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Scientific paper

The Effects of Some Botanical Insecticides and Pymetrozine on Life Table Parameters of Silver Leaf Whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae)

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SUMMARY

The objective of this research was to assess the effects of extracts of two medicinal plant species: *Allium sativum* (Linn) and *Calotropis procera* (Aiton), and a formulation containing azadirachtin on life table parameters of silver leaf whitefly (SLW), *Bemisia tabaci* biotype B (Gennadius) (Hemiptera: Aleyrodidae), grown on greenhouse tomato plants. The effects were compared to that of pymetrozine, a synthetic insecticide. Bioassays were carried out in a greenhouse under controlled conditions of 27 ± 2°C, R.H. of 55 ± 5% and 16:8 h (L:D) photo period.

All treatments significantly affected the survivorship and fertility of SLW female adults, reducing the net reproduction rate, mean generation time and intrinsic rate of increase of this insect.

The net reproductive rate [R_0] values for the populations treated with garlic extract, milkweed extract, pymetrozine, azadirachtin, control for extracts (ethanol + distilled water) and control for pesticides (distilled water) were 23.58, 19.32, 10.78, 8.23, 49.66, 57.55; the intrinsic rate of increases [r_m] were 0.134, 0.139, 0.110, 0.090, 0.177, 0.178; the mean generation times [T] were 23.49, 21.23, 21.66, 23.50, 22.06, 22.69; the doubling times [DT] were 5.14, 4.95, 6.27, 7.56, 3.91, 3.87, and the finite rates of increase [λ] were 1.144, 1.149, 1.116, 1.094, 1.193, 1.195, respectively. Azadirachtin had the highest effect on the life table parameters of SLW.

Our findings indicated that, although herbal extracts were not effective as much as the chemical insecticides, they can be effective in pest control. Therefore, they are suitable choices for replacing chemical insecticides and for alternative use with azadirachtin in SLW IPM program.

Keywords: Botanicals; Pymetrozine; *Bemisia tabaci*; Life tables

INTRODUCTION

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a polyphagous insect that attacks more than 700 plant species all over the world (Greathead, 1986). This species is known for its genetic diversity that includes a complex of biotypes (Perring, 2001; De Barro et al., 2005) or, as recently suggested, a complex of 11 well-defined groups with 24 distinct species (Dinsdale et al., 2010; De Barro et al., 2011). Biotype determination of Iranian populations of *B .tabaci* has indicated that the B biotype is widespread and dominant in Iran (Rajaei Shoorcheh et al., 2008). Plant damage is caused by transmission of plant viruses, direct feeding, plant physiological disorders, honeydew production and fungal growth (Oliveira et al., 2001). At present, chemical application is necessary to control this pest, which often results in overuse of these chemicals (Palumbo et al., 2001). Therefore, SLW has developed resistance to many conventional insecticides throughout the world, especially organophosphates and pyrethroids (Horowitz and Ishaaya, 1994; Palumbo et al., 2001; Byrne et al., 2003; Horowitz et al., 2004; Fernandez et al., 2009). Development of alternative methods other than chemical application is necessary for pest management to protect human health and the environment. Plant extracts may provide an alternative method to currently applied pesticides, as they constitute a rich source of bioactive chemicals (Daoubi et al., 2005; Kim et al., 2005). Studying the effects of pesticides on life table parameters has been discussed as the best approach regarding both lethal and sub-lethal effects (Daniels and Allan, 1981; Bechmann, 1994; Forbes and Calow, 1999; Stark and Banks, 2003; Stark et al., 2007). The high variety of substances present in different plants is very important and valuable for insect control, especially considering that just a small number of plants have been investigated for this goal (Lovatto et al., 2004). Neem *Azadirachta indica* (A. Juss.) is known for being resistant to attacks of a number of insects, acting as a growth regulator, feeding impediment and sterilizer (Silva et al., 2003). Its pesticidal potential allows up to 90% success in pest control within agroecological management, having the advantage of preserving natural enemies (Abreu Júnior, 1998). Several studies have shown that neem products are safe for beneficial insects (Saxena, 1987; Hoelmer, et al., 1990; Schmutterer, 1990).

