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Sublethal effects of indoxacarb and beta-cypermethrin on *Rhopalosiphum padi* (Hemiptera: Aphididae) under laboratory conditions

Yayun Zuo^{1,§}, Kang Wang^{1,§}, Fangfei Lin^{1,§}, Yuting Li¹, Xiong Peng¹, Jaime C. Piñero², and Maohua Chen^{1,*}

Abstract

Rhopalosiphum padi (L.) (Hemiptera: Aphididae) is a devastating pest of wheat worldwide. Beta-cypermethrin is widely used to control piercing-sucking pest insects, including *R. padi*. Field-applied insecticides gradually descend to sublethal concentrations with the passage of time. Sublethal concentrations of insecticide may affect physiology and behavior of pests, potentially leading to pest resurgence. This study investigated potential sublethal effects of beta-cypermethrin and indoxacarb on an experimental population of *R. padi*. Indoxacarb is a relatively new pesticide with a mechanism of action that is different from that of beta-cypermethrin. The leaf dip method was used to test the acute toxicity of these insecticides to adult aphids, and life tables were constructed to analyze the effects of 2 sublethal concentrations of beta-cypermethrin (B-LC10, B-LC30) and indoxacarb (I-LC10, I-LC30) on the development and reproduction of the F0 and F1 generations of *R. padi*. Results of these analyses showed that sublethal concentrations of the 2 insecticides can significantly reduce the fecundity and longevity of the F0 generation. Also, sublethal concentrations of both insecticides prolonged the development of nymphs in the subsequent generation. Life table parameters demonstrated that the evaluated insecticides at sublethal concentrations did not stimulate aphid reproductive potential. This information could be important to enable a more effective use of these insecticides in management programs for *R. padi*.

Key Words: bird cherry-oat aphid; pyrethroid; toxicity; life table parameter

Resumen

Rhopalosiphum padi (L.) (Hemiptera: Aphididae) es una plaga devastadora de trigo en todo el mundo. Beta-cipermetrina es ampliamente utilizado para controlar plagas de insectos chupadores, incluyendo *R. padi*. Los insecticidas aplicados en el campo descienden gradualmente a concentraciones subletales con el paso del tiempo. Las concentraciones subletales de insecticidas pueden afectar a la fisiología y el comportamiento de las plagas, que puede resultar en el resurgimiento de dichas plagas. Este estudio investigó los posibles efectos subletales de beta-cipermetrina y indoxacarb en una población experimental de *R. padi*. Indoxacarb es relativamente un nuevo pesticida con un mecanismo de acción que es diferente de la de los beta-cipermetrina. Se utilizó el método de inmersión foliar para probar la toxicidad aguda de estos insecticidas a los áfidos adultos, y las tablas de vida se construyeron para analizar los efectos de las 2 concentraciones subletales de beta-cipermetrina (B-LC10, B-LC30) y indoxacarb (I-LC10, I-LC30) sobre el desarrollo y la reproducción de los F0 y F1 generaciones de *R. padi*. Los resultados de estos análisis mostraron que las concentraciones subletales de los 2 insecticidas pueden reducir significativamente la fecundidad y la longevidad de la generación F0. Además, concentraciones subletales de los dos insecticidas prolongan el desarrollo de las ninfas en la generación siguiente. Los parámetros de vida demostraron que los insecticidas evaluados en concentraciones subletales no estimularon la capacidad de reproducción de áfidos. Esta información podría ser importante para permitir un uso más eficaz de estos insecticidas en los programas de manejo de *R. padi*.

Palabras Clave: áfido de avena de pájaro-cereza; piretroides; toxicidad; parámetro de tabla de vida

Rhopalosiphum padi (L.) (Hemiptera: Aphididae) is one of the most important pests of wheat because it causes direct crop damage through phloem feeding and indirect damage by honeydew contamination and associated fungal growth, or by vectoring plant viruses that cause yellow dwarf in winter cereals (Barley yellow dwarf virus, BYDV) (Savaris et al. 2013; Schliephake et al. 2013). Due to global warming, it is expected that insect-transmitted viruses will gain more importance (Schliephake et al. 2013). For instance, longer and warmer periods in autumn can lead to longer flight activities of the vectors

resulting in an increased risk of winter cereals to become infected by these viruses (Habekuß et al. 2009). In fact, the current population profile of wheat aphids differs from that recorded in past decades (1970–1990). In recent years, *R. padi* has become one of the most frequent species on cereal crops and is abundant throughout all development stages of wheat plants (Parizoto et al. 2013; Savaris et al. 2013). Increased levels of damage by *R. padi* have been recorded in wheat-growing regions in China, especially in southern China (Zhang et al. 2007; Lu et al. 2009).

