Sublethal effects of some synthetic and botanical insecticides on *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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Abstract: In addition to direct mortality caused by insecticides, some biological traits of insects may also be affected by sublethal insecticide doses. In this study, we used the age-stage, two-sex life table method to evaluate the sublethal effects of the four synthetic insecticides: abamectin, imidacloprid, diazinon, and pymetrozin as well as the botanical insecticide taken from *Calotropis procera* (Asclepiadaceae) extract, on eggs of the cotton whitefly, *Bemisia tabaci* (Hem.: Aleyrodidae). The lowest and highest survival rates and oviposition periods were observed in whiteflies treated by diazinon and imidacloprid, respectively. We found significant differences in the net reproductive rate (R_0), the intrinsic rate of increase (r), the finite rate of increase (λ), and the gross reproductive rate (GRR) among different insecticides. Altogether, our results showed that pymetrozin and *C. procera* induced the most sublethal effects, thus they may be suitable candidates for use in integrated pest management programs of *B. tabaci*.

Key words: *Bemisia tabaci*, cotton whitefly, pesticide, plant extract, two-sex life table

Introduction

The sweet potato whitefly, *Bemisia tabaci* (Hem.: Aleyrodidae), is an economically important pest of agricultural crops throughout the world (Brown *et al.* 1995; De Barro *et al.* 2011). Harm to the plant occurs when the pest directly feeds on plant phloem. The plant is also harmed by the high amounts of emitted honeydew as well as by transmission of plant pathogenic viruses (Jones 2003; Li *et al.* 2010). Growers in many countries rely largely on the use of chemical insecticides to reduce the damage caused by *B. tabaci* (Cameron *et al.* 2013). Various classes of insecticides, including organophosphates, carbamates, pyrethroids, and more recently, neonicotinoids are widely used to control *B. tabaci* (Liang *et al.* 2012). The use of pesticides, though, has been challenged due to some factors including the rapid development of resistance in exposed pests, resurgence of pest populations, environmental pollutions, and unwanted effects on non-target organisms such as pollinators, natural enemies, and humans (Weisenburger 1993; Biondi *et al.* 2012b; Lu *et al.* 2012; El-Naggar and Zidan 2013). Plant-derived (botanical) insecticides have long been considered as environmentally friendly agents which are alternatives to synthetic chemical pesticides for pest management (Agbenin and Marley 2006; Isman 2006; Aghaali *et al.* 2013). A large number of plant substances have been introduced for use as insect anti-feedants or repellents (Isman 2006; Ramos *et al.* 2006; Bacci *et al.* 2007; Mahdavi-Arab *et al.* 2008). Despite their safety and abundance, botanical insecticides have not yet achieved their rightful place due to the tremendous competition with the new generation of chemical insecticides (Isman 2006; El-Naggar and Zidan 2013).

The quantification of the acute toxicity of pesticides to target or non-target arthropods has been traditionally used to determine appropriate lethal doses for pest control (Biondi *et al.* 2013). However, the estimated lethal doses may only be a partial measure of the deleterious effects. Under field conditions, insects would be exposed to sublethal doses of insecticides, thus they may experience certain related sublethal effects (Desneux *et al.* 2007). In addition to direct mortality induced by pesticides, sublethal effects on arthropod physiology and behavior should also be considered for a comprehensive analysis of their impact (Desneux *et al.* 2007; Biondi *et al.* 2013). These effects are defined as the physiological and/or behavioral trait modifications in individuals that survive an exposure to insecticides (Desneux *et al.* 2007; He *et al.* 2013). An accurate assessment of sublethal effects is also required to estimate the overall efficacy of insecticides in pest control and to evaluate the selectivity towards non-target organisms (Biondi *et al.* 2012a).

For the past 15 years, the sublethal effects of pesticides on both beneficial and harmful insects have been the subject of an increasing number of studies (Desneux *et al.* 2007). For example, the sublethal effects of different insecticides on *B. tabaci* have been studied in depth, by different researchers (Byrne *et al.* 2003; Bacci *et al.* 2007; Esmaeily *et al.* 2012; He *et al.* 2013).

