Pestic. Phytomed. (Belgrade), 34(3-4), 2019, 201–209
DOI: https://doi.org/10.2298/PIF1904201D

UDC 632.951:632.7:632.937.1
Original scientific paper

Toxic and sublethal effects of buprofezin on the whitefly parasitoid *Encarsia formosa* Gahan

Tanja Drobnjaković[1]*, Dejan Marčić[1], Mirjana Prijović[1] and Slobodan Milenković[1]

[1] Institute of Pesticides and Environmental Protection, Bantska 31b, 11080 Belgrade, Serbia
[2] Megatrend University, Faculty of biofarming, Maršala Tita 39, 24300 Bačka Topola, Serbia
**Corresponding author: tanjadrobnjakovic@gmail.com*

SUMMARY

Acute toxicity of a buprofezin-based product (commercial product Elisa 440 SC) to pupae of the whitefly parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), and its effects on life history traits and population growth in F_1 generation 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 an acute toxicity bioassay, tobacco leaves carrying parasitoid pupae (20 pupae per replicate) were treated with a series of buprofezin concentrations covering a 10-90% mortality range, and mortality was calculated based on the number of emerging adults 9 days after treatment. The following LC_{50} (mg/l) estimates were obtained: 244.2, 281.5 and 199.5 (for B, N and D, respectively). The product based on buprofezin, applied to parasitoid pupae at concentrations within the LC_{50}s and 95% confidence limits (264 mg/l for B and N; 220 mg/l for D), significantly prolonged the duration of juvenile development (2, 1.7 and 2.2 days for B, N and D, respectively, compared to control data). Females from all tested populations that emerged from the treated pupae and were exposed to the residual action of buprofezin lived shorter than control females (B, N and D by 1.5, 0.7 and 1.7 days, respectively). Also, females that emerged from the treated pupae achieved a significantly reduced level of parasitism (B, N and D by 11.7, 17.7 and 17.6 %, respectively), total adult emergence (B, N and D by 11.6, 17.8 and 17.8 %, respectively) and instantaneous rate of increase (B, N and D by 8.2, 6.8 and 12.5 %, respectively), compared to control. More precise determination of risks involved in the use of buprofezin requires its more detailed field testing.

Keywords: *Encarsia formosa*; buprofezin; sublethal effects; life history traits; population growth

INTRODUCTION

The greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), a cosmopolitan and polyphagous species, has been widespread in Serbia since the 1970s and it is frequently found in greenhouses as a serious pest of vegetables and ornamentals (Prijović et al., 2014). The parasitic wasp *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) has been used for many years for biological control of *T. vaporariorum* and has been one of the most successful biological agents in greenhouse crops around the world (van Lenteren & Martin, 1999; Enkegaard & Brødsgaard, 2006), but it is rarely used for that purpose in Serbia. *E. formosa* is uniparental, its females are primary endoparasitoids of the greenhouse whitefly, and males occur only rarely

(Hoddle at al., 1998). Pesticide treatments are necessary when natural enemies fail to keep a pest population below its economic threshold (Albajes et al.,1999). The widespread use of chemical insecticides has caused whitefly resistance to compounds with various modes of action (Whalon et al., 2020).

Commercial field studies have shown that strategies of plant protection from pests based on initial use of insect growth regulators (IGR), such as buprofezin and pyriproxyfen, protect populations of natural enemies, unlike conventional insecticides (Naranjo et al., 2003). Buprofezin has a relatively small spectrum of activity against Hemiptera insects, including whiteflies (Cahill et al., 1996). This IGR has demonstrated efficacy in controlling *T. vaporariorum* (Masuda & Miyata, 2006), and has been effective in controlling *B. tabaci* in cotton (Natwick, 1993), even though less effective against *B. tabaci* biotype B (Masuda & Miyata, 2006) and *B. tabaci* biotype Q (Kobayashi, 2007). Buprofezin is not active against adults but reduces the viability of eggs of treated females, and it causes molting at younger larval stages, thus reducing the adult pest population only seven to 10 days after treatment (Horowitz & Ishaaya, 1996). Buprofezin, which inhibits chitin synthesis, is active during insect molting; it disturbs molting from larval stages to adulthood (interferes with embriogenesis in adults), affecting reproduction (Toscano et al., 2001; Yu, 2008).

Generally speaking, buprofezin is considered to be less harmful to parasitoids than to predators (Jones et al., 1998). Many studies have shown that different development stages of Hymenoptera parasitoids react in various ways to IGR treatments, showing a tendency of younger development stages to be more susceptible than the later and more mature stages (Gerling & Sinai, 1994; Darvas & Polgar, 1998). Parasitoids of the family Aphelinidae that survived the activity of buprofezin may suffer from harmful sublethal effects manifesting as shorter life span, lower fecundity, and wing deformation occurring after eclosion of parasitoid adults from the pupal stage (Stansly & Liu, 1997; Jones et al.,1998). Any indirect effect on natural enemies connected with buprofezin activity may occur as a consequence of its vaporability (De Cock et al., 1990).

