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Sublethal Effects of Some Conventional and Biorational Insecticides on Ectoparasitoid, *Habrobracon hebetor* Say (Hymenoptera: Braconidae)

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Abstract: This study was carried out to assess the effects of sublethal dose of profenofos, spinosad, thiodicarb and field recommended dose of hexaflumuron on demographic and biological parameters of *H. hebetor*. Gross reproductive rate in control (68.87) was significantly higher than insecticide treatments. The highest and the lowest gross reproductive rate between insecticides were related to the profenofos and spinosad, respectively. Higher intrinsic rat of increase in control (0.17) compared with insecticide treatments indicated harmful effects of insecticides on it. Hexaflumuron and spinosad had the highest (0.15) and the lowest (0.1) intrinsic rate of increase between insecticides, respectively. Number of laid eggs was significantly affected by insecticides and it was approximately 2 times more than insecticide treatments in control. In this study, hexaflumuron had tremendous sublethal negative effects on biological parameters of *H. hebetor* with no lethal effects on adult wasps. The female longevity in control (29.41) had no significant difference with the means of profenofos and hexaflumuron, but differences between spinosad and thiodicarb with control was significant. Spinosad had the lowest longevity (12.79). However hexaflumuron, profenofos and spinosad had lower generation time compared with control and thiodicarb but differences between treatments were not significant. Sex ratio of *H. hebetor* offsprings was significantly affected by insecticides. In control, it was lowest (39.23) which indicated that proportion of female to male was highest (≈2 time) and it was highest in spinosad (54.94) which means that spinosad caused higher male production in population. In all treatments, especially spinosad and thiodicarb, increase in female age caused increase in male production.

Key words: Population parameters, biological parameters, insecticides, *Habrobracon habetor*

INTRODUCTION

Insecticides are becoming increasingly harmful due to the development of resistance in many pests to the majority of the insecticides commonly used for their control (Biddinger and Hull, 1995; Guedes *et al*., 2006; Ahmad *et al*., 2007; Huang and Han, 2007). The most important factor disrupting biological control of arthropod pests in most cropping systems is the use of insecticides (Croft, 1990). Biological control agents are affected by insecticide applications. Integrated pest management systems have been increasing due to efficacy and applicability of natural enemies. Natural enemies are often

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small, with a short life cycle and a high reproductive potential. Integrating the application of the biocontrol agents and insecticides for pest management requires knowledge of the impact of the insecticides on the natural enemies and their selectivity on these (Croft, 1990; Dent, 1995; Banks and Stark, 1998).

Although the control of pests usually relied on insecticides but there are many natural enemies for biological control against lepidopteran pests. Among these, *Trichogramma* sp. and *Habrobracon* sp. have been used as common parasitoids showing ability to reduce pest populations significantly to low levels (Navaei *et al*., 2002). *Habrobracon hebetor* is a polyphagous ectoparasitoid that has been studied as a biological control agent for various lepidopteran pests in several countries (Gerling, 1971; Brower and Press, 1990; Youm and Gilstrap, 1993; Magro and Para, 2001). *Galleria mellonella* (L.) and *Anagasta kuehniella* Zeller are commonly used for mass rearing of *H. hebetor*; both are suitable for development and reproduction (Attaran, 1996; Amir-Maafi and Chi, 2006). In Iran, *H. hebetor* has been used to control *Helicoverpa* sp. and *Ostrinia nubilalis* and a mass rearing program has been initiated, but there is no information on lethal and sublethal effects of commonly used insecticides in cotton fields on this biocontrol agent (Navaei *et al*., 2002).

Lethal effects assays could not completely determine the effects of insecticides on organisms (Walthall and Stark, 1996). Also interest is in reducing of insecticides application rates. Thus, the evaluating sublethal effects of insecticides is very important (Stapel *et al*., 2000; Stark and Banks, 2003). Demographic toxicology is usually considered the best way to evaluate total effects of pesticides. Then, the parameter defined as the intrinsic rate of increase (r_m) has been recommended to evaluate total effects of pesticides, because it is based on both survivorship and fecundity parameters (Stark and Wennergren, 1995). This study was carried out at 2007-2008 to assess the sublethal effects of the insecticides to *H. hebetor* and to determine the possibility of integrating the application of this species with insecticides such as hexaflumuron, profenofos, spinosad and thiodicarb which are commonly used for pests' control.

