *Bemisia tabaci*.

Treatment	The intrinsic rate of increase (r)	The finite rate of increase (λ)	The net reproductive rate (R_0)	The gross reproductive rate (GRR)	The mean generation time (T)
Control	0.088 ± 0.008^{a}	1.092 ± 0.009^{a}	10.180 ± 1.864^{a}	38.590 ± 7.723^{b}	26.657 ± 0.647^{a}
Pymetrozin	0.016 ± 0.021^{b}	1.016 ± 0.021^{b}	1.393 ± 0.663^{b}	9.748 ± 3.608^{b}	28.289 ± 1.380^{a}
Neemarin	0.015 ± 0.012^{b}	1.015 ± 0.012^{b}	1.392 ± 0.391^{b}	4.299 ± 1.667^{b}	25.037 ± 1.341^{a}
T. polium	0.019 ± 0.014^{b}	1.019 ± 0.014^{b}	1.500 ± 0.482^{b}	12.194 ± 4.837^{b}	24.231 ± 0.749^{a}
Th. vulgaris	0.026 ± 0.012^{b}	1.026 ± 0.012^{b}	1.924 ± 0.631^{b}	7.394 ± 12.900^{b}	27.813 ± 3.485^{a}
F. parviflora	0.021 ± 0.017^{b}	1.021 ± 0.018^{b}	1.578 ± 0.611^{b}	7.118 ± 3.916^{b}	25.127 ± 0.962^{a}

Different letters show significant differences at 0.05 level.

In our study, the total number of offspring produced by all females was nearly equal to the net reproductive rate × the cohort size, and the minor difference was probably due to rounding-off. This relationship highlights the accuracy of the age-stage two-sex life table. These findings were in accordance to results obtained by other authors (Chi, 1988; Chi and Young, 2003; Yang and Chi, 2006). Yang and Chi (2006) found that the net reproductive rate was lower than the mean female fecundity ($R_0 \leq F$). This inequality may be explained by the presence of pre-adult mortality, a condition that was also demonstrated in our study.

Given the increasing knowledge on the importance of sustainable agriculture during the two last decades, efforts for detection and elaborate use of environmentally friendly agents for pest management has received growing attention. In this context, life table methods are valuable tools to evaluate and compare the sublethal effects of these agents on both target pests and non-target organisms such as natural enemies. This study showed that some biological aspects of *B. tabaci* are significantly affected at sublethal concentrations of the botanical insecticides and that these effects are comparable to those achieved from application of those chemical insecticides that are widely used for control of whiteflies. According to our current data, and our previous findings (Esmaeily et al., 2014), the extracts taken from *C. procera* and *T. polium* as well as the synthetic insecticide, pymetrozin, gave the best results in *B. tabaci* control. Given the safety of plant-derived insecticides for human and the environment, the two former insecticides may be considered as appropriate agents for use in integrated pest management of whiteflies. Although, both sublethal and lethal effects of these agents on non-target organisms, especially natural enemies, remain to be cleared in future studies.

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