

Sublethal effects of imidacloprid on the whitefly parasitoid *Encarsia formosa* Gahan

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SUMMARY

Acute toxicity of an imidacloprid-based product (Confidor 200 SL) to pupae of the whitefly parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), and its effects on life history traits and population growth in F₁ generation of the surviving parasitoid females of a commercial strain ("Dutch" strain, D) and two local populations from Serbia (Bujanovac, B; Negotin, N) were examined in laboratory bioassays. All trials were carried out at 27±1°C temperature and 60±10% relative humidity, and under 16/8 h daylight/darkness photoperiod in four replications. In acute toxicity bioassays, tobacco leaves carrying parasitoid pupae were treated with a series of symmetrical concentrations (800, 400, 200, 100, 50 and 25 mg a.i./l) covering a 10-90% mortality range. The product based on imidacloprid, applied directly onto parasitoid pupae at mean lethal concentrations (LC₅₀) determined in the acute toxicity assays (30 mg/l, 20 mg/l and 25 mg/l, for populations B, N and D, respectively), significantly affected the survival of females developed from the treated pupae, extended the duration of juvenile development (by 1.81, 1.59 and 1.73 days for populations B, N and D, respectively), significantly reduced total parasitism of females D (25.92 %), total female adult emergence in populations B (27.48 %) and D (17.92 %), and significantly reduced the instantaneous rate of increased only of females N (4.23 %). Considering the high acute toxicity of the imidacloprid product to the pupal stage of *E. formosa*, and significant reductions in life table and population parameters, imidacloprid is not considered compatible for simultaneous use with the parasitoid *E. formosa*. A more precise assessment of risks involved in the use of that insecticide requires a more detailed testing in the field. The implications of these results for the concept of integrated control of the greenhouse whitefly are discussed.

Keywords: *Encarsia formosa*; Imidacloprid; Sublethal effects; Life history traits; Population growth

INTRODUCTION

The greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) is a cosmopolitan and highly polyphagous pest species of greenhouse crops, especially tomato (Gerling, 1990). The incidence of established populations of *T. vaporariorum* has been documented in many countries of the world's temperate regions (Manzano & van Lenteren, 2009). It has been widespread in Serbia since the 1970s and frequently found in greenhouses as one of the most serious pests of vegetables and ornamentals (Perić et al., 2009; Prijović et al., 2014).

As the widespread use of chemical insecticides has caused an evolution of whitefly resistance to compounds with various modes of action, including newer chemical classes such as neonicotinoids (Whalon et al., 2008, 2017), durable and sustainable strategies for control of this pest species should be based on integration of chemical, biological, cultural and other measures (Gerling, 1990; Gentz et al., 2010). The parasitic wasp *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae; Coccophaginae) has been used for many years now for biological control of *T. vaporariorum* as one of the most successful biological agents for greenhouse and ornamental crops protection around the world (van Lenteren & Martin, 1999; Enkegaard & Brødsgaard, 2006).

Pesticides are necessary when natural enemies fail to keep a pest population below its economic threshold, as a result of adverse environmental conditions, inadequate host species or cultivar, excessive density of pest population, etc. (Hoddle et al., 1998; Albajes et al., 1999). Despite many restrictive factors, natural enemies can be effectively integrated with chemical treatments based on the knowledge of pesticides and their effects on natural enemy populations (Croft, 1990).

Imidacloprid is the first compound in the neonicotinoid class that has a systemic activity, so that its formulations for soil and foliar application have been developed for use in a variety of agricultural crops. Imidacloprid has demonstrated good efficacy in controlling a considerable number of pests in the order Hemiptera, including the greenhouse whitefly, which is usually treated with foliar applications in different crops (Cahill et al., 1996; Elbert & Nauen, 1996; Nauen et al., 1996, 1998; Horowitz et al., 2004; Grafton-Cardwell et al., 2008).

Most studies that have quantified the risk involved in imidacloprid applications in combination with

E. formosa have been based on the use of its recommended application rates and they were conducted as acute toxicity and/or persistence assays as proposed by the IOBC testing scheme (Yankova et al., 2011; Sterk et al., 2002; Richter et al., 2003). It has also been noted that different methods of imidacloprid application may also cause variable effects on *E. formosa* (van de Veire & Tirry, 2003) and other parasitoids (Stapel et al., 2000). Many laboratory tests have also assessed the safety of imidacloprid for different development stages of other parasitoids (Stapel et al., 2000; Morales, et al. 2006; Sabahi et al., 2011; Carvalho et al., 2012) and predators (Sclar et al., 1998; Torres et al., 2002). On the other hand, stimulation of reproduction of pests or natural enemies by imidacloprid, and its safety to beneficial insects have been documented in a number of studies (Elbert et al., 1991; Elzen, 2001; James & Vogege, 2001; James & Price, 2002; Sohrabi et al., 2012). However, only a few studies have focused on sublethal effects of imidacloprid by estimating population-level response of parasitoids (Saber et al., 2009; Saber, 2011; Kheradmand et al., 2012; Sohrabi et al., 2012, 2013) or predators (James, 1997). To the best of our knowledge, there are no data in literature focusing on the effects of imidacloprid on population growth of *E. formosa*.

Our previous examination of biological parameters of six local populations and a commercial "Dutch" strain (D) of *E. formosa* had shown that the local population Bujanovac (B) had the greatest potential for controlling greenhouse whitefly, while population Negotin (N) demonstrated the least ability (Drobnjaković et al., 2016). The present study focused on the effects of an imidacloprid-based insecticide on life history traits and population growth of F₁ generation, following treatment of pupae as the least susceptible development stage of the parasitoid. Data obtained in this study are discussed in terms of potentials for improving *T. vaporariorum* integrated management strategy.

MATERIALS AND METHODS

Test organism (origin and rearing)

Local populations of *E. formosa* have been established from insects collected at the pupal stage (parasitized whitefly larvae) in extensive crops of vegetables and ornamentals in locations known not to have a history

of biological control of greenhouse whiteflies by a commercial *E. formosa* strain so as to avoid possible mixing of populations. Specimens of population B were collected in Bujanovac, Southern Serbia (GPS: 42°30'27" N, 21°48'30" E) on *Solanum nigrum* Linnaeus, while population N was collected in Negotin, Eastern Serbia (GPS: 44°13'00" N, 22°31'00" E) on *Hibiscus* spp. The male and female parasitoid wasps issued from eclosed pupae in both populations were identified as *E. formosa* according to a determination key provided by Polaszek et al. (1992). All wasp populations demonstrated the asexual pattern noted also by other researchers (Zchori-Fein et al., 1992), i.e. males were very rare under regular conditions. A commercial strain of *E. formosa* (Koppert Biological Systems, Netherlands), referred thereafter as "Dutch" strain (D) of normal susceptibility to pesticides, was used as the reference strain.

