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Selectivity assessment of two biorational insecticides, azadirachtin and pyriproxyfen, in comparison to a neonicotinoid, acetamiprid, on pupae and adults of a Neotropical strain *Eretmocerus mundus* Mercet

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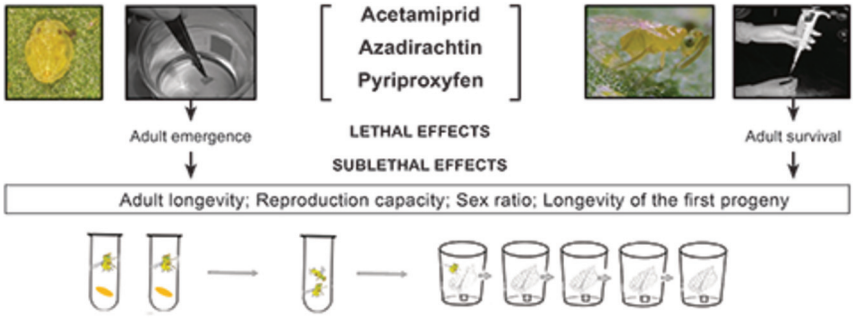
HIGHLIGHTS

- Acetamiprid, azadirachtin and pyriproxyfen were toxic to *Eretmocerus mundus*.
- Lethal and sublethal effects were observed in bioassays with this parasitoid.
- Pupal stage was more susceptible than adults for the three insecticides evaluated.
- Azadirachtin at lower concentration has not affected the reproduction parameters.
- The toxicity of acetamiprid highlights that neonicotinoids are not selective.

GRAPHICAL ABSTRACT



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ABSTRACT

Assessment of the susceptibility of natural enemies of pests to selective pesticides is relevant for a sustainable agriculture with low impact on the environment. The aim of this study was to assess the toxicity of two biorational insecticides, azadirachtin and pyriproxyfen in comparison to a neonicotinoid insecticide, acetamiprid, on pupae and adults of a Neotropical strain of *Eretmocerus mundus*. Adult emergence and survival were evaluated as lethal effects whereas the sublethal effects were assessed through the reproductive capacity, sex ratio, and longevity of the surviving first progeny. Adult emergence from treated pupae was reduced by all three insecticides, but azadirachtin at its maximum field recommended concentration (MFRC) proved the most toxic insecticide. The survival probability of emerged adults was reduced by the three insecticides below than 50% from 2 to 5 days after the adult emergence. Malformations in nonemerged adults from treated pupal hosts were observed at the MFRC of all three insecticides. Sublethal effects on survivors from pupal treatment could be evaluated at only the lowest azadirachtin concentration. At that concentration, though azadirachtin did not affect the reproductive capacity of females, the sex ratio and the longevity of the first progeny were disrupted. The survival of parasitoid adults after adult exposure was reduced by all three insecticides, pyriproxyfen at the MFRC being the most toxic. All insecticides at their half of MFRCs induced sublethal effects in the

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survivors' adults, with pyriproxyfen being the most harmful to the reproductive capacity of females. In conclusion, both biorational insecticides were toxic to *E. mundus*.

1. Introduction

Pesticides with a broad spectrum, as of several decades, have been used in agriculture as the main strategy for pests control in order to reduce crop damage by pests without considering, in most instances, the potential impact of these chemicals on the different environmental components (*i. e*., soil, water, and the atmosphere). The indiscriminate use of those compounds has caused environmental contamination as well as various several side effects to non-target organisms (Aktar, 2009; Botías et al., 2015; Desneux et al., 2007; Mac Loughlin et al., 2017; Stark et al., 2004, 2007). Current global trends in crop protection advocate to the implementation of integrated pest management (IPM) programs, incorporating reduced risk insecticides with a low impact on non-target species (Bueno et al., 2017; Guedes et al., 2016).

Biorational insecticides are designed as an alternative to conventional broad spectrum pesticides because of the associated selectivity against pests and lower impact on non-target organisms (Devine and Furlong, 2007; Villaverde et al., 2014). Several compounds are considered as biorational insecticides belonging to different groups on the basis of those products' chemical properties, modes of action, and other characteristics. A such, these chemicals have been grouped (Ware, 1983) as insect growth regulators (IGRs), lipid synthesis inhibitors, rianodyn modulators (neurotransmitters) and biopesticides (from botanical and microorganisms sources). The use of biorationally formulated insecticides in certain countries such as Argentina is still incipient, mainly because of the high cost of those compounds. Nevertheless, certain compounds such as azadirachtin and pyriproxyfen have been used on Argentine horticultural crops for controlling phytophagous pests and are considered as selective insecticides because of their reduced environmental impact respect to conventional pesticides (USEPA, 2012–2013).

The botanical biopesticide azadirachtin is a tetranortriterpenoid limonoid extracted from seeds of the Indian neem tree *Azadirachta indica* (Meliaceae). This compound may cause feeding deterrence, behavioral changes, incomplete ecdysis, altered developmental time, anatomical malformations, and hormonal disruption leading to sterility (Mordue Luntz and Nisbet, 2000). Pyriproxyfen is an insecticide belonging to the group of insect growth regulators IGRs—*i. e*., potent juvenile-hormone analogues mimicking the authentic hormones excreted by the *corpus allatum* in insects and thus causing a strong suppression of embryogenesis, metamorphosis, and adult reproduction (Ghanim and Ishaaya, 2010). Although the effectiveness of such compounds on several phytophagous pests has been widely documented (Bacci et al., 2007; Ishaaya et al., 1994, 2001; Koul, 1984; Palli and Cusson, 2007), but several lethal and sublethal effects of them on non target organisms have been also documented (Bernardes et al., 2017; Fogel et al., 2013, 2016; Sohrabi et al., 2012, 2013; Schneider et al., 2008).

Neonicotinoids insecticides were initially commercialized in '90 decade for aphids' control as alternative to broad spectrum conventional insecticides with good efficacy for suck-soap insects (Jeschke, 2007; Millar and Denholm, 2007). However, their registration was reevaluated in the last decade due to the potential environmental fate and effects on non-target of these compounds (USEPA, 2008–2012). Acetamiprid is a neonicotinoid that antagonizes the central nervous system of insects through a specific interaction with nicotinic acetylcholine receptors to produce excitation, paralysis, and death (Tomizawa and Casida, 2005). The selectivity of neonicotinoids, in general, is currently under discussion due to their high toxicity on several pollinators (Blacquiere et al., 2012; Decourtye et al., 2004; Thompson, 2003) as well as on other beneficial insects such as the predators and parasitoids of agricultural pests (Fogel et al., 2013, 2016; Rimoldi et al., 2017; Sohrabi et al., 2012, 2013).