An accurate understanding of pest life tables and plant phenology is crucial for successful application of

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control agents because it has been suggested that pest susceptibility to these agents alters between life stages (Chi 1990; Yang and Chi 2006). An increasing number of studies have focused on the identification and characterisation of the sublethal effects of pesticides on insect populations (Desneux *et al*. 2007). In this context, the analysis of life table parameters has been frequently used to predict both lethal and sublethal effects of pesticides on target and non-target organisms (Stark *et al*. 2004; Biondi *et al*. 2013; He *et al*. 2013). The traditional life table deals only with the female population and ignores two important issues in pest ecology: stage differentiation and variable developmental rates among individuals (Chi 1990). Chi and Liu (1985), however, developed an age-stage, two-sex life table to take into account the male populations as well as the variable developmental rate occurring among individuals. The simulations according to this life table suggest a possible way to predict the most suitable time for pest control (Chi 1988; Chi 1990; Yang and Chi 2006; Huang and Chi 2012).

In this study, we used the age-stage, two-sex method to evaluate the different life table parameters of *B. tabaci* as the main characters, to determine the sublethal effects of four commercially available insecticides – pymetrozin, diazinon, imidacloprid, and abamectin as well as extract taken from the Swallowwort, *Calotropis procera* (Asclepiadaceae). The important medicinal plant, *C. procera*, is distributed throughout the world, though it is commonly found in tropical, sub-tropical, and desert areas. In Iran, *C. procera* grows on the southern shores of the Oman Sea from Khuzestan through Macron, Baluchistan at 1,100 m above sea level (Rahman and Wilcock 1991). The essential oils and extracts of swallowwort have been known to be insecticidal agents in previous studies (Ramos *et al*. 2006; Mahdavi-Arab *et al*. 2008; Irannejad 2010).

Materials and Methods

Host plants

The seeds of cotton, *Gossypium hirsutum* cv. Varamin, and tomato, *Lycopersicum esculentum* cv. Bakker brothers, were cultured in transplant trays in greenhouses. Cotton plants were used as the main host for the mass rearing of *B. tabaci*, while tomato plants remained the experimental host plants. Some plants were transplanted separately in plastic pots (15 × 15 × 20 cm) containing a commercial Sterile Plant Growth Media, BAGA (Bastare Amade Giah Arganic, manufactured by Dashte Sabz Atie Co., UTSTP- Iran) and others were planted in hydroponic pots. To avoid the establishment of non-target insects including *B. tabaci*, all pots remained in 60 × 50 × 80 cm cages covered by a fine cloth mesh. Old pots were replaced monthly to prevent a pest population increase while mass rearing was taking place.

Insects

Adults of *B. tabaci* were collected from wild populations in Rafsanjan cotton fields (Kerman province, south east Iran) with an aspirator, during June 2011 and released on cotton plants in greenhouses of the College of Agriculture

(Vali-e-Asr University of Rafsanjan). Several puparia belonging to *B. tabaci* (biotype A) were selected from these colonies according to the method of Samih *et al*. (2006), and used to establish a stock colony in greenhouse conditions. All experiments were conducted in controlled environmental conditions at 27±2°C, 50±5% relative humidity (RH), and a photoperiod of 16 : 8 h L : D.

The whiteflies were reared similar to the method of Biondi *et al*. (2012a) with some modifications. Briefly, the young tomato leaves were put in small plastic glasses (10 cm diameter, 15 cm height) containing distilled water. A similar transparent glass was conversely placed on each glass to make the glass cages. Adult whiteflies were released into the cages through a small pore located in the upper glass. In order to produce the same aged 24-h adults, the eye-red puparia were detected daily in the colonies growing on tomato leaves and newborn adults were collected and released into the glass cages.

Pesticides

Commercial formulations of four insecticides, including pymetrozin (Chess®25% WP, Singenata Company), diazinon (Basudin®60% SC, Partonar Company under license of the Zagro Company), imidacloprid (Confidor® 35% SC, Behsam Company), and abamectin (Vertimec®1.8%, Singenata Company), were used in this study. In Iran, these compounds are among the most commonly used pesticides against *B. tabaci*.

C. procera extract preparation

The aerial parts (leaves and flowers) of *C. procera* were collected from its natural habitats in different parts of the Kerman province (southeastern Iran) during May and June 2011, when the plants were in the flowering stage. After air-drying for 4–5 days, 20 g of the dried materials were placed on filter paper and steeped in ethanol (90 ml) and water (210 ml) for 12 h. The extracts were prepared according to the Soxhlet extraction method (Pascual-villalobos and Robledo 1998). Afterwards, rotation was used to reach an extract amount of one third.