The commercial strain and two selected representatives of local *E. formosa* populations were reared on tobacco leaves infested with the host pest *T. vaporariorum* at 27±1°C temperature, 60±10% relative humidity, and under 16/8 h light/darkness photoperiod. A *T. vaporariorum* population was established in 2008 from insects collected from weed plants in the territory of Belgrade, and it has been reared ever since on tobacco plants, cv. Samsun, in ventilated muslin cages according to EPPO (2004) methodology.

Insecticide

The commercial product Confidor 200 SL (Bayer Crop Science, Germany); soluble concentrate; active ingredient – imidacloprid, content 200 g/l.

Bioassays

All assays were performed at 27±1°C temperature and 60±10% relative humidity, under 16/8 h photoperiod, and in four replications. All assays were conducted in Petri dishes (12 cm diameter) with four openings (1 cm) on the lids, covered with muslin for ventilation and to prevent condensation inside the dish, on tobacco leaves set upon 1% agar layer. Imidacloprid was applied as a dilution in distilled water by spraying the entire surface of both the lid and the dish containing a tobacco leaf. A Potter Precision Spray Tower (Burkard Scientific, UK) was used for spraying 2 ml liquid under 100 kPa air pressure to make a 2.7 ± 0.2 mg/cm² water deposit in each dish.

Acute toxicity bioassay

In an acute toxicity bioassay, tobacco leaves with parasitized whitefly nymphs (parasitoid pupae) were fixed onto tin aluminium foil with Tragant-kit (natural, non-toxic adhesive). After drying, the leaves were cut into pieces, each containing 20 parasitoid pupae (four days old, i.e. 12 days after egg laying) per replicate, and set onto filter paper inside plastic Petri dishes (filter paper was moistened to ensure that tobacco leaves stay in place during exposure). The Petri dishes (lids too) containing tobacco leaves with pupae were treated with serially diluted solutions (at least 6 concentrations: 800, 400, 200, 100, 50 and 25 mg a.i./l) to cover a 10-90% mortality range (determined in preliminary testing). Control pupae were treated with distilled water only. Two hours after treatment, the leaves were transferred into new Petri dishes to await emergence of parasitoid adults from pupae. Mortality was assessed from the number of emerged adults against the number of treated pupae, nine days after treatment (EPPO, 2004; Simmonds et al., 2002; Sohrabi et al., 2012).

Concentration-mortality data were processed by probit analysis (Finney, 1971) in Polo Plus software (LeOra Software, Berkeley, USA) calculating lethal concentrations (LC₅₀, LC₉₀) and the slope of regression line. A lethal dose ratio test was used for comparison of the calculated LC/EC values: when 95% confidence interval between two LC values included 1, those values were considered not significantly different (Robertson et al., 2007).

Bioassay testing effects on life history parameters and population growth in F₁ generation

The effects of the imidacloprid-based insecticide on life history parameters and population growth of *E. formosa* were assessed by focusing on the vitality and reproduction potential of females surviving treatment at the pupal stage. The bioassay had four replications. Concentrations of the imidacloprid insecticide (30, 20 and 25 mg/l for parasitoid populations B, N and D, respectively) were chosen based on data noted in the previous toxicity bioassay at the pupal stage of the parasitoid (concentrations were within 95 % confidence limits for the LC₅₀ calculated in acute toxicity bioassays). All surviving female adults that emerged from 40 treated pupae were transferred to Petri dishes containing third and fourth instar larvae/nymphs of the pest whiteflies that were offered for parasitizing at two day intervals until the death of the last female. Female adult survival,

longevity and total parasitism, total emergence of adults and instantaneous rate of increase in F_1 generation of the parasitoid from all three populations were noted (Gholamzadeh et al., 2012).

To determine the development time of surviving juveniles, when parasitoid adults were just before emergence from pupae, the number of eclosed adults was noted at 12 h intervals (Enkegaard, 1993). Development time was calculated as the total number of days from egg laying to adult emergence from the parasitoid pupae. To determine the longevity of survived adults, the number of surviving parasitoid females was checked every other day and the longevity of females was calculated as the total number of days a female was alive assuming that their ultimate death occurred at the midpoint of 48 h. Before analysis, data on juvenile development time and female longevity were \sqrt{x} transformed. Parasitism was determined based on the number of parasitized host nymphs (black pupae) in each inspection interval (parasitism/48 h period), as well as the total number of parasitized host nymphs (total, lifetime parasitism) (Stouthamer & Mak, 2002). After determining the number of parasitoid pupae, leaves carrying them were transferred into new Petri dishes to monitor the emergence of parasitoid adults from the pupae. The data were then used to calculate the number of parasitized host nymphs (parasitism/48 h period and total parasitism), and total adult emergence from parasitized host nymphs (total fertility). Parasitism was calculated as the number of parasitized host nymphs per female alive at the midpoint of 48 h (parasitism/48 h period) and during the entire life span of females (total, lifetime parasitism). Adult emergence was calculated as the number of wasps that emerged from parasitized nymphs and reached adult stage. Parasitism and adult emergence data were

transformed by $\sqrt{(x+0.1)}$. Parasitism and adult emergence data were used to calculate at the end of each interval the instantaneous rate of increase (r_i), using the formula:

$$r_i = \frac{\ln\left(\frac{N_f}{N_0}\right)}{\Delta t}$$

where N_0 is the initial number of wasps (e.g. 40 parasitoid pupae per replicate), N_f – final number of wasps at the end of t -day (number of black parasitized pest larvae – parasitoid pupae and number of eclosed adults), Δt – number of days that elapsed from the beginning of bioassay (in our assay Δt was determined as the shortest oviposition period in the examined population in each bioassay to make the most reliable and uniform comparison of population status). Positive r_i indicated a population growth, negative r_i was indicative of a population decline, while $r_i = 0$ showed a stable population (Stark et al., 1997; Stark & Banks, 2003). Arcsin \sqrt{x} transformation was applied to the instantaneous rate of increase data.

