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Original scientific paper

Acute toxity and sublethal effects of pymetrozine on the whitefly parasitoid *Encarsia formosa* Gahan

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SUMMARY

Sublethal effects of a pymetrozine-based product (commercial product Chess 50 WP) on life history traits and population growth of one commercialized strain ("Dutch" strain) and two local populations (Bujanovac and Negotin) of the whitefly parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) were evaluated in laboratory bioassays. All trials were carried out at 27±1°C temperature and under 60±10 % relative humidity and 16/8 h daylight/darkness photoperiod in four replications. Longevity of wasps exposed for 48 h to residues of the pymetrozine insecticide (LC_{50}, 280 mg a.i./l) was shorter (by 2.7-3 days) than that of control wasps. Total parasitism of Negotin wasps was significantly reduced (by 8.2 %), as well as total parasitism and adult emergence of the Dutch strain (by 7.3 and 8.2 %, respectively), compared to control wasps. The instantaneous rate of increase (r_i) of surviving adult wasps was also significantly reduced (by 6.6, 6.3 and 7.6 % in populations Negotin, Bujanovac and Dutch strain, respectively). Direct treatment of wasps at their pupal stage (LC_{50}, 300 mg a.i./l) reduced total parasitism of Negotin wasps (by 8 %), and reduced r_i levels, but the reduction was significant only for the Bujanovac (by 6.7 %) and Negotin (by 4.6 %) populations. Juvenile development of the parasitoid in treated pupae was significantly extended (by 0.3-1.1 days), compared to control wasps. The implications of these results on integrated control of the greenhouse whitefly are discussed.

Keywords: *Encarsia formosa,* pymetrozine, sublethal effects, life history traits, population growth

INTRODUCTION

The parasitic wasp *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae; Coccophaginae) has been used for many years for biological control of the cosmopolitan and polyphagous species of greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), as one of the most successful biological agents in greenhouse and ornamental crops around the world (Enkegaard & Brdsøgaard, 2006; Pilkington et al., 2010). In order to achieve sustainable integration of biological and chemical measures, evaluation of the effect of pesticides, especially at population level, on any parasitoid used in integrated control programmes is needed (Stark et al., 1997; Stark & Banken, 1999; Stark & Banks, 2003). Despite many restrictive factors, natural enemies can be effectively integrated through adequate knowledge of the pesticides to be used (selective pesticides and/or rates or timing of applications) and their effects (lethal or sublethal) on natural enemy populations (Croft, 1990; Greathead, 1995).

Pymetrozine is a relatively new insecticide, the only representative of pyridine azomethine, which is highly effective and specific in controlling insects that suck plant sap (Flückiger et al., 1992a,b; Nicholson et al., 1996). Various authors have demonstrated the efficacy of pymetrozine in controlling whiteflies, primarily *T. vaporariorum* and *Bemisia tabaci* biotype B (Ferron & Deguine, 2005; Kobayashi, 2007). Pymetrozine is also considered a valuable tool in the integrated protection management (IPM) of greenhouse whitefly and other pest species (Acheampong & Stark, 2004; Ferron & Deguine, 2005). Pymetrozine is a modulator of chordotonal organs and its mechanism of action differs from the mechanism of action of insecticides in other chemical groups (IRAQ, 2017). This insecticide is not directly toxic to insects that suck plant sap, but it has an irreversible effect on their ability to feed, i.e. it acts by interfering with the nervous regulation of behavior related to insect nutrition. By causing inhibition of the neuromuscular system, which participates in food assimilation, pymetrozine can also cause a so-called "knock-down" effect in exposed insects, similar to most conventional neurotoxic insecticides (Harrewijn & Kayser, 1997).

Several studies have examined the potential toxicity of this relatively new insecticide to parasitoids of the order Hymenoptera. These studies focused mainly on direct effects of pymetrozine on adults and/or pupae after its topical application (residual contact bioassay or direct treatment of adults and pupae). In general, pymetrozine and its commercial formulations are considered to be either slightly toxic or non-toxic to parasitoids (Tran et al., 2005; Medina et al., 2007).

Studies evaluating the risk of using the commercial pymetrozine-based formulation Chess WP for *E. formosa* and/or other parasitoid and predator species were mainly based only on the use of its recommended dose, and on acute toxicity and persistence tests performed according to the IOBC phase-up scheme (van de Veire & Tirry, 2003; Abu-Tara et al., 2008). There are a number of studies that have classified the effects of other pymetrozine formulations on other species of parasitoids (Hoddle et al., 2001; Torres et al., 2003; Acheampong & Stark, 2004) and predators (Torres et al., 2002; Yoshizawa & Aizawa, 2007; Cabral et al., 2008, 2011). A small number of studies can be found in literature that address the profile of pymetrozine through quantification of life parameters of predator and parasitoid population growth (Rezaei et al., 2007; Kheradmand et al., 2012), and none of them concerns the parasitoid *E. formosa*.

As implementation of the principles of integrated control of whitefly is in its initial stage in Serbia at present, the goal of this study was to evaluate the effects of pymetrozine on life history traits and population growth of two local populations and a commercial Dutch strain of the parasitoid. The results of this study are discussed in terms of *T. vaporariorum* integrated management strategies.

MATERIALS AND METHODS

Parasitoid and whitefly populations

Two local populations of *E. formosa* were started from pupae collected from tunnel greenhouses for vegetables and ornamentals in locations in Serbia with no known use of commercial parasitoid strains for biological control of greenhouse whiteflies. Population Bujanovac was collected in Bujanovac (Southern Serbia; 42°30′27″ N, 21°48′30″ E) from *Solanum nigrum* L., and population Negotin was collected in Negotin (Easthern Serbia; 44°13′00″ N, 22°31′00″ E) from *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* was purchased from the company 'Zeleni hit', which is the local agent of Koppert Biological Systems Inc., The Netherlands, and it was successfully cultured as a reference strain. The Dutch strain of *E. formosa* and the two local populations of this parasitoid wasp were reared on *T. vaporariorum* at 27 ± 1°C, and under 60 ± 10 % RH and 16/8 h light/darkness photoperiod. All whiteflies were reared on tobacco plants, cv. Samsun, in ventilated muslin cages and according to the recommended European Plant Protection Organisation (EPPO, 2004) methodology.

Insecticide

The commercial product Chess 50 (manufactured by Syngenta, Germany) is formulated as water dispersible granules (WG). Its content of pymetrozine as the leading product ingredient is standardised to 500 g/l.