In this study, the effects of two plant extracts, garlic *Allium sativum* (Linn) and milkweed *Calotropis procera* (Aiton), as herbal insecticides (in a first report) and a neem-based insecticide on life table parameters of SLW were evaluated. The effects were compared to that of pymetrozine, a pyridine azomethine compound, a synthetic insecticide with selective activity against sucking insect species.

MATERIAL AND METHODS

Insecticides

Commercial formulations of the synthetic insecticide pymetrozine and botanical insecticide azadirachtin used in this study are given in Table 1.

Table 1. Pesticides used on adults of SLW

Common name	Commercial name	Formulation	Company	Country
Pymetrozine	Chess	25% WP	Syngenta	Australia
Azadirachtin	Neemarin	EC1500	Biotech International	India

Plant material

Two plants known to have medicinal activity, garlic and milkweed, were collected from their natural habitats at different localities in Iran. The parts of these plants used in the experiments are given in Table 2.

Table 2. Plant species whose extracts were used on adult SLW

Plant	Common name	Family	Parts used
Allium sativum	garlic	Alliaceae	fruits
Calotropis procera	milkweed	Asclepiadaceae	leaves

Preparation of plant extracts

Powdered plant materials were extracted in a Soxhlet apparatus for 8 h (Vogel, 1978). Ethanol (70%) and distilled water (30%) were used for extraction. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator at 40° C. Plant extracts prepared this way were used in bioassay tests.

Insect culture

Adult SLW males and females were obtained from UT Source of the Abureihan College, Tehran University, and were reared on tobacco plants in the greenhouse (27 ± 2° C, R.H. 55 ± 5% and 16:8 h (L:D) photo period). Female adults of the same age were collected from red-eye pupae and moved to separate tomato plant cages for further experiments.

Bioassay

To determine the toxicity of insecticides and herbal extracts to silver leaf whiteflies, two leaves of tomato (var. 'Bakker Brothers') were treated by immersing in insecticide solutions for 20 seconds (leaf dip method). The same method was used for control treatments and the leaves were immersed either in distilled water, or in a mixture of ethanol (70%) and distilled water (30%). Two clear plastic glasses (10 cm in diameter, 15 cm in height) were put together to form plant cages. The upper one was covered with a fine mesh and the lower one filled with distilled water. The treated plants were air dried and females were released on leaves under the clear plastic glasses. The number of dead adults was recorded for 48 hours after application. The experiments were carried out with three replicates while each replicate had 15 female adults. Lethal concentrations of 25 percent (LC_{25}) were calculated for each plant extract and pesticide, and used for further studies.

Life table analysis

To determine the effects of these insecticides and herbal extracts on whitefly life table parameters, leaflets of tomato plants at the 2-4 leaf stage were dipped for 20 seconds in insecticide solutions prepared as LC_{25} concentrations. For the controls, leaflets were dipped in distilled water and ethanol (70%) + distilled water (30%). Thirty same-aged females were released on tomato leaflets in plant cages. Every day, live females were transferred to new cages and the number of eggs was recorded until all females were dead.

Life tables, using daily age-specific survival [l_x] and fertility [m_x] rates were produced for estimating the life table parameters in treated and control cohorts. Values of the intrinsic rate of increase [r_m] were obtained by the Euler equation and other life table parameters were calculated (Birch, 1948) (Table 3).

Table 3. Identification and formula of stable population parameters (Carey, 1993)

Parameters	Formula	Identification
R_0 (*NRR*)	$R_0 = \sum l_x m_x$	The mean number of daughters produced per cohort of females over their lifetimes
r_m	$1 = \sum e^{-rx} l_x m_x$	The maximum exponential rate of increase by a population growing within defined physical conditions
λ	$\lambda = e^r$	The ratio of population sizes at each time step
T	$T = (\ln R_0 / r)$	The average interval separating the births of one generation from the births of the next
DT	$DT = (\ln 2 / r)$	The time needed for a population to double in size from a fixed point in time

R_0 (*NRR*): Net reproductive rate; r_m: Intrinsic rate of population increase; λ: Finite rate of population increase; *T*: Generation time; *DT*: Doubling time.

Statistic analysis

Mean population growth parameters of adults were compared by analysis of variance, followed by Duncan's test (SAS Institute, 2001). Jackknife pseudo values for the r_m, the mean generation time [T], the doubling time [DT], and the finite rate of increase [λ] for each treatment were analyzed by ANOVA. The jackknife method removes one observation at a time from the original data set and recalculates the statistic of interest from the truncated data set. These new estimates, or pseudo values, form a set of numbers from which mean values and variances can be calculated and compared statistically (Meyer et al., 1986).