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Although chemical control is still the main means of *R. padi* management, the escalating use of chemical pesticides had caused resistance of this pest to various insecticides (Zhang et al. 2007; Lu et al. 2009; Zuo et al. 2016). Hence, *R. padi* may probably be exposed to sublethal concentrations of insecticides under field conditions, and this may lead to sublethal effects. It is known that sublethal concentrations of insecticides can impair fundamental behavioral and physiological traits of the target insects such as activity, appetite, foraging behavior, and fecundity (Wang et al. 2008a; Qu et al. 2014). Sublethal concentrations of insecticides leading to pest outbreaks in the field have been reported in many pests (Reissig et al. 1982; Tripathi & Sachan 1990; Zhang et al. 2010). For example, Bartlett (1968) reported that a sublethal level of organochlorine and organophosphorus insecticides could cause aphid resurgence. Sublethal concentrations of imidacloprid and azadirachtin have been reported to increase survival and reproductive ability of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Cutler et al. 2006; Wang et al. 2008b). However, sublethal effects can also be manifested as reductions in lifespan, development rate, fertility, fecundity and other demographic parameters (Stark & Banks 2003; Lashkari et al. 2007), thereby potentially influencing the efficacy of pest control tactics. Sublethal concentrations of azadirachtin significantly reduced the longevity and fecundity of the pea aphid, *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae) (Stark & Rangus 1994). Imidacloprid was shown to disrupt *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) population dynamics, slow population increase, and reduce infestation levels at low concentrations (He et al. 2011).

Beta-cypermethrin is classified as a toxicity class II (pyrethroid) insecticide, and it represents one of the main options for controlling aphids on cereals in China (Zhang et al. 2008; Shuai & Wang 2005). Rotational use of various insecticides or use of new insecticides that have different mechanisms of action can potentially reduce onset of resistance of *R. padi* to pyrethroids and maintain toxicity of pyrethroids to *R. padi*. Indoxacarb is an oxadiazine insecticide effective against a broad spectrum of insect pests and has low toxicity to non-target organisms (Dinter & Wiles 2000). These 2 kinds of pesticides are nerve agents, but their mechanisms of action are different. Beta-cypermethrin targets the voltage-gated sodium channels of insect neurons where they alter the activation/inactivation kinetics of the channel and cause disruption to normal nerve signaling, leading to paralysis and death (Soderlund & Bloomquist 1989). The metabolite of indoxacarb, so-called DCJW, is known to block the sodium channel of the target's nerve cells and to inhibit sodium currents in cultured insect neurons (Tsurubuchi et al. 2001).

Choosing pesticides for controlling pests based on integrated pest management requires information on the efficacy and potential risks of insecticides in populations of insect pests and non-target organisms (Qu et al. 2014). Life table parameters are recommended as a means to assess the sublethal effects and toxicological studies at the population level (Stark & Rangus 1994; Stark & Banks 2003). However, potential sublethal effects of indoxacarb and beta-cypermethrin on *R. padi* are still unknown. The objective of this study was to evaluate the sublethal effects of indoxacarb and beta-cypermethrin on 2 successive generations of *R. padi*.

Materials and Methods

INSECT CULTURE

The relatively susceptible clone of *R. padi* was collected from Yangling, Shaanxi, in China. Insects were reared on wheat seedlings in the laboratory using the caging method at 25 ± 1 °C with a 16:8 h L:D pho-

toperiod and relative humidity >80%. The colony had been reared in the laboratory for more than 3 yr.

CHEMICALS

Two insecticides were used in the present study: 96% beta-cypermethrin (Yancheng Nongbo Bio-technology Co. Ltd., Jiangsu, China) and 95% indoxacarb (Huzhou Nanxun Nonghuida Chemical Co. Ltd., Jiangsu, China).