Bioassays

All bioassays were conducted in four replications in a climate chamber at 27±1°C and under 60±10 % RH and 16/8 h light/darkness photoperiod. The bioassays were performed in Petri dishes (12 cm diameter), each

having four lid openings (1 cm diameter) with muslin covers on top to provide ventilation and prevent internal condensation. Each Petri dish contained a 1 % agar layer onto which a tobacco leaf was settled. Parasitoid adults, pupae or whitefly nymphs were released into Petri dishes, as required for each experiment. The pesticide was diluted in distilled water and applied by spraying onto the entire area of each Petri dish (i.e. the lid and lower dish containing a tobacco leaf on top of agar medium). The insecticide was applied by a Potter spray tower (2 ml of spray liquid, 100 kPa air pressure, aqueous deposit 2.7 ± 0.2 mg/cm^2).

(A) Acute toxicity bioassay with adults. Acute insecticide toxicity to *E. formosa* adults was assessed by spraying a series of pymetrozine concentrations, covering a range of 10-90 % mortality: 400, 350, 300, 250, 225, 200 and 100 mg/l. Adults in a control treatment were sprayed with distilled water. The insecticide was applied to tobacco leaves placed onto agar and to the inner lid of each Petri dish. Petri dishes with tobacco leaves were left for 2 h to air dry at room temperature, and then parasitoid adults were released inside the dishes. Twenty adult wasps (12-24 h old) were released into each Petri dish that also contained a few droplets of honey on a piece of tinfoil (0.5 × 0.5 mm) fixed to the lid of each Petri dish by Traganth-kit (a natural, non-toxic adhesive, manufactured by C.E. ROEPER, Germany). Honey was applied after the insecticide deposit had dried in order to avoid possible contamination of honey drops and ingestion of insecticide residues by parasitoid wasps. Mortality was calculated based on the number of live wasps in relation to the number of treated wasps 48 h after their release (EPPO, 2004).

(B) Acute toxicity bioassay with pupae. Tobacco leaves with parasitised whitefly nymphs (pupae) were fixed to tinfoil with Traganth-kit. After drying, the leaves were cut into pieces, each bearing about 25 parasitoid pupae (4 days old, i.e. 12 days after parasitoid oviposition in host nymphs) and placed on filter paper in plastic Petri dishes (filter paper was moistened with water to fix them in place during exposure). Then the pieces of tobacco leaves were treated with a series of pymetrozine concentrations: 500, 400, 350, 300, 250, 200, and 100 mg/l. Two hours after treatment, the leaves were transferred to new Petri dishes and remained there until adults emerged from the pupae. Mortality was assessed as the emerged adult count in relation to the number of treated pupae nine days after treatment (EPPO, 2004).

Parasitism bioassays

(A) Parasitism bioassay with insecticide-exposed parasitoid adults was carried out by releasing 40 wasps into Petri dishes (10 adults aged 0-24 h per each of four dishes) already containing 200-250 third- or fourth-instars of whitefly nymphs on tobacco leaves settled upon agar medium previously treated with 280 mg/l of pymetrozine. This concentration was within the 95 % confidence limits (CLs) of the LC_{50} value estimated in the previous acute toxicity bioassay with adults (Table 1). Approximately 12 whitefly third- or fourth-instars were present per cm^2 (EPPO, 2004) because a higher number of nymphs would cause premature withering of tobacco leaves, while a lower number would lead to low parasitism. The Petri dishes with tobacco leaves were left for 2 h to air dry, and the wasps were then left to lay eggs over the next 48 h before they were transferred to new Petri dishes with hosts. The transfering of wasps to new leaves with host nymphs at 48 h intervals continued until the last wasp died. Control dishes were sprayed only with distilled water. Parasitised hosts were counted after turning black in appearance. After counting, such pupae were transferred to new clean Petri dishes to monitor the survival of treated pupae (Stouthamer & Mak, 2002). Parasitism was calculated as the number of parasitised pupae per female alive during each 48 h period (parasitism/48 h) summed over the female wasp lifetime (total parasitism). Adult emergence was calculated as the total number of adults that emerged from parasitised whitefly nymphs. Longevity was calculated as the total number of days that each wasp lived, assuming that wasps died at the midpoint of each 48 h interval.

(B) Parasitism bioassay with F_1 generation wasps was carried out in which 40 pupae (4 days old, i.e. 12 days after parasitoid oviposition) from each tested population were treated in Petri dishes with 300 mg/l of pymetrozine. The concentrations were within 95 % CLs for the LC_{50}s calculated in the acute toxicity bioassay (Table 1). After adults emerged from treated pupae, the development time, parasitism/48 h, total parasitism, adult emergence and longevity of the F_1 generation were calculated using the same method as described in the bioassay with exposed wasps. Development time was calculated as the total number of days that elapsed from parasitoid egg laying to adult emergence from pupae.

In both parasitism bioassays (with insecticide-exposed adult parasitoids and with insecticide-exposed

F_1 generation wasps), parasitism and survival data were used to calculate the instantaneous rates of increase (r_i) using the equation:

$$r_i = [\ln(N_f / N_0)] / \Delta t$$

where N_0 is the initial number of individuals (i.e. 40 adult wasps per replicate), N_f is the final number of individuals (i.e. the number of surviving adult wasps, black parasitised pupae and adults emerged), and Δt is the number of days elapsed between the start and the end of a 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). In order to standardise the influence of different oviposition durations on r_i values, N_f values were determined at the end of the 14th and 16th days of oviposition in the first and second parasitism bioassays, respectively, i.e. at the time intervals that corresponded to the shortest oviposition period achieved by tested females. In the parasitism bioassay with insecticide-exposed adult parasitoids, Negotin and Dutch strain wasps had the shortest oviposition period (14 days), while Negotin wasps had the shortest oviposition period (16 days) in the parasitism bioassay with insecticide-exposed F_1 generation wasps.

Statistical analysis

Concentration-mortality data from both acute toxicity bioassays were subjected to probit analysis using the POLO Plus software (LeOra Software, Berkeley, CA). A pairwise comparison of LC_{50}s was performed using the lethal dose ratio test: when 95 % CLs for LC ratios included 1, the LCs were not significantly different (Robertson et al., 2007). Kaplan-Meier analysis was used to estimate wasp longevity (SPSS Statistics, Version 17), and survival curves were analyzed by the Log-rank test. Development, parasitism/48 h, total parasitism and adult emergence, and r_i data were analysed by two-way ANOVA (insecticide treatment and population were factors), with means separated by Fisher's LSD test ($p < 0.05$). Parasitism/48 h was also analysed by repeated measures ANOVA. Means of all parameters for treatment and control, for each population, were separated by Student's t-test ($p < 0.05$). Parasitism and adult emergence data were transformed by $\sqrt{(x + 0.1)}$ to normalise data and eliminate zero values.