MATERIALS AND METHODS

Biological Sources

Adults of *H. hebetor* were obtained from an insectarium maintained by Plant Protection Bureau of Bilehsavar in Ardabil Province, Iran and reared on 5th instars of *Anagasta kuehniella* in the laboratory. *Anagasta kuehniella* cultures were maintained in clear plastic boxes (40×25×15 cm) containing 1 kg of wheat flour. Fifth instars *A. kuehniella* were used for both colony maintenance and experiments. Rearing conditions were 26±2°C, 70±5% RH and a photoperiod of 16:8 h (L:D).

Insecticides

Insecticides tested were hexaflumuron (Consult® 10EC), spinosad (SpinTor® 25SC), profenofos (40EC) and thiodicarb (80DF).

Life Table Parameters Study

Exposure cages (100×100×10 mm) were used for experiments (Saber *et al*., 2005). Glass plates of cages were sprayed with 2 mL of aqueous solutions of LC_{25} of profenofos, spinosad, thiodicarb and field recommended dose of hexaflumuron using Potter Spray Tower (Burkard µfg, Co. ltd, uxbridge, UK). The operating pressure was 0.5 bar and the mean spray deposit was 1.68±0.04 µL cm^{-2}. The concentrations were 4.45, 5.04, 23.46 and 250 mg AI L^{-1}, respectively. Triton x100 was used as the surfactant at a concentration of 555 ppm in this experiment. The control plates were sprayed with distilled water plus Triton X-100. Since, adult wasps emerged, 50 female and 50 male *H. hebetor* were left to mate up to 24 h in glass tubes. Adults female were exposed to LC_{25} of profenofos, spinosad,

Table 1: Identification and formula of stable population parameters (Carey, 1993)

Parameters	Formula	Identification
GRR	Σm_x	The total number of daughters produced per cohort of females over their lifetimes
R_0 (NRR)	$\Sigma l_x m_x$	The mean number of daughters produced per cohort of females over their lifetimes
r_m	$1=\sum_{x=\alpha}^{\beta} e^{-rx} L_x m_x$	The maximum exponential rate of increase by a population growing within defined physical conditions
λ	$\lambda = e^r$	The ratio of population sizes at each time step
T	$T = (\ln R_0/r)$	The average interval separating the births of one generation from the births of the next
DT	$DT = (\ln 2/r)$	The time needed for a population to double in size from a fixed point in time

GRR: Gross reproductive rate, R_0 (NRR): Net reproductive rate, r_m: Intrinsic rate of population increase, λ: Finite rate of population increase, T: Generation time, DT: Doubling time

thiodicarb and field recommended dose of hexaflumuron. After 24 h, randomly selected 24 alive females were transferred individually to plastic Petri dishes (60 mm in diameter). Each female wasp was presented 3 pyralid larvae and provided with honey. Three host larvae were supplied daily for wasp oviposition in new Petri dishes. The daily survival of each individual and fecundity of each female were recorded. Females were moved to Petri dishes every 24 h for parasitoid life time to determine daily and lifetime fecundity (the number of eggs laid by a female wasp over her lifetime). Daily schedules of mortality and fecundity were integrated into a life table format (Carey, 1993; Table 1) and used to calculate life table parameters.

All demographic statistics were analyzed by the Jack-knife technique (Myer *et al*., 1988) to calculate the standard error as a measure of variance for these demographic parameters. Data were analyzed using the ANOVA procedures with SAS for Windows® release 9.0 (SAS Institute, 2002).

RESULTS

Gross reproductive rate (GRR) in control (68.87 females) was significantly higher than insecticide treatments. The highest and the lowest GRR between insecticides were related to the profenofos and spinosad, respectively (Table 2). Net reproductive rates (NRRs) were very lower from GRRs because of l_x interference. Control NRR (51 females) was higher than insecticide treatments. Hexaflumuron and spinosad had the highest and the lowest NRRs between insecticides, respectively. On the other hand, survivorship (l_x) was hardly affected by spinosad and was low, compared with other treatments (Fig. 1).