INSECTICIDE BIOASSAYS

Toxicity bioassays were conducted with apterous adult aphids by using the leaf dip method (Han et al. 2007). Serial dilutions (mg/L) of the active ingredient of the test compounds were prepared using 0.1% Triton X-100 surfactant in water. Wheat leaves with apterous adult aphids were dipped in the dilutions of those compounds for 10 s. After 10 s, the leaves were removed from the solution and residual solution droplets on the leaves were adsorbed off with clean, dry filter paper pieces. The treated leaves were then transferred to plastic Petri dishes lined with moistened filter paper. Three replicates of 30 to 50 aphids were used for each insecticide concentration and for each of 5 serial concentrations. Leaves dipped in 0.1% Triton X-100 were used as controls. During and after the treatment, aphids were kept at a constant temperature of 25 ± 1 °C with a photoperiod of 16:8 h L:D. At 24 h post-treatment, mortality was scored. Aphids failing to exhibit movement after a gentle probe were considered dead. Data were analyzed by probit analysis using SPSS software (SPSS Inc. 2008).

SUBLETHAL CONCENTRATIONS OF INDOXACARB AND BETA-CYPERMETHRIN

According to the results of the toxicity bioassays, sublethal LC10 and LC30 concentrations of beta-cypermethrin were used at 0.39 and 0.92 mg/L, respectively, and those of indoxacarb were used at 0.14 and 0.42 mg/L, respectively. Wheat leaves with 100 to 120 aphids of mixed stages were dipped in LC10 and LC30 concentrations of indoxacarb and beta-cypermethrin for 10 s and the control, as described above. Twenty-four h after treatment, 30 survivors of wingless 4th instar nymphs or newly molted adults that had not undergone reproduction were selected and transferred to fresh, untreated leaves placed in plastic Petri dishes lined with moistened filter paper. Each Petri dish received only 1 aphid. A high relative humidity was maintained in the plastic Petri dish by periodically adding water to the filter paper. Leaves were replaced on a daily basis until the death of the last individual. The development, survival, and fecundity of individual aphids were recorded every 12 h until death. Fifty F1 nymphs were randomly selected from each leaf of each treatment and moved to separate plastic Petri dishes containing leaves. The development, survival, and fecundity of F1 individual aphids were recorded as described above.

DATA ANALYSES

Data were corrected for control mortality using Abbott's formula (Abbott 1925) before analysis, and analyzed by probit analysis (Finney 1971) using the software SPSS (SPSS Inc. 2008). The raw life history data of all individuals of this study were pooled and analyzed according to the age-stage, two-sex life table and the method described by Chi & Su 2006. The development time, longevity, and fecundity, and their mean values and standard errors were estimated using the bootstrap method included in the computer program TWOSEX-MSChart (Chi 2005). Treatment means were compared statistically using analysis of variance (ANOVA) and separated, whenever appropriate, by the Tukey

test at the $P = 0.05$ level in SPSS (SPSS Inc. 2008). Population parameters were also calculated, and their means, standard errors, and significance differences were estimated with the bootstrap technique using TWSEX-MSChart (Chi 2005). Means were compared using ANOVA and separated, whenever appropriate, using the Tukey test ($P < 0.05$) in SPSS (SPSS Inc. 2008).

Results

TOXICITY BIOASSAY

The results of the leaf dip method with 95% confidence limits were obtained for apterous adults of *R. padi* at 24 h after treatment (Table 1). The LC10 and LC30 values of indoxacarb were 0.14 and 0.42 mg/L, respectively, after 24 h. The LC10 and LC30 values of beta-cypermethrin were 0.39 and 0.92 mg/L, respectively, after 24 h. For both insecticides, the LC10 and LC30 values were used to evaluate the sublethal effects on *R. padi* (Table 1).

SUBLETHAL EFFECTS TO PARENTS (F0 GENERATION) OF *R. PADI*

The fecundity and longevity of adults of *R. padi* were significantly affected by the various beta-cypermethrin and indoxacarb concentrations that were evaluated (Table 2). The fecundity and longevity of *R. padi* exposed to B-LC30 were lowest. There were significant differences between the 4 treatments and the control group in mean longevity and reproduction of aphids ($F = 13.15$; $df = 4,145$; $P < 0.001$ and $F = 23.55$; $df = 4,145$; $P < 0.001$, respectively). Mean adult longevity of the *R. padi* F0 generation decreased by 50.75, 68.96, 30.68, and 40.61%, whereas mean fecundity of the *R. padi* F0 generation decreased by 65.93, 76.67, 47.70, and 54.30% after treatment with B-LC10, B-LC30, I-LC10 and I-LC30, respectively.