RESULTS

Acute toxicity bioassays

Toxicity parameters of the insecticide Chess 50 WP after adult exposure to its residues and after direct treatment of *E. formosa* pupae are shown in Table 1. Pymetrozine demonstrated a significantly higher toxicity to the adult than pupal stage of the parasitoid. The obtained LC_{50} values for both developmental stages of the parasitoid, were close to the maximum recommended concentration of Chess 50 WP (0.06 %= 300 mg a.i./l) for use in protected cultivation systems.

Table 1. Acute toxicity of pymetrozine to *Encarsia formosa* adults and pupae from local populations Bujanovac (B) and Negotin (N), and a commercial Dutch strain (D)

Life stages	Populations	*n*	LC_{50} (mg/l) (95% CLs)	Slope (± SE)	χ^2	df
Adults	Bujanovac	640	281.654 a (259.252- 304.962)	10.387 (±0.44)	15.267	5
	Negotin	640	270.793 a (243.478-297.104)	10.115 (±0.45)	19.297	5
	Dutch strain	640	277.021 a (253.975-299.494)	10.447 (±0.46)	14.425	5
Pupae	Bujanovac	560	317.082 a (280.951-345.331)	9.807 (±0.54)	14.092	5
	Negotin	560	307.650 a (267.915-336.077)	9.824 (±0.55)	14.843	5
	Dutch strain	560	325.903 a (285.925-356.678)	10.117 (±0.57)	16.221	5

n = number of treated adults/pupae; CLs = confidence limits; df = degrees of freedom
LC_{50}s at stages marked by different letters are significantly different (lethal dose ratios test, p=0.05)

A LC_{50} ratio test showed that female adults and pupae of all tested populations of *E. formosa* wasps were equally susceptible to pymetrozine treatment.

Parasitism bioassay with exposed adult wasps

Longevity of *E. formosa* wasps after exposure to pymetrozine residues are shown in Figure 1. Exposure to pymetrozine residues had a significant impact on the longevity of *E. formosa* adult wasps ($F_{1,18}$=142.06, p<0.001). Besides treatment, longevity reduction was also related to population ($F_{2,18}$=8.17, p<0.01), unlike the interaction of these two factors, which caused no statistically significant influence ($F_{2,18}$=0.27, p=0.763). The exposed wasps of Bujanovac population lived 2.7 days shorter than control wasps, which was shown to be statistically significant ($F_{1,6}$=84.24, p<0.001). Pymetrozine residues shortened the lifetime of exposed Negotin wasps by 2.71 days ($F_{1,6}$=30.392, p<0.01), compared to control wasps. A significant difference ($F_{1,6}$=52.249, p<0.001) was also revealed regarding the longevity of Dutch strain wasps from treatment, which lived 2.96 days briefer than control wasps.

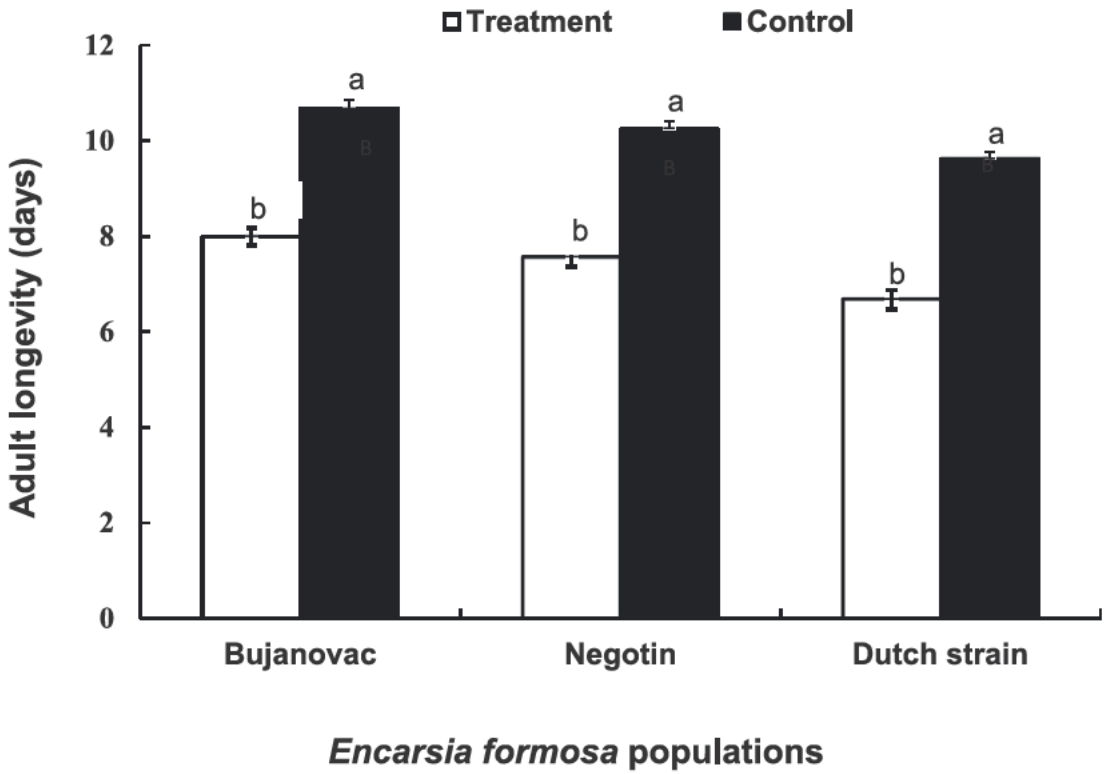

Figure 1. Longevity (means ± SE, days) of exposed *E. formosa* parasitoid wasps from the local populations Bujanovac and Negotin, and the Dutch strain; Control = distilled water; Treatment = pymetrozine 280 mg/l. Means for treatment and control (for each population individually) marked by different letters are significantly different (t- test, p<0.05)

The survival curves for wasps from residual pymetrozine bioassay are shown in Figure 2. Wasps of all three populations survived for longer periods of time than those exposed only to distilled water: Bujanovac treatment vs. Bujanovac control (ww=60.766, p<0.001); Negotin treatment vs. Negotin control (ww=65.174, p<0.001) and Dutch strain treatment vs. Dutch strain control (ww=67.827, p<0.001).

Parasitism/48 h of wasps in all test populations exposed to pymetrozine residues was under a significant influence of observation periods ($F_{8,144}$=199.05, p<0.001). Between and within observation periods, all main effects and their related interactions were shown to be statistically significant at the significance level p=0.05, except the interaction of treatment and population between observation periods, which was not significant ($F_{18,2}$=3.41, p=0.055).