Insecticides had harmful effects on the daily female offspring (m_x) production by wasps (Fig. 2). Trends of m_x curves at different days shows alternately decreases and increases in female production that may be caused by females' energy resources production. Because of interference of age (X), survival rate (l_x) and female offspring's (m_x) in calculation of intrinsic rate of increase (r_m), it is most important parameter for evaluating of populations development. Higher r_m in control (0.17 day^{-1}) compared with insecticide treatments indicated harmful effects of insecticides on r_m. Hexaflumuron and spinosad had the highest (0.15 day^{-1}) and the lowest (0.1 day^{-1}) r_m between insecticides, respectively. Extreme increase rate (λ) indicator of population increase in each day compared with day before also was adversely affected by the insecticides. Similar to r_m, spinosad had the lowest rate of λ. There was no significant difference in generation time (T) between control, profenofos and thiodicarb. Hexaflumuron and thiodicarb had the lowest and the highest T, respectively. The lower T is an advantage for parasitoids compared with their hosts, because of generation number that they could produced on certain time. If insecticides cause increase in parasitoid generation time, it would be harmful effect on parasitoid. Doubling time (DT) is also important parameters that indicate when population would be double. DT was affected adversely by insecticides; it was lowest in control and highest in spinosad.

Table 2: The mean rates of stable population parameters (±SE) of adults *H. hebetor* treated with LC_{25} of profenofos, spinosad, thiodicarb, field recommended dose of hexaflumuron

Parameters	Control	Spinosad	Profenofos	Thiodicarb	Hexaflumuron	df	F-value
GRR	68.87±5.31^{a}	27.37±5.31^{c}	58.72±5.27^{ab}	30.93±4.32^{bc}	43.49±8.41^{abc}	4	9.09**
R_0 (NRR)	51.00±3.56^{a}	9.78±1.71^{c}	21.20±1.99^{bc}	16.39±1.94^{bc}	27.41±3.25^{b}	4	36.86**
r_m	0.17±0.004^{a}	0.10±0.01^{d}	0.13±0.002^{bc}	0.12±0.05^{cd}	0.15±0.006^{ab}	4	18.05**
λ	1.18±0.005^{a}	1.11±0.01^{c}	1.14±0.004^{bc}	1.12±0.006^{c}	1.17±0.007^{ab}	4	18.45**
T	22.68±0.46^{ab}	21.58±0.67^{ab}	22.28±0.5^{ab}	23.19±0.71^{a}	20.77±0.44^{b}	4	2.73*
DT	4.00±0.10^{a}	6.63±0.61^{c}	5.06±0.15^{ab}	5.77±0.26^{bc}	4.36±0.17^{ab}	4	4.36**

Values within rows followed by the different letters are significantly different based on the Tukey test. **and *indicate that the parameters means are significantly different at p = 1 and 5%, respectively. GRR: Gross reproductive rate, R_0 (NRR): Net reproductive rate, r_m: Intrinsic rate of population increase, λ: Finite rate of population increase, T: Generation time, DT: Doubling time

Table 3: The mean rates of biological parameters (±SE) of adults *H. hebetor* exposed to LC_{25} of profenofos, spinosad, thiodicarb and field recommended dose of hexaflumuron

Parameters	Control	Spinosad	Profenofos	Thiodicarb	Hexaflumuron	df	F-value
Eggs	306.29±31.13^{a}	114.95±31.12^{b}	172.66±24.45^{b}	155.33±22.98^{b}	162.66±21.62^{b}	4	7.47**
Hatched eggs	279.95±28.59^{a}	99.54±27.00^{b}	157.58±22.80^{b}	133.41±20.46^{b}	146.80±19.53^{b}	4	8.13**
Longevity	29.41±2.50^{a}	12.79±2.82^{b}	19.41±2.2^{ab}	16.70±2.23^{b}	19.75±1.914^{ab}	4	6.74**
Sex ratio	39.23±3.34	54.94±11.21	46.06±1.51	51.19±6.18	46.80±3.38	4	1.18^{ns}

Values within rows followed by the different letters are significantly different based on the Tukey test. **and ^{ns} indicate that the parameters means are significantly different at p = 1% and non significant, respectively. GRR: Gross reproductive rate, R_0 (NRR): Net reproductive rate, r_m: Intrinsic rate of population increase, λ: Finite rate of population increase, T: Generation time, DT: Doubling time