SUBLETHAL EFFECTS TO THE SUBSEQUENT (F1) GENERATION OF *R. PADI*

Development times of 1st to 4th instar nymphs were compared between all treatments (Fig. 1). The total development time of nymphs treated with sublethal concentrations of both insecticides was significantly longer compared with the control treatment ($F = 12.14$; $df = 4,240$; $P < 0.001$) (Fig. 1). Compared with the control, the B-LC10, B-LC30, I-LC10 and I-LC30 treatments significantly increased the average development period of the nymphal stage of the *R. padi* F1 generation by 0.86, 0.67, 0.45, and 0.46 d, respectively (Fig. 1). Development time of 1st instar nymphs was significantly affected by beta-cypermethrin treatments and I-LC30 treatment, but no differences were noted between the I-LC10 treatment and the control group ($F = 36.05$; $df = 4,245$; $P < 0.001$) (Fig. 1). Development time of 2nd instar nymphs was not significantly affected by beta-cypermethrin and indoxacarb, except

Table 1. Toxicity of beta-cypermethrin and indoxacarb to apterous adults of *Rhopalosiphum padi*.

Insecticide	n^a	Slope \pm SE	χ^2	df	Concentration (95% CI)
					(mg/L)
Beta-cypermethrin	658	2.06 \pm 0.17	2.13	3	LC10 = 0.39 (0.32–0.46)
					LC30 = 0.92 (0.81–1.08)
Indoxacarb	616	1.64 \pm 0.18	0.36	3	LC10 = 0.14 (0.07–0.24)
					LC30 = 0.42 (0.27–0.58)

^a Number of apterous adult aphids tested.

Table 2. Effect of exposure of parent adults (F0 generation) of *Rhopalosiphum padi* to beta-cypermethrin (B) and indoxacarb (I) at LC10 and LC30 on their fecundity and longevity (mean \pm SE).

Treatment	Adult longevity (d)	Number of offspring per female
Control	19.33 \pm 1.13a	51.57 \pm 3.16a
B-LC10	9.52 \pm 1.50bc	17.17 \pm 3.17bc
B-LC30	6.00 \pm 1.54c	12.03 \pm 3.47c
I-LC10	13.40 \pm 1.09b	26.97 \pm 2.41b
I-LC30	11.48 \pm 1.50b	23.57 \pm 3.42bc

Data followed by the same lower case letter in the same column were not significantly different based on Tukey's tests ($P > 0.05$).

for the I-LC30 treatment, in which development time was shorter than in other treatments ($F = 5.85$; $df = 4,238$; $P < 0.001$) (Fig. 1). Development time of 3rd instar nymphs was significantly different between the I-LC10 treatment and the other treatments ($F = 3.66$; $df = 4,238$; $P = 0.006$). The total development time of 4th instar nymphs was significantly prolonged in indoxacarb treatments ($F = 21.73$; $df = 4,235$; $P < 0.001$). The B-LC30 treatment exerted significant effects on the longevity and reproduction of the *R. padi* F1 generation compared with the control treatment (Table 3) ($F = 2.65$; $df = 4,238$; $P = 0.034$ and $F = 9.02$; $df = 4,233$; $P < 0.001$, respectively).

Age-specific survival rate (lx) and age-specific fecundity of the total population (mx) were also measured (Fig. 2). The curves of lx in the B-LC30 treatment group declined significantly from day 6. However, the curves of lx in other treatment groups declined significantly from day 23. The mx curve of the treatment groups is consistent with that of the control group, wherein all curves display a tendency of ascending first around the day 4 and going up and down in succession.