In some cases, buprofezin performed poorly as regards its selectivity, which depends on the test species of insects (Stansly & Liu, 1997; Jones et al., 1998). Buprofezin classification based on quantification of life parameters and population growth of *E. formosa* was at the focus of research by Heydari et al. (2006) and Southwood and Handerson (2000). The present study focused on the effects of a buprofezin-based insecticide on life history traits and population growth of F_1 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

Two local populations of *E. formosa* were started from pupae collected in tunnel greenhouses of vegetables and ornamentals in locations without a history of using commercial parasitoid strains for biological control of greenhouse whiteflies: population B, collected in Bujanovac (GPS: 42°30'27'' N, 21°48'30'' E) on *Solanum nigrum* L, and population N, collected in Negotin (GPS: 44°13'00'' N, 22°31'00'' E) on *Hibiscus* sp. The emerged female wasps of each population were identified as *E. formosa* using the key given by Polaszek et al. (1992). The Dutch strain of *E. formosa* (D) was purchased from Zeleni hit d.o.o., the Serbian agent of Koppert Biological Systems Inc., The Netherlands, and it was successfully cultured as a reference strain. The Dutch strain and two Serbian populations of the parasitoid wasp *E. formosa* were reared on *T. vaporariorum* hosts at 27 ± 1°C and 60 ± 10% R.H. in 16L:8D h photoperiod. Whiteflies were reared on tobacco plants, cv. Samsun, in ventilated muslin cages according to the European Plant Protection Organisation (EPPO, 2004) methodology.

The commercial insecticide product Elisa 440 SC (manufactured by Galenika Fitofarmacija, Serbia) is formulated as a suspension concentrate (SC). Its content of buprofezin, as the leading product ingredient, is standardised to 440 g/l.

All bioassays were performed in a climate chamber at 27 ± 1°C and 60 ± 10% R.H. with a 16/8 h light/dark photoperiod and in four replicates. The bioassays were performed in Petri dishes (12 cm diameter), each having four (1 cm diameter) lid openings with muslin covers on top to provide ventilation and prevent internal condensation, and containing 1% agar layer upon which a tobacco leaf was set. The insecticide was diluted in distilled water and applied by spraying onto the entire area of each Petri dish (i.e. upturned lid and lower dish with a tobacco leaf placed on top of the agar layer). The insecticide was applied using a Potter spray tower (2 ml of spray liquid, 100 kPa air pressure, aqueous deposit 2.7 ± 0.2 mg/cm2).

In the acute toxicity bioassay with pupae, tobacco leaves with parasitised whitefly pupae were fixed to tin foil with Traganth-kit. After drying, the leaves were cut to pieces that carried about 20 parasitoid pupae (4 days old, i.e. 12 days after egg laying) and then placed on filter paper in plastic Petri dishes (filter paper was moistened with water to fix leaves in place during exposure). The pieces of tobacco leaves were then treated with a series of insecticide concentrations (8800, 4400, 440, 220, 110, 80, 55 and 27.5 mg a.i./l). Two hours after treatment, tobacco leaves were transferred to new Petri dishes, and they remained there until adults emerged from the pupae. Mortality assessment was based on the counts of emerged adults 9 days after treatment, compared to the number of treated pupae (EPPO, 2004). Concentration-mortality data were subjected to probit analysis using the POLO Plus software (LeOra Software, Berkeley, CA). A pairwise comparison of the LC_{50}s was performed using the lethal dose ratio test: when 95% confidence limits (CLs) for LC ratios included 1, the LCs were not significantly different (Robertson et al., 2007).

A parasitism bioassay with F_1 generation wasps was carried out in which 20 pupae (4 days old, i.e. 12 days after egg laying) were treated in Petri dishes with the following buprofezin concentrations (mg/l): B and N with 264 mg/l, and population D with 220 mg/l (concentrations were within 95% confidence limits for the LC_{50} calculated in acute toxicity bioassays, Table 1). All surviving female adults that emerged from the 20 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. The development time, longevity, parasitism/48 h 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). 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 its ultimate death occurred at the midpoint of 48 h. Parasitism was determined based on the number of parasitized host nymphs (black pupae) in each inspection interval (parasitism/48 h period) and the total number of parasitized host nymphs (total, lifetime parasitism). Adult emergence was calculated as the number of wasps that emerged from parasitized nymphs and reached adult stage.

In the parasitism bioassay, data on parasitism and survival of treated pupae were used to calculate the instantaneous rates of increase (r_i) using the equation: $r_i = [ln\ (N_f/No)]/\Delta t$, where N_0 is the initial number of individuals (i.e. 20 pupae per replicate), N_f is the final number of individuals, i.e. black (parasitised) pupae and adults emerged, and Δt is the number of days elapsed between the start and the end of the bioassay. Positive r_i values indicate a growing population, negative r_i values indicate a population in decline and $r_i = 0$ indicates a stable population (Walthall & Stark, 1997; Stark & Banks, 2003). The N_f was determined at the end of the 14th day of oviposition, the time interval that corresponds to the shortest oviposition period (period during which wasps oviposited over their lifetime). In parasitism bioassays, population N wasps had the shortest oviposition period.