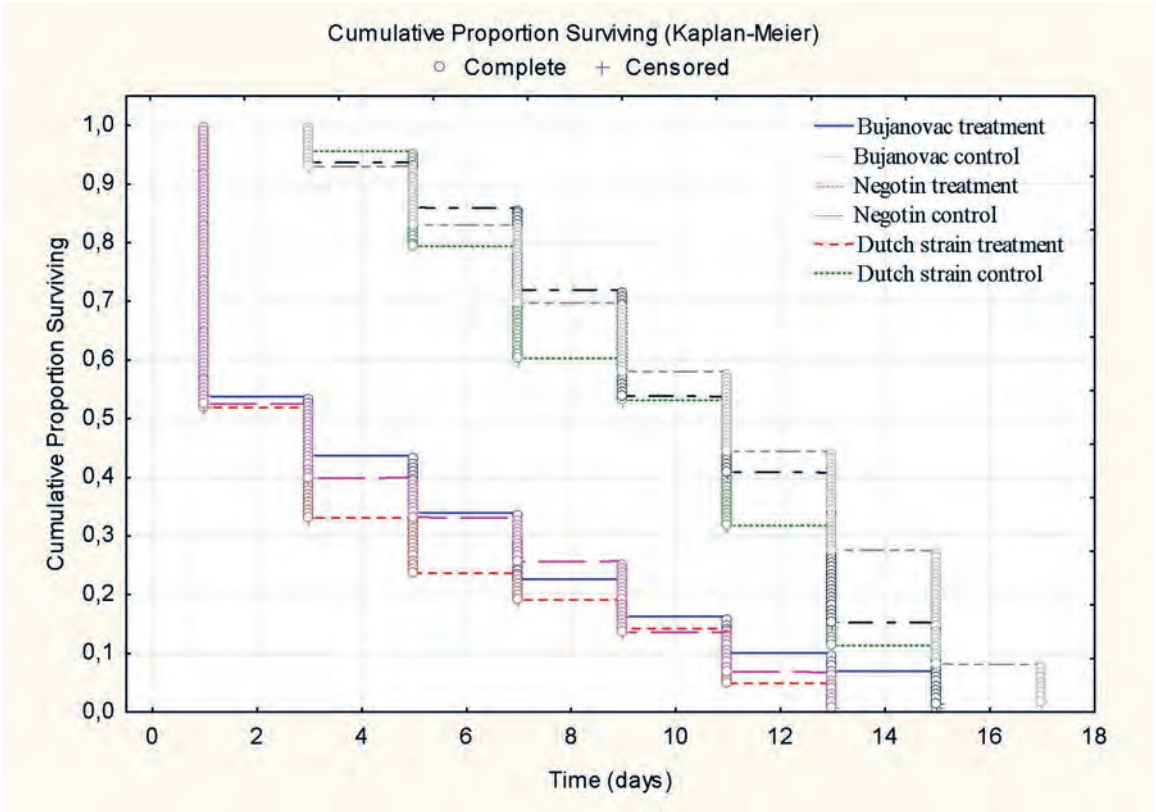

Figure 2. Survival curves for *E. formosa* parasitoids from local populations Bujanovac (B) and Negotin (N), and commercial Dutch strain (D); c = control (distilled water); t = treatment (pymetrozine 280 mg/l)

Parasitism/48 h of wasps from the test populations of *E. formosa* are presented in Figure 3. Bujanovac wasps and the Dutch strain achieved lower parasitism/48 h than control females, but not statistically significant. Regarding Negotin population wasps, pymetrozine caused a significant reduction only in the last observation interval (12-14 days of oviposition) ($F_{1,6}$=12.400, p<0.05). In all test populations, the exposure of adult wasps to pymetrozine activity resulted in shorter oviposition periods by two days, compared to control wasps. Bujanovac population had the longest oviposition period, both treated (16 days) and control wasps (18 days). Wasps from Negotin and the Dutch strain laid eggs for two days less than Bujanovac females both under treatment conditions (14 days) and control (16 days).

Total parasitism (Figure 3d) of wasps from all test populations that survived exposure to pymetrozine residues, was significantly lower under the influence of treatment ($F_{1,18}$=10.99, p<0.01) and population ($F_{2,18}$=40.41, p<0.001), while the interaction of these two factors ($F_{2,18}$=0.17, p=0.85) was not significant. The highest total parasitism was achieved by Bujanovac wasps (143.95 pupae/female), which parasitised the highest average number of whitefly nymphs even under control conditions (151.28 pupae/female). Population Negotin suffered the greatest reduction in total parasitism under treatment conditions and achieved the lowest value of that parameter. Total parasitism of the treated Bujanovac wasps was reduced by 4.8 % ($F_{1,6}$=3.76, p=0.100), which proved to be statistically insignificant, while the parasitism of Negotin and Dutch strain wasps was significantly reduced by 8.2 % ($F_{1,6}$=8.67, p<0.05) and 7.3 % ($F_{1,6}$=22.31, p<0.01), respectively, compared to control.

Similar to total parasitism, the reduction in total emergence of adults from Dutch strain pupae in F_1 generation (Table 2) was significantly affected by treatment ($F_{1,18}$=5.45, p<0.05) and population ($F_{2,18}$=23.04, p<0.001), while the interaction of these two factors was not significant ($F_{2,18}$=0.13, p=0.877). Adult emergence of the Dutch strain population was significantly reduced ($F_{1,6}$=10.33, p<0.05) by 8.2 %.