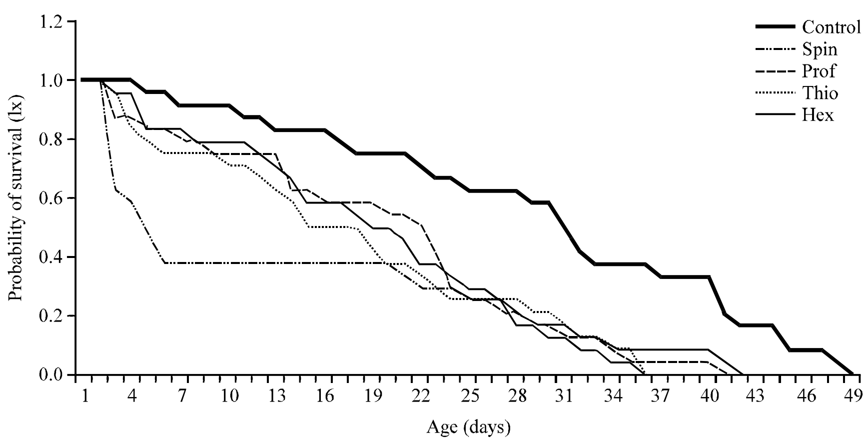

Fig. 1: Female survival (l_x) of adults *H. hebetor* treated with LC_{25} of profenofos, spinosad, thiodicarb and field recommended dose of hexaflumuron over their life time

Number of laid eggs was significantly affected by insecticides and it was approximately 2 times more than insecticide treatments in control which indicated very harm effects of sublethal doses on wasps (Table 3). O'Brien *et al.* (1985) reported that chlordimeform had very high LC_{50} on *Bracon mellitor* compared with azinphosmethyl but their sublethal adverse effects on the parasitoid fecundity were similar. In this study, hexaflumuron had hard sublethal negative effects on biological parameters of *H. hebetor* with no lethal effects on adult wasps. The mean of female life span in control (29.41 days) had no significant difference with the means of profenofos and hexaflumuron, but differences between spinosad and thiodicarb with control was significant. Spinosad had the lowest life span (12.79 days). Sex ratio (male/male+female) of *H. hebetor* offsprings was affected by insecticides, but their differences were not significant. In control, it was the lowest (39.23) which means that

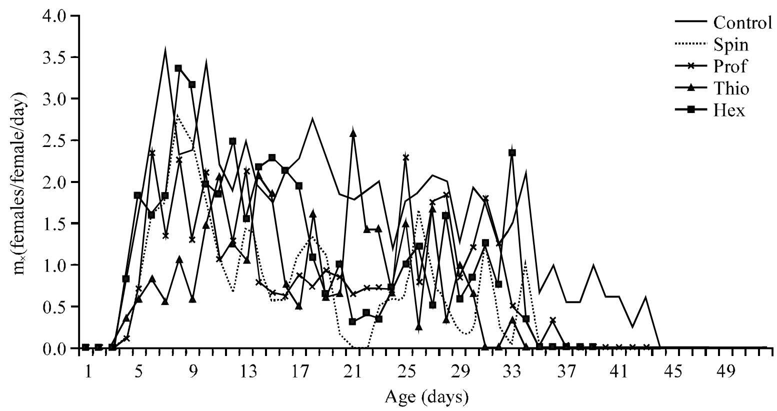

Fig. 2: Distribution of daily female offspring (m_x) of adults *H. hebetor* treated with LC_{25} of profenofos, spinosad, thiodicarb, field recommended dose of hexaflumuron and distillated water over their life time

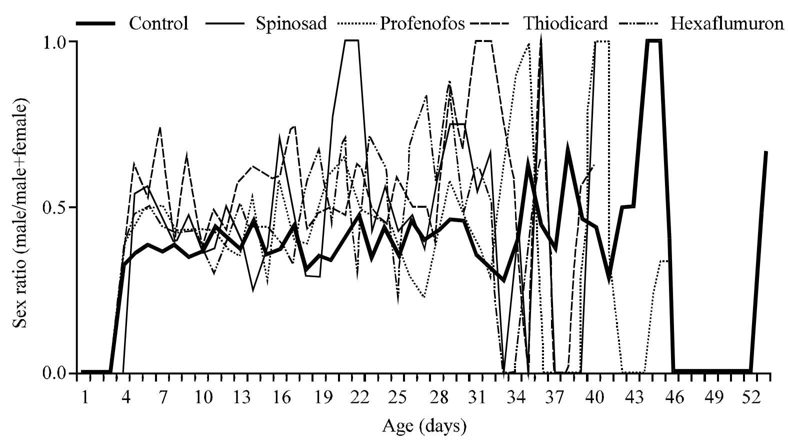

Fig. 3: Offspring sex ratio (male/male+female) of adults *H. hebetor* treated with LC_{25} of insecticides and distillated water

proportion of female to male was highest (≈2 time) and it was highest in spinosad (54.94) which means that spinosad caused higher male production in population. In all treatments, especially spinosad and thiodicarb, increase in female age caused increase in male production (Fig. 3).