SUBLETHAL EFFECTS ON LIFE TABLE PARAMETERS OF F1 OF *R. PADI*

The effects of beta-cypermethrin and indoxacarb on the life table of the F1 population of *R. padi* are shown in Table 4. Compared with the control treatment, the intrinsic rate of increase (r) decreased for all treatments, except for the LC10 concentration of the indoxacarb treatment ($F = 16.72$; $df = 4,245$; $P < 0.001$) (Table 4). The finite rate of increase (λ) was 1.527, 1.432, 1.422, 1.491, and 1.474 d^{-1} for control, B-LC10, B-LC30, I-LC10, and I-LC30 treatments, respectively, and significantly decreased after beta-cypermethrin and indoxacarb treatment ($F = 23.09$; $df = 4,245$; $P < 0.001$). In terms of gross reproductive rate (GRR), the beta-cypermethrin treatments had negative effects (53.76 and 51.03 offspring per individual for B-LC10 and B-LC30, respectively), as compared with the other treatment (61.37, 59.45, and 59.57 offspring per individual for control, I-LC10 and I-LC30, respectively) ($F = 16.06$; $df = 4,245$; $P < 0.001$). The net reproductive rate (R_0) of *R. padi* was significantly depressed in the LC30 concentration of the indoxacarb treatment (Table 4). The mean generation time (T) in the control was significantly shorter than in the insecticide treatments ($F = 4.45$; $df = 4,245$; $P = 0.0017$).

Discussion

In this study, indoxacarb and beta-cypermethrin were similarly effective on apterous aphids, but sublethal effects of both insecticides on *R. padi* varied, possibly due to the different mechanisms of action of beta-cypermethrin and indoxacarb. Beta-cypermethrin behaves as a fast-acting neurotoxin in insects (Song et al. 2013), whereas indoxacarb blocks the insect voltage-gated sodium channel by the N-decarbome-