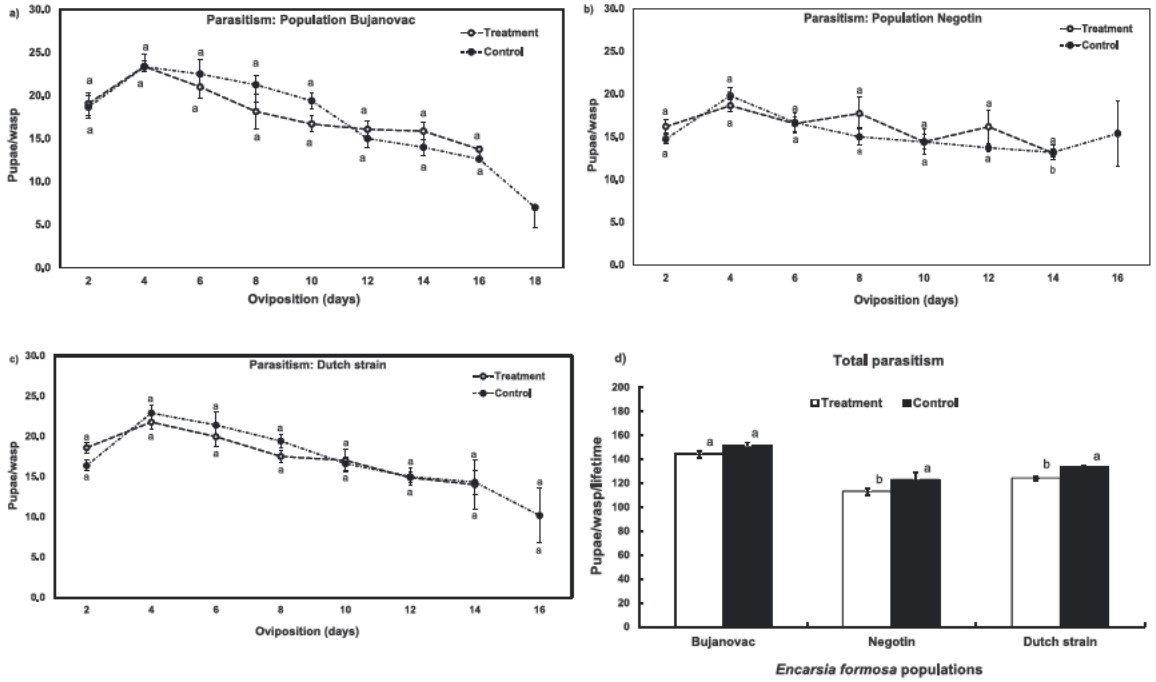

Figure 3. Parasitism/48h (means ± SE, pupae/wasp/48h) and total parasitism (means ± SE, pupae/wasp/ lifetime) of *E. formosa* parasitoid wasps from local populations (a) Bujanovac and (b) Negotin, and (c) the Dutch strain; Control = distilled water; Treatment = pymetrozine 280 mg/l. Means marked by different letters are significantly different (t-test, p<0.05)

Total adult emergence of Negotin adults was reduced by 5.6 % ($F_{1,6}$= 0.629, p=0.458), and Bujanovac adults by 5.3 % ($F_{1,6}$= 1.707, p=0.239), but the reductions were not statistically significant. Similar to total parasitism, the highest average adult emergence in both variants (treatment and control) was achieved by Bujanovac wasps.

As a consequence of significantly reduced survival and/or parasitism 14 days after oviposition started (the duration of the shortest oviposition period of exposed Negotin and Dutch strain adult wasps), the instantaneous rate of increase (r_i) of surviving female wasp adults was also significantly lower: 6.64 % ($F_{1,6}$=51.41, p<0.001), 6.34 % ($F_{1,6}$=14.73, p<0.01) and

Table 2. Adult emergence (mean ± SE, adults/wasp/lifetime) and the instantaneous rate of increase (mean ± SE, day^{-1}) of *Encarsia formosa* wasps from local populations Bujanovac and Negotin, and the Dutch strain, in F_1 generation; Control = distilled water; Treatment = pymetrozine 280 mg/l (Bujanovac and Dutch strain), 225 mg/l (Negotin)

Population	Adults	Adult emergence F_1 generation	Instantaneous rate of increase, F_1 generation
Bujanovac	Treatment	120.71±4.58 a	0.267±0.002 b
	Control	127.42±2.45 a	0.286±0.002 a
Negotin	Treatment	95.66±3.33 a	0.251±0.003 b
	Control	101.30±6.18 a	0.268±0.003 a
Dutch strain	Treatment	104.74±2.05 b	0.256±0.002 b
	Control	114.12±2.07 a	0.277±0.000 a

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

7.58 % ($F_{1,6}$=139.06, p<0.001) for Bujanovac, Negotin and Dutch strain populations, respectively (Table 2). Reductions in r_i were significantly affected by treatment ($F_{1,18}$=107.77, p<0.001) and population ($F_{2,18}$=28.38, p<0.001), unlike the interaction of these two factors ($F_{2,18}$=0.41, p=0.669). Concerning treatment and control, the highest rate was achieved by wasps of the Bujanovac population.

Parasitism bioassay with F_1 generation wasps

It took juveniles 0.3-1.1 days longer to develop from parasitoid pupae treated with pymetrozine than those that developed from untreated parasitoid pupae (Table 3). The extention of juvenile development period was significantly due to treatment ($F_{1,18}$=26.9, p<0.001) and population ($F_{2,18}$=7.3, p<0.01), while interaction of the two factors was not found to have a significant

Table 3. Juvenile development time (mean ± SE, days), adult emergence (mean ± SE, adults/wasp/lifetime), and instantaneous rate of increase (mean ± SE, day^{-1}) in F_1 generation of *Encarsia formosa* wasps from local populations Bujanovac and Negotin, and the Dutch strain; Control = distilled water; Treatment = pymetrozine 320 mg/l (Bujanovac and Dutch strain), 310 mg/l (Negotin)

Population	Pupae	Juvenile development time	Adult emergence, F_1 generation	Instantaneous rate of increase, F_1 generation
Bujanovac	Treatment	15.74±0.24 b	148.65±3.38 a	0.278±0.001 b
	Control	14.73±0.30 a	144.57±3.15 a	0.298±0.001 a
Negotin	Treatment	14.79±0.14 b	111.24±2.54 a	0.271±0.002 b
	Control	14.46±0.12 a	119.27±3.09 a	0.284±0.001 a
Dutch strain	Treatment	15.46±0.12 b	129.04±1.99 a	0. 286±0.001 a
	Control	14.46±0.15 a	125.54±5.63 a	0. 282±0.001 a

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

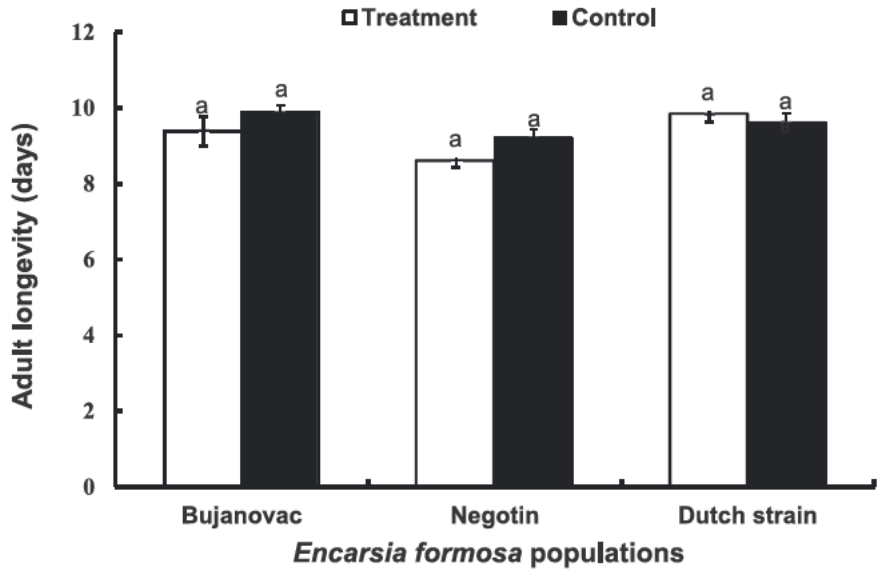

Figure 4. Longevity (means ± SE, days) in F1 generation of *E. formosa* parasitoid wasps from the local populations Bujanovac and Negotin, and the Dutch strain; Control = distilled water; Treatment = pymetrozine 300 mg/l. Means for treatment and control (for each population individually) marked by different letters are significantly different (t-test, p<0.05)

influence on that parameter ($F_{2,18}$=0.2, p=0.794). It took juveniles of population Bujanovac 1.07 days longer to develop, ($F_{1.6}$ = 4.91, p < 0.05), Negotin juveniles 0.33 days longer ($F_{1.6}$= 15.25, p <0.01) and juveniles of the Dutch strain one day longer ($F_{1.6}$= 26.53, p<0.01) than control juveniles.