Kaplan-Meier analysis (SPSS for Windows, Version 17) was used to calculate the average female longevity and survival curves were constructed (Enkegaard, 1993; Qiu et al., 2004), which were analyzed by Long-rank test. Development time, longevity, parasitism/48 h, total parasitism and adult emergence, and instantaneous rate of increase data were analyzed by two-way ANOVA (azadirachtin treatment and population were the factors) with means separated by Fisher's LSD test ($p < 0.05$). Means of all parameters for treatment and control, for each population individually, were separated by Student's t -test ($p < 0.05$), using the software Statsoft Statistica 7.0.

Table 1. Parameters of imidacloprid (mg/l) toxicity to pupae of *E. formosa* originating from local populations Bujanovac (B) and Negotin (N), and a commercial "Dutch" strain (D)

Population	n	LC ₅₀ (mg/l) (95% CLs)	LC ₉₀ (mg/l) (95% CLs)	b (± SE)	χ^2	df
B	560	30.66 a (20.46 – 44.72)	491.80 a (304.71 - 900.16)	1.06 (± 0.09)	3.06	5
N	560	18.50 a (6.61 - 42.60)	325.60 a (125.18 - 1703.86)	1.03 (± 0.05)	8.64	5
D	560	24.00 a (11.15 - 45.47)	394.28 a (184.88 - 1266.55)	1.05 (± 0.05)	5.26	5

LC data marked with different letters columnwise are significantly different (lethal dose ratio test, $P=0.05$, Robertson et al., 2007)

n = number of treated pupae

CLs = confidence limits

b = slope of regression line

df = degree of freedom

RESULTS

Acute toxicity to parasitoid pupal stage

Acute toxicity parameters of imidacloprid after direct treatment of *E. formosa* pupae originating from two local populations and a commercial strain are shown in Table 1. The resulting lethal concentrations were considerably below the insecticide maximum recommended concentration (0.1 % = 200 mg a.i./l). A lethal dose ratio test revealed no significant difference between the calculated LC₅₀ and LC₉₀ values for the parasitoid pupae.

Effects on life history parameters and population growth of F₁ generation

The juvenile development time was significantly affected by treatment ($F_{1,18}=97.1, p<0.001$), while the examined population ($F_{2,18}=2.0, p=0.165$), and interaction between treatment and population ($F_{2,18}=2.0, p=0.932$) had no significant effect. Juvenile development of the parasitoid wasps in treated pupae of all three populations of *E. formosa* was 1.59-1.81 days longer than the development in untreated pupae (Table 2). Regarding treated juveniles, population B needed 1.81 days more, population N 1.55 days more and juveniles D 1.73 days more for development than control juveniles.

Table 2. Juvenile development (mean±SE, days) of *E. formosa* local populations Bujanovac (B) and Negotin (N), and commercial “Dutch” strain (D); Control = distilled water; Treatment = imidacloprid 30 mg/l (B), 20mg/l (N) and 25 mg/l (D)

Population	Treatment	Control
B	16.15 a (± 0.09)	14.34 b (±0.10)
N	15.93 a (±0.30)	14.38 b (±0.23)
D	16.39 a (±0.21)	14.66 b (±0.28)

Means marked with different letters in rows are significantly different (*t*-test, $P<0.05$).

Female wasps that emerged from parasitoid pupae after treatment with the insecticide LC₅₀ in the acute toxicity bioassay lived either shorter or longer than control females, but the difference in longevity was not significant ($F_{1,18}=44.31, p=0.083$) (Figure 1). Females B lived 0.35 days shorter, females N 0.47 days longer,

while females D lived as long as control females. Both treated and control females B had the leading longevity (Bt:Bc = 9.61 days: 9.96 days).

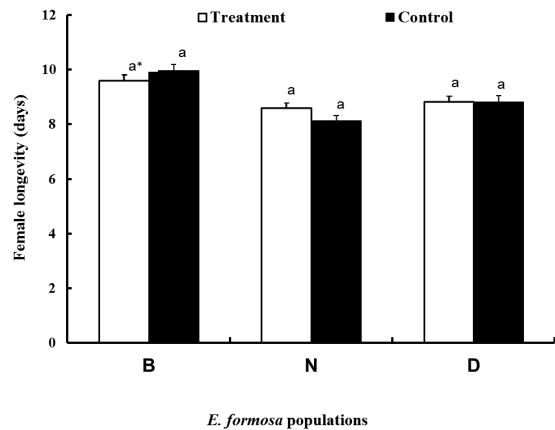


Figure 1. Longevity (Mean±SE, days) of *E. formosa* F₁ females from local populations Bujanovac (B) and Negotin (N), and commercial “Dutch” strain (D); Control = distilled water; Treatment = imidacloprid 30 mg/l (B), 20 mg/l (N) and 25 mg/l (D)

(Means marked with different letters at the top of bars are significantly different, *t*-test, $P<0.05$)

The survival curves for female wasps of the examined populations are shown in Figure 2. Females that emerged from pupae treated with imidacloprid had lower survival rates than females which emerged from pupae treated only with distilled water (Bt vs. Bc: $ww=38.00, p<0.001$; Dt vs. Dc: $ww=46.94, p<0.001$; Nt vs. Nc: $ww = 42.522, p<0.001$). Considering only the treated females, there were no significant differences in survival between D and N females (Dt vs. Nt: $ww=-6.214, p=0.355$), nor between B and D females (Bt vs. Dt: $ww=194.06, p=0.055$), while B females had better survival data than N females (Bt vs. Nt: $ww=-16.41, p<0.05$). Considering control females, population B had a better survival than populations D (Bc vs. Dc: $ww=-16.60, p<0.05$), and N (Bt vs. Nt: $ww=-20.61, p<0.01$). Control females D had a higher survival rate than control females N, but the difference is not statistically significant (Dc vs. Nc: $ww=-4.305, p=0.527$).

Repetitive ANOVA analysis showed that parasitization rates of the females that emerged from pupae treated with imidacloprid in all test populations were significantly affected by the observation period ($F_{7,126}=195.41, p<0.001$). All main effects and associated interactions were found to be statistically significant between and within observation period, at $p=0.05$ significance level (Table 3).