RESULTS

The age-specific survivorship (l_x) and age-specific fertility (m_x) of SLW populations exposed to each treatment are presented in figure 1. The females exposed to azadirachtin and milkweed extract had the lowest survivorship. The maximum value of m_x was 4.9 females/female/day. This value was observed in the population treated with azadirachtin. Maximum values of m_x for populations exposed to milkweed extract, garlic extract and pymetrozine were 4.5, 4.3, and 2.6, respectively.

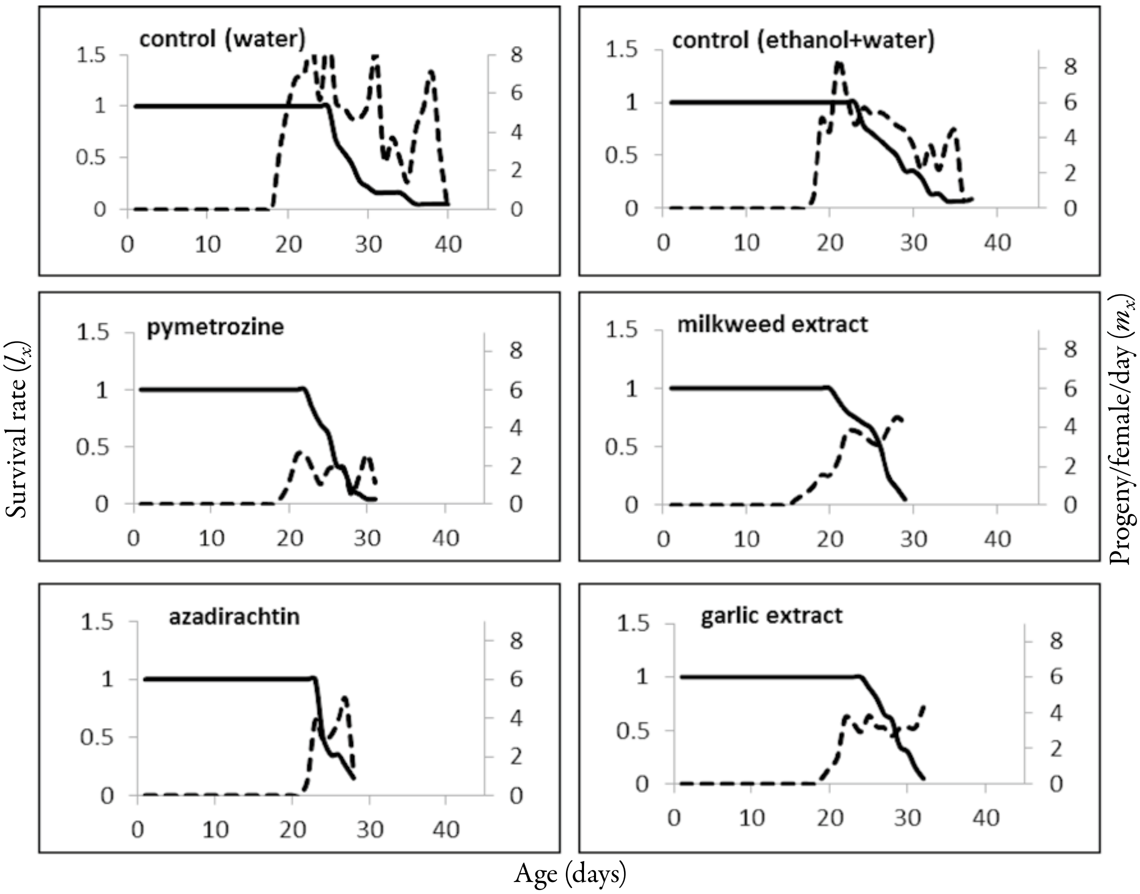

Figure 1. Age-specific survivorship, l_x, (solid line) and fertility, m_x, (dashed line) of SLW cohort exposed to pymetrozine, azadirachtin, garlic extract and milkweed extract compared with the controls

The effects of treatments on daily net reproductive rate of adults of SLW are presented in figure 2. Adults exposed to pymetrozine had the lowest maximum net reproductive rate (2.5 egg/day) that occurred 20 days after treatment. This value was followed by azadirachtin, milkweed extract and garlic extract.