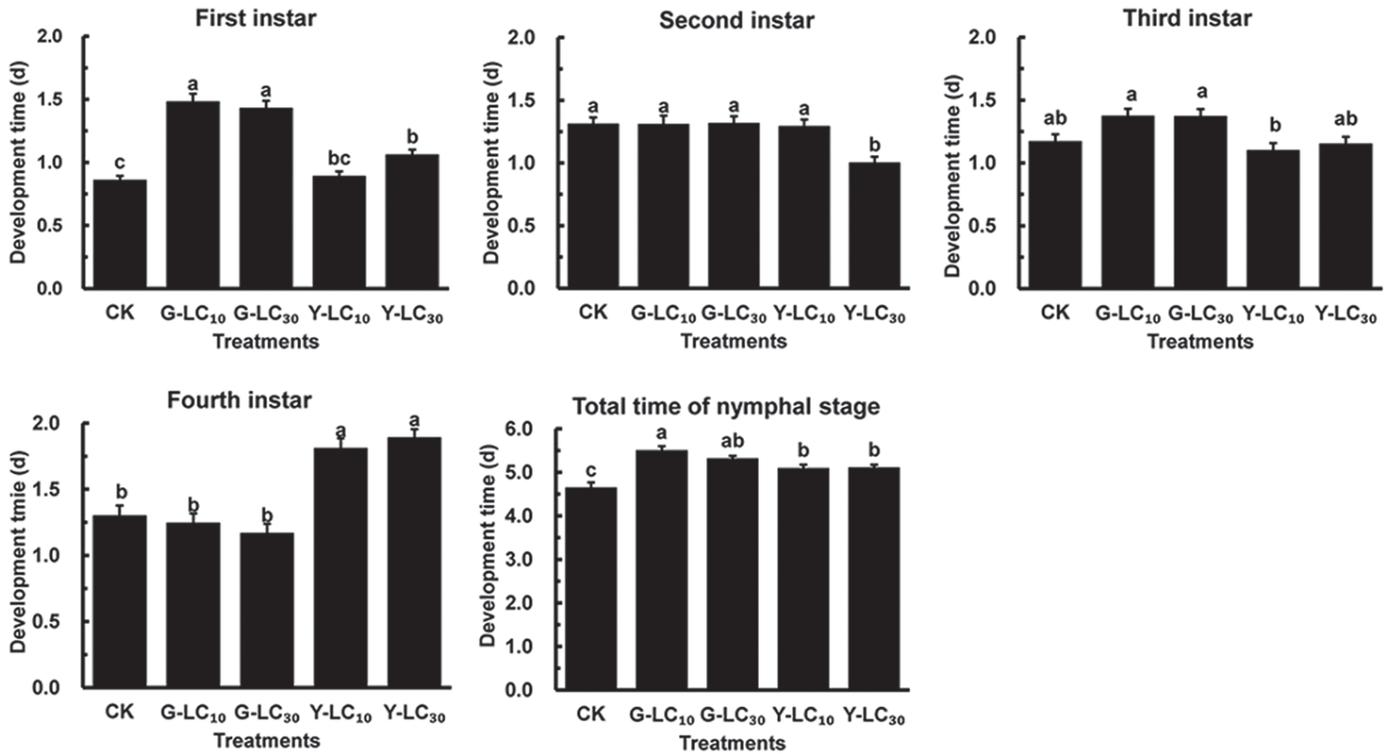


Fig. 1. Comparisons of development times of nymphs (mean ± SE) across all treatments (different letters indicate significant differences between populations at the $P < 0.05$ level). CK: control treatment; B-LC10: LC10 of beta-cypermethrin; B-LC30: LC30 of beta-cypermethrin; I-LC10: LC10 of indoxacarb; I-LC30: LC30 of indoxacarb.

thoxylated metabolite (Wing et al. 2000). Indoxacarb is a new insecticide not only with strong field activity against Lepidoptera but also with high efficiency in controlling certain important sucking insects such as leafhopper species and aphids (Wing et al. 2000). Results of this study support the claim that indoxacarb can be used as a substitute for older insecticide classes to manage populations of *R. padi*.

In addition to direct mortality induced by pesticides, sublethal concentrations of insecticides may cause behavioral and physiological effects on the target pest (Desneux et al. 2007). Increased fecundity and reproduction after exposure to sublethal concentrations of insecticides have been reported (Morse 1998; Zanoncio et al. 2003; Cutler et al. 2009; Cho et al. 2011). This may be one of the mechanisms of pest resurgence (Cutler et al. 2009; James & Price 2002). Insect pest resurgence induced by sublethal effects of insecticides could lead to the application of additional pesticide treatments, thereby potentially exacerbating insecticide resistance development (Qin et al. 2013). However, our study found no clear evidence for stimulation of growth or reproduction in *R. padi* following the application of the sublethal

concentrations of the evaluated insecticides. The *R. padi* population did not increase, at least in the F1 generation, after application of sublethal concentrations of beta-cypermethrin and indoxacarb. On the contrary, longevity and fecundity of parent adults of *R. padi* significantly decreased after treatment. The decreased longevity and fecundity of female parent adults was similar to the result obtained in the cabbage aphid, *Brevicoryne brassicae* (L.) (Homoptera: Aphididae), treated with imidacloprid and pymetrozine (Lashkari et al. 2007) and the pea aphid, *A. pisum*, treated with imidacloprid (Hui et al. 2009). The reduced fecundity of *R. padi* observed in our study might be due either to a shorter life span or to detrimental effects on the female's sexual organs or ovipositing mechanism; however, mechanisms involved were not studied here. Longevity and F1 generation were also affected by sublethal

Table 3. Effect of exposure of parent adults of *Rhopalosiphum padi* to beta-cypermethrin (B) and indoxacarb (I) at LC10 and LC30 on fecundity and longevity (mean ± SE) of the F1 generation of *R. padi*.

Treatment	Life span (d)	Number of offspring per female
Control	25.16 ± 0.93ab	55.88 ± 3.41a
B-LC10	23.28 ± 1.39ab	46.37 ± 2.24ab
B-LC30	20.57 ± 1.64b	37.13 ± 2.93b
I-LC10	25.76 ± 0.89a	48.96 ± 3.40a
I-LC30	23.73 ± 1.13ab	48.86 ± 3.85a

Data followed by the same lower case letter in the same column were not significantly different based on Tukey's tests ($P > 0.05$).