Dose-mortality response

The effects of different concentrations of pesticides, and *C. procera* extract on adult *B. tabaci* were assayed by using the leaf-dip method. Tomato stalks with two leaves were dipped in different dilutions for 5 sec (Heydari *et al*. 2003), then put separately in glass cages. Fifteen coetaneous adults of *B. tabaci* were released on each leaf after drying the treated leaflets. Cages were maintained as under the previous controlled conditions and the numbers of dead adults counted after 24 h. Adults were considered dead when they were not able to move properly; when their legs were probed with a soft camel-hair brush. The average values for mortality percentages were calculated and the data corrected by Abbott's formula (Abbott 1925). This assay was carried out in three replicates, each containing 15 adult whiteflies. An ethanol/water solution (3%) was used as the control.

Sublethal effects on life history traits

The LC_{25} value obtained from the previous assay was used to evaluate the sublethal effects of the four insecticides, and *C. procera* extract, on *B. tabaci* in a completely randomised design. The leaflets of tomato plants were dipped in the prepared solutions of each insecticide for 5 sec (Heydari *et al*. 2003) and allowed to air dry. Thirty coetaneous adults were released in cages containing the treated leaflets. After 24 h, adults were removed and the eggs laid on plant surfaces were kept for life table studies. The population parameters including the development time of different stages, oviposition period, longevity, and number of progeny produced per females, were recorded daily. After adult emergence, male and female whiteflies, were differentiated according to the method of Gerling (1990), and were transferred to separate cages. The longevity of the whiteflies as well as the number of eggs laid by females were recorded daily until adult death. This experiment was carried out in six replicates.

Data analysis

Probit analysis was used to estimate the LC_{50} and LC_{25} values using Polo-Plus 2.00 software. The data for population parameters were analysed using SPSS computer software (version 16). The raw data of the insecticides' sublethal effects were normalised by square root transformation, then compared using one-way analysis of variance (ANOVA) and Duncan's multiple range at $p < 0.05$ level.

In the age-stage, two-sex life table, developmental time of all individuals, including males, females, and those dying before the adult stage, and female daily fecundity were analysed according to the age-stage, two-sex life table developed by Chi and Liu (1985) and Chi (1988). Processing of raw data analysis was facilitated by the computer program, TWOSEX-MSChart (Chi 2010). The age-stage specific survival rate (s_{xj}, where x – age, and j – stage), the age-stage specific fecundity (f_{xj}), the mean fecundity (F) (i.e. the contribution of an individual of age x and stage j to the future population), the age-specific survival rate (l_x), the age-specific fecundity (m_x), and the population parameters including the intrinsic rate of increase (r), the finite rate of increase (λ) ($\lambda = e^r$), the net reproductive rate (R_0), and the mean generation time (T) were calculated according to the Chi method (Chi 1988).

The intrinsic rate of increase was appraised using the iterative bisection method as:

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

with age indexed from 0, where $l_x m_x$ – the age-specific maternity (Goodman 1982). The mean generation time was explained as the time length that a population requires to increase to R_0-times its size as the firmly fixed age distribution, to obtain the stable population increase rate. In other words:

$$e^{rT} = R_0 \text{ or } \lambda^T = R_0.$$

The mean generation time (T) and the gross reproductive rate (GRR) were calculated as, respectively:

$$T = (\ln R_0)/r,$$

and

$$GRR = \sum m_x .$$

Chi (1988) demonstrated that the relationship among the mean female fecundity (F) and the net reproductive rate (R_0) can be explained as:

$$R_0 = F(N_f/N),$$

where: N – the total number of individuals used at the start of the life table study, and N_f – the number of emerged female adults from the N eggs. This also means:

$$N_f \times F = R_0 \times N.$$

On the other hand, the total numbers of offspring produced by all females equal by the net reproductive rate, multiply the cohort size. This relationship shows the accuracy in the age-stage, two-sex life table analysis (Chi 1988; Chi and Yang 2003; Yang and Chi 2006).