Table 1. Acute toxicity of Elisa 440 SC (mg/l) to *Encarsia formosa* pupae from local populations Bujanovac (B) and Negotin (N), and commercial Dutch strain (D).

Life stage	Populations	n	LC_{50} (mg/l) (95% CLs)	b (± SE)	χ^2	df
Pupae	B	640	244.221 a (109.000-505.047)	0.942 (±0.04)	12.994	5
	N	640	281.457 a (131.685-576.551)	0.984 (±0.55)	13.040	5
	D	640	199.553 a (79.795-430.819)	0.895 (±0.05)	13.986	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

Kaplan-Meier analysis (SPSS for Windows, Version 17) was used to calculate the average female longevity, and survival curves were constructed (Enkegaard, 1993), which were analyzed by Log-rank test. Development time, longevity, parasitism/48 h period, total parasitism and adult emergence and instantaneous rate of increase data were analysed by two-way ANOVA (buprofezin 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.

RESULTS

The insecticide Elisa 440 SC caused similar levels of mortality to pupae of all three populations of *E. formosa* and no significant differences were noted among their LC_{50} values (Table 1).

Juvenile developmental time of the parasitoid in nymphs treated with the insesticide was prolonged by 1.71-2.15 days, compared to the control. A two-way analysis of variance showed that the development period depended on treatment ($F_{1,18}=84.18$, $p<0.001$), while population ($F_{2,18}=1.37$, $p=0.280$) and interaction of the two ($F_{2,18}=0.33$, $p=0.721$) had no significant effect (Table 2).

The surviving parasitoid wasps that emerged from pupae exposed to the insecticide LC_{50} (obtained from acute toxicity biossays) lived significantly shorter than control females. The relevant two-way analysis showed that the insecticide treatment of parasitised pupae ($F_{1,18}=28.69$, $p<0.001$) and parasitoid populations ($F_{2,18}=46.21$, $p<0.01$), considered as factors, had significant effects on the longevity of parasitoid wasps in any test population, while the interaction of treatment and population ($F_{2,18}=1.09$, $p=0.358$) was not significant. Females B lived 1.52 days shorter ($F_{1,6}=11.98$, $p<0.05$) than control females, females N 0.74 days shorter ($F_{1,6}=12.99$, $p<0.05$) and females D 1.71 days shorter ($F_{1,6}=19.76$, $p<0.05$), compared to control females.

Table 2. Juvenile development time (means ± SE, days), adult longevity (means ± SE, days), adult emergence (means ± SE, adults/wasp/lifetime), and instantaneous rate of increase (means ± SE, day-1) in F_1 generation of *Encarsia formosa* parasitoids from local populations Bujanovac (B) and Negotin (N), and Dutch strain (D); Control = distilled water; Treatment = buprofezin 264 mg/l (B and N), 220 mg/l (D).

Population	Pupae	Juvenile development time	Adult longevity	Adult emergence F_1 generation	Instantaneous rate of increase F_1 generation
B	Treatment	15.96 ± 0.14 b	9.18 ± 0.21 b	115.07 ± 2.22 b	0.255 ± 0.001 b
	Control	14.00 ± 0.09 a	10.70 ± 0.15 a	130.22 ± 2.51 a	0.278 ± 0.002 a
N	Treatment	15.78 ± 0.37 b	6.81 ± 0.17 b	86.90 ± 1.56 b	0.246 ± 0.002 b
	Control	14.07 ± 0.32 a	7.55 ± 0.12 a	105.70 ± 3.21 a	0.264 ± 0.002 a
D	Treatment	16.40 ± 0.20 b	8.23 ± 0.21 b	105.97 ± 3.46 b	0.246 ± 0.002 b
	Control	14.25 ± 0.20 a	9.94 ± 0.16 a	128.54 ± 5.63 a	0.281 ± 0.001 a

Means for treatment and control (for each population individually) marked by different letters are significantly different (*t*-test, $p<0.05$).

Table 3. Repeated measures ANOVA parameters for the main effects and their associated interactions for parasitism of *Encarsia formosa* parasitoids that emerged from buprofezin treated pupae from local populations Bujanovac and Negotin, and commercial Dutch strain; Control = distilled water; Treatment = buprofezin 264 mg/l (B and N), 220 mg/l (D).

	df	Buprofezin	
		F	*p*
Between observation periods			
Treatment/control	1	28.25	0.000
Population of *E. formosa*	2	33.52	0.000
Treatment/control x population	2	0.16	0.851
Error	18		
Within observation period			
Observation period	7	67.15	0.000
Observation period x treatment/control	7	0.38	0.910
Observation period x population	14	3.86	0.000
Observation period x treatment/control x population	14	0.70	0.770
Error	126		