The SLW cohorts exposed to various treatments showed different net reproductive rates [R_0] (Table 4). The lowest value of R_0 was in cohorts treated with azadirachtin, while the highest related to garlic extract treatment, but these treatments did not differ from each other significantly ($F = 34.62, P < 0.0001$).

The r_m was significantly different among the cohorts that were exposed to different pesticides and plant extracts ($F = 31.32, P < 0.0001$). The r_m values in cohorts treated with garlic and milkweed extracts did not differ significantly (Table 4). The analysis revealed that there was no significant difference between the mean generation times (T) of cohorts that were exposed to azadirachtin and garlic extract ($F = 5.26, P > 0.0002$). The shortest T belonged to the cohort exposed to milkweed extract, and the highest was in the cohort treated with azadirachtin (Table 4).

Among the cohorts treated, DT was affected ($F = 9.71, P < 0.0001$). The shortest duration of this statistic was recorded in the cohort exposed to milkweed, while the longest duration occurred after exposure to azadirachtin. In cohorts treated with garlic extract and pymetrozine, DT was 5.14 ± 0.13 and 6.27 ± 0.33, respectively, which did not have any significant difference (Table 4).

A significant difference was observed in the λ ($F = 35.16, P < 0.0001$) among the cohorts treated. The highest λ was in the cohort exposed to milkweed extract, whereas the lowest one was in azadirachtin-treated cohort. There were no significant differences between cohorts treated with garlic and milkweed extracts (Table 4).