The lack of toxicity of biorational insecticides to beneficial organisms and other non-target organisms is expected to reduce the environmental risks occasioned by those compounds. Moreover, the selectivity action of them against phytophagous pests must be thoroughly assessed before their inclusion in IPM programs, and the evaluation of low impact of the pesticides to the natural enemies of pests involves an assessment of both the lethal (short-term) and the sublethal (long-term) effects of those compounds (Stark et al., 2004). Nowadays the impact of the sublethal effects of pesticides on the fitness of the natural enemies of pests is considered more relevant than the lethal effects. Sublethal effects have been defined as toxicities occurring in individuals that surviving exposure to a given compound (Desneux et al., 2007) that are manifested as a reduction in life span, developmental rate, fecundity, and/or fertility or as changes in the sex ratio and/or changes in behavior (Stark and Banks, 2003).

Parasitoids are ecologically effective natural enemies of several phytophagous pests through controlling increases in their populations. *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) is the solitary parasitoid of the whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) biotype complex that is a relevant pest of several crops worldwide (Taggar and Gill, 2016). The parasitoid life cycle takes placed inside of whitefly host nymph. Larval instars and pupae develop within to the host and the adult emerges through a circular hole. It is native of the Mediterranean region, however has also been found in association with *B. tabaci* on Argentine horticultural crops since 2002 (López and Evans, 2008). The establishment and conservation of this hymenopteran in vegetable crops in Argentina is, however, now being compromised by the high level of pesticide contamination in those agroecosystems because such chemical control currently constitutes the main approach to pest regulation (Defensor del Pueblo Prov. Bs As-UNLP, 2015; Gerling and Fried, 2000; López and Botto, 1997; López and Andorno, 2009; Urbaneja and Stansly, 2004; Stansly et al., 2005).

Within this context, the aim of the work reported here was to determine, under laboratory conditions, the selectivity of two biorational insecticides azadirachtin and pyriproxyfen, in comparison to the neonicotinoid acetamiprid, with respect to their action on pupae and adults of *E. mundus*, through an evaluation of both the lethal and the sublethal effects those compounds on that parasitoid. The final objective of these experiments was focussed on the need to redesign pest-control strategies in order to minimize the impact of these insecticides on non-target organisms such as *E. mundus*.

2. Materials and methods

The insect rearing and all the bioassays were carried out in growth chambers under controlled environmental conditions (25 ± 2 °C, 70 ± 5% relative humidity, and a 16: 8-h light: dark photoperiod).

2.1. Neotropical insect strains

Individuals of *B. tabaci* and *E. mundus* organisms were collected from greenhouses in La Plata, Argentina (34°56′04″S, 58°10′14″W) on organically grown vegetable crops. Samples of whitefly nymphs found on the leaves and whiteflies with evidence of parasitism were collected in the summer and maintained in quarantine until the whiteflies and adult parasitoids were recorded. The whiteflies and parasitoids were observed by binocular stereomicroscopy to identify the species through the use of the taxonomical keys of Viscarret and Botto (1996) for *B. tabaci*, and of Rose and Zolnerowich (1997) for *E. mundus*. The progeny of both insects were used to start the respective laboratory colonies. The whiteflies were reared on sweet pepper (*Capsicum annuum* L.) seedlings without a history of pesticide treatment. Sweet-pepper plants were grown on fertile soil mixed with per liter (1:1 [*v/v*]). The parasitoid colony was likewise maintained on sweet-pepper seedlings, but containing nymphal stages of *B. tabaci* (with most being in the second nymphal instar). Both colonies were maintained in ventilated cages (26 cm width, 40 cm length, 50 cm height). New plant material containing whiteflies was added weekly in the parasitoid rearing to maintain the colony.

2.2. Insecticides

The formulated insecticides tested were Mospilan® (20% [*w/v*] acetamiprid; Summit-Agro S.A, Argentina), Neem-Azal® (1.2% [*w/v*] azadirachtin; Agristar, Argentina) and Epingle® (10% [*w/v*] pyriproxyfen; Summit-Agro S.A, Argentina). Each insecticide was tested at 100% of the maximum field recommended concentration (MFRC)—*i. e.*, 200, 40, and 75 mg active ingredient per liter [a.i. L^{-1}], respectively—and 50% of the MFRC (100, 20 and 37.5 mg a.i. L^{-1}, respectively) as registered in Argentina (CASAFE, 2013/2015). In the adult treatment involving exposure to residues, concentrations from 0.007 to 0.017 mg a.i. L^{-1} were applied per tube (cf. the following section on toxicity bioassays on *E. mundus* adults for more details). Insecticide test solutions were prepared with distilled water for pupal exposure or with analytical-grade acetone for adult exposure as solvents. Controls were treated with solvent alone.

2.3. Toxicity bioassays on E. mundus pupae

Pupae of *E. mundus* that had developed within fourth instar *B. tabaci* nymphae (N_4) were glued on a piece of double-sided tape (1 cm width by 1 cm length) after the method developed by M. I. Schneider (unpublished data), then dipped into the insecticide solutions for 10 s and dried in a fume hood for 30 min. Thereafter, the treated insects were kept in plastic Petri dishes (6 cm diameter, 1 cm depth) and checked daily until the emergence of *E. mundus* adults. Five replicates of six pupa per insecticide, at each concentration and the same number in the controls were used. A reduction in adult emergence and/or survival of adult emerged were regarded as evidence of lethality.

Eretmocerus mundus survivors emerged from treated *B. tabaci* were followed to evaluate the sublethal effects only after exposure to half the MFRC of azadirachtin because at the full MFRC of this compound and at both concentrations evaluated for acetamiprid and pyriproxyfen the adult emergence was lower than 20% and the longevity of the survivors fewer than 5 days—these conditions constituting the minimal criteria for survival. The male and female parasitoid survivors were paired for 24 h to insure the occurrence of mating. Thereafter, the females were placed individually in plastic cylinders (6.5 cm diameter, 8.5 cm height) containing a pepper-plant leaf along with *B. tabaci* host nymphs (mainly second instars) and a 5 mL plastic vial with tap water to prevent leaf dehydration. The females were left with the host (25-30 nymphs) for 24 h, then removed and placed in another cylinder containing a second leaf prepared in the same way. This procedure was repeated for 5 consecutive days. The sublethal endpoints analyzed were: the effective parasitism (number of nymphs showing signs of parasitism), the offspring size (number of adults emerging from parasitized nymphs), the sex ratio ([number of females]/[number of females + number of males]) after the first day of host exposure, and the cumulative parameters after 5 consecutive days of host exposure. The transgenerational effect of the insecticides was estimated through the longevity of the F_2 progeny.