Results

Dose-mortality response

Log-probit regression analyses of dose-mortality data showed that after 24 h of exposure to abamectin, diazinon, imidacloprid, pymetrozin, and *C. procera*, the LC_{25} values for different concentrations of abamectin (0.0002, 0.002, 0.02, 0.25, 2.7 µg/ml), diazinon (61, 96, 150, 235, 367, 575, 900 µg/ml), imidacloprid (0.1, 0.17, 0.31, 0.56, 1 µg/ml), pymetrozin (106, 150, 210, 298, 420, 593 µg/ml), and *C. procera* (200, 283, 400, 566, 800 mg/ml) were 0.00017, 41.7, 0.11, 89.95 µg/ml and 250.5 mg/ml, respectively (Table 1).

Sublethal effects

In figure 1 has been illustrated s_{xj}, that shows the probability that a newborn egg will survive to age x and stage j. Particularly, the figure shows the specific survival rate and overlapping in different developmental stages (Chi 1988; Yang and Chi 2006; Hu *et al*. 2010). The results showed that the amount of s_{xj} of *B. tabaci* in populations treated by pymetrozin and *C. procera* were reduced compared to other treatments. The survival rate of populations treated by pymetrozin particularly decreased in the nymphal stage, while the nymphs did not suffer mortality in the other treatments (Fig. 1). The male whiteflies treated by abamectin survived longer than the females. The parameters f_{xj}, l_x, m_x and $l_x m_x$ are shown in figure 2. The whiteflies lived for 32 days in the control, while the survival duration of adults (from birth to death) in whiteflies treated by pymetrozin, diazinon, imidacloprid, abamectin, and *C. procera* extract was 40, 33, 41, 45, and 39

Table 1. The LC_{25} ratio (mg/ml), lower and upper 95% confidence intervals (CI), slope ±SE, df and χ^2 for four synthetic pesticides, and one plant-derived pesticide

Treatment	LC_{25} [mg/ml]	95% CI	Slope ±SE	df	Chi-square
Abamectin	0.00017	(0.00003–0.00052)	0.626±0.086	3	0.665
Diazinon	41.760	(18.34–66.75)	1.400±0.220	5	4.253
Imidacloprid	0.1117	(0.013–0.207)	1.820±0.289	3	4.7348
Pymetrozin	89.95	(53.99–120.47)	2.236±0.357	4	1.936
C. procera	250.532	(130.37–338.92)	2.100±0.498	3	0.762
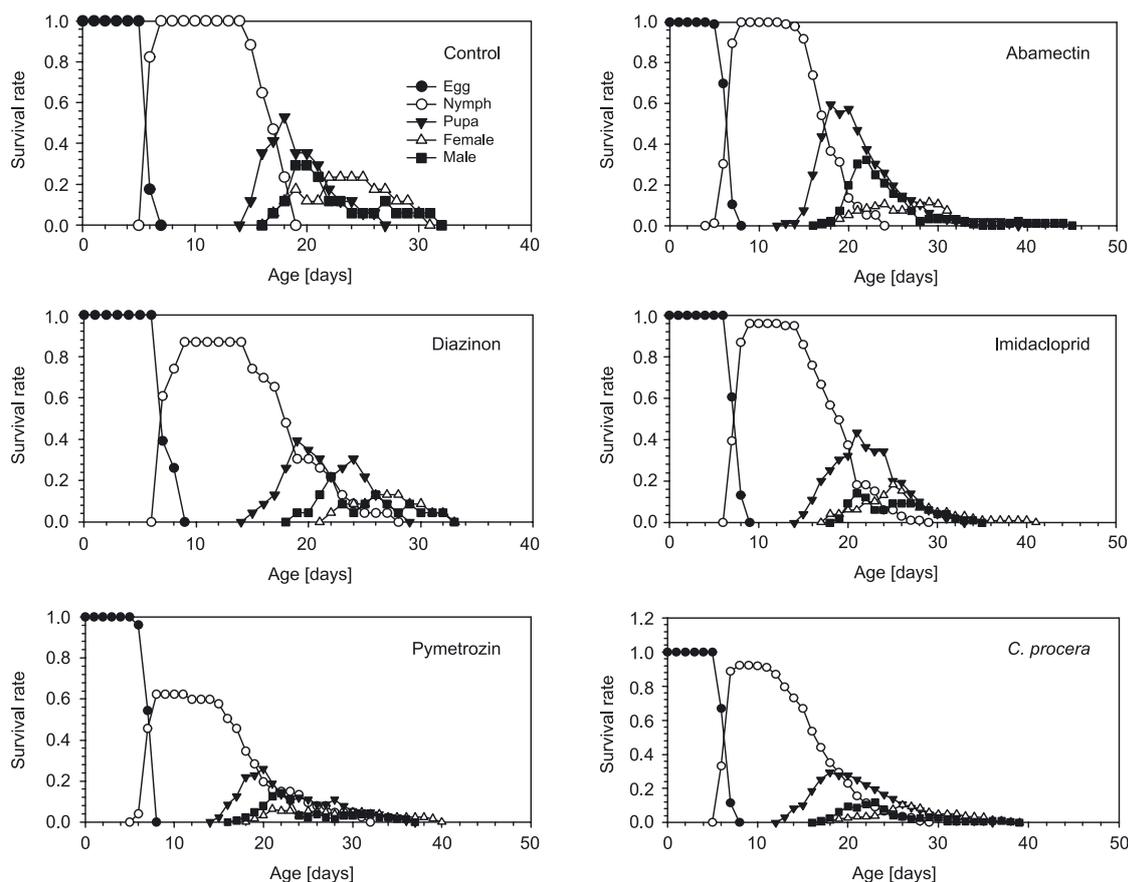
Fig. 1. Age-stage specific survival rate (s_{xj}) of *B. tabaci* exposed to different insecticides