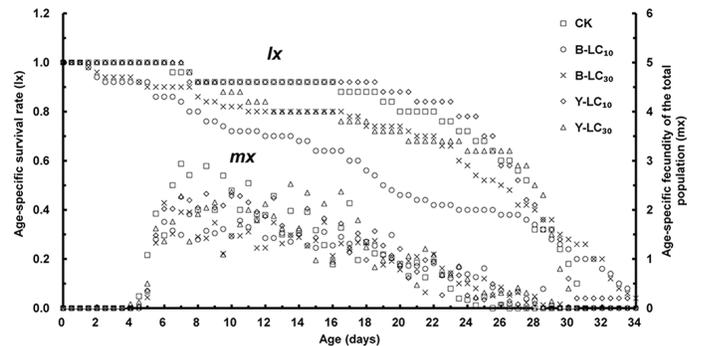


Fig. 2. Effects of beta-cypermethrin and indoxacarb sub-lethal concentrations on age-specific survival rate and age-specific fecundity of *Rhopalosiphum padi* F1 generation. CK: control treatment; B-LC10: LC10 of beta-cypermethrin; B-LC30: LC30 of beta-cypermethrin; I-LC10: LC10 of indoxacarb; I-LC30: LC30 of indoxacarb.

Table 4. Effect of exposure of parent adults of *Rhopalosiphum padi* to beta-cypermethrin (B) and indoxacarb (I) at LC10 and LC30 on various population parameters (mean \pm SE) of the F1 generation of *R. padi*.

Treatment	Intrinsic rate of increase (r) (d^{-1})	Finite rate of increase (λ) (d^{-1})	Net reproduction rate R_0 (offspring per individual)	Mean generation time (T) (d^{-1})	Gross reproduction rate GRR (offspring per individual)
Control	0.4235 \pm 0.0053a	1.527 \pm 0.0081a	55.88 \pm 2.280a	9.51 \pm 0.103b	61.37 \pm 1.10a
B-LC10	0.3593 \pm 0.0083c	1.432 \pm 0.0001c	41.82 \pm 2.759bc	10.39 \pm 0.165a	53.76 \pm 1.02b
B-LC30	0.3517 \pm 0.0094c	1.422 \pm 0.0133c	33.42 \pm 3.031c	9.98 \pm 0.158ab	51.03 \pm 1.35b
I-LC10	0.3994 \pm 0.0050ab	1.491 \pm 0.0074b	54.44 \pm 2.158a	10.01 \pm 0.096ab	59.45 \pm 0.95a
I-LC30	0.3883 \pm 0.0070b	1.474 \pm 0.0103b	48.96 \pm 2.579ab	10.02 \pm 0.195ab	59.57 \pm 1.04a

Data followed by the same lower case letter in the same column were not significantly different based on Tukey's tests ($P > 0.05$).

concentrations of beta-cypermethrin and indoxacarb, particularly by the B-LC10 treatment, which significantly reduced the longevity and fecundity of the aphid. These results may play an important role in the control of aphid pests by prolonging the efficacy of beta-cypermethrin and indoxacarb throughout the growing season.

The various insecticide treatments had different sublethal effects on the aphids. The differences were possibly caused by the different mechanisms of action of beta-cypermethrin and indoxacarb. Additionally, differences in residue levels in the bodies of mother aphids could also influence the results. Beta-cypermethrin treatments had greater influence on the development time of 1st instar nymphs than indoxacarb treatments. It is possible that beta-cypermethrin may suppress embryogenesis indirectly through adults and more effectively than indoxacarb (Tan et al. 2012). It is therefore conceivable that the sublethal effects observed on nymphs of the F1 generation may be due to penetration of the insecticides into the embryo.

Life table assays provided more detailed information on the side effects of the insecticides evaluated. Application of beta-cypermethrin and indoxacarb to parent aphids reduced r , R_0 , and GRR of the F1 generation and prolonged T of the F1 generation in this study (Table 4). This finding is in agreement with results by Song et al. (2013) with *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) after treatment with beta-cypermethrin and indoxacarb.

This study also showed that aside from lethal effects, beta-cypermethrin and indoxacarb cause multiple sublethal effects. Beta-cypermethrin and indoxacarb not only had marked effects on parent adults of *R. padi* but also had adverse effects on development and reproduction of individuals in the F1 generation. These findings indicate that indoxacarb could be used as an alternative or rotational pesticide option targeting pyrethroid-resistant *R. padi* populations in the field. It not only has a unique mechanism of action but also does not seem to stimulate aphid reproductive potential, potentially resulting in good control of *R. padi*. However, further studies are necessary to investigate the possible effect on digestion and ovaries and the potential mechanisms of physiological and behavioral effects by pesticides.

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