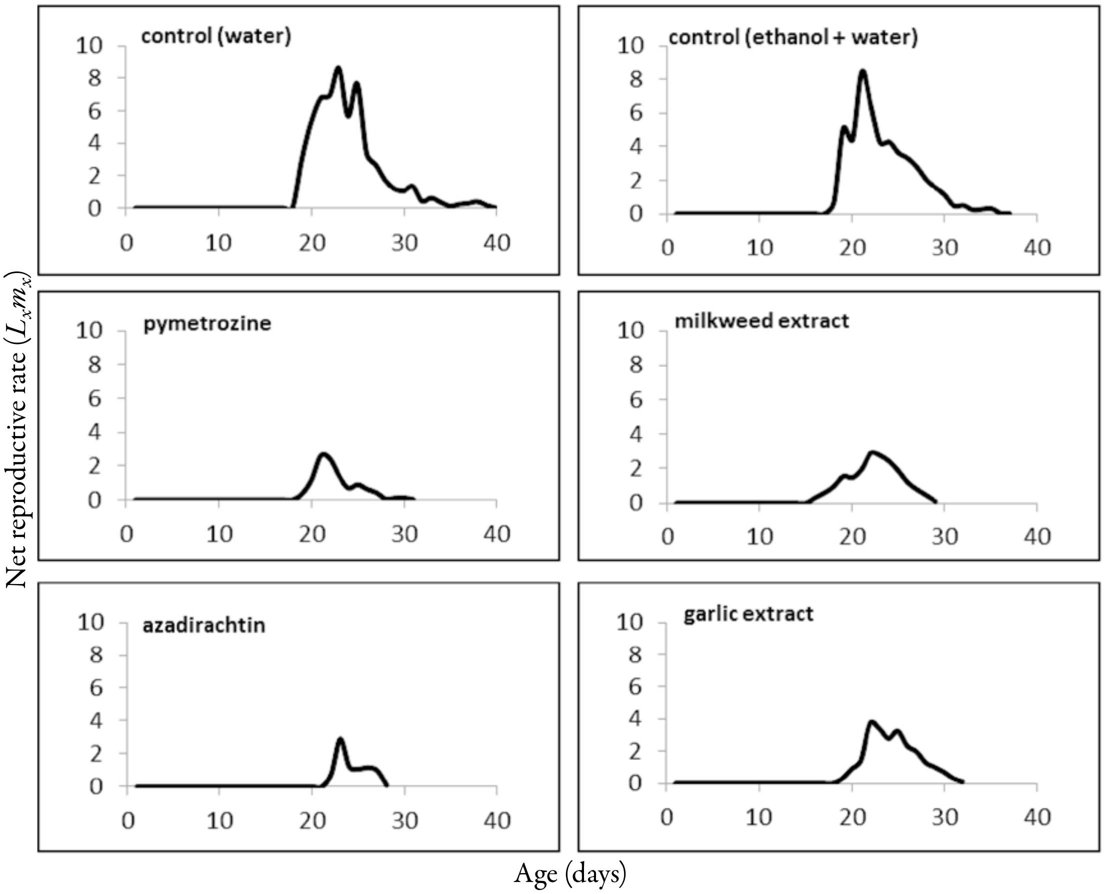

Figure 2. The effects of treatments on daily net reproductive rate ($L_x m_x$) of SLW adults

Table 4. Life table parameters (means ± SE) of SLW females

Parameter	Control (water)	Control (ethanol & water)	Milkweed	Garlic	Azadirachtin	Pymetrozine
R_0 (female/female)	57.55±6.8 a	49.66 ± 3.19 ab	19.32± 2.20 b	23.58± 1.17 b	8.23 ± 1.79 b	10.78 ± 1.38 b
r_m (female/female/day)	0.178 ± 0.003 a	0.177 ± 0.003 a	0.139 ± 0.006 b	0.134 ± 0.003 b	0.090 ±0.008 d	0.110 ± 0.005 c
T (days)	22.69 ± 0.42 ab	22.06 ± 0.45 bc	21.23 ± 0.59 c	23.49 ± 0.41 a	23.50 ± 0.30 a	21.66 ± 0.34 bc
DT (days)	3.87 ± 0.08 d	3.91 ± 0.07 d	4.95 ± 0.27 c	5.14 ± 0.13 bc	7.56 ± 0.77 a	6.27 ± 0.33 ab
λ (female/female/day)	1.195 ± 0.004 a	1.193 ±0.003 a	1.149 ± 0.006 b	1.144 ± 0.004 b	1.094 ± 0.009 d	1.116 ± 0.006 c

R_0 = Net reproductive rate; r_m = Intrinsic rate of population increase; λ = Finite rate of population increase; T = Generation time; DT = Doubling time.
Means within each row followed by the same letter are not significantly different (P > 0.01).
Duncan's Multiple Range Test (Confidence limit = 99%)

DISCUSSION

The present study clearly demonstrates that the life table parameters of SLW were affected significantly after exposure to the pesticides and herbal extracts. In a similar study, *B. tabaci* ovipositions confined to cotton treated with neem seed extracts were 80% fewer than on control untreated cotton for up to 7 days after treatment (Coudriet et al., 1985). In another study, *B. tabaci* adults previously exposed for 72 h to an extract of *Melia azedarach*, which belongs to the same family as neem, laid fewer eggs on untreated plants than unexposed adults but adult emergence from the laid eggs was comparable between the extract and control treatments (Abou-Fakhr Hammad et al., 2001). Also, aqueous and methanol extracts of *M. azedarach* fruits were found to have repellant activity against *B. tabaci* adults (Abou-Fakhr Hammad et al., 2000).

The r_m values of SLW ranged from 0.090 ± 0.008 to 0.139 ± 0.006 female/female/day in different treatments. This statistic reached maximal value in the cohort exposed to milkweed extract that significantly differed from the control, whereas minimal value was related to the azadirachtin-treated cohort. The value of r_m for control was 0.178 ± 0.003 female/female/day.

The fecundity of homopterous insects is strongly influenced by neem extracts and azadirachtin (Schmutterer, 1990). Also, exposure to azadirachtin has been found to reduce the fertility and fecundity of adults of green peach aphid *Myzus persicae* (Sulzer), lettuce aphid *Nasonovia ribisnigri* (Mosley) and strawberry aphid *Chaetosiphon fragaefolii* (Cockerell) in a linear concentration-dependent manner (Lowery and Isman, 1996). In another experiment, toxicity of pymetrozine on the pupa stage of *B. tabaci* biotype B on poinsettia was reported (Hoddle et al., 2001).

Mean generation time [T] is a close estimate of the average age of reproduction (Omer et al., 1992). This study indicates that all treatments used at a sub-lethal rate decreased the fertility period of SLW. We found values for this parameter ranging from 22.69 ± 0.42 days for control to 23.50 ± 0.30 days for azadirachtin-treated cohorts.