2.4. Toxicity bioassays on adults of E. mundus

Parasitoid adults (1–3 days old) were exposed to insecticide residues, according to Desneux et al. (2004). The three insecticides were evaluated at each compound's MFRC and half the MFRC. Fresh acetone solutions of acetamiprid, azadirachtin, and pyriproxyfen were prepared before the bioassays and then applied (0.7 μL solution/tube, 0.016 μL/cm^2) to the surface of glass tubes (1 cm diameter, 7 cm length, 43.96 cm^2 internal surface). On the basis of the surface area of the glass tube, the amount applied per tube corresponded to 0.14 and 0.07 mg a.i. for acetamiprid; 0.03 and 0.015 mg a.i. for azadirachtin and 0.05 and 0.025 mg a.i. for pyriproxyfen at the MFRC or the half MFRC of each insecticide, respectively. The glass tubes were next rotated to insure an equal deposit of the residues and then dried for 45 min in a fume hood for complete solvent evaporation. Finally, adults of *E. mundus* were exposed to the insecticides residues (at one adult per tube per replicate) for 1 h before transfer to an untreated tube, where a trace of pure organic bee honey was added as food. Thereafter, the tube was maintained in a rearing chamber. The adults were inspected daily until the time of death. The experiment was performed a total of 30 times per treatment (*i. e.*, at each insecticide concentration along with a control). The individual survival was assessed as the lethal endpoint, whereas the different aspects of the reproductive capacity of the females—*i. e.*, the effective parasitism, the offspring size, and sex ratio—were considered as sublethal effects. On the basis of the adult survival obtained in these assays, half of the MFRCs for three insecticides were chosen to evaluate the sublethal effects on the reproductive capacity of the adult female survivors by means of the methodology outlined for pupal bioassays.

2.5. Statistical analysis

The evaluation of the adult-emergence data was performed through a difference-of-proportions test to compare emergence proportions; but in instances where the conditions for the application of this test were not met, an exact Fisher test was conducted. For the multiple comparisons, an experimental error of $\alpha \leq 0.06$ was taken by adjusting the individual confidence levels according to the number of comparisons involved (Lyman Ott and Longnecker, 2010).

The probability of adult survival after pupal and adult exposure was estimated by the Kaplan-Meir method along with the log-rank test for treatment comparisons, while the Bonferroni correction was used for paired comparisons between treatment methods. For

the sublethal-effects data of the pupae and adults of *E. mundus* the statistical evaluation was carried out through the one-way analysis of variance (ANOVA), and the differences between by a least-significant-difference multiple-range test ($p \leq 0.05$). If the assumptions of ANOVA were not met —*i. e.*, the data did not fall into a normal distribution —either the, raw data sets were transformed to [log (x +1) or arcsine $\sqrt{x}$] or a nonparametric test was performed for the data analysis.

The interface R-Studio (version 1.0.136) software R (version 3.3.3) (RStudio Team, 2016) was used for the analyses.

The percent of reduction in each endpoint selected to measure the lethal and sublethal effects of azadirachtin, acetamiprid, and pyriproxyfen on the pupae and adults of *E. mundus,* was estimated by the following the formula:

$$\%\ of\ reduction = [(C - T) \div C] \times 100;$$

where **C** is the mean endpoint for the control and **T** is the mean endpoint for each treatment.

3. Results

3.1. Toxicity bioassays on E. mundus pupae

The emergence of *E. mundus* from parasitized hosts exposed to insecticides by dipping was significantly disrupted by all three of the insecticides tested at their MFRC and at half of MRFC, though azadirachtin 40 mg a.i.L^{-1}(the MFRC) was the most toxic insecticide ($X^2 = 32.59$, $df = 2$, $p < 0.0001$; Fig. 1). In this last treatment, the adult emergence was around 17% (a reduction of 82% relative to control values), with the pupae developing inside of pupae host becoming dark and dehydrated at 72 h posttreatment. Acetamiprid also caused a high reduction in the adult emergence relative to the control (64–75% at 100 [half the MFRC], and 200 mg a.i.L^{-1} [the MFRC], respectively), though the impact of the compound on adult emergence was somewhat lower that of azadirachtin at the MFRC. In comparison, pyriproxyfen 37.5 mg a.i. L^{-1} (half the MFRC) was the insecticide with the lowest toxicity to parasitoid pupae, reducing the adult emergence to around 22% relative to the control value. A dissection of the pupae from which no adult emerged was observed (at the MFRC) revealed the presence of malformations and teratologic abnormalities (Fig. 2a–d)—for example, an increase in size and a darkening of the compound eyes as observed in pupae treated with acetamiprid (Fig. 2b). Moreover, a thinning of the abdominal region in individuals developing inside the pupal host treated with azadirachtin was likewise recorded (Fig. 2c). Finally, a disruption in the normal development of the antennae and wings occurred upon pyriproxyfen treatment (Fig. 2d).

The probability of survival of *E. mundus* adults emerged from such treated pupae was significantly affected by the three insecticides evaluated (log rank = 160.31, $df = 6$, $p < 0.0001$; Fig. 3). In the control, the probability of survival for adults emerged from untreated pupae was 100% approximately 7 consecutive days after adult emergence. Nevertheless, the survival probability of adults emerging from pupae treated with the three insecticides was reduced by more than 50% between 2 and 5 days after adult emergence. In particular, pyriproxyfen at 75 mg a.i. L^{-1} (the MFRC) reduced the survival by 60% of survival at two days, while 100% of the adults died at 3 days after adult emergence. Although this insecticide at the lower concentration reduced the adult survival by around 80% at 4 days after emergence, by Day 5 all the adults had died. Acetamiprid at 200 mg a.i. L^{-1} (the MFRC) and 100 mg a.i. L^{-1} (half the MFRC) reduced the survival of adults by 43% and 20% at 2 and 3 days after emergence, whit 100% dying at 3 and 4 days after emergence, respectively. In addition, azadirachtin at 40 mg a.i. L^{-1} (the MFRC) reduced survival by around 60% by the third day after adult emergence, whit 100% of adults having died by Day 4. In contrast, with this last insecticide, at the lower concentration tested, the survival recorded by Day 5 was around 45%, though all of those adults were dead by Day 7—leaving, nevertheless, more emerged adults initially alive than with the other insecticide treatments.

