

The effect of sublethal concentrations of malathion on some biological parameters of the ectoparasitoid wasp, *Habrobracon hebetor* (Say, 1836)

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ABSTRACT

The ectoparasitoid wasp, *Habrobracon hebetor* (Say, 1836) (Hym.: Braconidae) is one of the most important natural enemies of many pests in Iran. In this study, the effects of sublethal concentrations of malathion (LC₁₀ and LC₂₅) on biological parameters of the adult wasp including parasitism percentage, male production and longevity of the parasitoid in different growth stages (larvae, pupae and adult) were investigated. The experiments were conducted at 26 ± 1 °C, 60 ± 5 % RH, and 16L:8D h photoperiods. The effect of sublethal concentrations of malathion on adults was determined by exposure of wasp to insecticide, and in the case of larvae and pupae, immersion method was used. Based on the results, with the increase of toxic dose, the percentage of parasitism in adult decreased from 95.65 % to 87.78 %, compared to the control treatment. Male production increased from 22.73 % in control to 56.38 % at LC₂₅ concentration. Also, the percentage of parasitism of the malathion-impregnated wasp larvae decreased from 87.78 to 22.92%, compared to the control treatment. Male production from 30.94 % in the control increased to 39.63 % at the LC₂₅ concentration. In addition, the percentage of parasitism in the wasp pupae impregnated with malathion was reduced from 93.33 to 88.89 %, respectively. Male production increased from 22.98 in the control to 37.23 % in the LC₂₅ concentration. The data showed that pupae were more susceptible to sublethal concentrations than the larvae.

Key words: malathion; sublethal concentration; *Habrobracon hebetor*; biology; biological parameters

IZVLEČEK

UČINEK SUBLETALNIH KONCENTRACIJ MALATIONA NA NEKATERE BIOLOŠKE PARAMETRE EKTOPARAZITSKE OSICE *Habrobracon hebetor* (Say, 1836)

Ektoparazitska osica *Habrobracon hebetor* (Say, 1836), (Hymenoptera: Braconidae) je eden izmed najpomembnejših naravnih sovražnikov mnogih škodljivih žuželk v Iranu. V raziskavi so bili preučevani učinki subletalnih koncentracij malationa (LC₁₀ in LC₂₅) na biološke parametre odraslih osic vključno z odstotkom parazitiranja, razvojem samcev in življenjsko dobo parazitoida v različnih razvojnih stadijih (ličinke, bube in imagi). Poskus je bil izveden pri 26 ± 1 °C, 60 ± 5 % RH in osvetlitvi 6 : 8 dan/noč. Učinek subletalnih koncentracij malationa na image je bil določen z izpostavitvijo osic insekticidu, v primeru ličink in bub je bila uporabljena metoda potapljanja. Rezultati so pokazali, da je z naraščanjem koncentracije insekticida odstotek parazitiranja upadel iz 95,65 % na 87,78 % v primerjavi s kontrolo. Razvoj samcev se je iz 22,73 % v kontroli povečal na 56,38 % pri koncentraciji LC₂₅. Tudi odstotek parazitiranja z malationom impregniranih ličink osic se je zmanjšal iz 87,78 % na 22,92 % v primerjavi s kontrolo. Razvoj samcev se je iz 30,94 % v kontroli povečal na 39,63 % pri koncentraciji LC₂₅. Dodatno se je odstotek parazitiranja bub impregniranih z malationom zmanjšal iz 93,33 na 88,89 %. Razvoj samcev se je povečal iz 22,98 v kontroli na 37,23 % pri koncentraciji LC₂₅. Podatki so pokazali, da so bile bube bolj občutljive na subletalne koncentracije kot ličinke.

