

ARTICLE

## Insecticidal activity of isolated essential oils from three medicinal plants on the biological control agent, *Habrobracon hebetor* Say (Hymenoptera: Braconidae)

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**ABSTRACT** The effects of *Allium sativum* L. (Alliaceae), *Piper nigrum* L. (Piperaceae) and *Glycyrrhiza glabra* L. (Fabaceae) essential oils were investigated on the biological control agent, *Habrobracon hebetor* Say. The female wasps of *H. hebetor* were treated by LC<sub>30</sub> concentrations of the tested essential oils for 24 h and their demography was investigated. Results indicated that the adult longevity, survival, fecundity, fertility, hatch rate, offspring sex ratio and the other demographic parameters negatively were affected by these essential oils. At the same time, our findings indicated that *G. glabra* essential oil has the less severe effect on *H. hebetor*. Accordingly, *G. glabra* essential oil seems to be a compatible botanical compound with *H. hebetor* for applying in integrated pest management programs.

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### Introduction

The synthetic pesticides concern environmental pollution, emergence of resistance in insect pests and negative effects on non-target organisms in agricultural ecosystems (Sagheer et al. 2014; Yazdgerdian et al. 2015; Asadi et al. 2018a). During the last decades, application of various natural compounds were seriously considered for the effective control of insect pests (Mwine et al. 2013; Rafiee-Dastjerdi et al. 2013; Kramer et al. 2016; Asadi et al. 2018a). According to this, the compounds of plant origin including essential oils and extracts have been recommended as natural and effective compounds in integrated pest management (IPM) programs (Koul et al. 2008; Manzoor et al. 2011; Asadi et al. 2018a; Razmjou et al. 2018). The essential oils are a chemically diverse group of volatile plant metabolites (Bakkali et al. 2008; Isman et al. 2008; Rafiee-Dastjerdi et al. 2013). Most of these odor compounds have fumigant, contact, repellent and antifeedant properties and, therefore, they are frequently recommended for control of insect pests due to their high insecticidal activity and low negative effects on non-target organisms (Isman 2000; Yildirim et al. 2011; Rafiee-Dastjerdi et al. 2013; Shiva Parsia and Valizadegan 2015; Asadi et al. 2018a).

Application of any compounds of synthetic or natural

origin potentially can have harmful effects on demography of the natural enemies of pests including predators and parasitoids (Stark et al. 2004; Mahmoudvand et al. 2011). The acute toxicity of different compounds on insect populations previously have been investigated in detail, but the sublethal effects of them have not been considered completely (Motazedian et al. 2012; Mwine et al. 2013). The negative sublethal effects can change the demographic parameters and finally will reduce the efficiency of natural enemies for control of their hosts (Croft 1990; Stark and Wennergren 1995; Salerno et al. 2002; Stark and Banks 2003; Desneux et al. 2007; Hamedi et al. 2010 and 2011).

There are several reports describing the harmful effects of some plant essential oils and the other compounds of plant origin on non-target organisms especially on the natural enemies of pests (Abramson et al. 2006; Asadi et al. 2018a). If the application of plant essential oils will be widespread, they can disrupt the biological control agents in IPM programs (Desneux et al. 2007). Accordingly, co-application of biological control agents with plant-based compounds requires suitable knowledge about their obvious and hidden interactions (Dent 1995; Banks and Stark 1998; Desneux et al. 2007). These researches are defined as demographic toxicology that is a useful tool for understanding the effects of different compounds on populations of different insects and their natural enemies (Walthall and Stark 1996; Stapel et al. 2000; Stark and

Banks 2003; Desneux et al. 2007).

*Habrobracon hebetor* Say is one of the important natural enemies of different destructive lepidopteran larvae especially from families Pyralidae and Noctuidae in agricultural crops (Gerling 1971; Youm and Gilstrap 1993; Magro and Parra 2001; Navaei et al. 2002). The projects on the mass rearing of this ectoparasitoid wasp have been started in Iran in different commercial insectariums and it has been released for the control of some invasive lepidopteran pests (Navaei et al. 2002; Ahmadpour 2017). However, little information is available regarding the lethal and sublethal effects of plant essential oils and the other plant compounds on this important ectoparasitoid wasp (Seyyedi 2011; Hashemi et al. 2014; Ahmadpour 2017; Asadi et al. 2018a; Razmjou et al. 2018).