Neither the sublethal effects of the insecticides on the reproductive capacity of female *E. mundus* survivors emerging from treated pupae nor the transgenerational effects could be assessed either for acetamiprid or pyriproxyfen at both concentrations of those compounds tested or for azadirachtin at the higher concentration tested because of the extremely low adult emergence and longevity those agents at those concentrations. Accordingly, the sublethal effects on reproduction and transgenerational modifications were evaluated at just the lowest concentration of azadirachtin (Table 1). At this concentration the reproductive capacity of the females was not affected, but the sex ratio of the progeny (after five cumulative days of host exposure) became biased toward the males (with only 27% of the offspring being females). In addition, a significant reduction in the longevity of the first offspring was also registered.

3.2. Toxicity bioassays on E. mundus adults

The survival of *E. mundus* adults after exposure to insecticides residues demonstrated that the three agents significantly reduced this endpoint, although the impact was quantitatively different among the three compounds (log rank = 110.514, $df = 6$, $p < 0.0001$; Fig. 4). In particular, pyriproxyfen at 75 mg a.i. L^{-1} (the MFRC) and 37.5 mg a.i. L^{-1} (half the MFRC) reduced the adult survival to around 50% at 2 and 3 days after exposure to residues, whit 100% of those adults subsequently died by 6 and 7 days after exposure, respectively. Acetamiprid at 200 mg a.i. L^{-1} (the MFRC) reduced the adult survival to 52% at 3 days after exposure to residues, whit 100% dying at the end of 7 days. This insecticide, however, at half that concentration reduced the adult survival to around 20% at 3 day after whit no adult surviving after the same number of days. In contrast, azadirachtin at 40 mg a.i. L^{-1} (the MFRC) reduced the adult survival by around 45% by third day after exposure to residues, with 100% of the adults dying by 7 days; but, at the lower concentration of 20 mg a.i.L^{-1} (half the MFRC), this compound allowed a survival of around 80% between days 4 and 5, though with death nevertheless occurring in all of the adults by Day 6.

Table 2 summarizes the sublethal effects of acetamiprid, azadirachtin and pyriproxyfen on the reproductive capacity of *E. mundus* female survivors and the transgenerational effects on their progeny. As stated above, these parameters were evaluated at the half of the MFRC owing to the low probability of survival obtained at the full MFRC. Acetamiprid at 100 mg a.i. L^{-1} did not reduce any reproductive parameter tested by the first day, but after five cumulative days of host exposure the compound drastically reduced the effective parasitism (*i. e.*, by some 54%), the offspring size (by 15%) and the sex ratio (by a full 72%) compared to the control values. Azadirachtin at 20 mg a.i. L^{-1} affected significantly the effective parasitism after five consecutive days, reducing this parameter by 63% relative to the values obtained for the control. Pyriproxyfen at 37.5 mg a.i. L^{-1} affected only the reproductive capacity of females and the sex ratio, though both parameters were reduced on the first as well as the fifth day of host exposure, thus manifesting a high impact on the female *E. mundus* parasitoids.

The longevity of the progeny was affected by all three insecticides, as indicated by a reduction in that parameter by around a respective 36, 34, and 34% by those three compounds at these their respective half-MFRC levels.

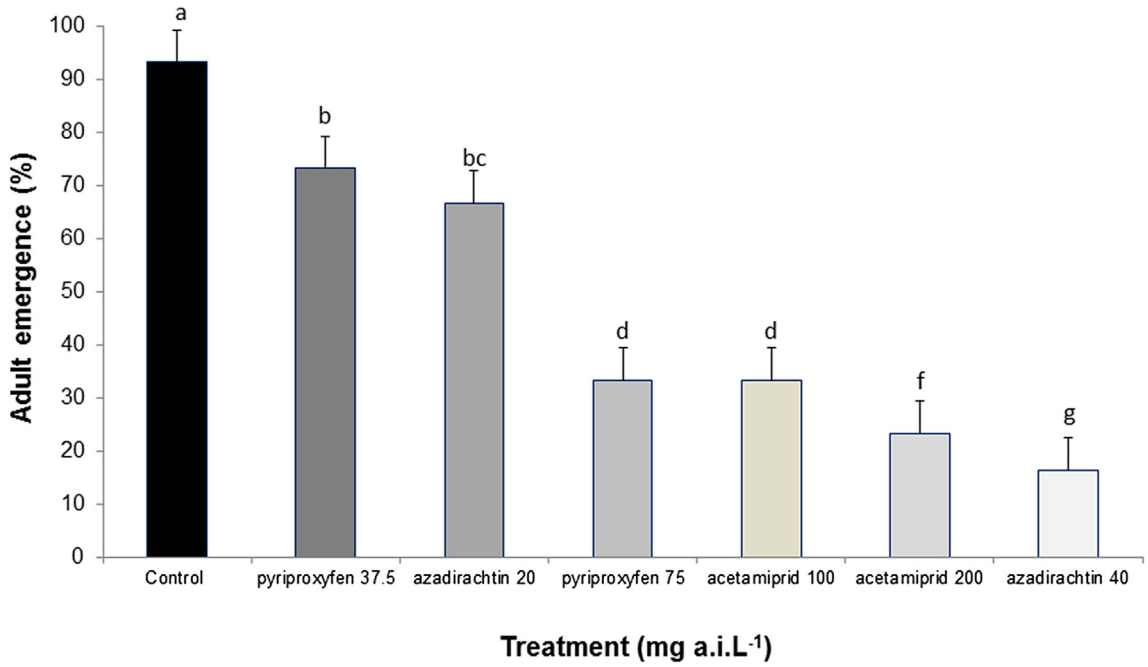

Fig. 1. Effects of insecticides on the emergence of *Eretmocerus mundus* adults emergence exposed to insecticides during the pupal stage of development inside the host *Bemisia tabaci*. In the figure, the percent adult emergence is plotted on the *ordinate* after pupal exposure to acetamiprid, azadirachtin, or pyriproxyfen at the concentrations in mg of active ingredient per liter indicated on the *abscissa*. Arrows indicate the significant differences between treatments (Proportion test at a significance level $p < 0.0001$).