Ključne besede: malation; subletalna koncentracija; *Habrobracon hebetor*; biologija; biološki parametri

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1 INTRODUCTION

Most insecticides have harmful effects on non-target organisms, especially natural enemies (Croft, 1990). Biocontrol agents are commonly susceptible to insecticide applications. In recent years, integrated pest management systems attempt to use natural enemies in combination with lower doses of insecticides for pest control. The combined use of biocontrol agents and insecticides to manage pests requires adequate knowledge about the aspects and selective effects of insecticides on natural enemies (Croft, 1990; Dent, 1995; Banks & Stark, 1998; Bajc et al., 2017; Laznik & Trdan, 2014). Several natural enemies can be used for biological control of lepidopteran pests. Among these, *Habrobracon* spp. are used as effective parasitoids of different pests specially in stored products and crop plants (Navaei et al., 2002).

The bollworms, *Helicoverpa* spp. (Lepidoptera: Noctuidae) moths have a variety of key pests in the world that are polyphagous and have a wide range of host plants for the crops, vegetables and etc. in the world and Iran. Three species of *Helicoverpa armigera* (Hubner, (1808)), *Heliothis virescens* (Hufnagel, 1766) and *Heliothis peltigera* (Denis & Schiffermüller, 1775) have been reported in chickpea fields of Iran. The dominant species, *H. virescens* is reported in the chickpea fields of most regions of Iran. Farmers every year spray one or two times to prevent damage to *Helicoverpa* spp. by chemical insecticides such as Carbaryl (Sevin), Malathion and Phosalone. However, some species of this genus, such as *H. virescens*, have shown resistance to the major types of organophosphate, carbamate, and pyrethroid insecticides (Kranthi et al., 2001; Murray et al., 2005).

Habrobracon hebetor Say is an ectoparasitoid and has been studied as a biocontrol agent of various lepidopteran pests in several countries (Gerling, 1971; Youm & Gilstrap, 1993; Magro & Parra, 2001). In recent years, the mass rearing program of *H. hebetor* has been initiated in Iran and it has been used to control *H. armigera*, *H. virescens* and *Ostrinia nubilalis* (Hübner, 1796) specially in different regions of Iran (Navaei et al., 2002; Rafiee-Dastjerdi, 2008; Sarmadi et al., 2010). Limited information is available on the sublethal effects of commonly used insecticides on this biocontrol agent (Rafiee-Dastjerdi et al., 2008 and 2012). Estimation of lethal and sublethal effects of pesticides on a natural enemy is necessary to recognize their effects of it (Walthal & Stark, 1997; Stapel et al., 2000; Stark & Banks, 2003). Demographic toxicology is usually considered to be the best way to evaluate total effects of pesticides on a target insect. So, stable population parameters have been recommended to evaluate the overall effects of pesticides, because it is based on both survivorship and fecundity parameters (Stark & Wennergren, 1995).

Considering the importance of recognizing the susceptibility of various growth stages of parasitoid to insecticides, and in order to determine the best time to release them in the field and to perform the spraying, we try to evaluate the *H. hebetor* wasp susceptibility in the adult stages in comparison with the recommended dosage. The main objective of this study was to evaluate the sublethal effects of the malathion, commonly used insecticide against *H. virescens* in the chickpea fields of most regions of Iran and to determine the possibility of integrating the application of this species with insecticides in IPM program of this key pest.

2 MATERIALS AND METHODS

2.1 Insects rearing

Insects *H. hebetor* was obtained in adult stage from the insectarium in Lorestan province in Iran. The colony of *H. hebetor* was reared on 5th instar larvae of the flour moth, *Ephesia kuehniella* Zeller, 1879 reared on wheat flour in semi-clear plastic boxes (40×25×15 cm) in the laboratory. Fifth instar larvae of *E. kuehniella* were used for both rearing the colony of wasp and applying experiments. Bioassay and rearing conditions were 26 ± 1°C, 60 ± 5% RH and a photoperiod of 16 : 8 h (L: D).

2.2 Bioassay experiments

2.2.1 Preliminary bioassay

Malathion (EC 57 %, Partonar Co., Iran) was used in this experiment. Serial dilutions were prepared and concentrations (determined after preliminary tests) ranged from 5 to 200 µl l⁻¹ used immediately after preparation. Distilled water alone was used as a control. Contact toxicity of the insecticide was assessed on the adult, larvae and pupae of *H. hebetor*. Five concentrations of the insecticide were prepared to treat the insect. First, a number of parasitoid adult wasps were placed in a refrigerator at +5 °C for five minutes to freeze and do not move. Then for each concentration (treatment), 80-100 insects were used. Then using the

POLO PC software, the concentrations of LC₁₀, LC₂₅, LC₅₀, and LC₉₀ were obtained. The adult, larvae and pupae of parasitoid were exposed to sublethal concentrations (LC₁₀ and LC₂₅) and compared with the control.