Three medicinal plants were selected for this study, including garlic (*A. sativum*), black pepper (*P. nigrum*) and liquorice (*G. glabra*). It is known from previous studies that they have sufficient amount of essential oil with high insecticidal activity (Koul et al. 2008; Ajayi and Olonisakin 2011; Rafiee-Dastjerdi et al. 2013). In this research we investigated the effects of isolated essential oils from above-mentioned medicinal plants on *H. hebetor* for evaluation the possibility of their integration with this important biocontrol agent in IPM programs.

## Materials and Methods

### Essential oils isolation and analysis

The mature plants of garlic (*Allium sativum* L., Alliaceae), black pepper (*Piper nigrum* L., Piperaceae) and liquorice (*Glycyrrhiza glabra* L., Fabaceae) were collected in different regions of Islam-Abad Gharb city (34.11° N and 46.52° E) (Kermanshah province, Iran) during May to June 2016. The collected plants were dried in shadow conditions under room temperature (approximately 25 °C). Parts of the collected plants (bulbs of *A. sativum*, seeds of *P. nigrum* and leaves of *G. glabra*) that contained the highest insecticidal activity were considered. Analyses of the isolated essential oils were carried out with a Gas Chromatography-Mass spectroscopy (GC-MS; Agilent, 7890 B) as described earlier (Asadi et al. 2018b). Investigation of fumigant toxicity on the young female wasps of *H. hebetor* (less than 24 h old) also was carried out as described earlier (Asadi et al. 2018b).

### Rearing of biological control agent

The primitive adult wasps of *H. hebetor* were obtained from a private insectarium (registered name: Kesht-Gostar Pishgam) in Islam-Abad Gharb city (Kermanshah province, Iran), during 2017. The obtained parasitoids urgently were transferred to laboratory conditions at 25

**Table 1.** The biological parameters of *H. hebetor* females treated by the isolated essential oils.

Treatment	Female longevity (day)	Daily fecundity (egg)	Daily fertility (egg)
Control	25.60 ± 0.56 <sup>a</sup>	17.23 ± 0.57 <sup>a</sup>	11.54 ± 0.38 <sup>a</sup>
<i>A. sativum</i>	14.12 ± 0.65 <sup>c</sup>	12.58 ± 0.68 <sup>b</sup>	6.41 ± 0.35 <sup>c</sup>
<i>P. nigrum</i>	15.84 ± 0.69 <sup>c</sup>	11.88 ± 0.69 <sup>b</sup>	6.89 ± 0.40 <sup>c</sup>
<i>G. glabra</i>	21.88 ± 0.79 <sup>b</sup>	15.96 ± 0.73 <sup>a</sup>	10.22 ± 0.47 <sup>b</sup>

Values in each column that characterized by different letters showed significant difference (Tukey test; P < 0.05).

± 1 °C, 60 ± 5% relative humidity (RH) and photoperiod of 16:8 (L:D) h and were reared on the larvae of flour moth (*Ephesia kuehniella* Zeller) as its laboratory host. During the rearing processes, the honey solution (10%) was used for feeding of this biocontrol agent (Rafiee-Dastjerdi et al. 2008, 2009a, 2009b; Mahdavi 2013; Abedi et al. 2012, 2014; Asadi et al. 2018a).

### Demography of *H. hebetor*

For studying the demography of *H. hebetor* on its laboratory host, first one-hundred young female wasps (less than 24 h old) were exposed to sublethal concentration (LC<sub>30</sub>) of each essential oil consist of *A. sativum* (2.22 µl/l air), *P. nigrum* (5.41 µl/l air) and *G. glabra* (8.72 µl/l air) on filter paper (2×2 cm) in glass Petri dish with approximately volume of 60 ml. The selection of LC<sub>30</sub> values for these testes was based on the results of earlier studies of IPM programs with synthetic and plant-derived compounds (Borzoui et al. 2016). This concentration (LC<sub>30</sub>) was calculated from the analyses of bioassay experiments by using SPSS version 20 software. In the control experiments, the wasps were treated by distilled water. After 24 h of exposure, twenty-five live females randomly were selected and released singly to Petri dishes (80 mm in diameter) and were paired with untreated males (less than 24 h old) (Ahmadpour 2017; Asadi et al. 2018a; Razmjou et al. 2018). The wasps were fed daily with honey solution (10%) and seven larvae of *E. kuehniella* as host for oviposition. The biological features of the treated wasps (total 25 paired wasps) including survival and fecundity were recorded daily to death time of all treated females. Then, among deposited eggs by them, one-hundred eggs were selected randomly and the number of emerged larvae, pupae and adult wasps (male and female) were recorded. The life table of Carey (1993) that is the base of one-six life table analysis was used for studying the demography of this parasitoid wasp.