4. Discussion

The impact of pesticides on the environment is nowadays one of the main concerns worldwide, where a modern agricultural chemistry for sustainable agricultural systems is needed (Jeschke, 2016; Roubos et al., 2014). The effect of pesticides on aquatic organisms has been more thoroughly studied than those on terrestrial organisms. Since the natural enemies of pests are terrestrial, a determination of their role as biological control agents for pest control has required biological studies to evaluate their fitness and susceptibility to pesticides. The selectivity of pesticides usually assesses the lethal (short-term) and sublethal (long-term) effects on natural enemies (Desneux et al., 2007; Stark et al., 2004, 2007). The present study provides novel ecotoxicological data for the insecticides acetamiprid, azadirachtin and pyriproxyfen on the hymenopteran *E. mundus*. All insecticides evaluated affected several biological parameters in both the immature pupal and adult stages of this parasitoid even though the three insecticides manifested differing degrees of toxicity.

4.1. Toxic effects of insecticides on pupae of E. mundus

In the present study, azadirachtin (at the MFRC) caused adverse effects on the treated pupae of *E. mundus* that reduced the emergence and survival of adults. The mode of action of this insecticide, however, is still not clear because the compound acts on several sites with IGR, antifeedant, and repellent or deterrent effects among others. Azadirachtin disrupted the normal development of *E. mundus*, acting mainly as an antagonist of the ecdysone hormone mainly (Schmutterer, 1990). Our results are consistent with those reported by Zuazúa et al. (2003), who observed that the compound attained a certain degree of penetration through the host cuticle and disrupted the normal development of the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) within the mummified aphids of the phytophagous *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae). Likewise, Saber et al. (2004) reported an adverse effect of this insecticide on the adult emergence of the parasitoid *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) from treated pupae. In contrast, Luna Cruz et al. (2011) reported that azadirachtin had a negligible effect on the adult emergence of the parasitoid *Tamarixia triozae* Burks (Hymenoptera: Eulophidae) exposed to the insecticide in the pupal stage, and the authors hypothesized that the effects observed could be related to the insecticide's mode action on that species being more as an antifeedant or repellent.

Acetamiprid was toxic to the pupae of *E. mundus* at even the half of the MFRC, reducing the survival probability of the emerged adults to below 50% by three days after treatmentat both concentrations evaluated. This action may be directly related to the persistent activation of nicotinic receptors caused by the neurotoxic insecticides, such as acetamiprid, that leads to continuous synapse overstimulation resulting in hyperexcitation, convulsion, paralysis, and death (Ishaaya et al., 2007). Our results agree with those reported by Sugiyama et al. (2011), who found a low percentage of adult emergence from pupae of different species of the genus *Eretmocerus* treated by immersion in this same insecticide and observed similar results with *Encarsia formosa* Timberlake (Hymenoptera: Aphelinidae). Likewise, other adverse effects have also been reported for similar neurotoxins—*i. e.*, a decrease in the emergence rate of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) adults caused by imidacloprid, another neonicotinoid insecticide, as well as toxicity to the pupae of the parasitoids *Encarsia inaron* Walker (Hymenoptera: Aphelinidae) and *E. mundus* (Saber, 2011; Sohradi et al., 2012, 2013) —thus underscoring the high toxicity of the neonicotinoids in general towards the natural enemies of pests.

Pyriproxyfen reduced the emergence and survival of adults from treated pupae and in addition caused an abnormal molting and subsequent emergence during pupae-adult intermolting period. This effect may be associated with the mode of action of the insecticide in mimicking the juvenile hormone, causing a suppression of embryogenesis, disruptions in metamorphosis, and

