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Sublethal effects of indoxacarb, imidacloprid and deltamethrin on life table parameters of *Habrobracon hebetor* (Hymenoptera: Braconidae) in pupal stage treatment

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Abstract: *Habrobracon hebetor* Say (Hymenoptera: Braconidae) as an ectoparasitoid of larval stage of lepidopterous pests is widely used in biological control programs. In the present research, the effect of field recommended doses of imidacloprid, indoxacarb and deltamethrin were studied on life table parameters of *H. hebetor* in pupal stage treatment. One hundred, two-day-old pupae were treated with 1 micro liter insecticide solution using topical method. The pupae were treated with acetone in the control. Thirty emerged adults in each treatment were transferred individually to a Petri dish along with a male for mating. Three last instar larvae of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) were presented to each female wasp daily as host. The numbers of eggs produced per female per day were counted until all of the females were dead. The gross and net reproductive rates in control, imidacloprid, indoxacarb and deltamethrin were 204.6, 207.7, 209.1 and 112.1 and also 75, 41.3, 64.6 and 14.9, respectively. Intrinsic rates of increase were estimated to be 0.215, 0.154, 0.205 and 0.14 female offspring/female/day, respectively. Deltamethrin and imidacloprid had the most adverse effects on life table parameters of *H. hebetor*. Intrinsic rate of increase was not significantly affected by indoxacarb. These findings indicated that indoxacarb was relatively safe for *H. hebetor* and could be an appropriate candidate in integrated chemical and biological control.

Keywords: Life table parameters, demography, insecticides, natural enemy

Introduction

The application of insecticides is the most important factor disrupting biological control of insect pests in most agricultural systems (Croft, 1990). Arthropod natural enemies are commonly more susceptible to insecticide applications compared to target pests. Integrated pest management systems attempt to use natural enemies besides insecticides for pest control. Integrating the application of insecticides and biocontrol agents for pest management requires knowledge about impacts of insecticides on natural enemies (Croft, 1990; Dent, 1995; Banks and Stark, 1998). *Habrobracon hebetor* is used as effective parasitoid of different lepidopteran pests on field crops and stored products (Navaei *et al.*, 2002).

Habrobracon hebetor has been studied as a biocontrol agent of lepidopteran pests in some countries (Gerling, 1971; Brower and Press, 1990; Youm and Gilstrap, 1993; Magro and para, 2001; Abdi-Bastami *et al.*, 2011). Also,

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the mass rearing of *H. hebetor* has been initiated in Iran and it has been used to control *Helicoverpa* sp. and *Ostrinia nubilalis* (Hübner) (Navaei *et al.*, 2002). Little information is available on lethal and sublethal effects of commonly used insecticides on *H. hebetor* (Rafiee-Dastjerdi *et al.*, 2008, 2009a, 2009b, Mahdavi *et al.*, 2011). To understand the overall effects of insecticides on organisms it requires not only an estimation of lethal concentration of insecticides, but also evaluation of their sublethal effects (Walthal and Stark, 1996; Stapel *et al.*, 2000; Stark and Banks, 2003). Ecotoxicology especially demographic toxicology is usually considered as the best way to evaluate effects of insecticides on organisms. The parameters defined for the stable population have been recommended to evaluate effects of pesticides, because it is based on both survivorship and fecundity parameters (Stark and Wennergren, 1995). There is less knowledge about sublethal effects of chemical insecticides on predators and parasites compared with pest arthropods (Croft, 1990). In present study, sublethal effects of imidacloprid, indoxacarb and deltamethrin were assessed on ectoparasitoid, *H. hebetor*.

Materials and Methods

Insects' resources

Adults of *H. hebetor* were obtained from an insectarium maintained by Plant Protection Bureau of Bilehsavar in Ardabil province, Iran. *Habrobracon hebetor* was reared on fifth instar larvae of *Anagasta kuehniella* in the laboratory. The colony of *A. kuehniella* was reared on wheat flour in plastic boxes (40 × 25 × 15 cm). Fifth instar larvae of *A. kuehniella* were used for all experiments and rearing the colony. Conditions for rearing and bioassay were 26 ± 1 ˚C, 60 ± 5 % RH and a photoperiod of 12: 12 h (L: D).

Insecticides

The effect of imidacloprid (Confidor® 350 SC, Gyah company, Iran), indoxacarb (Avaunt® 150 SC, DuPont company, France) and deltamethrin (Decis® 2.5 EC, Gyah company, Iran) on life table parameters of *H. hebetor* in pupal stage treatment was determined.