### Data analysis

For statistical analysis, jackknife and pseudovalues were

**Table 2.** The stable population parameters of *H. hebetor* females treated by the isolated essential oils.

Treatment	GRR (female offspring)	R <sub>0</sub> (female offspring)	λ (day <sup>-1</sup> )	T (day)	DT (day)
Control	275.24 ± 17.72 <sup>a</sup>	144.68 ± 4.83 <sup>a</sup>	1.32 ± 0.003 <sup>a</sup>	18.14 ± 0.16 <sup>b</sup>	2.53 ± 0.02 <sup>c</sup>
<i>A. sativum</i>	55.34 ± 3.33 <sup>c</sup>	19.32 ± 1.07 <sup>d</sup>	1.17 ± 0.003 <sup>d</sup>	19.19 ± 0.12 <sup>a</sup>	4.49 ± 0.08 <sup>a</sup>
<i>P. nigrum</i>	90.38 ± 6.01 <sup>c</sup>	40.96 ± 2.39 <sup>c</sup>	1.23 ± 0.004 <sup>c</sup>	17.93 ± 0.13 <sup>b</sup>	3.34 ± 0.05 <sup>b</sup>
<i>G. glabra</i>	182.48 ± 10.70 <sup>b</sup>	88.51 ± 4.05 <sup>b</sup>	1.30 ± 0.004 <sup>b</sup>	17.10 ± 0.12 <sup>c</sup>	2.64 ± 0.03 <sup>c</sup>

Values in each column that characterized by different letters showed significant difference (Tukey test;  $P < 0.05$ ). GRR: Gross reproductive rate, R<sub>0</sub>: Net reproductive rate, λ: Finite rate of increase, T: Mean generation time, DT: Doubling time

calculated for the control and each essential oil treatment and then the obtained pseudovalues were tested for normal distribution. The square-root and logarithmic transformations were performed on those parameters that had not normal distribution. Finally the normal data were analyzed by one-way ANOVA and the means were compared by Tukey test at probability level ( $p < 0.05$ ) by using SPSS version 20 software (Meyer et al. 1986; Maia et al. 2000).

## Results

### Biological parameters

The biological parameters from treated females of *H. hebetor* by three isolated essential oils are shown in Table 1. The shortest and longest females' longevity was obtained in *A. sativum* essential oil and the control, respectively ( $F_{3,96} = 40.18$ ,  $P < 0.05$ ). The daily fecundity also significantly was affected by the tested essential oils; but, there was no significant difference between the control and *G. glabra* essential oil and also between the essential oils of *A. sativum* and *P. nigrum* ( $F_{3,96} = 14.92$ ,  $P < 0.05$ ). Moreover, the lowest and highest daily fertility were observed in *A. sativum* essential oil (6.41 eggs) and the control (11.54 eggs), respectively ( $F_{3,96} = 39.01$ ,  $P < 0.05$ ).

### Stable population parameters

The stable population parameters from treated females of *H. hebetor* by the isolated essential oils are given in Table 2. The gross reproductive rate (GRR) significantly was

affected by the isolated essential oils, being the lowest in *A. sativum* essential oil and the highest in the control ( $F_{3,96} = 82.01$ ,  $P < 0.05$ ). The R<sub>0</sub> value in *A. sativum* essential oil (19.32 female offspring) was significantly lower than the control and the other essential oils ( $F_{3,96} = 266.06$ ,  $P < 0.05$ ). The finite rate of increase (λ) also varied under different essential oils treatments compared with the control. The lowest λ value being in *A. sativum* essential oil and the highest was in the control ( $F_{3,96} = 376.05$ ,  $P < 0.05$ ). Moreover, the shortest and longest values of mean generation time (T) were obtained in *G. glabra* and *A. sativum* essential oils, respectively ( $F_{3,96} = 42.13$ ,  $P < 0.05$ ). In addition; the shortest and longest values of doubling time (DT) were seen in the control and *A. sativum* essential oil, respectively ( $F_{3,96} = 296.77$ ,  $P < 0.05$ ).