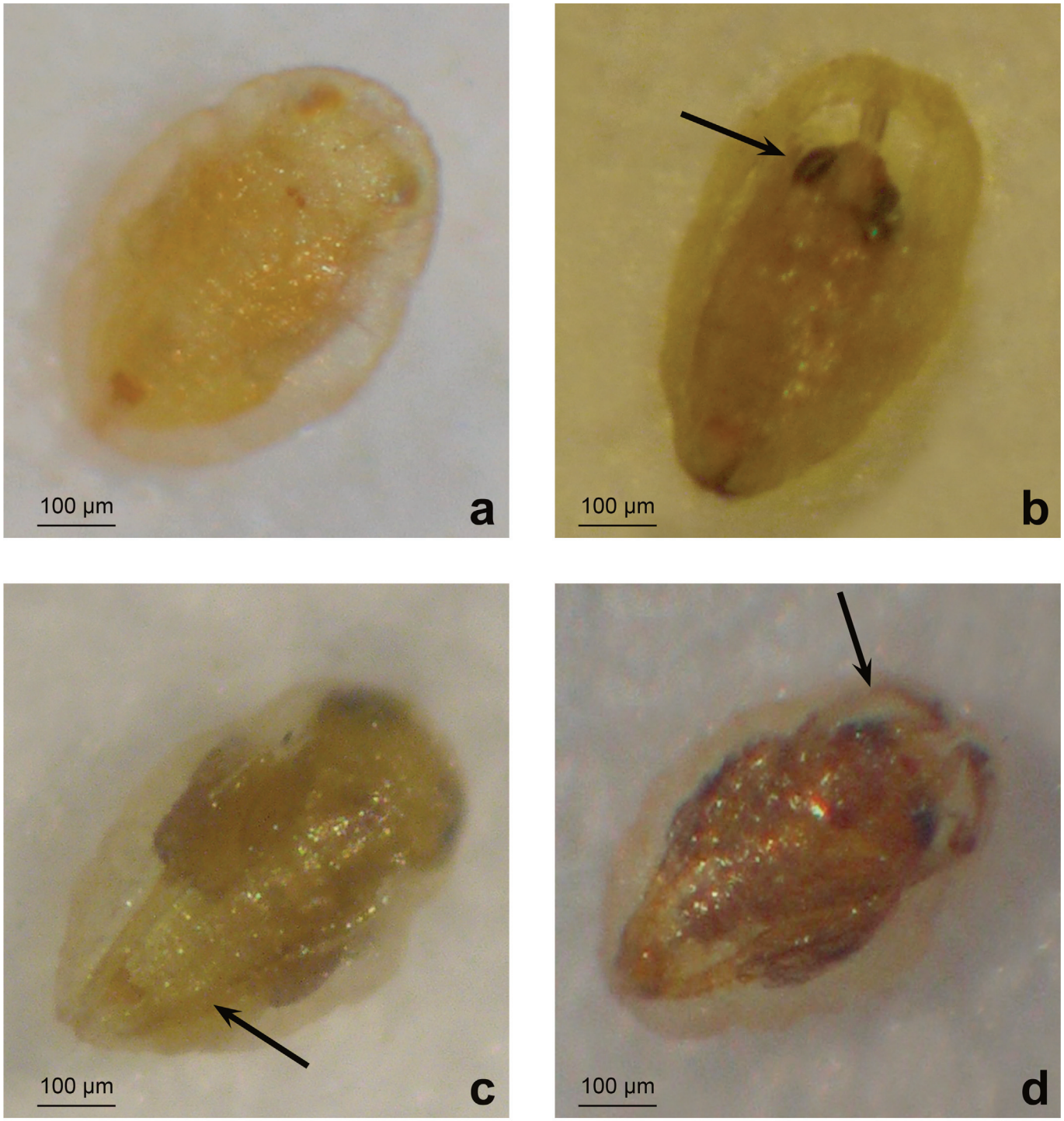

Fig. 2. Anatomical malformations observed in *Eretmocerus mundus* pupae after exposure host of pupae by dipping. a: Control (healthy pupae treated with solvent alone, without insecticides),b: acetamiprid (200 mg a.i L^{-1}), c:azadirachtin (40 mg a.i L^{-1}), d: pyriproxyfen (75 mg a.i L^{-1}). The arrows point to the main malformations observed.

aberrations in the formation of adults (Ishaaya et al., 2007). A decrease in the percentage of adult emergence and in malformations in the adults emerged from pupae treated by immersion in this insecticide were also reported for certain other aphelinid parasitoids (Liu and Stansly, 1997; Sohrabi et al., 2013). In contrast, Hoddle et al. (2001) found no deleterious effect of pyriproxyfen on the adult emergence from treated pupae of the parasitoid *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae). The differences in these last studies with respect to our results could be attributed to the use of different species and parasitoid developmental stages (*i. e.*, the first and third instar larvae of *E. eremicus*).

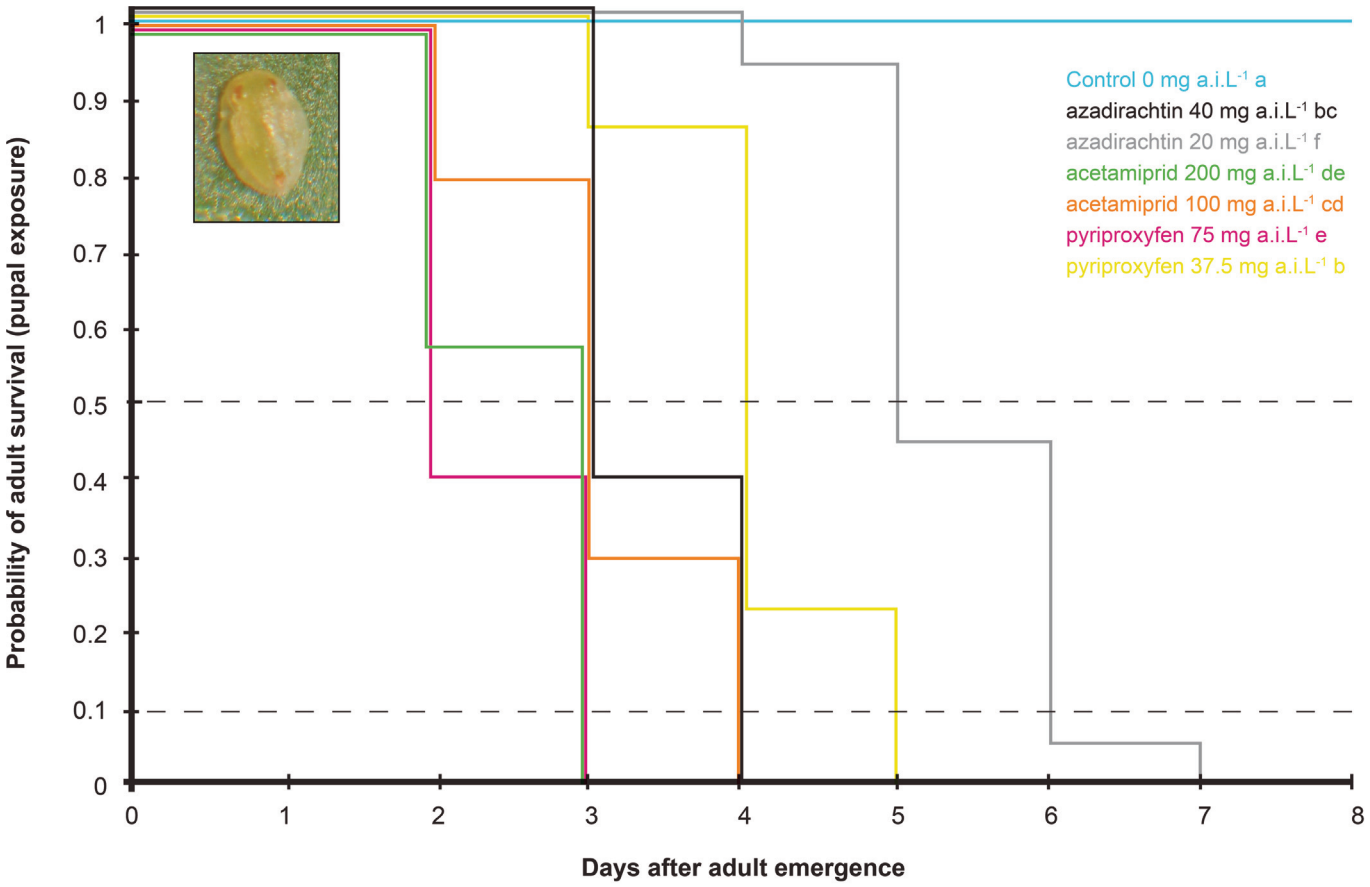

Fig. 3. Effect of insecticides on the survival of *Eretmocerus mundus* adults emerged from the *Bemisia tabaci* host pupae (one show in the *Inset*) exposed to insecticides through dipping. In the figure, the fractional probability of adult survival is plotted on the *ordinate* as a function of time in days after exposure to the insecticide concentrations indicated after the different curves. Different letters at the end of the survival curves indicate significant differences between the treatments as assessed by the Bonferroni method ($p < 0.05$).

Table 1
Sublethal effects of azadirachtin on the reproductive capacity of *Eretmocerus mundus* females emerging from treated pupae and transgenerational effects on the first progeny of the survivors. The data correspond to the means ± the standard error.