Biological and life table parameters

Pupae were treated with aqueous solutions of field recommended doses of imidacloprid, indoxacarb, and deltamethrin by topical method (290, 125, and 83 ppm based on active ingredient, respectively). Triton X-100 was used as the surfactant at a concentration of 555 ppm in the experiment. The controls were treated with distilled water plus Triton X-100. The treated pupae were transferred to 90 mm diameter Petri dishes. After the emergence of adult wasps, 60 females and 60 males were let to mate for 24 h in glass tubes. After 24 h, 30 alive females were randomly selected and transferred individually to plastic Petri dishes (60 mm in diameter). Each female wasp was presented three pyralid larvae and provided with honey. The host larvae were supplied daily for wasp oviposition in new Petri dishes. The survival of each individual female wasp and its fecundity was recorded daily. Females were moved to new Petri dishes every 24 h to determine daily and lifetime fecundity (the number of eggs laid by a female wasp daily and over her lifetime). Daily schedules of mortality and fecundity were integrated into a life table format (Carey, 1993) and used to calculate life table parameters. All demographic parameters were analyzed by the jackknife technique (Meyer *et al.*, 1988) to calculate the standard error as a measure of variance for these demographic parameters. The parameters were analyzed using SAS for Windows® release 9.0 (SAS Institute, 2002).

Results

Biological parameters of females emerged from treated pupa and their progeny

The longevity and fecundity data of female wasps of *H. hebetor* emerged from pupa exposed to the field recommended dose of the used insecticides were recorded and are presented in Table 1. Laboratory exposure of pupae to the field recommended

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concentrations of the insecticides significantly affected female longevity (P < 0.05) and the emerged female wasps from the treated pupae with deltamethrin showed the shortest longevity (Table 1). However two other insecticides did not significantly affect the female longevity. Similarly, the fecundity, i.e., the total number of eggs per female during its life span, was affected significantly (P < 0.05). The parasitoids exposed to recommended dose of deltamethrin produced less eggs (98.08 eggs) than the control parasitoids (430.60 eggs), whereas the difference between the indoxacarb and imidacloprid-treated and control parasitoids was not significant (Table 1). The highest and lowest egg survivorship was recorded in control (0.98) and deltamethrin treatment (0.91), respectively. No significant difference was observed between in sex ratio of offsprings in the three insecticide treatments and control (Table 1).

Life table parameters of offspring

The life table parameters of the *H. hebetor* females emerged from treated pupae are shown in Table 2. The gross reproductive rate (*GRR*) was significantly affected by insecticide treatments and the lowest *GRR* value was detected in deltamethrin treatment (Table 2) (F = 5.04; df = 3; P = 0.01). The highest *GRR* value was on control; however it was not significantly influenced by imidacloprid and indoxacarb compared to control. The female offspring from treated and control pupae also showed significant difference in net reproductive rate value (R_0). The R_0 value in deltamethrin treatment (14.9) was significantly lower than that of control and the two other insecticide treatments. The respective descending order of R_0 value was in control, indoxacarb, imidacloprid and deltamethrin treatments. The intrinsic rate of increase (r_m) was strongly affected in offspring from the treated pupae with recommended doses and there were two statistical groups for intrinsic rate of increase (Table 2). In control and offspring from the pupae treated with indoxacarb, imidacloprid and deltamethrin, the population increased daily by 1.24, 1.23, 1.17 and 1.15 times, respectively. The mean generation time (*T*) was also found to be significantly different between treatments and the longest mean generation time was observed in imidacloprid (Table 2). In addition, the doubling time (*DT*) significantly differed among treatments and its value in deltamethrin and imidacloprid treatments was higher than those observed in indoxacarb and control (Table 2).

Table 1 Biological parameters (mean ± SE) of *Habrobracon hebetor* females emerged from the pupae treated by the field recommended doses of insecticides.

Parameters Treatments	Female longevity (day)	Fecundity (egg number)	Sex ratio	Egg survival (%)
Control	29.56 ± 2.34^a	430.60 ± 56.02^a	0.57 ± 0.03^a	0.98
Indoxacarb	31.24 ± 2.27^a	397.56 ± 54.75^a	0.41 ± 0.05^a	0.97
Imidacloprid	24.18 ± 3.03^a	325.45 ± 62.55^a	0.47 ± 0.04^a	0.96
Deltamethrin	11.37 ± 1.85^b	98.08 ± 22.14^b	0.48 ± 0.05^a	0.91

Values within each column followed by different letters are significantly different (Tukey test, P < 0.05)

Table 2 The life table parameters (mean ± SE) of *Habrobracon hebetor* exposed to the field recommended doses of insecticides in pupal stage.