days, respectively. The survival rate and the oviposition period of female whiteflies in the control and populations treated by pymetrozin, diazinon, imidacloprid, abamectin, and *C. procera* extract were 15, 22, 12, 24, 17, and 22 days and 11, 17, 9, 20, 13, and 18 days, respectively. The lowest and highest survival rate and oviposition period were observed in whiteflies exposed to diazinon and imidacloprid, respectively.

The age-stage reproductive value (v_{xj}) of *B. tabaci* is shown in figure 3. The main peaks in the reproductive parameters of females in the control, pymetrozin, diazinon, imidacloprid, abamectin, and *C. procera* treatments were at the age of 21 ($v_{21} = 36.54$), 24 ($v_{24} = 14.91$), 28 ($v_{28} = 8.21$), 35 ($v_{35} = 22.24$), 20 ($v_{20} = 12.70$), and 22 days ($v_{22} = 12.02$), respectively (Fig. 3). The reproductive value of a newborn (v_{01}) was definitely the finite rate of increase. The reproductive value significantly increased when reproduction was initiated (Fig. 3). A newborn egg in the pymetrozin

treatment had a reproductive value of 1.03 but a 21-day female had the much higher reproductive value of 14.91 (Fig. 3). This implies that those individuals at peak reproduction could contribute to enhanced fitness much better than a newly laid egg.