Doubling time, which is a function of rate of increase, varied from 3.87 ± 0.08 days in control to 7.56 ± 0.77 days in the cohort exposed to azadirachtin, showing a significant adverse effect of this insecticide on the whitefly cohort.

The insecticidal effect of plant extracts on adults of *B. tabaci* may indirectly reduce whitefly oviposition (Gómez et al., 1997; Cubillo et al., 1999). Feeding deterrence without previous contact with the compound has also been reported in experiments with neem (Saxena, 1989).

Our results indicated that maximum R_0 was obtained in the cohort treated with garlic extract, while the shortest DT and highest L_x, r_m and λ were obtained in the cohort treated with milkweed extract. Also the lowest r_m was obtained for azadirachtin.

No report has ever been made on the effects of milkweed and garlic extract as herbal insecticides on the life table parameters of SLW. Some authors have worked on extracts other than these. For example, antitermitic properties of *Calotropis procera* latex (Giridhar et al., 1988), and fruit borer and whitefly control by *Calotropis giganta* leaf extract (Narayanasamy, 2006) have been reported.

Application of neem oil is considered safe for the environmental, human health and natural enemies (Raguraman and Rajasekaran, 1996; Dash et al., 2001). However, neem reduces the oviposition of some parasitoid species (Price and Schuster, 1991; Raguraman and Singh, 1999). Thus, as any other chemical product with insecticidal properties, neem should be cautiously used and only when strictly necessary.

These results showed that, although garlic and milkweed extracts were not effective as much as chemical insecticides in decreasing the cohort growth parameters, they may be effective in pest control and can be a suitable choice for replacing chemical insecticides and can alternatively be applied with azadirachtin in a *B. tabaci* biotype B IPM program.

Final evaluation of the utility of these compounds should be reserved until semi-field and field trials have been conducted.

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Efekat nekih botaničkih insekticida i pimetrozina na životne parametre leptiraste vaši duvana *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae)

REZIME

Cilj ovog istraživanja bio je da se proceni delovanje ekstrakta dve lekovite biljne vrste, *Allium sativum* (Linn) i *Calotropis procera* (Aiton), kao i formulacije azadirahtina na životne parametre leptiraste vaši duvana *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) gajene na biljkama paradajza u stakleniku. Delovanje je upoređeno sa sintetičkim insekticidom pimetrozinom. Biotest je urađen u stakleniku pod kontrolisanim uslovima, na temperaturi od 27 ± 2°C, pri relativnoj vlažnosti 55±5% i sa fotoperiodom 16:8 h (D:N).

Svi tretmani su imali značajan uticaj na preživljavanje i plodnost odraslih ženki leptiraste vaši duvana, smanjujući neto stopu reprodukcije, srednje vreme generacije i prirodnu stopu rasta ovog insekta.

Vrednosti neto stope reprodukcije [R_0] za populaciju tretiranu ekstraktom belog luka, ekstraktom kalotropisa (sodomske ili mrtvomorske jabuke), pimetrozinom i azadirahtinom, kao i kontrolom za ekstrakte (etanol + destilovana voda) i kontrolom za pesticide (destilovana voda) bile su 23,58; 19,32; 10,78; 8,23; 49,66; 57.55; vrednosti prirodne stope rasta [r_m] su bile 0,134; 0,139; 0,110; 0,090; 0,177; 0,178; srednje vreme generacije [T] bilo je 23,49; 21,23; 21,66; 23,50; 22,06; 22,69; vreme udvostručenja [DT] 5,14; 4,95; 6,27; 7,56; 3,91; 3,87; i konačna stopa rasta [λ] 1,144; 1,149; 1,116; 1,094; 1,193; 1,195; respektivno. Azadirahtin je imao najveće dejstvo na životne parametre leptiraste vaši duvana.

Rezultati ispitivanja su pokazali da biljni ekstrakti, iako nisu imali izraženo delovanje kao hemijski insekticidi, mogu biti efikasno upotrebljeni u suzbijanju štetnih insekata. Otuda su oni pogodni kao alternativa hemijskim insekticidima, a mogu se upotrebiti i sa azadirahtinom u integralnom upravljanju leptirastom vaši duvana.

Ključne reči: Botanički insekticidi; pimetrozin; *Bemisia tabaci*; tablice života