The survival curves for female wasps of the examined populations are shown in Figure 1. Females that emerged from pupae treated with buprofezin had lower survivorship than females which emerged from pupae treated only with distilled water (Bt vs. Bc: WW [sum of survival scores for the first group] = 54.751, p<0.001; Dt vs. Dc: WW=55.977, p<0.001; Nt vs. Nc: WW = 48.303, p<0.001). Considering only the treated females, populations B and D had higher survivorship than population N (Bt vs. Nt: WW=-21.84, p<0.01; Dt vs. Nt: WW=-14.95, p<0.05). Treated females B had a higher survivorship than treated females D (Bt vs. Dt: WW=-7.015, p=0.293), but the difference was not statistically significant. Considering control females, populations B and D had higher survivorship than population N (Bc vs. Nc: WW=-54.67, p<0.001; Dc vs. Nc: WW=-44.73, p<0.001). Control females B had higher survivorship than control females D, but the difference is not statistically significant (Bc vs. Dc: WW=-11.23, p=0.993).

Repetitive ANOVA analysis showed that parasitism/48 h periods of the females that emerged from pupae treated with buprofezin in all test populations were significantly affected by the observation period ($F_{7,126}$=67.15, p<0.001). Between observation periods, all main effects and their associated interactions were significant, except the interaction of treatment and population ($F_{2,18}$=0.16, p=0.851). Within each observation period, all main effects and their associated interactions were significant, except the interaction of treatment and observation period ($F_{7,126}$=0.38, p=0.910), the 3-way interaction of treatment, population and observation period, which did not show a significant influence ($F_{7,126}$=0.70, p=0.770) (Table 3).

Parasitism/48 h of wasps that emerged from treatment and control pupae are shown in Figure 2a-c. The insecticide Elisa 440 SC did not shorten oviposition periods in any of the test populations, i.e. wasps of all populations that emerged from the pupae treated with the mean lethal concentration of the insecticide oviposited over the same period of time as control wasps treated only with water (populations B and D for 16 days, population N for 14 days).

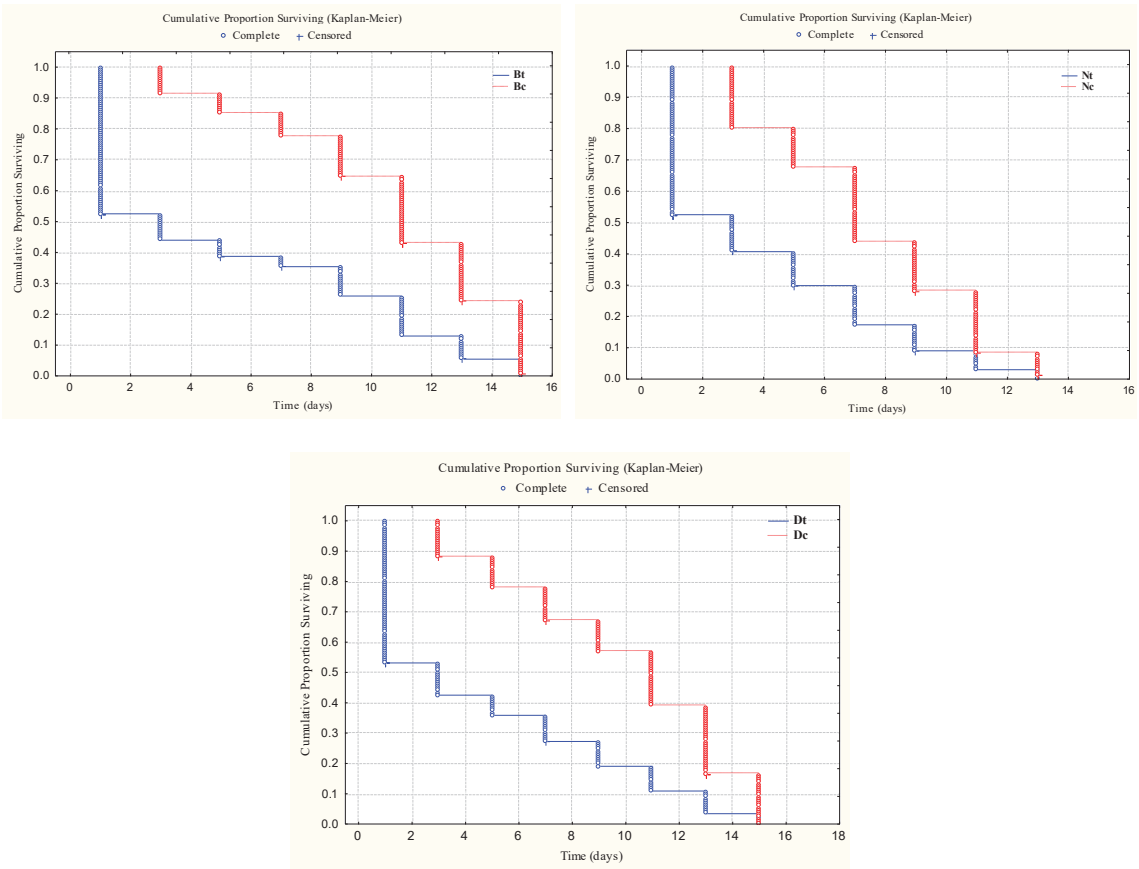

Figure 1. Survival curves of *Encarisa formosa* F_1 females from populations Bujanovac (B), Negotin (N) and commercial Dutch strain (D); Control (c) = distilled water; Treatment (t) = buprofezin 264 mg/l (B and N), 220 mg/l (D).