Parameters Treatments	*GRR*	R_0	r_m	λ	*T*	*DT*
Control	204.62 ± 13.97[a]	75.03 ± 6.08[a]	0.215 ± 0.003[a]	1.240 ± 0.004[a]	20.03 ± 0.21[b]	3.21 ± 0.05[b]
Indoxacarb	209.09 ± 24.71[a]	64.56 ± 8.33[ab]	0.205 ± 0.005[a]	1.228 ± 0.006[a]	20.25 ± 0.40[b]	3.37 ± 0.09[b]
Imidacloprid	207.67 ± 29.63[a]	41.27 ± 8.37[b]	0.154 ± 0.009[b]	1.166 ± 0.010[b]	24.01 ± 0.76[a]	4.51 ± 0.27[a]
Deltamethrin	112.06 ± 11.87[b]	14.90 ± 3.57[c]	0.140 ± 0.010[b]	1.151 ± 0.010[b]	19.00 ± 0.24[b]	4.96 ± 0.44[a]

Values within each column followed by different letters are significantly different (Tukey test, $P < 0.05$); *GRR*: Gross reproductive rate, R_0: Net reproductive rate, r_m: Intrinsic rate of population increase, λ: Finite rate of population increase, *T*: Generation time, *DT*: Doubling time

Discussion

The pests and their natural enemies usually occur in fields simultaneously, so natural enemies would be subjected to insecticide applications and acquire toxicant by direct contact, consuming contaminated food and walking over surfaces that have pesticide residues. For successful implementation of an IPM program specially an integration of biological and chemical control, an understanding of the effects of pesticides on biocontrol agents and insecticides compatibility would be valuable tools (Croft, 1990). Hence, toxicological studies on natural enemies are necessary (Stark *et al*., 2004). Natural enemies surviving after an exposure to a pesticide dose may suffer sublethal effects that influence their biological parameters like fecundity, longevity, life span, sex ratio or behavioral parameters like host searching ability, subsequently reduce their effectiveness as biocontrol agents, resulting in decreased pest control (Croft, 1990; Stark *et al.*, 1995; Staple *et al*., 2000; Salerno *et al.*, 2002, Stark and Banks, 2003; Desneux *et al.*, 2007; Hamedi *et al.,* 2010; Hamedi *et al.*, 2011).

Different life stages of natural enemies are target to insecticides, hence all stages of natural enemies should be considered in researchers' studies. *H. hebetor* is a very important control agent of lepidopterous pests in a variety of agricultural crops and stored products. It is an ectoprasitoid, hence all its developmental and adult stages are subjected to insecticides. There are some investigations on sublethal effects of insecticides on *H. hebeotr* (Rafiee *et al.*, 2008; 2009a; Sarmadi *et al.*, 2010, Mahdavi *et al.*, 2011), and in related studies mostly adult wasps have been used while other stages like pupae may also be affected. The results of the present study showed the remarkable sublethal effects of insecticides on females emerging from the treated pupae and their progeny. Application of recommended concentrations of deltamethrin on pupal stage had a considerable effect on the fecundity and longevity of emerged females and that of their progeny (Table 2). The results about deltamethrin effects on female's fecundity of the present study were in agreement with the findings reported by Sarmadi *et al.* (2010). They concluded that fecundity of treated adult females with deltamethrin was significantly decreased compared with control; but the adult female longevity was not. This finding is in disagreement with our results. The different reactions of pupa and adult could be attributed to differences in their physiological status, subsequently their different sensitivity. Importantly, the female longevity and fecundity were not affected by two other tested insecticides, indoxacarb and imidacloprid. These findings reveal that the latter two insecticides may be relatively compatible with *H. hebetor*.