The results of statistical analyses revealed significant differences between the effect of *C. procera* extract and the four used insecticides on the net reproductive rate ($F_{5,695} = 12.925$; $p < 0.001$), the intrinsic rate of increase ($F_{5,695} = 3.110$; $p < 0.01$), the finite rate of increase ($F_{5,695} = 3.369$; $p < 0.01$), and the gross reproductive rate ($F_{5,695} = 3.119$; $p < 0.01$). There was no significant difference in the mean generation time ($F_{5,695} = 0.551$; $p > 0.05$) of whiteflies exposed to these insecticides. The effects of the insecticides on the mean values of r, λ, GRR, R_0, and T/day parameters were evaluated (Table 2). The results showed that imidacloprid had no negative effect on the gross reproductive rate in comparison to other treatments, but in-
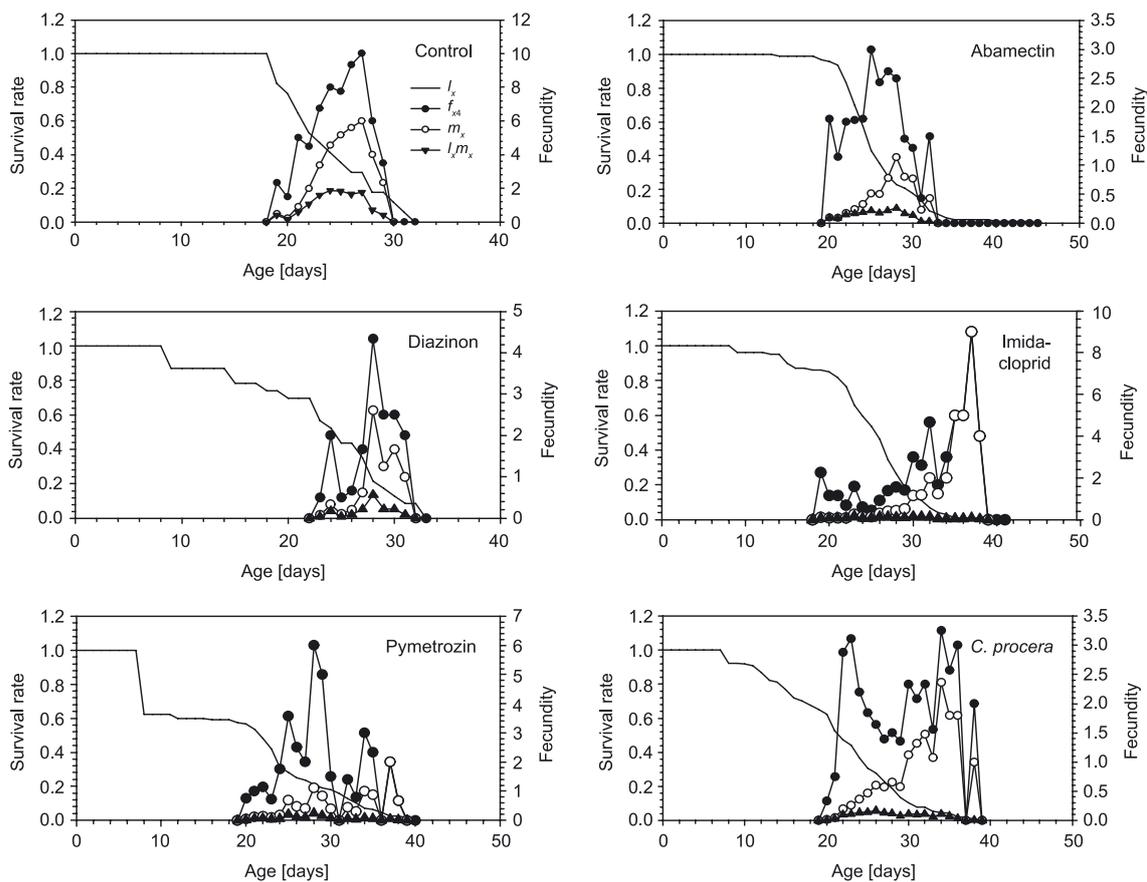
Fig. 2. Age-specific survival rate (l_x), female age-specific fecundity (f_{x4}) (eggs/female), and age-specific maternity ($l_x m_x$) of *B. tabaci* exposed to different insecticides
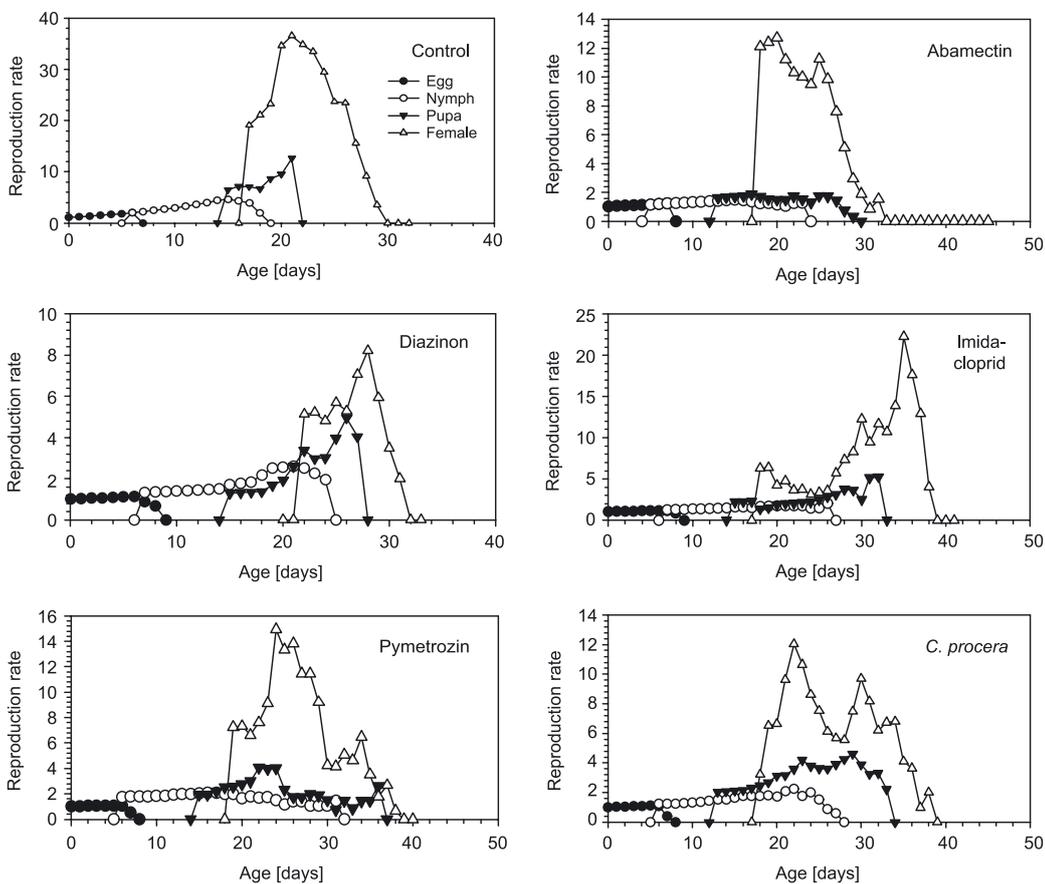