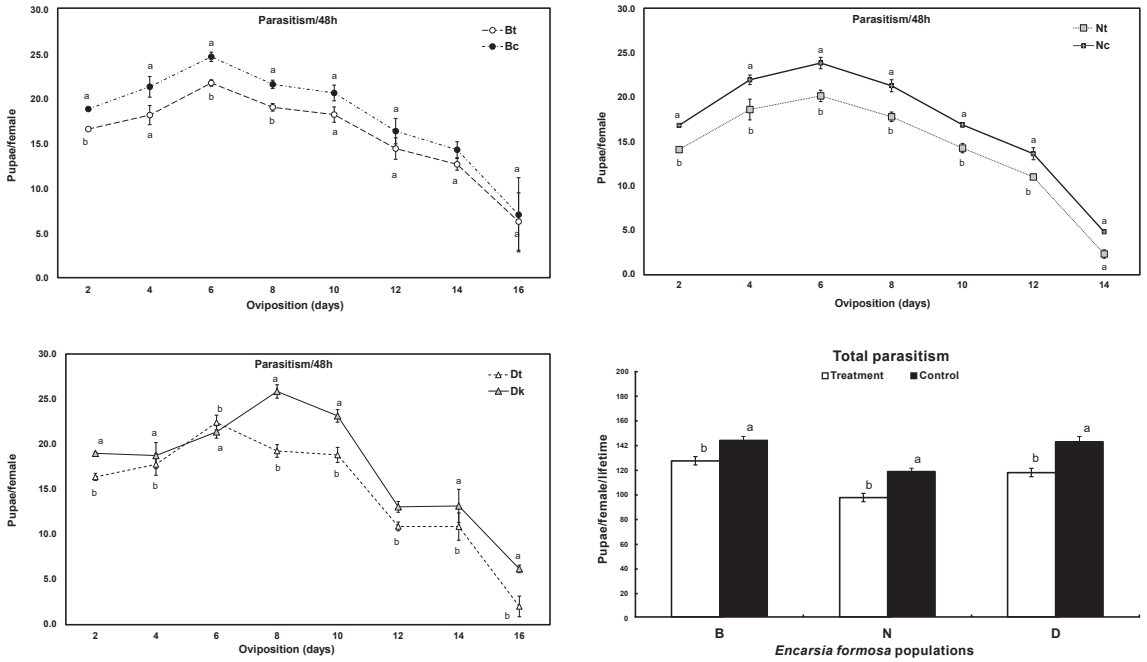

Figure 2. Parasitism/48 h periods (means ± SE, pupae/wasp/48 h) and total parasitism (means ± SE, pupae/wasp/lifetime) of *Encarsia formosa* parasitoids from local populations Bujanovac (B) and Negotin (N), and commercial Dutch strain (D); Control (c) = distilled water; Treatment (t) = buprofezin 264 mg/l (B and N), 220 mg/l (D).
(Means marked by different letters are significantly different, t-test, P < 0.05).

Total parasitism of wasps that survived insecticide treatment at the pupal stage was significantly lower than parasitism of control wasps by 11.7 ($F_{1,6}$=11.71, p<0.05), 17.7 ($F_{1,6}$=23.42, p<0.01) and 17.6 % ($F_{1,6}$=24.69, p<0.01) in populations B, N and D, respectively (Figure 1d). The relevant two-way analysis of variance showed that treatment ($F_{1,18}$=62.25, p<0.001) and population source ($F_{2,18}$=40.7, p<0.01) significantly affected total parasitism.

The counts of F_1 generation adults that emerged from treated and untreated pupae did not differ significantly between treatments (Table 2). Similar to total parasitism, the two-way analysis of variance showed that population source significantly influenced the adult F_1 counts ($F_{1,18}$=72.83, p<0.001) and treatment $F_{2,18}$= 55.08, p<0.001), while the interaction of these two factors ($F_{2,18}$=0.99, p=0.389) was not significant. Total adult emergence in treatments was reduced for B by 11.6 % ($F_{1,6}$=20.39, p<0.01), for N by 17.8 % ($F_{1,6}$=13.26, p<0.05), and for D by 17.6 % ($F_{1,6}$=24.29, p<0.01), compared to control wasps.