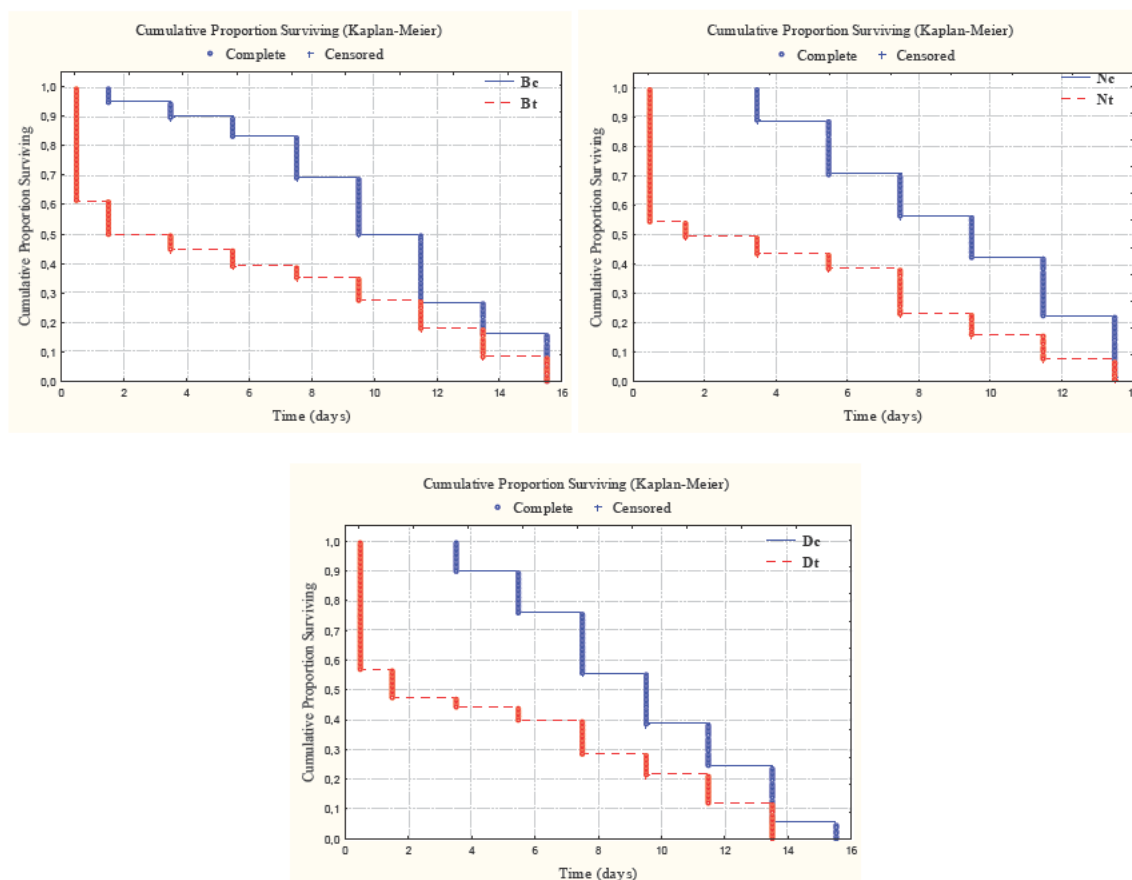


Figure 2. Survival curves of *E. formosa* F₁ females from populations Bujanovac (B), Negotin (N) and commercial “Dutch” strain (D); Control (c) = distilled water; Treatment (t) = imidacloprid 30 mg/l (B), 20 mg/l (N) and 25 mg/l (D)

Table 3. Repetitive ANOVA parameters for main effects, and associated interactions, on parasitization rates of *E. formosa* females that emerged from imidacloprid-treated pupae, local populations Bujanovac (B) and Negotin (N), and commercial “Dutch” strain (D); Control = distilled water; Treatment = imidacloprid 30 mg/l (B), 20 mg/l (N) and 25 mg/l (D)

	df	Imidacloprid	
		F	p
Between observation periods	1		
Treatment/Control	1	11.53	0.003
<i>E. formosa</i> population	2	92.30	0.000
Treatment/Control x population	2	7.17	0.005
Error	18		
Within observation period			
Observation period	7	195.41	0.000
Observation period x treatment/control	7	2.11	0.047
Observation period x population	14	11.52	0.000
Observation period x treatment/control x population	14	1.88	0.034
Error	126		

Parasitization rates (parasitism/48 h period) of the tested *E. formosa* females that emerged from treated or untreated parasitoid pupae are shown in Figure 3. Considering the examined populations, imidacloprid treatment affected only females D by shortening their oviposition period by two days (14 days) in comparison with control females. Females of the local populations B and N that emerged from treated pupae laid eggs as long as control females (B females 16 days, N females 14 days). Parasitization rate was not reduced significantly by imidacloprid in any of the three populations, except during the oviposition interval of 0-2 days of females B, and the interval of 12-14 days of females D. Females B achieved the highest parasitism within 48 h period; maximum parasitization was noted on

the fifth and sixth days of oviposition (25.2 pupae/female/48 h in treatment, and 25.93 pupae/female/48 h in control).

The results of the two-way ANOVA analysis revealed that reduction in total parasitism was significantly affected by treatment ($F_{1,18}=5.02$, $p<0.05$), population ($F_{2,18}=69.37$, $p<0.001$) and their interaction ($F_{2,18}=6.25$, $p<0.01$). Total parasitism (Figure 3) of wasp females from treated pupae B and N showed no significant difference from control females, in contrast to females D, whose total parasitism was significantly lower by 25.92 %. In both cases, i.e. treatment and control, the highest average number of pest nymphs was parasitized by females B (Bt:Bc = 143.79 pupae/female:145.15 pupae/female).