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Determining the life table parameters has been considered as a better measure of response to insecticides (Forbes and Calow, 1999). In our study, the life table parameters showed significant differences in performance and population growth parameters among the treated and untreated females of *H. hebetor* (Table 2). Lower female's fecundity in deltamethrin treatment resulted lowest gross and net fecundity among the treatments. In all treatments, the net reproductive rates (R_0) were considerably lower than the gross reproductive rates (*GRR*) indicating that the survivorship (*lx*) was strongly affected by insecticides. The highest mean generation time (*T*) was observed at imidacloprid treatment. It would be harmful effect on parasitoid, if an insecticide causes an increase in its generation time. The mean generation time was not affected by deltamethrin and indoxacarb compared with control. Among the life table parameters, the intrinsic rate of increase (r_m) has been recommended to be used for evaluating the sublethal effects of pesticides, because it reflects the overall effects on both survivorship and fecundity (Stark and Wennergren, 1995). Higher intrinsic rate of increase (r_m) in control compared with the insecticide treatments indicated the adverse effects of insecticides on this parameter. The considerable reduction value of R_0 in females treated with deltamethrin followed by a great reduction in r_m value. Moreover, the longest mean generation time (*T*) in imidacloprid treatment caused that r_m values between imidacloprid and deltamethrin treatments were not significantly different. Sarmadi *et al*., (2010) studied the population parameters of *H. hebetor* when adult females were treated with the same insecticides and reported that r_m values were reduced in imidacloprid and deltamethrin treatments. These results were relatively in agreement, but the r_m value in indoxacarb treatment was significantly lower than control in their study. In our study the r_m value was not significantly affected by indoxacarb. These findings indicated that indoxacarb is relatively safe for *H. hebetor* and could be an appropriate candidate in integrating chemical and biological control in an IPM program.

In conclusion, this research showed that the three used insecticides had sublethal effects on *H. hebetor*. Deltamethrin and imidacloprid negatively affected female wasps' performance and population growth. But Indoxacarb caused no major noxious effect on biological parameters and all measured population parameters compared with control. Therefore, indoxacarb has been considered as IPM-compatible insecticide due to its relatively low negative effects on the effectiveness of *H. hebetor*. Such information can be used to predict the potential of indoxacarb in combination with *H. hebetor* in an integrated pest management program. After laboratory studies, further attention should be devoted on semifield and field experiments to find more applicable results under field conditions.

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اثرات زیرکشندگی ایندوکساکارب، ایمیداکلوپرید و دلتامترین روی پارامترهای جدول زندگی زنبور پارازیتوئید *Habrobracon hebetor* (Hymenoptera: Braconidae) در تیمار مرحله شفیرگی

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چکیده: زنبور *Habrobracon hebetor* Say پارازیت خارجی لاروهای تعداد زیادی از بال‌پولکدارن آفت می‌باشد که در برنامه‌های کنترل بیولوژیک استفاده وسیعی دارد. در مطالعه حاضر اثرات دز توصیه شده مزرعه‌ای حشره‌کش‌های ایندوکساکارب، ایمیداکلوپرید و دلتامترین روی پارامترهای جدول زندگی زنبور پارازیتوئید *H. hebetor* در تیمار مرحله شفیرگی مورد بررسی قرار گرفت. ۱۰۰ شفیره دو روزه هر کدام با یک میکرولیتر از محلول‌های سمی به‌روش موضعی مورد تیمار قرار گرفتند. شاهد تنها با استون تیمار شد. تعداد ۳۰ عدد زنبور کامل ماده ظاهر شده در هر تیمار انتخاب و به‌همراه یک حشره نر به‌صورت جداگانه به داخل ظروف پتری پلاستیکی منتقل شدند. در هر روز تعداد ۳ عدد لارو سن آخر بید آرد *Anagasta kuehniella* (Zeller) در اختیار هر زنبور قرار گرفت. تعداد تخم‌های گذاشته شده به ازای هر ماده تا مرگ آخرین فرد شمارش شدند و تعداد تخم‌های تفریخ شده، تعداد لاروها، شفیره‌ها و حشرات کامل تولیدی در هر تیمار نیز مشخص شد. سپس جداول زندگی به روش کری (۱۹۹۳) تشکیل و پارامترهای جدول زندگی محاسبه شدند. دلتامترین و ایمیداکلوپرید بیشترین اثر سوء را روی پارامترهای جدول زندگی زنبور داشتند ولی ایندوکساکارب تأثیر معنی‌داری نداشت. با توجه به نتایج به‌دست آمده می‌توان گفت که ایندکساکارب برای زنبور نسبتاً امن بوده و می‌تواند در برنامه‌های مدیریت تلفیقی آفات به‌همراه زنبور پارازیتوئید *H. hebetor* مورد استفاده قرار گیرد.

کلمات کلیدی: جدول زندگی، دشمنان طبیعی، اثرات زیرکشندگی، دموگرافی

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