The relevant two-way analysis of variance showed that 14 days after oviposition began (duration of the shortest oviposition period observed in wasps of population N), insecticide treatment ($F_{1,18}$=254.8, p<0.001) and population ($F_{2,18}$=20.7, p<0,001) as the main factors, and interaction of the two ($F_{2,18}$=10.6, p<0.001), caused significantly different data of the instantaneous rate of increase (r_i) of wasp populations treated at the pupal stage. The insecticide Elisa 440 SC resulted in significant reductions in r_i values of wasps from all test populations: females B by 8.17 % ($F_{1,6}$=75.93, p<0.001), females N by 6.82 % ($F_{1,6}$=30.18, p<0.01), and females D by 12.46 % ($F_{1,6}$=241.01, p<0.001), compared to control wasps (Table 2).

DISCUSSION

After the recommended dose of buprofezin-based product (Elisa 440 SC) was applied, around 60 % of wasp adults failed to eclode from directly treated pupae of the parasitoid *E. formosa*. The LC_{50}s ranged from 199.55 mg a.i./l (D) to 281.46 mg a.i./l (N), which is somewhat lower and higher, respectively, than the recommended buprofezin concentration for field treatment (264 mg a.i./l).

Buprofezin selectivity to beneficial insects was indicated more than three decades ago (Mullin & Croft, 1985; Hassan et al. 1994). In a similar study of buprofezin effects (formulation NNI-750; concentrations 125 and 250 ppm) on immatures of *E. formosa* and *C. noacki*, based on IOBC criteria (impact on survival, reproduction and parasitoid capacity) Garrido et al. (1985) graded buprofezin

as harmless, noting no sterilization effects on either test parasitoid. Jones et al. (1998) reported different levels of mortality caused by a buprofezin test product, depending on parasitoid development stage (i.e. young larva or pupa), as it caused mortality among the early larval stages of *E. luteola* Howard, *Eretmocerus eremicus* and *E. tejanus* Rose and Zolnerowich. Similar studies have noted that parasitoids treated with buprofezin at later stages of larval development were less affected than those treated at earlier stages (Gerling & Sinai, 1994; Hoddle et al., 2001).

In our own research, the buprofezin product that was applied directly onto parasitoid pupae at concentrations of 264 mg/l (B and N) and 220 mg/l (D), prolonged juvenile development significantly (1.96, 1.71 and 2.15 days in B, N and D wasps, respectively), and reduced significantly the longevity (1.52, 0.74 and 1.71 days in B, N and D wasps, respectively), total parasitism (11.66, 17.72 and 17.59 % in B, N and D wasps, respectively), total adult emergence (11.63, 17.79 and 17.56 % in B, N and D wasps, respectively) and instantaneous rate of increase of all test populations (8.17, 6.82 and 12.46 % in B, N and D wasps, respectively). In the present study, treated B females achieved better results than D females regarding nearly all observed parameters.

In another study, Gholamzadeh et al. (2012) treated *E. formosa* pupae directly with buprofezin (Applaud 40 SC, 1000 μg a.m./ml) and classified the insecticide into group I as harmless, based on its total effect (10.9 % reduction in beneficial capacity), and also based on its toxicity (23.14 % reduction in beneficial capacity). Besides, buprofezin showed no significant impact on parasitoid fecundity and longevity. Gerling and Sinai (1994) reported consistent data as they detected no harmful effects of buprofezin on one-day fecundity of *E. luteola* and *Eretmocerus* sp. females when the insecticide was applied to parasitised tobacco whitefly nymphs. Heydari (2004) also noted no detrimental effects of buprofezin on the longevity or fecundity of *E. formosa.*

Other studies, however, have reported results similar to ours regarding buprofezin selectivity below expectation. In a demographic study by Heydari et al. (2006), direct treatment of *E. formosa* pupae with the recommended concentration of buprofezin did not affect significantly the parasitoid population parameters, apart from net reproductive rate (R_0), which was significantly lower than control data. In another study by Southwood & Handerson (2000), life table data indicated a minor negative influence on the intrinsic rate of increase, which was reduced by 7.03 %. In contrast, Prabhaker et al. (2007) found buprofezin treatment (Applaud 40 SC, 400 g a.i./l; application by soaking leaves with parasitized larvae into buprofezin solution) to have significantly affected population stability parameters (R_0, intrinsic rate of increase - r_m; final rate of increase – l, and mean generation time - T) of the parasitoid wasp *E. inaron*. Sohrabi et al. (2013) reported data consistent with our present results, showing an adult eclosion rate of *E. mundus* pupae that was significantly reduced by the recommended buprofezin concentration and not dependent on the parasitoid development stage (larva or pupa).

It is possible to infer from the acquired data that the test product based on buprofezin (Elisa 440 SC) demonstrated a certain level of toxicity to *E. formosa* pupae, and that besides its lethality, buprofezin may also have sublethal effects on the parasitoid's physiology. Application of this growth regulator in early stages of whitefly development and parasitoid wasp release at a later stage of pest development (or in its next generation) may allow for biological and chemical control measures to coexist in practice. More precise determination of risks involved in the use of buprofezin (especially the toxicity of its gas phase) requires its more detailed field testing.