DISCUSSION

In this study, stable population parameters such as gross and net reproductive rates, intrinsic and finite rates of population increase, generation time and doubling time, also biological parameters such as fecundity, fertility and longevity were significantly and adversely affected by insecticides. Only offspring's sex ratio was not significantly affected. Saber *et al*. (2005) have reported that fenitrothion and deltamethrin had no significant effects on biological parameters of *Trissolcus* wasps such as adult span and offspring sex ratio, but they adversely affected r_m and λ related to wasps' population. Currently, great efforts are towards a reduction in the application of pesticides and towards an increase in the use of integrated pest management (IPM) strategies.

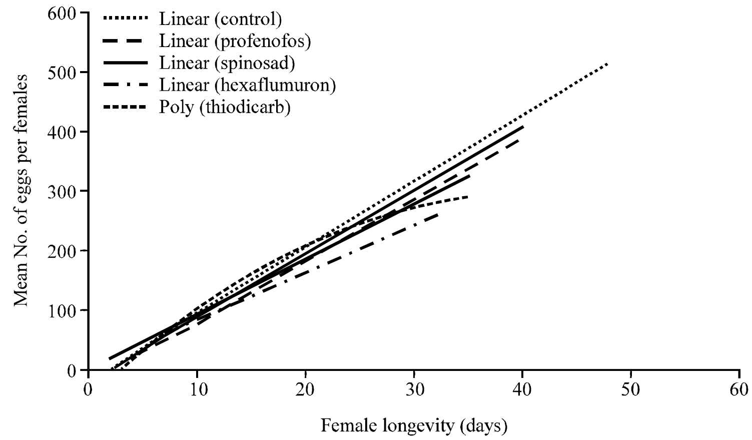

Fig. 4: Correlations between fecundity and longevity of adults *H. hebetor* treated with LC_{25} of insecticides and distillated water

Armenta *et al*. (2003) reported that orghanophosphate (chlorpyrifos and methamidophos) and carbamate (carbaryl) had adverse effects on insect natural enemies on maize in southern Mexico. In this study, organophosphate (profenofos) and carbamate (thiodicarb) had adverse sublethal effects on *H. hebetor*. Therefore, the search for pesticides that are compatible with IPM programs, such as benzoylphenil ureas (BPUs) is an interesting approach. BPUs inhibit chitin synthesis in a wide range of insect groups, resulting in abortive moulting. They act mainly as larvicides and ovicides (Retnakaran and Wright, 1987). Naranjo and Akey (2005) reported that the IGRs negatively affected four of 17 taxa of arthropod predators, However, the overall impact of the IGRs was less than that caused by either acetamiprid or mixtures of conventional materials.

In this study, recommended field rate of hexaflumuron had little effects on biological and demographical parameters of *H. hebetor*.

Spinosad is very effective insecticide against pests specially stored products pests (Nayak *et al*., 2005). Many data have been published on compatibility of spinosad with natural enemies. Toews and Subramanyam (2003), Maxwell and Fadamiro (2006) and Mendez *et al*. (2002) reported that application of reduced rate of spinosad (3 ppm) on *Spodoptera frugiperda* had very little effects on abundance of natural enemies on maize plants. Williams *et al*. (2003) indicated that spinosad was compatible with predators but it had adverse effects on parasitoids. But we found that spinosad was not safe to *H. hebetor*. In contrast, Cisneros *et al*. (2002) reported that spinosad could not be considered to have safety to some predators. Schneider *et al*. (2004) reported that, in sublethal concentrations, spinosad was harmful to *Hyposoter didymator* an important larval parasitoid of several lepidopteran pests. Also some researchers have reported that spinosad significantly decreased *C. insularis* and *Trichogramma* wasps' longevity (Suh *et al*., 2000; Penagos *et al*., 2005). In this study, correlations between fecundity and life span were linear in control and insecticide treatments except by thiodicarb, indicate that decrease in span cause directly decrease at fecundity (Fig. 4). Spinosad hardly affected females span and mostly caused negative effects on fecundity. Penagos *et al*. (2005) have reported that most females of *C. insularis* died in earliest days after treatment with spinosad, thus their fecundity was hardly decreased. At all, between insecticides tested, hexaflumuron and spinosad had the lowest and highest adverse sublethal effects on population and biological parameters of *H. hebetor*. Profenofos and thiodicarb had moderately toxic effects on wasp.

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