The lifetime of wasps that ecloded from pymetrozine-treated pupae was not significantly different from control wasps (Figure 4) ($F_{1,6}$=3.36, p=0.116; $F_{1,6}$=6.85, p=0.059; $F_{1,6}$=4.69, p=0.060, for Bujanovac, Negotin and Dutch strain populations, respectively).

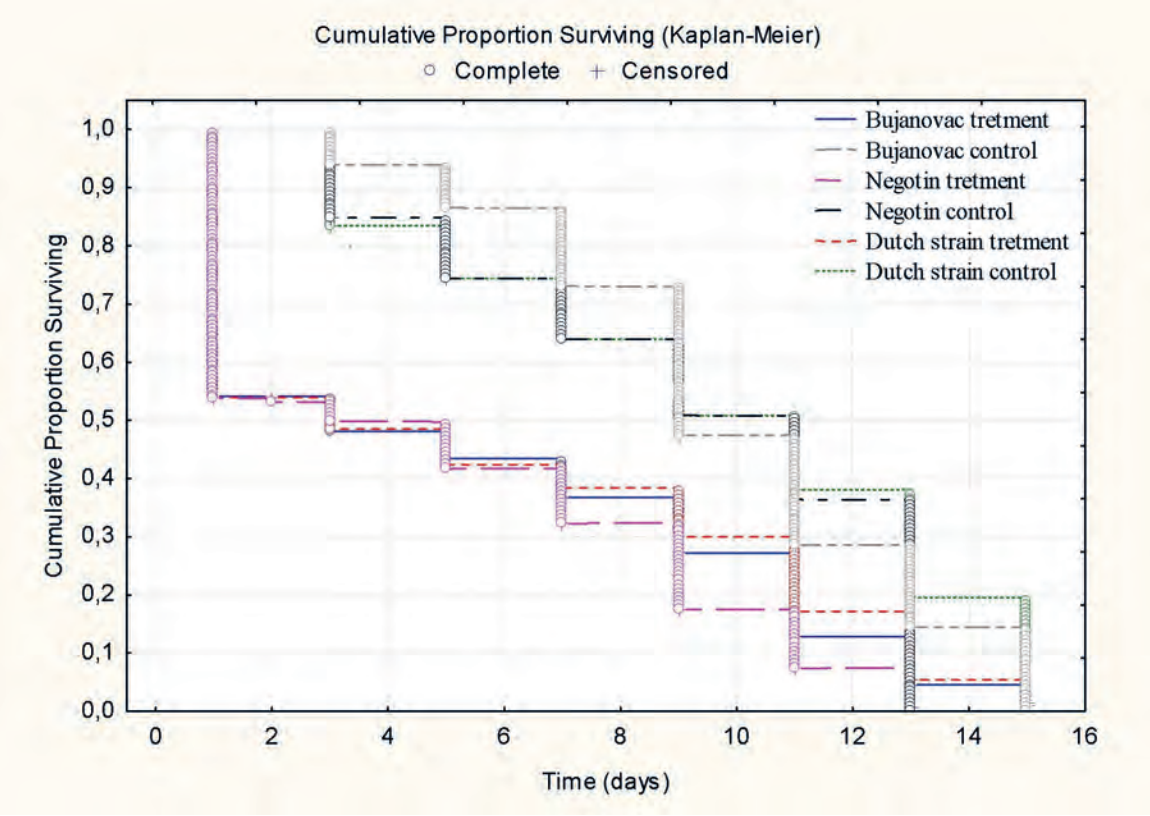

Figure 5. Survival curves of *E. formosa* parasitoid wasps in F_1 generation from local populations Bujanovac (B) and Negotin (N), and the commercial Dutch strain (D); c = control (distilled water); t = (pymetrozine 300 mg/l)

The survival curves for parasitoid wasps of all test populations are shown in Figure 5. Wasps that ecloded from pupae treated with pymetrozine survived better than females that ecloded from pupae treated only with distilled water (Bujanovac treatment vs. Bujanovac control: ww=-10.675, p=0.105; Negotin treatment vs. Negotin control: ww=40.026, p<0.001; Dutch treatment vs. Dutch control: ww=50.610, p<0.001). Regarding treatment, Negotin wasps survived significantly better than Dutch wasps (Negotin treatment vs. Dutch treatment: ww=-14.472, p<0.05). Contrary to treatment, control females of the Dutch strain survived better than Negotin wasps (Negotin control vs. Dutch control: ww=-15.2, p<0.05).

Parasitism/48 h of female wasps that survived direct pupal treatment was significantly affected by the observation period ($F_{7,126}$=372.17, p<0.001). All main effects and related interactions were found to be significant both between and within observation periods, except treatement between observation periods ($F_{7,126}$=2.69, p=0.118), and the interaction of treatment and population, which had no significant effect ($F_{7,126}$=2.57, p=0.104).

Parasitism/48 h of wasps that had ecloded from treated and control pupae is presented in Figure 6. Pymetrozine caused no shortening of oviposition period in any test population (Bujanovac and Dutch strain females oviposited eggs for 16 days, and Negotin

wasps for 14 days). Parasitism/48 h of the treated wasps of Bujanovac population differed significantly from control wasps over all observation intervals except 10-12 ($F_{1,6}$=0.072, p=0.797) and 12-14 days of oviposition ($F_{1,6}$=1.722, p=0.237), when the differences were not statistically significant. In wasps of the Dutch strain population, pymetrozine caused no significant reduction in parasitism/48 h in any observation interval during oviposition, as it did in Negotin wasps.