Treatment	Concentration (mg a.i.L^{-1})	First day of host exposure (%)			Cumulative exposure for 5 consecutive days (%)			Longevity [a] of offspring
		Effective parasitism [a]	Offspring size [a]	Sex ratio [a, b]	Effective parasitism [a]	Offspring size [a]	Sex ratio [a, b]	
Control	0	35.33 (±2.15) **a**	52.28 (±2.01) **a**	0.32 (±0.04) **a**	33.92 (±3.32) **a**	65 (±14.17) **a**	0.58 (±0.01) **a**	6.97 (±0.05) **a**
Azadirachtin	20	28.66 (±4.16) **a**	18.99 (±18.69) **a**	0.14 (±0.09) **a**	25.27 (±7.34) **a**	56.93 (±11.28) **a**	0.27 (±0.09) **b**	4.71 (±0.14) **b**
Statistical Analysis		$F = 9.38$ $df = 1, 8$ $p = 0.15$	$F = 2.56$ $df = 1, 8$ $p = 0.148$	$F = 2.95$ $df = 1, 8$ $p = 0.12$	$F = 1.15$ $df = 1, 8$ $p = 0.31$	$F = 0.22$ $df = 1, 8$ $p = 0.65$	$F = 0.38$ $df = 1, 8$ $p = 0.0117$	$F = 209.68$ $df = 1, 8$ $P < 0.0001$

Within the columns, different letters denote significant differences between treatments ($p \leq 0.05$).
[a] *ANOVA* and *LSD test*.
[b] Sex ratio: the number of females divided by (the number of females + the number of males).

4.2. Toxic effects of insecticides on adults of E. mundus

Pyriproxyfen had a strong impact on *E. mundus* in terms of adult survival: a higher toxicity was observed at the MFRC that caused a strong decrease to around fifty percent at 2 days after exposure in comparison to half the MFRC, where that same lethality took one further day. Our results agree with those reported by several authors who have pointed out that at low concentrations pyriproxyfen could cause a moderate short-term toxicity, but permitted a higher overall survival in several hymenopteran parasitoids (Prabhaker et al., 2007; Vanaclocha et al., 2013).

Acetamiprid was toxic to *E. mundus* adults, thus demonstrating once again the high toxicity of neonicotinoid insecticides to natural enemies. Similar effects have been cited by Prabhaker et al. (2007) and by Sugiyama et al. (2011), who reported a low survival in adults of *Aphytis melinus* De Bach (Hymenoptera: Aphelinidae), *E. eremicus*, *E. formosa*, and *E. mundus* exposed to residues of this insecticide.

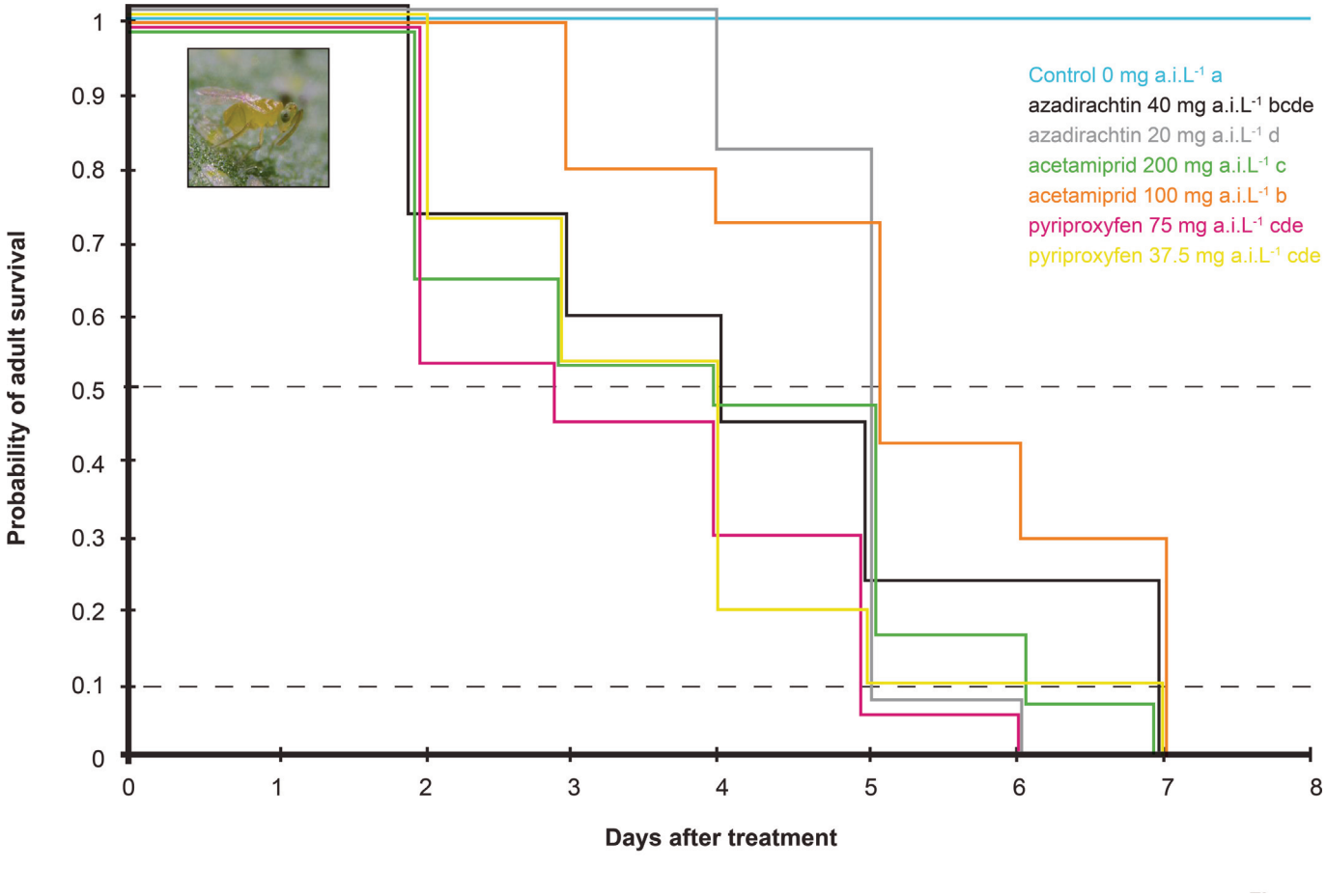

Fig. 4. Effect of insecticides on the survival of *Eretmocerus mundus* adults (one show in the *Inset*) exposed to insecticides during the adult stage. In the figure, the fractional probability of adult survival is plotted on the *ordinate* as a function of time in days after exposure to the insecticide concentrations indicated after the different curves. Different letters at the end of the survival curves indicate significant differences between the treatments as assessed by Bonferroni method ($p < 0.05$).