Fig. 3. Age-stage reproductive value (v_{xj}) of *B. tabaci* exposed to different insecticides

Table 2. The population parameters (±SE) of cotton whiteflies exposed to four synthetic insecticides, and one plant-derived insecticide

Treatments	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)
The control	0.088±0.008 a	1.092±0.009 a	10.180±1.864 a	38.590±7.723 ab	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
Diazinon	0.026±0.026 b	1.026±0.026 b	1.652±0.960 b	7.852±4.822 b	28.819±0.942 a
Imidacloprid	0.025±0.013 b	1.025±0.013 b	1.888±0.675 b	55.918±26.058 a	29.01±2.245 a
Abamectin	0.026±0.013 b	1.027±0.014 b	1.916±0.656 b	6.078±1.981 b	26.612±0.891 a
C. procera	0.018±0.011 a	1.018±0.011 b	1.628±0.495 b	15.701±4.895 b	28.817±1.128 a

Different letters show significant differences within each column at 0.05 level; SE – standard error

creased *GRR* in comparison with the control. Abamectin, diazinon, pymetrozin, and *C. procera* were placed in the same statistical group, meaning that they had the most negative effects on *GRR*. The highest amounts of R_0, λ, and r parameters were observed in the control treatment followed by abamectin, imidacloprid, *C. procera*, diazinon, and pymetrozin, respectively. The differences between these parameters were statistically significant for all the studied treatments. The T parameter was not statistically different where all treatments were placed in the same group. Our results showed that R_0 of the control, pymetrozin, diazinon, imidacloprid, abamectin, and *C. procera* were 10.18, 1.39, 1.65, 1.88, 1.91, and 1.62 and F were 41, 7.7, 6.33, 5.19, 9.68, and 7.98 egg per adults, respectively.

Discussion

In the current study, the sublethal effects of four commonly used synthetic pesticides as well as a plant-derived insecticide were evaluated on the biological characteristics of the cotton whitefly, *B. tabaci* in semi-field conditions. Insecticides have been predicted to remain the cornerstones upon which pest management practices are based as long as effective and inexpensive chemicals are available (Haynes 1988). However, with the increasing anxieties related to environmental pollution and health dangers, environmentally friendly biopesticides have received greater attention during the last few decades. We used a plant-derived compound whose insecticidal properties had been approved in previous studies (Ramos *et al*. 2006; Mahdavi-Arab *et al*. 2008; Irannejad 2010; Esmaeily *et al*. 2012; Jafarbeigi *et al*. 2012). Our current study also showed that *C. procera* extract can potentially be used as an alternative to the prevalent synthetic insecticides for control of *B. tabaci* (Fig. 1).