ACKNOWLEDGEMENT

This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No.: TR31043).

REFERENCES

Albajes, R., Lodovica Gullino, M., van Lenteren, J.C., & Elad, Y. (eds.) (1999). Integrated pest and disease management in greenhouse crops. Dordrecht, The 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 of Pests Diseases*, *2B-3* (pp. 75-81). Brighton, UK: University of Brighton Press.

Darvas, B., & Polgar, L.A. (1998). Novel type insecticides: specificity and effects on non-target organisms. In I. Ishaaya, & D. Degheele (Eds.), *Insecticides with novel modes of action: mechanisms and applications* (pp. 188-259). Berlin, Germany: Springer Publishing.

De Cock, A., Ishaaya, I., Degheele, D., & Veierov, D. (1990). Vapor toxicity and concentration-dependent persistence of buprofezin applied to cotton foliage for controlling the sweetpotato whitefly (Homoptera: Aleyrodidae). *Journal of Economic Entomology*, *83(4)*, 1254-1260. doi:10.1093/jee/83.4.1254

Enkegaard, A. (1993). *Encarsia formosa* parazitizing 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 and H.M.T. Hokkanen (eds.), *An ecological and society approach to biological control* (pp. 91-122). The Netherlands: Springer.

EPPO (Europian Plant Protection Organisation) (2004). Side effects on *Encarsia formosa*. In: *EPPO standards,* PP1/142(2). Paris, France: EPPO.

Garrido, A., Beitia F., & Gruenholz, P. (1985). Incidence of the growth regulator NNI-750 on immature stages of *Encarsia formosa* Gahan and *Cales noacki* How (Hymenoptera: Aphelinidae). *Annual INIA Agricola*, *28*, 137-145.

Gerling D., & Sinai, P. (1994). Buprofezin effects on two parasitoid species of whitefly(Homoptera: Aleyrodidae). *Journal of Economic Entomology*, *87(4)*, 842-846. doi:10.1093/jee/87.4.842

Gholamzadeh, M. Ghadamyari, M., Salehi L., & Hoseininaveh, 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.

Hassan, S. A., Bigler, F., Bogenschütz, H., Boller, E., Brun, J., Calis, J. N. M. ... Vogt, H. (1994). Results of the sixth joint pesticide testing programme of the IOBC/WPRS-working group „Pesticides and beneficial organisms". *Entomophaga*, *39***,** 107-119 (1994). doi: 10.1007/BF02373500

Heydari, A. (2004). *Effects of insect growth regulators on the bionomic of Trialeurodes vaporariorum (Westwood) (Homoptera: Aleyrodidae) and its parasitoid wasp, Encarsia formosa (Hymenoptera: Aleyrodidae) in the laboratory conditions*. (Ph.D. thesis). Tarbiat Modares University, Tehran, Iran.

Heydari, A., Moharramipour, S., Pourmirza, A.A., & Talebi, A.A. (2006). Effects of buprofezin, pyriproxyfen and fenpropathin on the reproductive parameters of *Encarsia formosa* (Hymenoptera: Aphelinidae). *Journal of Entomological Society of Iran*, *25(2)*, 17-34.

Hoddle, M.S., van Driesche, R.G., Lyon, S.M., & Sanderson, J.P. (2001). Compatibility of insect growth regulators with *Eretmocerus eremicus* for whitefly control on poinsettias: I. Laboratory assays. *Biological Control*, *20(2)*, 122-131. doi:10.1006/bcon.2000.0885

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*, 645-649. doi:10.1146/annurev.ento.43.1.645

Horowitz, A.R., & Ishaaya, I. (1996). Chemical control of *Bemisia*: management and application. In D. Gerling & R.T Mayer (Eds.), *Bemisia 1995: taxonomy, biology, damage, control and management* (pp. 537-556). Andover, Hants: Intercept.

Jones, W.A., Ciomperlik, M.A., & Wolfenbarger, D.A. (1998). Lethal and sublethal effects of insecticides on two parasitoids attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Biological Control*, *11(1)*, 70-76. doi:10.1006/bcon.1997.0581

Kobayashi, M. (2007). Susceptibility of whitefly against several kinds of insecticides. *Plant Protectione*, *61*, 21-26 (in Japanese).

Masuda, T., & Miyata, M, (2006). Effect of insecticides on whitefly, *Trialeurodes vaporariorum*. *Annual Report of the Society of Plant Protection of North Japan*, *57*, 167-170 (in Japanese).

Mota-Sanchez, D., & Wise, J.W. (2019). *Arthropod pesticide resistance database*. Retrieved from www.pesticideresistance.com (Accessed: December 30, 2019)

Mullin, C.A., & Croft, B.A. (1985). An update on development of selective pesticides favoring arthropod natural enemies. In M.A. Hoy & D.C. Herzog (Eds.). *Biological control in agricultural IPM systems* (pp. 123-150). Orlando, FL: Academic Press.