2.2.2 Effect of sublethal concentrations on the adults of *H. hebetor*

For each concentration of malathion, three 1000 ml plastic glasses were considered separately, then the walls of the glasses were sprayed with (LC₁₀ = 10.5 and LC₂₅ = 20.2 µl l⁻¹) concentrations, after the treated glasses were completely dried, in each glass, about 80 to 100 adult insects were released. After 24 hours, twenty impregnated adults (12 female and 8 male) from each glass were transferred into new 150 ml plastic glasses, then each one was reversed on a piece of paper containing 30-days old host larvae. After 48 hours, the insects were removed and the parasitized larvae were subsequently transferred and kept in germinator at the same conditions as used for their respective parental adults. The number of parasitized larvae of the host and the percentage of parasitism was recorded. Upon emergence, the number and sex of the progeny and longevity of adults were recorded. This experiment was replicated three times for each concentration, separately. The control treatment was only with distilled water (Abedi et al., 2014).

2.2.3 Effect of sublethal concentrations on the larvae of *H. hebetor*

First, in order to obtain *H. hebetor* larvae, a large number of male and female wasps were randomly placed in ten clear cylindrical plastic containers (30 cm height and 14 cm diameter) with a valve at the bottom covered with fine mesh gauze for ventilation and with a 2.5 cm diameter hole for feeding with 10 % honey solution. Then, on five A₄ size papers, a large number of *E. kuehniella* larvae were placed and exposed to adult parasitoids in plastic containers. After 48 hours, adult parasitoids were removed and the parasitized host larvae were kept under controlled at the same conditions as used for their respective parental adults. After appearance of *H. hebetor* larvae on the host, they were exposed to sublethal concentrations of the insecticide, so that A₄ paper containing wasp larvae sticking to host larvae was cut to smaller size. On each paper cut, about 10 wasp larvae were attached to the host larvae, each cut piece was dipped separately into the insecticide solutions and immediately removed, and then the impregnated larvae were placed under rearing conditions to adult stage.

In this experiment, twenty adults (12 female and 8 male recognized by genitalia) emerged from impregnated larvae were transferred into new 150 ml plastic glasses, then each one was reversed on a piece of paper containing 30-days old host larvae. After 48 hours, the insects were removed and the host larvae were kept under the same conditions as used for their respective parental adults. The number of parasitized larvae of the host and the percentage of parasitism, number of emerged males and females and longevity of adults were calculated. This experiment was replicated three times for each concentration, separately. The control was treated only with distilled water (Mahdavi et al., 2011).

2.2.4 Effect of sublethal concentrations on the pupae of *H. hebetor*

The rearing of a large number of *H. hebetor* pupae was carried out similar to the previous experiment for larvae. After the pupae were formed, they were exposed to sublethal concentrations, so that the paper containing the pupae was cut to smaller sizes. On each paper cut, about 40 pupae were placed, each cut was individually dipped in containers containing different concentrations of the insecticide and quickly pulled out and then the impregnated pupae were placed under rearing conditions to adult stage (Rafiee-Dastjerdi et al., 2012).

In this experiment, twenty adults (12 female and 8 male) emerged from impregnated pupae were transferred into new 150 ml plastic glasses, then each one was reversed on a piece of paper containing 30-days old host larvae. After 48 hours, the insects were removed and the host larvae were kept under the same conditions as used for their respective parental adults. The number of parasitized larvae of the host and the percentage of parasitism, number of emerged males and females and longevity of adults were calculated. This experiment was replicated three times for each concentration, separately. The control was treated only with distilled water (Rafiee-Dastjerdi et al., 2012).