In addition to direct mortality caused by pesticides, those insects that are exposed to a pesticide in which the pesticide did not directly cause death, may experience some behavioral or physiological alterations, commonly known as sublethal or non-lethal effects (Stark *et al*. 2004; Desneux *et al*. 2007; Biondi *et al*. 2013). An increasing number of studies on both beneficial and noxious arthropods have demonstrated that synthetic insecticides impose some negative effects on fecundity, longevity, development time, searching behaviors, learning capabili-

ties, etc. (Studebaker and Kring 2001). Many of the newly developed classes of insecticides are believed to have less noxious effects on non-target organisms due to their specificity against the pests of interest (Studebaker and Kring 2001; Grafton-Cardwell *et al*. 2005). Surprisingly, the sublethal effects are particularly prevalent in the case of the newly developed insecticides which have a slower mode of action and may produce a higher rates of physiological and behavioral effects rather than acute ones (Studebaker and Kring 2001; He *et al*. 2013). Therefore, before any decisions are made concerning integrated pest management programs, the non-visible (sublethal) effects of insecticides on both target pest and beneficial organisms should be evaluated.

We used the recently developed age-stage, two-sex life table to evaluate the biological characteristics of whiteflies exposed to the studied insecticides. The traditional age-specific life tables (Chi and Liu 1985) concentrate only on the survival and the fecundity of the female population, while ignoring the male population, the stage differences, and stage overlapping, leading to miscalculation in the survival and fecundity curves (Chi 1988; Chi and Yang 2003; Chi and Su 2006). The age-stage, two-sex life table has been widely used in population dynamics of different insects including whiteflies (Chi and Su 2006; Yang and Chi 2006; Hu *et al*. 2010; Farhadi *et al*. 2011; Huang and Chi 2012; Azimi *et al*. 2013). Yet, as far as we know, this study is the first one that uses this model to evaluate the non-lethal effects of insecticides on the life table parameters of *B. tabaci*. Despite being a relatively time-consuming process, a deep understanding of the life table parameters of pests is necessary to set an elaborate integrated pest management program (Chi 1990; Yang and Chi 2006; Hu *et al*. 2010).

The results of life table analysis showed that, the survival rate of *B. tabaci* in treatments of pymetrozin and *C. procera* was reduced more forcefully than in other treatments (Fig. 1). The male whiteflies treated by abamectin, survived longer than the females. In field conditions without any insecticidal treatment, the female whiteflies appear to live longer than males (Yang and Chi 2006; Azimi *et al*. 2013). For example, Azimi *et al*. (2013) and Yang and Chi (2006) found that female whiteflies have a higher longevity than males on different cotton varieties and in different temperatures, respectively. The reduced longev-

ity of female whiteflies observed in abamectin-treated populations, indicates that females suffer more sublethal effects compared to males.

The age-stage, two-sex life table reveals the variable developmental rate and stage overlapping that is present in insect populations (Chi and Su 2006; Hu *et al.* 2010). The overlapping in s_{xj} curve (Fig. 1) displays the stage dissociation of *B. tabaci* due to variable developmental rate between individuals. The life table also gains a correct relationship between R_0 and F (Chi 1988; Chi and Yang 2003; Yang and Chi 2006). The total number of offspring produced by all females is equal to the net reproductive rate multiplied by the cohort size. The minor difference is probably related to rounding-off. This relationship shows the accuracy in the age-stage, two-sex life table analysis.

According to Yang and Chi (2006), if there is pre-adult mortality, the net reproductive rate would be expected to be lower than the mean female fecundity (i.e. $R_0 \leq F$). In our study, R_0 was slightly lower than F, implying that pre-adult mortality was caused by the studied insecticides. Liu and Stansly (1998) reported that net reproductive rate was higher than the mean fecundity. This difference may be related to the method of the R_0 and F calculation.

Altogether, the current study revealed that some biological traits of the cotton whitefly are affected at non-lethal concentrations of all the studied insecticides. Our results clearly showed that susceptibility to pesticides, and *C. procera* varied between the different developmental stages of *B. tabaci*. These results are in agreement with Liu and Stansly (1995) on *B. argentifolii*. In our study, the pymetrozin and *C. procera* extract showed the most sublethal effects on *B. tabaci* where they significantly reduced r_m compared to the control. Our results suggest that pymetrozin, and *C. procera* can be potentially used as effect agents against pests in the integrated pest management programs. However, before a decision is made about widely applying these insecticides in integrated pest management programs, their negative effects on a broader community of insects, especially non-target ones such as natural enemies and pollinators, are required to be clearly evaluated.

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