Table 2
Sublethal effects ofacetamiprid, azadirachtin and pyriproxyfen on reproductive capacity of *Eretmocerus mundus* females that survivors to adult treatment and transgenerational effects on progeny. The data correspond to means ± standard error.

Treatment	Concentration (mg a.i.L^{-1})	First day of host exposure (%)			Cumulative exposure for 5 consecutive days (%)			Longevity[b] of offspring
		Effective parasitism[a]	Offspring size[a]	Sex ratio[a, c]	Effective parasitism[a]	Offspring size[a]	Sex ratio[b, c]	
Control	0	41.33 (±9.22) **a**	96 (±4.01) **a**	0.31 (±0.03) **a**	40.71 (±5.41) **a**	89.99 (±1.26) **a**	0.53 (±0.01) **a**	7 (±0.04) **a**
Acetamiprid	100	25.33 (±6.87) **ab**	64.66 (±18.43) **ab**	0.10 (±0.09) **ab**	18.77 (±4.43) **b**	76.34 (±5.36) **b**	0.14 (±0.06) **b**	4.49 (±0.36) **b**
Azadirachtin	20	17.46 (±14.25) **ab**	53.99 (±26.22) **ab**	0.14 (±0.10) **ab**	15.03 (±4.22) **b**	88.77 (±2.08) **a**	0.26 (±0.22) **ab**	4.61 (±0.23) **b**
Pyriproxyfen	37.5	11.33 (±8.65) **b**	29.33 (±18.08) **b**	0.00 (±0.00) **b**	20.67 (±4.12) **b**	77.94 (±3.17) **b**	0.23 (±0.03) **b**	4.61 (±0.23) **b**
Statistical Analysis		$F = 2.25$ $df = 3, 16$ $p = 0.012$	$F = 2.68$ $df = 3, 16$ $p = 0.0082$	$F = 3.24$ $df = 3, 16$ $p = 0.0437$	$F = 7.46$ $df = 3, 16$ $p = 0.0024$	$F = 4.71$ $df = 3,16$ $p = 0.01$	$K = 8.93$ $df = 3, 16$ $p = 0.030$	$K = 10.83$ $df = 3, 16$ $p = 0.013$

Within the columns, different letters denote significant differences between treatments ($p \leq 0.05$).
[a] ANOVA and LSD test.
[b] Kruskal-Wallis and Dunn tests.
[c] Sex ratio: the number of females divided by (the number of females + the number of males).

With azadirachtin treatments, as with pyriproxyfen, we observed a low adult survival—*i. e.*, with fifty percent of the individuals dying within 4 days after exposure to the MFRC—but with an increase in that endpoint at the half of the MFRC. Luna Cruz et al. (2011) had also observed an increase in the adult survival of *T. triozae*, after exposure of parasitoids to residues of this same

insecticide at the medium and low concentrations evaluated. Nevertheless, the selectivity of azadirachtin towards beneficial insects is currently under discussion worldwide because of the high toxicity mainly to several pollinators that has been documented (Barbosa et al., 2015; Bernardes et al., 2017).

4.3. Sublethal effects of insecticides on E. mundus survivors

The sublethal effects of acetamiprid, azadirachtin, and pyriproxyfen were assessed on *E. mundus* survivors. Although the results demonstrated that the application of azadirachtin at the pupal stage produced no detrimental effects on the reproductive capacity of females, both the sex ratio (*i. e.*, with fewer females in the offspring) and the longevity of the first progeny were reduced by this insecticide. Although the sublethal effects of azadirachtin on hymenopteran parasitoids has not been extensively documented, our laboratory results agree with those reported by Saber et al. (2004), who reported a slightly detrimental effect on the reproductive capacity of females of *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae).

When parasitoid adults were exposed to any one of the three insecticides evaluated, the reproductive capacity of the female survivors was affected as well as the sex ratio and the longevity of the first progeny. The present results demonstrated that the application of azadirachtin reduced the effective parasitism (after five cumulative days of host exposure) and the longevity of the first progeny (*i. e.*, the transgenerational effect). We could hypothesize that the effect of azadirachtin could be related to a hormonal disruption leading to sterility, which action had previously been cited for different insect species (Schmutterer, 1990). Likewise, Abedi et al. (2014) reported that azadirachtin caused a reduction in the fecundity of females of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) parasitoids, and Barbosa et al. (2015) documented a significant inhibition of egg-laying along with a decrease in the length of the ovaries in the females of *Bombus terrestris* L. (Hymenoptera: Apidae) effected by that same pesticide.

Pyriproxyfen (by the first and fifth days after host exposure) and acetamiprid (by the fifth day after host exposure) reduced the reproductive capacity and the sex ratio of female survivors as well as the longevity of the first progeny. These results agree, in part, with the findings reported by Sohrabi et al. (2013), who observed that imidacloprid (neonicotinoid) reduced the fecundity of *E. mundus* female parasitoids, whereas buprofezin (an analogue of the juvenile hormone) had no effect on this parameter. These same insecticides, however, did not alter the reproductive capacity of *E. inaron* parasitoid females (Sohrabi et al., 2012).The differences between our results and those observations could be attributable to the use of a different neonicotinoid and IGRs insecticides or to the species and strains studied. In view of the overall findings from our studies, we could hypothesize that the pyriproxyfen acts by interfering with the normal reproductive development of *E. mundus* females, while acetamiprid interacts with the central nervous system, causing a disruption of the normal activity of the neurohormones that stimulate the main systems involved in insect development, growth, and reproduction in insects (*i. e.*, the *corpora allata*, *corpora cardiac*, and prothoracic glands; Nation, 2001).

On the basis of our results, azadirachtin, and pyriproxyfen—in their recognized role as biorational control insecticides—were definitely toxic to *E. mundus.* The present work clearly demonstrates that, in addition to the lethal effects observed, both insecticides caused several sublethal alterations in the parasitoid physiology that disrupted the normal development and reproduction of the surviving adults to a high degree. Besides, as in previous studies by our research group, acetamiprid show high short-term toxicity as well as long-term effects demonstrating that this neonicotinoid insecticide was non-selective for *E. mundus*.

These results reported here provide novel and significant information on the lethal and sublethal effects of acetamiprid, azadirachtin and pyriproxyfen on *E. mundus* and underscore the relevance of pesticide-selectivity studies before including those compounds in IPM programs involving horticultural crops within the Neotropical Region. Nevertheless, the present results should be supplemented with further studies under semifield and field conditions to corroborate or not the toxicity of these insecticides on *E. mundus* before their compatible use with this parasitoid.

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