2.2.5 Data analysis

The biological data were submitted to analysis of variance and the means were compared by the Tukey test, using SPSS 14.0 software program (SPSS, 2004). The demographic parameters and their corresponding standard errors were estimated by the Jackknife technique (Meyer, 1986; Sokal & Rohlf, 1981), and the means were compared by the Tukey test, using SPSS 14.0 software program (SPSS, 2004).

3 RESULTS

3.1 Effect of sublethal concentrations on the adults of *H. hebetor*

According to analysis variance of the the data obtained from *H. hebetor* adult, rate of parasitism percentage was found to have a significant difference at 1 % level in the sublethal concentrations of malathion ($F_{2, 6} = 6.7$; $P < 0.01$). The results showed that the highest percentage of parasitism in the control treatment was 95.56 ± 1.11 % and the lowest in the LC₂₅ concentration was 87.88 ± 1.11 %. Also, results of *H. hebetor* male production were found to be significantly different

between treatments ($F_{2, 6} = 5.26$; $P < 0.01$). The highest percentage of males was appeared in LC₂₅ concentration that was 38.56 ± 5.26 % and the lowest in control treatment was 22.73 ± 2.62 %. Also, based on the results of the analysis of variance, the adult longevity of *H. hebetor* under the influence of different concentrations of malathion was significant ($F_{2, 6} = 20.73$; $P < 0.01$). The results showed that the maximum adult longevity was 16.00 ± 0.58 days for the control treatment and the lowest for the LC₂₅ concentration for 10.67 ± 0.33 days (Table 1).

Table 1: Mean comparison (\pm SE) of percentage of parasitism, male production and longevity of *Habrobracon hebetor* adult wasps effected by sublethal concentrations of malathion

Concentration	Parasitism (%)	Male production (%)	Longevity (day)
Control	92.22 ± 1.11 a*	30.94 ± 2.17 a	16.67 ± 0.88 a
LC ₁₀	92.22 ± 1.11 a	29.33 ± 2.13 a	12.23 ± 0.88 b
LC ₂₅	87.78 ± 1.11 b	39.63 ± 0.37 b	11.67 ± 0.33 b

Values within the columns followed by different letters are significantly different based on Duncan Multiple Range test.

3.2 Effect of sublethal concentrations on the larvae of *H. hebetor*

According to analysis variance of the data obtained from *H. hebetor* adults emerged from impregnated larvae, rate of parasitism percentage was found to have a significant difference at 1 % level in the sublethal concentrations of malathion ($F_{2, 6} = 9.8$; $P < 0.01$). The results showed that the highest percentage of parasitism in the control and LC₁₀ treatments was 92.22 ± 1.11 % and the lowest in the LC₂₅ concentration was 87.78 ± 1.11 %. Also, results of *H. hebetor* male produced from impregnated larvae were found to be significantly

different between treatments ($F_{2, 6} = 9.83$; $P < 0.01$). The highest percentage of males was appeared in LC₂₅ concentration that was 39.63 ± 0.37 % and the lowest in LC₁₀ treatment was 29.33 ± 2.13 %. Also, based on the results of the analysis of variance, the longevity of *H. hebetor* adults emerged from impregnated larvae under the influence of different concentrations of malathion was significant ($F_{2, 6} = 13.27$; $P < 0.01$). The results showed that the maximum adult longevity was 16.67 ± 0.88 days for the control treatment and the lowest for the LC₂₅ concentration for 11.67 ± 0.33 days (Table 2).

Table 2: Mean comparison (\pm SE) of percentage of parasitism, male production and longevity of *Habrobracon hebetor* adult wasps emerged from impregnated larvae effected by sublethal concentrations of Malathion

Concentration	Parasitism (%)	Male production (%)	Longevity (day)
Control	92.22 ± 1.11 a*	30.94 ± 2.17 a	16.67 ± 0.88 a
LC ₁₀	92.22 ± 1.11 a	29.33 ± 2.13 a	12.23 ± 0.88 b
LC ₂₅	87.78 ± 1.11 b	39.63 ± 0.37 b	11.67 ± 0.33 b

Values within the columns followed by different letters are significantly different based on Duncan Multiple Range test.