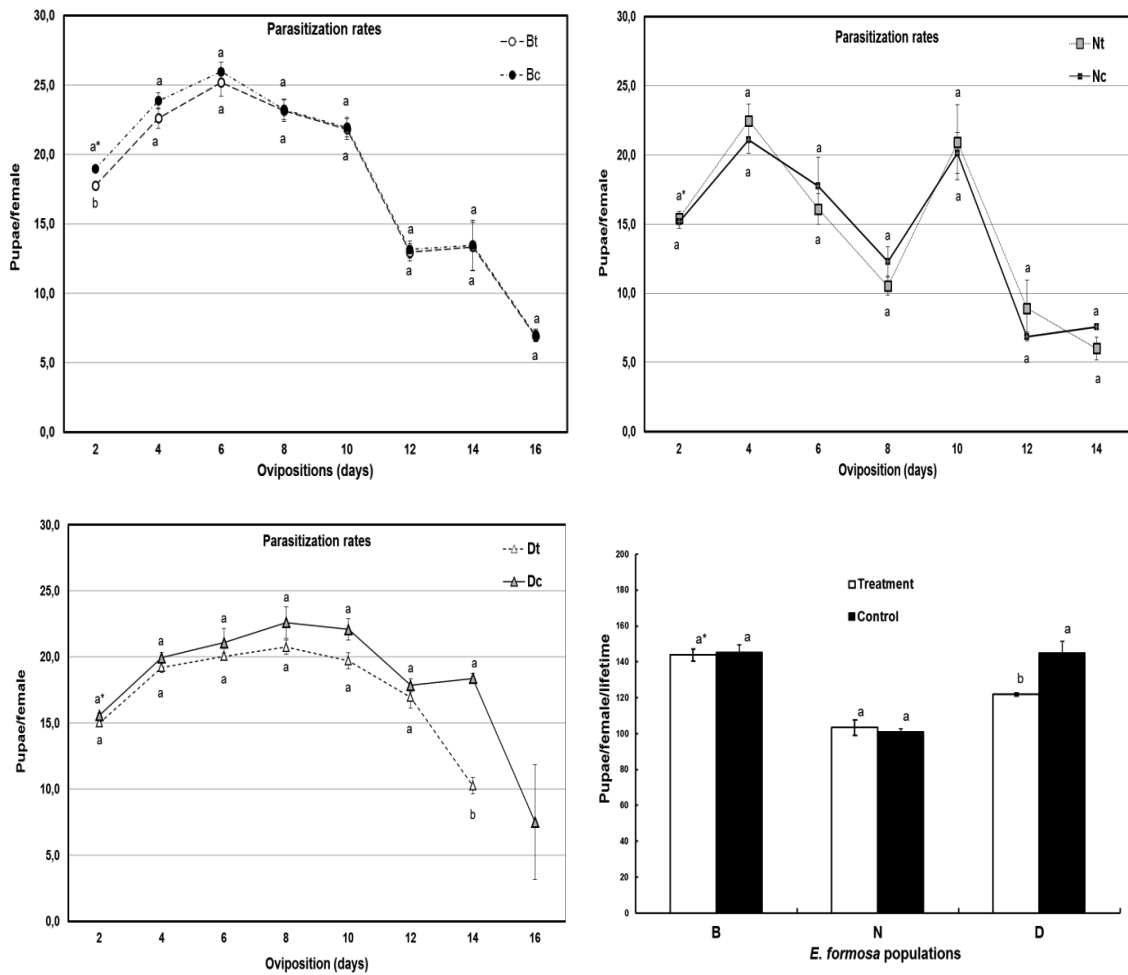


Figure 3. Parasitization rates (Mean±SE, pupae/female/48 h) and total parasitism (Mean±SG, pupae/female/lifetime) of *E. formosa* F₁ females from local populations Bujanovac (B) and Negotin (N), and commercial “Dutch” strain (D); Control (c) = distilled water; Treatment (t) = imidacloprid 30 mg/l (B), 20 mg/l (N) and 25 mg/l (D)

(Means marked with different letters are significantly different, *t*-test, $P < 0.05$).

Reductions in adult emergence in populations B (27.48 %) and D (17.52 %) were significantly affected by treatment ($F_{1,18}=43.09$, $p<0.001$), population ($F_{2,18}=51.98$, $p<0.001$) and their interaction ($F_{1,18}=14.01$, $p<0.001$). The emergence of F_1 female adults from pupae treated with imidacloprid in populations B and D differed from the emergence of control adults, while no such difference was noted for population N (Table 4). Control population B had the highest value of this parameter (131.61 adults/female), while population D had the highest value considering treatment data (105.52 adults/female).

Table 4. Total adult emergence (Mean \pm SE, adults/female) of *E. formosa* in F_1 generation of local populations Bujanovac (B) and Negotin (N), and commercial "Dutch" strain (D); Control = distilled water; Treatment = imidacloprid 30 mg/l (B), 20 (N) and 25 mg/l (D)

Population	Treatment	Control
B	95.45 <i>b</i> (± 2.71)	131.6 <i>a</i> (± 1.51)
N	84.87 <i>a</i> (± 1.25)	85.73 <i>a</i> (± 2.55)
D	105.52 <i>b</i> (± 2.06)	127.93 <i>a</i> (± 2.54)

Means marked with different letters in rows are significantly different (*t*-test, $P<0.05$).

Table 5. Instantaneous rates of increase (Mean \pm SE, females/day) of *E. formosa* females in F_1 generation, local populations Bujanovac (B) and Negotin (N), and commercial "Dutch" strain (D); Control = distilled water; Treatment = imidacloprid 30 mg/l (B), 20 (N) and 25 mg/l (D)

Population	Treatment	Control
B	0.283 <i>a</i> (± 0.002)	0.286 <i>a</i> (± 0.008)
N	0.249 <i>b</i> (± 0.001)	0.260 <i>a</i> (± 0.001)
D	0.270 <i>a</i> (± 0.001)	0.273 <i>a</i> (± 0.002)

Means marked with different letters in rows are significantly different (*t*-test, $P<0.05$).

The reductions in instantaneous rates of increase (r_i) of females that survived treatment with imidacloprid at the pupal stage were caused by treatment ($F_{1,18}=19.3$, $p<0.001$) and population ($F_{2,18}=172.9$, $p<0.001$), while their interaction did not result in a statistically

significant influence on the r_i values ($F_{2,18}=3.0$, $p=0.074$). Rates of increase of treated females N, compared to control females, were significantly lower, by 4.23 %, while the rates of increase in populations B and D were not significantly reduced (Table 5). Both treated and control females of population B (Bt:Bc = 0.283 females/day:0.286 females/day) had the highest rate of increase.

DISCUSSION

The imidacloprid product applied in our present study directly to parasitoid pupae at the concentrations of 30 mg/l (B), 20 mg/l (N) and 25 mg/l (D) significantly affected female survival after pupal treatment, extended juvenile development, significantly reduced total parasitism of females D, total adult emergence of females B and D, and significantly reduced the instantaneous rate of increase in females N.