Total parasitism of Negotin wasps that survived treatment as pupae was significantly reduced ($F_{1,6}$=9.09, p<0.05) by 8 %, compared to control wasps (Figure 6d). Total parasitism of Bujanovac ($F_{1,6}$=2.15, p=0.193) and Dutch strain wasps ($F_{1,6}$=0.07, p=0.794) showed no significant deviation from total parasitism found in wasp survivors from control pupae. A two-way analysis showed that total wasp parasitism was significantly affected by the test population ($F_{2,18}$=71.96, p<0.001) and interaction of treatment and population ($F_{2,18}$=5.07, p<0.05), while treatment had no significant effect ($F_{1,18}$=0.78, p=0.388). In both cases, treatment and control, the highest average number of whitefly nymphs was parasitized by Bujanovac wasps.

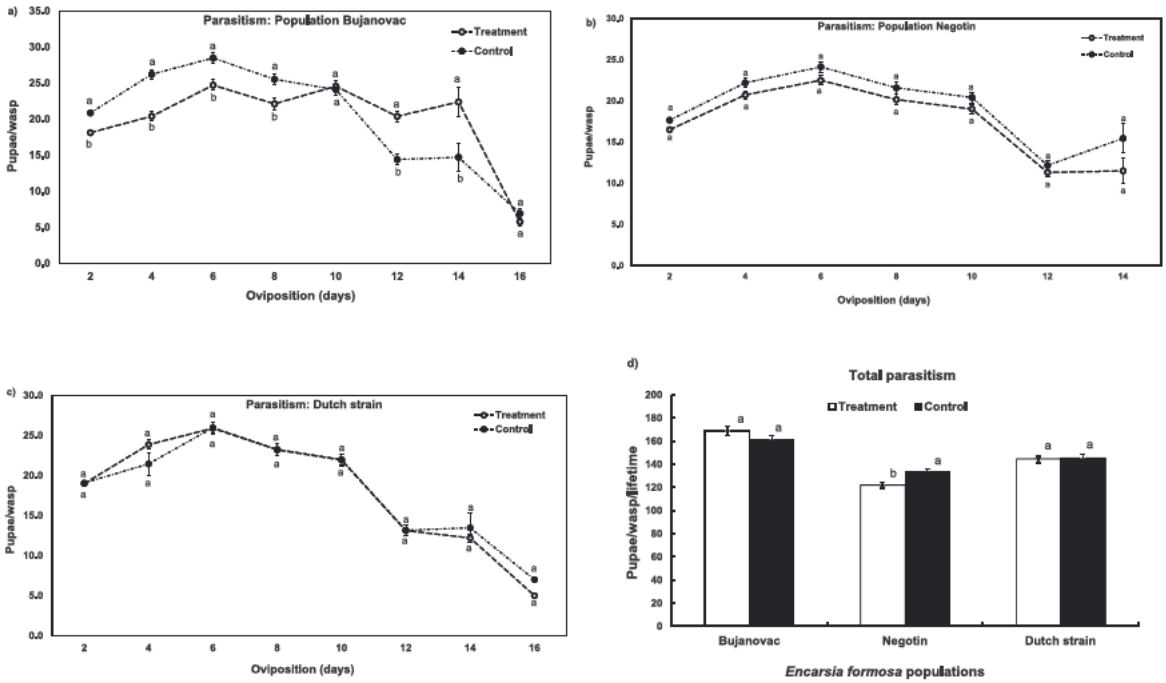

Figure 6. Parasitism/48 h (means ± SE, pupae/wasp/48 h) and total parasitism (means ± SE, pupae/wasp/lifetime) of *E. formosa* parasitoid wasps from local populations: (a) Bujanovac and (b) Negotin, and (c) Dutch strain; Control = distilled water; Treatment = pymetrozine 300 mg/l. Means marked by different letters are significantly different (t-test, p<0.05)

Total adult emergence from pymetrozine-treated parasitoid pupae was not significantly different from the emergence from untreated pupae of Bujanovac ($F_{2,9}$=0.79, p=0.409), Negotin ($F_{2,9}$=0.79, p=0.409) and Dutch strain populations ($F_{2,9}$=0.37, p=0.567) (Table 3). Wasps of the Bujanovac population, both in treatment and control, had the highest scores regarding this parameter.

Fourteen days after oviposition began (the duration of the shortest oviposition period of population Negotin in treatment and control), the r_i of treated Bujanovac ($F_{1,6}$=133.4, p<0.001) and Negotin ($F_{1,6}$=27.35, p<0.01) wasps decreased by only 6.7 and 4.6 %, respectively, which nevertheless turned out to be statistically significant. The r_i of the Dutch strain was not significantly lower than the rate of control wasps (Table

3). Reduction in r_i was caused by treatment ($F_{1,18}$=62.8, p<0.001), population ($F_{2,18}$=26.8, p<0,001), and their interation ($F_{2,18}$=33.5, p<0,001). Considering treatment conditions, the highest r_i value was achieved by wasps of the Dutch strain, while Bujanovac wasps had the highest rate in the control.

DISCUSSION

The pymetrozine formulation Chess 50 WP did not show high acute toxicity to adults or pupae of any of the three examined populations of *E. formosa*. The obtained LC_{50} values for both parasitoid development stages were close to pymetrozine concentration recommended for use in protected cultivation systems (300 mg/l). In adult bioassays, pymetrozine applied at mean lethal concentrations obtained in acute toxicity studies significantly reduced wasp survival, life longevity, total parasitism of Negotin and Dutch strain populations, total emergence of Dutch strain wasps, and instantaneous rate of increase of all three test populations. Applied directly to the parasitoid pupal stage, pymetrozine significantly extended juvenile development, reduced total parasitism of Negotin wasps and significantly reduced the instantaneous rate of increase of Bujanovac and Negotin populations.

A large number of studies have estimated pymetrozine as non-toxic to different development stages of *E. formosa*, some other parasitoid species and predators, classifying it as compatible for use with these beneficial organisms. In a laboratory residual test, van de Veire and Tirry (2003) showed that pymetrozine (Chess 25 % WP, 200 g a.i/1) did not cause any mortality of *E. formosa* adults (one, three and seven days after exposure), proving also to be harmless to another two beneficials tested, the predators *Macrolophus caliginosus* Wagner and *Amblyseius* (*Neoseiulus californicus*) McGregor, while causing toxicity to the predator *Orius laevigatus* (Fieber). Laboratory research by Abu-Tara et al. (2008) showed that pymetrozine (applied at two recommended concentrations) reduced (<10 %) adult emergence from pupae of the endoparasitoids *Bemisia tabaci* (Gennadius), *E. formosa* and *Eretmocerus mundus* Mercet. Studying the compatibility of different insect growth regulators in the control of *B. argentifolii* Bellows and Perring, Hoddle et al. (2001) noted that pymetrozine caused the lowest mortality of juveniles of the parasitoid *Eretmocerus eremicus* Rose and Zolnerowich, but also the lowest mortality rate of whiteflies as their hosts five days after parasitism. In a laboratory study conducted by Morales et al. (2006), in which the formulation of pymetrozine Plenum® WP150 (25 mg a.i./l) was tested on all development stages of *Hyposoter didymator* (Thunberg), a solitary endoparasitoid of *Spodoptera littoralis* larvae, there was no statistically significant difference in eclosion between treated and untreated pupae. In a study by Medina et al. (2007) the recommended dose of pymetrozine was shown to be totally harmless to *H. didymator* pupae after its uptake with artificial food treated topically. The results of these authors are in congruence with a laboratory research by Krespi et al. (1991), who noted that there was no significant difference in the emergence of adult parasitoids *Aphidius uzbekistanicus* Luzhetzki after direct treatment of parasitoid pupae.