3.3 Effect of sublethal concentrations on the pupae of *H. hebetor*

According to analysis variance of the data obtained from *H. hebetor* adults emerged from impregnated pupae, rate of parasitism was found to have a significant

difference at 1 % level in the sublethal concentrations of malathion ($F_{2, 6} = 5.6$; $P < 0.01$). The results showed that the highest percentage of parasitism in the control and LC₁₀ treatments was 95.56 ± 1.11 % and the lowest in the LC₂₅ concentration was 88.89 ± 1.11 %. Also,

results of *H. hebetor* male produced from impregnated pupae were found to be significantly different between treatments ($F_{2, 6} = 34.68$; $P < 0.01$). The highest percentage of males was appeared in LC₂₅ concentration that was 37.12 ± 1.49 % and the lowest in control treatment was 22.98 ± 0.53 %. Also, based on the results of the analysis of variance, the longevity of *H.*

hebetor adults emerged from impregnated pupae under the influence of different concentrations of malathion was significant ($F_{2, 6} = 11.55$; $P < 0.01$). The results showed that the maximum adult longevity was 16.33 ± 0.33 days for the control treatment and the lowest for the LC₂₅ concentration for 12 ± 0.58 days (Table 3).

Table 3: Mean comparison (\pm SE) of percentage of parasitism, male production and longevity of *Habrobracon hebetor* adult wasps emerged from impregnated pupae effected by sublethal concentrations of malathion

Concentration	Parasitism (%)	Male production (%)	Longevity (day)
Control	93.33 ± 1.92 b*	22.98 ± 0.53 b	16.33 ± 0.33 a
LC ₁₀	95.56 ± 1.11 a	23.91 ± 1.70 b	14.33 ± 0.88 a
LC ₂₅	88.89 ± 1.11 b	37.12 ± 1.49 a	12.00 ± 0.58 b

Values within the columns followed by different letters are significantly different based on Duncan Multiple Range test.

4 DISCUSSION

Parasitism is a practice where the parasitoid needs to have searching efficiency, eggs in the ovary, and the ability to access the host. Therefore, reducing each of the above factors can lead to reduction in the percentage of parasitism. Chemical pesticides may be more or less able to do so. In natural condition, natural enemies first find their host habitat through kairomones that comes from the plant (Blassioli-Moraes et al., 2016). According to Cloyd (2012), the smell of the insecticide and the material produced from the plant at low doses may cause confusion of the natural enemy and reduce parasitism. Although it is easy to find the host in the laboratory condition, but the smell of insecticide may lessen the ability to search and access the host. Research has also shown that some insecticides reduce the number of eggs in the ovary (Daniel, 1975). The results of our experiments showed that there is a significant difference between the percentage of parasitism of adult wasps exposed to sublethal concentrations of malathion. Parasitism rate of *H. hebetor* has increased with decreasing sublethal concentrations. The percentage of parasitism in exposed wasps at the mentioned concentrations (LC₂₅, LC₁₀ and control) was 87.78, 92.22 and 95.56 %, respectively.

Rafiee-Dastjerdi et al. (2009) examined the susceptibility of the immature stages of *H. hebetor* parasitoid wasp to the pesticides in order to investigate the effect of the recommended dose of Spinosad, Profenofos, Thiodicarb and Hexaflumuron in two methods of spraying and placement on the immature stages of the wasp and the emergence of adults. Based on the International Organization for Biological Control, Profenofos, Spinosad and Thiodicarb, with a

mean decrease of 73.62, 39 and 29.25 %, respectively, of the emergence of insects were placed in in the less toxic group and Hexaflumuron with a reduction of 87.8 % in the adult emergence in the harmless insecticide group. It seems that the difference between the results of this study and Rafiee Dastjerdi et al. (2009) is due to difference in the laboratory conditions, host as well as differences in the stage of life of the wasp. Because the susceptibility of the adult stages have been studied in this study. It seems that the larval growth stage of *H. hebetor* is less affected by the sublethal concentration of the insecticide, because until the adult stage most of insecticide decomposes inside the insect's body. It also seems that due to the fact that wasp has been fed with water, honey and vitamin C until death, less fluctuations has occurred over their lifetime (Soyelu, 2013).