Only sporadic studies have examined imidacloprid sublethal effects by quantifying life history parameters and/or population growth of parasitoids (Saber et al., 2009; Saber, 2011; Kheradmand et al., 2012; Sohrabi et al., 2012, 2013) and predators (James, 1997). Sohrabi et al. (2012) used female longevity, fecundity, gender ratio and population parameters such as r_m to evaluate sublethal effects of imidacloprid at population level of the parasitoid wasp *E. inaron*. They immersed larvae and pupae of *E. inaron* together with cotton leaves in an imidacloprid solution ($LC_{25}=62.5$ ppm). The study showed that treatment of that parasitoid at the pupal stage caused net reproductive rate (R_0) and mean generation time (T) of *E. inaron* to decrease significantly, while intrinsic (r_m) and final (l) rates of increase did not change significantly. Similarly, Saber (2011) found imidacloprid to reduce significantly the longevity, fecundity and population parameters of adults of the egg parasitoid *Trichogramma cacoeciae* Marchall that survived treatment at the pupal stage.

Although the treated females of population D had the highest number of emerged adults, females B lived longer, laid eggs longer, and had higher values of instantaneous rate of increase. Treated females N had less desirable results of all parameters, compared to commercial females D.

Based on high acute toxicity of imidacloprid to the pupal stage of *E. formosa* in our study and a significant reduction in its life history and population parameters, imidacloprid cannot be considered as compatible for integrated use with this parasitoid. Precise estimation

of risks, including an assessment of different ways in which the parasitoid may be exposed to the compound, require further testing in the laboratory, as well as in the field. Before more data have been obtained in field trials under more realistic conditions, our conclusions need to be considered with rational caution. Imidacloprid may exert different undesirable effects on *E. formosa* and be coactive with other factors apart from its insecticidal effect. Although these laboratory data should not be immediately used in field testing, they provide a sound illustration of potential negative effects that the insecticide may have on the parasitoid in practice and commercial use, while also providing a basis for careful selection of the most compatible insecticide for complementary use with this beneficial organism.

Data on the potential of local parasitoid populations of *E. formosa*, and lethal and sublethal effects of imidacloprid on the pupal stage of this biological agent provide a basis for further research towards improved integrated programmes for sustainable management of *T. vaporariorum* populations in Serbia.

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REFERENCES

- Albajes, R., Gullino, M.L., van Lenteren, J.C., & Elad, Y. (1999): *Integrated Pest and Disease Management in Greenhouse Crops*. Dordrecht, Netherlands: Kluwer Academic Publishers.
- Cahill, M., Denholm, I., Byrne, F.J., & Devonshire, A.L. (1996). Insecticide resistance in *Bemisia tabaci* - current status and implications for management. In *Proceedings of Brighton Crop Protection Conference: Pests & Diseases, Volume 1* (pp 75-80). Farnham, UK: BCPC.
- Carvalho, S.S. Vendramim, J.D., Pitta, R.M., & Forim, M.R. (2012). Efficiency of neem oil nanoformulations to *Bemisia tabaci* (Genn.) biotype B (Hemiptera: Aleyrodidae). *Semina: Ciências Agrárias*, 33(1), 193-202. doi:10.5433/1679-0359.2012v33n1p193
- Croft, B.A. (1990). *Arthropod biological control agents and pesticides*. New York, NY: John Wiley.
- Drobnjaković, T., Marčić, D., Prijović, M., Perić, P., Milenković, S., & Bošković, J. (2016). Life history traits and population growth of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) local population from Serbia. *Entomologia Generalis*, 35(4), 281-295. doi: 10.1127/entomologia/2016/0183
- Elbert, A., Becker, B., Hartwig, J., & Erdelen, C. (1991). Imidacloprid - a new systemic insecticide. *Pflanzenschutz Nachrichten Bayer*, 44, 113-116.
- Elbert, A., & Nauen, R. (1996). Bioassays for imidacloprid for a resistance monitoring against the whitefly *Bemisia tabaci*. In *Proceedings of Brighton Crop Protection Conference: Pests & Diseases, Volume 2* (pp 731-738). Brighton, UK: BCPC.
- Elzen, G.W. (2001). Lethal and sublethal effects of insecticide residues on *Orius incidiolus* (Hemiptera: Anthocoridae) and *Geocoris punctipes* (Hemiptera: Lygaeidae). *Journal of Economical Entomology*, 94 (1), 55-59. doi:10.1603/0022-0493-94.1.55
- Enkegaard, A. (1993). *Encarsia formosa* parasitizing the Poinsettia-strain of the cotton whitefly, *Bemisia tabaci*, on Poinsettia: bionomics in relation to temperature. *Entomologia Experimentalis et Applicata*, 69(3), 251-261. doi: 10.1111/j.1570-7458.1993.tb01748.x
- Enkegaard, A., & Brødsgaard, H.F. (2006). Biocontrol in protected crops: is lack of biodiversity a limiting factor? In J. Eilenberg & H.M.T. Hokkanen (Eds.), *An ecological and societal approach to biological control* (pp 91-122). Dordrecht, Netherlands: Springer.
- EPPO (European Plant Protection Organisation) (2004). Side effects on *Encarsia formosa*. *EPPO, Paris, PPI/142(2)*.
- Finney, D.J. (1971): *Probit analysis* (2nd ed.). Cambridge, UK: Cambridge University Press.
- Gentz, M.C., Murdoch, G., & King, G.F. (2010). Tandem use of selective insecticides and natural enemies for effective, reduced-risk pest management. *Biological Control*, 52(3), 208-215. doi:10.1016/j.biocontrol.2009.07.012
- Gerling, D. (1990). *Whiteflies: Their bionomics, pest status and management*. Andover, UK: Intercept.
- Gholamzadeh, M., Ghadamyari, M., Salehi, L., & Hoseinaveh, V. (2012). Effects of amitraz, buprofezin and propargite on some fitness parameters of the parasitoid *Encarsia formosa* (Hym.: Aphelinidae), using life table and IOBC methods. *Journal of Entomological Society of Iran*, 31(2), 1-14.
- Grafton-Cardwell, E.E., Lee, J.E., Robillard, S.M., & Gorden, J.M. (2008). Role of imidacloprid in integrated pest management of California citrus. *Journal of Economic Entomology*, 101(2), 451-460. doi: 10.1603/0022-0493
- Hoddle, M.S., van Driesche, R.G., & Sanderson, J.P. (1998). Biology and use of the whitefly parasitoid *Encarsia formosa*. *Annual Review of Entomology*, 43, 645-669. doi:10.1146/annurev.ento.43.1.645