Naranjo, S.E., Hagler, J.R., & Ellsworth, P.C. (2003). Improved conservation of natural enemies with selective management systems for *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton. *Biocontrol Science and Technology*, *13(6)*, 571-587. doi:10.1080/0958315031000151828

Natwick, E.T. (1993). Silvedeaf whitefly control in cotton using various insecticides in the Imperial Valley of California. In D. J. Herber & D. A Richter (Eds.), *Proceedings of Beltwide Cotton Production Research Conference* (pp. 680-68.). Memphis, Tennessee: National Cotton Council.

Polaszek, A., Evans, G.A., & Bernett, F.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

Prabhaker, N., Morse, J.G. Castle, S.J., Naranjo, S.E., Henneberry, T.J., & Toscano, N.C. (2007). Toxicity of seven foliar insecticides to four insect parasitoids attacking citrus and cotton pests. *Journal of Economic Entomology*, *100(4)*, 1053-1061. doi: 10.1603/0022-0493(2007)100[1053:tosfit]2.0.co;2

Prijović, M., Drobnjaković, T., Marčić, D., Perić, P., Petronijević, S., & Stamenković, S. (2014). Efficacy of insecticides of natural origin in whitefly (*Trialeurodes vaporariorum*) control in tomato. *Acta Horticulturae*, *960*, 359-364. doi:10.17660/ActaHortic.2012.960.52

Robertson, J.L., Russell, R.M., Preisler, H.K., & Savin, N.E. (2007). Bioassays with Arthropods (2nd edition). Boca Raton, FL: CRC Press.

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: https://doi.org/10.1016/j.cropro.2012.11.024

Southwood, T.R.E., & Handerson, P.A. (2000): Ecological methods, with particular reference to the study of insect populations (3rd edition). Oxford, UK: Blackwell Scientific Press.

Stansly, P.A., & Liu, T.X. (1997). Selectivity of insecticides to *Encarsia pergandiella*, an endoparasitoid of *Bemisia argentifolii*. *Bulletin of Entomological Research*, *87(5)*, 525-531. doi:10.1017/S0007485300041390

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

Toscano, N.C., Prabhaker, N., Castle, S.J., & Henneberry, T.J. (2001). Inter-regional differences in baseline toxicity of *Bemisia argentifolii* to the two insect growth regulators, buprofezin and pyriproxyfen. *Journal of Economic Entomology*, *94(6)*, 1538-1546. doi:10.1603/0022-0493-94.6.1538

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 menagement in greenhouse crops* (pp 202-216). Dordrecht, Netherlands: Kluwer Academic Publishers.

Walthall, W.K., & Stark, J.D. (1997). Comparison of two population-level ecotoxicological endpoints: the intrinsic (r_m) and instantaneous (r_i) rates of increase. *Environmental Toxicology and Chemistry*, *16(5)*, 1068-1073. doi:10.1002/etc.5620160529

Yu, S.J. (2008). The toxicology and biochemistry of insecticides. Boca Raton, FL: CRC Press. doi:10.1201/9781420059762

Letalni i subletalni efekti buprofezina na parazitoida bele leptiraste vaši *Encarsia formosa* Gahan

REZIME

Akutna toksičnosti preparata na bazi buprofezina (komercijalni produkt Elisa 440 SC) 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±1°C i relativnoj vlažnosti vazduha od 60±10%, uz fotoperiod 16:8h, u četiri ponavljanja. U biotestu akutne toksičnosti, listovi duvana sa lutkama parazitoida (20 lutki po ponavljanju) tretirani su serijom simetrično raspoređenih koncentracija buprofezina, u rasponu koji pokriva 10-90% smrtnosti, i smrtnost je utvrđivana na osnovu broja eklodiranih adulta iz tretiranih lutki, devet dana nakon tretmana. U testovima akutne toksičnosti dobijene su sledeće srednje letalne koncentracije: 244.2, 281.5 and 199.5 (za B, N i D, respektivno). Preparat na bazi buprofezina, primenjen direktno na lutke parazitoida, u srednjim letalnim koncentracijama (264 mg/l za B i N; 220 mg/l za D), kod svih ispitivanih populacija značajno je umanjio preživljavanje ženki iz tretiranih lutki, produžio dužinu juvenilnog razvića (za 2, 1.7 i 2.2 dana, za B, N i D, respektivno), redukovao ukupni parazitizam ženki (za 11.7, 17.7 i 17.6 % za B, N i D, respektivno), ukupnu pojavu adulta ženki (11.6, 17.8 i 17.8 % za B, N i D, respektivno) i statistički značajno redukovao trenutnu stopu rasta ženki (za 8.2, 6.8 i 12.5 % za B, N i D, respektivno), u odnosu na kontrolu. Tačna determinacija rizika primene ovog preparata, zahteva njegovo dalje testiranje u poljskim uslovima.

Ključne reči: *Encarsia formosa*; buprofezin; subletalni efekti; životni parametri; populacioni rast