Results of timed bioassay of Baker et al. (1995) showed that field strain of *B. hebetor* was significantly more tolerant of malathion compared to the laboratory strain. Also, triphenyl phosphate (TPP) substantially delayed the toxicity of malathion. The results of Mahdavi & Saber (2013) showed that malathion rather than diazinon had the lowest adverse effects on the functional response of *H. hebetor*. After conducting advanced field studies, it was found that malathion may be used as a compatible chemical material with biological control in IPM programs.

The results of this study showed that there was a significant difference between male production of *H. hebetor* adult wasps and sublethal concentrations of malathion. In the LC₂₅ concentration, male production

was increased in adult wasps and was reduced by decreasing sublethal concentrations. Amir-Maafi et al. (2002) stated in their study on *Trissolcus grandis* (Thompson, 1861) that the female wasps in the first five days lay 26 % of their total eggs and 51 % of the hatched eggs of the female wasp is related to its first five days of activity. Also, 63.66 % of the total produced females were related to this period, but after 5 days from the evaluation period, the male production and mortality of the female wasps increased. In fact, in the male production of insecticide-exposed wasps, the age of the wasps is also important. Also, based on the growth characteristics of parasitoid wasps of the sunn pest, male parasitoids appear earlier than females and work around un-emerged parasitized eggs (Amir Majafi, 2000; Safavi, 1974; Asgari, 1996). For this reason, chemical insecticides appear to effect on male wasps and cause mating ability to reduce and inoculate the egg, resulting in the production of haploid (male) individuals.

Based on the results of the effect of sublethal concentrations of malathion on the longevity, the results were significantly different between treatments. According to the results, with the increase of sublethal dose of malathion, the longevity of *H. hebetor* progeny has decreased compared to control treatment. The results of Shishehbor and Faal-Mohammad-Ali (2013) showed that the effects of Flufenoxuron (Cascade) and Lufenuron insecticides on different stages of *H. hebetor* larvae were not significantly different in terms of longevity and egg oviposition of females. But different growth stages had a significant effect on the longevity of female parasitoid. According to their results, the highest longevity was related to the lufenuron-treated pupal stage. Interaction effects of insecticides and

different growth stages did not have a significant effect on the longevity of the female. The results of our study were not similar to this study. It seems that the difference in the results is due to the type of insecticide used in the treatment. It may also be possible that reduction of longevity of the wasps at high concentrations of insecticide is due to reduction in wasp body water content. Wasps seem to need more water to eliminate toxic compounds in their body, resulting in more activity to eliminate poisonous compounds, lose their body water and die.

The results showed that the percentage of parasitism decreased with increasing sublethal concentration of malathion. The results of Faal-Mohammad-Ali et al. (2015) in evaluating the effects of chlorpyrifos and fenpropathrin in the sublethal concentration of LC₂₅ on the parameters of stable population of *H. hebetor* parasitoid wasp in the larval stage showed that fenpropathrin insecticide had the most effect on the parameters of stable population growth. There was a significant difference between the treatments and the control. There was no significant difference between chlorpyrifos and fenpropathrin except for the population growth rate. The results of these researchers are similar to the results of the present study, and the numerical differences in the data are related to the test conditions and insecticide concentrations.

Abedi et al. (2014) evaluated the sublethal effects of azadirachtin and cypermethrin insecticide and the adult wasps were exposed to the LC₃₀ concentration of the insecticides, and then demographic parameters of the surviving wasps were determined. Fecundity, fertility, and parameters including the intrinsic rate of increase (r_m) were affected negatively.

5 CONCLUSION

Even though results obtained in small laboratory arenas may not be realized under natural conditions (Kareiva, 1990), these kinds of investigations under the laboratory conditions can be helpful in selecting insecticides for

additional studies under more natural conditions and for application of suitable insecticides along with natural enemies in the pest management programs.

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