- Horowitz, A.R., Kontsedalov, S., & Ishaaya, I., (2004). Dynamics of resistance to the neonicotinoids acetamiprid and thiamethoxam in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Journal of Economic Entomology*, 97(6), 2051-2056. Retrieved from <https://doi.org/10.1093/jee/97.6.2051>
- James, D.G. (1997). Imidacloprid increases egg production in *Amblyseius victoriensis* (Acari: Phytoseiidae). *Experimental and Applied Acarology*, 21(2), 75-82. doi:10.1023/a:1018493409832
- James, D.G., & Price, T.S. (2002). Fecundity in twospotted spider mite (Acari: Tetranychidae) is increased by direct and systemic exposure to imidacloprid. *Journal of Economic Entomology*, 95(4), 729-732. doi:10.1603/0022-0493-95.4.729
- James, D.G., & Vogebe, B. (2001). The effect of imidacloprid on survival of some beneficial arthropods. *Plant Protection Quarterly*, 16(2), 58-62.
- Kheradmand, K., Khosravian, M., & Shahrokhi, S. (2012). Side effect of four insecticides on demographic statistics of aphid parasitoid, *Diaeretiella vappae* (McIntosh) (Hym., Braconidae). *Annals of Biological Research*, 3(7), 3340-3345.
- Manzano, M.R., & van Lenteren, J.C. (2009). Life history parameters of *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) at different environmental conditions on two bean cultivars. *Neotropical Entomology*, 38(4), 452-458. doi:10.1590/S1519-566X2009000400002
- Morales, J.J., Medina, P., & Viñuela, E. (2006). Compatibility of *Hyposoter didymator*, an endoparasitoid of *Spodoptera littoralis*, with several insecticides used on horticultural crops. *IOBC/WPRS Bulletin*, 29, 345-350.
- Nauen, R., Koob, B., & Elbert, A. (1998). Antifeedant effects of sublethal dosages of imidacloprid on *Bemisia tabaci*. *Entomologia Experimentalis et Applicata*, 88(3), 287-293. doi:10.1046/j.1570-7458.1998.00373.x
- Nauen, R., Strobel, J., Tietjen, K., Otsu, Y., Erdelen, C., & Elbert, A. (1996). Aphicidal activity of imidacloprid against a tobacco feeding strain of *Myzus persicae* (Homoptera: Aleyrodidae) from Japan closely related to *Myzus nicotianae* and highly resistant to carbamates and organophosphates. *Bulletin of Entomological Research*, 86(2), 165-171. doi:10.1017/S0007485300052408
- Perić, P., Marčić, D., & Stamenković, S. (2009). Natural enemies of whitefly (*Trialeurodes vaporariorum* Westwood) in Serbia. *Acta Horticulturae*, 830, 539-544. doi:10.17660/ActaHortic.2009.830.77
- Polaszek, A., Evans, G.A., & Bennett, E.D. (1992). *Encarsia* parasitoids of *Bemisia tabaci* (Hymenoptera: Aphelinidae, Homoptera: Aleyrodidae): a preliminary guide to identification. *Bulletin of Entomological Research*, 82(3), 375-392. doi:10.1017/S0007485300041171
- Prijović, M., Škaljac, M., Drobnjaković, T., Žanić, K., Perić, P., Marčić, D., & Puizina, J. (2014). Genetic variation of the greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), among populations from Serbia and neighbouring countries, as inferred from COI sequence variability. *Bulletin of Entomological Research*, 104(3), 357-366. doi:10.1017/S0007485314000169.
- Qiu, Y.T., van Lenteren, J.C., Drost, Y.C., & Posthuma-Doodeman, J.A.M. (2004). Life history parameters of *Encarsia formosa*, *Eretmocerus eremicus* and *E. mundus*, aphelinid parasitoids of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *European Journal of Entomology*, 101(1), 83-94. doi:10.14411/eje.2004.017
- Richter, S.E., Albert, R., Jaeckel, B., & Leopold, D. (2003): *Encarsia formosa* – a parasitoid for biological control under influence of insecticides and changing hosts. *Nachrichtenbl Deut Pflanzenschutzd Journal*, 55, 161-172.
- Robertson, J.L., Russell, R.M., Preisler, H.K., & Savin, N.E. (2007): *Bioassays with Arthropods* (2nd edition). Boca Raton, FL, USA: CRC Press, Taylor & Francis Group.
- Sabahi, Q., Rasekh, A., & Michaud, J.P. (2011). Toxicity of three insecticides to *Lysiphlebus fabarum*, a parasitoid of the black bean aphid, *Aphis fabae*. *Journal of Insect Science* 11, 104. doi:10.1673/031.011.10401
- Saber, M. (2011). Acute and population level toxicity of imidacloprid and fenpyroximate on an important egg parasitoid, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). *Ecotoxicology*, 20(6), 1476-1484. doi:10.1007/s10646-011-0704-3
- Saber, M., Hassan, S.A., & Hejazi, M.J. (2009). The effect of a neonicotinoid insecticide, imidacloprid, on an important egg parasitoid *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae). In *Proceedings of German Society of Basic and Applied Entomology (DGaE)* (pp 16-19). Göttingen, Germany: Georg-August University.
- Sclar, D.C., Gerace, D. & Cranshaw, W.S. (1998). Observations of population increases and injury by spider mites (Acari: Tetranychidae) on ornamental plants treated with imidacloprid. *Journal of Economic Entomology*, 91(1), 250-255. doi:10.1093/jee/91.1.250
- Simmonds, M.S.J., Manlove, J.D., Blaney, W.M., & Khambay, B.P.S. (2002). Effects of selected botanical insecticides on the behaviour and mortality of the glasshouse whitefly *Trialeurodes vaporariorum* and the parasitoid *Encarsia formosa*. *Entomologia Experimentalis et Applicata*, 102(1), 39-47. doi:10.1046/j.1570-7458.2002.00923.x
- Sohrabi, F., Shishebor, P., Saber M., & Mosaddegh, M.S. (2012). Lethal and sublethal effects of buprofezin and imidacloprid on the whitefly parasitoid *Encarsia inaron* (Hymenoptera: Aphelinidae). *Crop Protection*, 32, 83-89. doi:10.1016/j.cropro.2011.10.005