Similar to the results of our study, a study conducted by Joseph et al. (2011) showed that the development of juvenile stages of the endoparasitoid *Aphidius ervi* Haliday (even at concentrations that were sublethal for the aphid host) was significantly compromised by the use of pymetrozine. Pymetrozine had a negative effect on the development of *A. ervi* larvae, causing changes in the sex ratio of that species in male favor (Joseph et al. 2011), and increased mortality and reduced life longevity, compared to control wasps (Tran et al. 2005). Similarly, although the pymetrozine formulation Plenum® 50 WG (500 mg a.i /l) has been known by its good reputation of being selective for insects that suck plant sap (Flückiger et al. 1992a, b; Sechser et al., 2002), Harrewijn and Kayser (1997), classifying the effects of this formulation on *A. ervi* juveniles, noted a significant reduction in the growth and development success (40 %) of the parasitoid in the contaminated body of its host. Only the egg and larval stages of the parasitoid were significantly affected by the presence of sublethal doses of the formulation in bodies of their hosts, while adult emergence and nymph development did not differ from controls in uncontaminated hosts. This may be due to either direct or indirect effect of the contaminated host on the parasitoid. Insecticides can be directly absorbed by parasitoids that develop inside the contaminated bodies of their hosts (the insecticide is moved to the third trophic level) (Harrewijn & Kayser, 1997).

Adverse effects of pymetrozine on biological and/or population parameters of beneficial organisms have been documented in a small number of studies. Very little is still known about sublethal effects of this compound on the parasitoid/host and prey/predator interactions within the trophic system. In the study by Joseph et al. (2011), pymetrozine did not reduce the number of offspring of *A. ervi* females (Haliday) but, as in our study, it impacted negatively the development of parasitoid larvae. Similarly, Morales et al. (2006) noted that despite a low acute toxicity of pymetrozine to the parasitoid *H. didymator*, the parasitoid lifetime was significantly reduced, regardless of the insecticide mode of action; in contrast, pymetrozine did not affect parasitoid survival, nor did it reduce parasitism.

In a demographic study conducted by Kheradmand et al. (2012), pymetrozine caused 27 % mortality of adults of the aphid parasitoid *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae), and significantly reduced its life table parameters (intrinsic rate of increase - r_m, net reproductive rate - R_0, and finite rate of increase - λ), compared to the control. No mortality was observed in pupae treated with pymetrozine and this insecticide had no significant effects on life table parameters of wasps emerged from treated pupae. In a laboratory study by Rezaei et al. (2007), pymetrozine caused a 34 % reduction in r_m value of the common green lacewing, *Chrysoperla carnea* (Stephens).

Pymetrozine has been shown compatible for use with some species of whitefly predators, another important group of biological agents (Torres et al., 2002; Rezaei et al., 2007; Yoshizawa & Aizawa, 2007; Cabral et al., 2008).

After exposing adult *E. formosa* wasps to pymetrozine residues, Bujanovac population was found to show more favorable values of all examined parameters than the Dutch strain population of wasps. Regarding treated parasitoid pupae, the surviving Dutch population wasps achieved the highest values of the instantaneous rate of increase, while Bujanovac wasps lived and oviposited longer, and achieved higher values of total parasitism and total emegence. Similar to our previous findings, Bujanovac population wasps were again shown to be more promising for integrated control of whitefly (Drobnjaković et al., 2018, 2019).

Considering the obtained lethal concentrations for both examined stages of the parasitoid, and taking into account the reduction in reproductive and demographic parameters, which is (lower than 10 %), the application of the recommended concentration of pymetrozine would show limited adverse effects on parasitoid population, so that pymetrozine can be used together with the parasitoid *E. formosa* without causing a great impact on its effectiveness in integrated whitefly control. Disadvantages of this insecticide include its significant impact on wasp lifetime (in bioassays with adults) and juvenile development (in bioassays with pupae).

These results offer a starting point for further investigation of local *E. formosa* populations, in comparison to the commercial Dutch strain, as biological agents intended for integrated control of *T. vaporariorum* in vegetable and ornamental protected crops. Further research should focus on evaluating pymetrozine in greenhouse trials with an emphasis on population-level responses.

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Akutna toksičnost i subletalni efekti pimetrozina na parazitoida bele leptiraste vaši *Encarsia formosa* Gahan

REZIME

U laboratorijskim uslovima, utvrđivani su subletalni efekti preparata na bazi pimetrozina (Chess 50 WP) na parametre životne istorije i populacioni rast preživelih ženki komercijalizovane („Dutch" rase) i dve lokalne populacije (Bujanovac i Negotin) parazitoida Encarsia formosa Gahan (Hymenoptera: Aphelinidae). Svi ogledi su izvedeni na temperaturi 27±1°C i relativnoj vlažnosti vazduha od 60±10 %, uz fotoperiod 16:8 h, u četiri ponavljanja. U biotestu sa adultima, preparat na bazi pimetrozina, primenjen u srednjim letalnim koncentracijama (280 mg a.s./l) značajno je redukovao dužinu života (za 2,7-3 dana) i preživljavanje ženki iz svih ispitivanih populacija. Rezidualno delovanje pimetrozina smanjilo je ukupni parazitizam osa populacije Negotin i komercijalizovane populacije (za 8,2 i 7,3 %), ukupnu ekloziju adulta komercijalizovane populacije (za 8,2 %), kao i trenutnu stopu rasta sve tri ispitivane populacije (za 6,6, 6,3 i 7,6 %, za Bujanovac, Negotin i „Dutch" populaciju, respektivno). Primenjen direktno na lutke parazitoida (300 mg a.s./l), preparat na bazi pimetrozina je značajno produžio razviće juvenila (za 0,3-1,1 dan) svih ispitivanih populacija, smanjio ukupni parazitizam ženki populacije Negotin (za 8 %) i statistički značajno redukovao trenutnu stopu rasta populacija Bujanovac (6,7 %) i Negotin (4,6 %). 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.

Ključne reči: *Encarsia formosa,* pimetrozin, subletalni efekti, životni parametri, populacioni rast