- Sohrabi, F., Shishehbor, P., Saber, M., & Mosaddegh, M.S. (2013). Lethal and sublethal effects of imidacloprid and buprofezin on the sweetpotato whitefly parasitoid *Eretmocerus mundus* (Hymenoptera: Aphelinidae). *Crop Protection*, 45, 98-103. doi:10.1016/j.cropro.2012.11.024
- Stapel, J.O., Cortescero, A.M., & Lewis, W.J. (2000). Disruptive sublethal effects of insecticides on biological control: altered foraging ability and lifespan of a parasitoid after feeding on extrafloral nectar of cotton treated with systemic insecticides. *Biological Control*, 17(3), 243-249. doi:10.1006/bcon.1999.0795
- Stark, J.D., & Banks, J.E. (2003). Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology*, 48, 505-519. doi:10.1146/annurev.ento.48.091801.112621
- Stark, J.D., Tanigoshi, L., Bounfour, M., & Antonelli, A. (1997). Reproductive potential: its influence on the susceptibility of a species to pesticides. *Ecotoxicology and Environmental Safety*, 37(3), 273-279. doi:10.1006/eesa.1997.1552
- Sterk, G., Heuts, F., Merck, N. & Bock, J. (2002). Sensitivity of non-target arthropods and beneficial fungal species to chemical and biological plant protection products: Results of laboratory and semi-field trials. In *First International Symposium on Biological Control of Arthropods* (pp 306-313). Retrieved from USDA, USFS at <https://www.fs.fed.us/foresthealth/technology/webpubs/FHTET-2003-05/day4/sterk.pdf>
- Stouthamer, R., & Mak, F. (2002). Influence of antibiotics on the offspring production of the *Wolbachia*-infected parthenogenetic parasitoid *Encarsia formosa*. *Journal of Invertebrate Pathology*, 80(1), 41-45. doi:10.1016/S0022-2011(02)00034-4
- Torres, J.B., Silva-Torres, C.S.A., Silva, M.R., & Ferreira, J. (2002). Compatibilidade de inseticidas e acaricidas com o percevejo predador *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) em algodoeiro. *Neotropical Entomology*, 31(2), 311-317. doi:10.1590/S1519-566X2002000200020
- van Lenteren, J.C., & Martin, N.A. (1999). Biological control of whiteflies. In R. Albajes, M. L. Gullino, J. C. van Lenteren & Y. Elad (Eds.), *Integrated pest and disease management in greenhouse crops* (pp 202-216). Dordrecht, Netherlands: Kluwer Academic Publishers.
- van de Veire, M., & Tirry, L. (2003). Side effects of pesticides on four species of beneficials used in IPM in glasshouse vegetable crops: "worst case" laboratory tests. *IOBC/WPRS Bulletin* 26(5), 41-50.
- Whalon, M.E., Mota-Sanchez, D., & Hollingworth, R.M. (2008): *Global Pesticide Resistance in Arthropods*. Wallingford, UK: CAB International.
- Whalon, M.E., Mota-Sanchez, D., Hollingworth, R.M., & Duynslager, L. (2017): Arthropod Pesticide Resistance Database. Retrieved from <https://www.pesticideresistance.org/>
- Yankova, V. Masheva, S. Boev, B., & Toskov, K. (2011). Toxicity of plant protection products towards the imago of *Encarsia formosa* Gah. *Agricultural Science and Technology*, 3(4), 374, 377. Retrieved from http://www.nhm.ac.uk/resources/research-curation/projects/chalcidoids/pdf_X/YankovMaBo2011.pdf
- Zchori-Fein, E., Roush, R.T., & Hunter, M.S. (1992). Male production induced by antibiotic treatment in *Encarsia formosa* (Hymenoptera: Aphelinidae), an asexual species. *Experientia*, 48(1), 102-105. doi:10.1007/BF01923619

Subletalni efekti imidakloprida na parazitoida bele leptiraste vaši *Encarsia formosa* Gahan

REZIME

Akutna toksičnosti preparata na bazi imidakloprida (Confidor 200 SL) po stadijum lutke parazitoida *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) i efekti na parametre životne istorije i populacioni rast preživelih ženki parazitoida komercijalizovane („Dutch“ rase, D) i dve lokalne populacije iz Srbije (Bujanovac, B; Negotin, N) utvrđivani su u laboratorijskim uslovima. Svi ogledi su izvedeni na temperaturi $27\pm 1^{\circ}\text{C}$ i relativnoj vlažnosti vazduha od $60\pm 10\%$, uz fotoperiod 16:8h, u četiri ponavljanja. U biotestu akutne toksičnosti, listovi duvana sa lutkama parazitoida tretirani su serijom simetrično raspoređenih koncentracija (800, 400, 200, 100, 50 i 25 mg a.m./l) u rasponu koji pokriva 10-90% smrtnosti. Preparat na bazi imidakloprida, primenjen direktno na lutke parazitoida, u srednjim letalnim koncentracijama (LC50) dobijenim

u testovima akutne toksičnosti (30 mg/l, 20 mg/l i 25 mg/l, za B, N i D, respektivno), značajno je uticao na preživljavanje ženki iz tretiranih lutki, produžio je dužinu juvenilnog razvića (1.81, 1.59 i 1.73 dana, za B, N i D, respektivno), značajno redukovao ukupni parazitizam ženki D (25.92 %), ukupnu pojavu adulta ženki B (27.48 %) i D (17.92 %) i statistički značajno redukovao samo trenutnu stopu rasta ženki N (4.23 %). S obzirom na visoku akutnu toksičnost preparata na bazi imidakloprida po stadijum lutke parazitoidea, i značajne redukcije životnih i populacionih parametara, imidaklopid se ne smatra kompatibilnim za zajedničku primenu sa parazitoidom *E. formosa*. Tačna determinacija rizika primene ovog preparata, zahteva njegovo dalje testiranje u poljskim uslovima. Razmatrane su mogućnosti praktične primene dobijenih rezultata u okviru integralnog koncepta zaštite biljaka od bele leptiraste vaši.

Gljučne reči: *Encarsia formosa*; Imidaklopid; Subletalni efekti; Životni parametri; Populacioni rast