### Population growth parameters

The population growth parameters from treated females of *H. hebetor* by the isolated essential oils are shown in Table 3. About the intrinsic birth rate (b), there were no significant differences among the control, *P. nigrum* and *G. glabra* essential oils ( $F_{3,96} = 234.28$ ,  $P < 0.05$ ). The highest intrinsic death rate (d) also was observed in *P. nigrum* essential oil that showed significant differences with the other essential oils ( $F_{3,96} = 772.20$ ,  $P < 0.05$ ). The intrinsic rate of increase (r<sub>m</sub>) was strongly affected in offsprings of the treated females. The lowest r<sub>m</sub> value was determined in the treated wasps of *H. hebetor* by *A. sativum* essential oil and the highest was in the control ( $F_{3,96} = 381.23$ ,  $P < 0.05$ ). The gross hatch rate (h<sub>x</sub>) in *A. sativum* essential oil (0.51) was lower than the control and the other isolated

**Table 3.** The population growth parameters of *H. hebetor* females treated by the isolated essential oils.

Treatment	Birth rate (day <sup>-1</sup> )	Death rate (day <sup>-1</sup> )	r <sub>m</sub> (day <sup>-1</sup> )	Hatch rate (%)	Sex ratio (%)
Control	0.38 ± 0.003 <sup>a</sup>	0.11 ± 0.001 <sup>c</sup>	0.27 ± 0.002 <sup>a</sup>	0.67	0.63
<i>A. sativum</i>	0.26 ± 0.003 <sup>b</sup>	0.11 ± 0.001 <sup>c</sup>	0.15 ± 0.003 <sup>d</sup>	0.51	0.34
<i>P. nigrum</i>	0.39 ± 0.005 <sup>a</sup>	0.18 ± 0.002 <sup>a</sup>	0.21 ± 0.003 <sup>c</sup>	0.58	0.56
<i>G. glabra</i>	0.38 ± 0.003 <sup>a</sup>	0.12 ± 0.001 <sup>b</sup>	0.26 ± 0.003 <sup>b</sup>	0.64	0.57

Values in each column that characterized by different letters showed significant difference (Tukey test;  $P < 0.05$ ). r<sub>m</sub>: Intrinsic rate of increase

**Table 4.** The reproductive parameters of *H. hebetor* females treated by the isolated essential oils.

Treatment	Gross fertility rate (egg)	Net fertility rate (egg)	Gross fecundity rate (egg)	Net fecundity rate (egg)
Control	292.72 ± 18.84 <sup>a</sup>	152.80 ± 5.07 <sup>a</sup>	436.90 ± 28.12 <sup>a</sup>	228.06 ± 7.56 <sup>a</sup>
<i>A. sativum</i>	83.01 ± 5.00 <sup>c</sup>	27.94 ± 1.52 <sup>d</sup>	162.77 ± 9.81 <sup>c</sup>	54.78 ± 2.98 <sup>c</sup>
<i>P. nigrum</i>	93.60 ± 6.23 <sup>c</sup>	41.60 ± 2.41 <sup>c</sup>	161.39 ± 10.74 <sup>c</sup>	71.72 ± 4.15 <sup>c</sup>
<i>G. glabra</i>	204.89 ± 12.01 <sup>b</sup>	97.95 ± 4.47 <sup>b</sup>	320.14 ± 18.77 <sup>b</sup>	153.05 ± 6.99 <sup>b</sup>

Values in each column that characterized by different letters showed significant difference (Tukey test;  $P < 0.05$ ).

essential oils. Also, the offspring sex ratio ( $S_x$ ) value based on the number of emerged females on total number of emerged male and female wasps was affected in offsprings of *H. hebetor*; indicating that *A. sativum* essential oil significantly have decreased the emergence of female offspring in *H. hebetor*.

### Reproductive parameters

The reproductive parameters from treated wasps of *H. hebetor* by the isolated essential oils are given in Table 4. The lowest and highest gross fertility rate were determined in *A. sativum* essential oil and the control and the difference between *A. sativum* and *P. nigrum* essential oil wasn't significant ( $F_{3,96} = 70.26$ ,  $P < 0.05$ ). The net fertility rate also significantly was lower in *A. sativum* essential oil than the control and the other essential oils ( $F_{3,96} = 243.17$ ,  $P < 0.05$ ). Moreover, the results of gross fecundity rate indicated that there was no significant difference between *A. sativum* and *P. nigrum* essential oils ( $F_{3,96} = 52.82$ ,  $P < 0.05$ ). In addition, the net fecundity rate showed significant differences among the control and the essential oils treatments ( $F_{3,96} = 193.41$ ,  $P < 0.05$ ).

### Discussion

The demographic parameters based on the demographic toxicology are very useful tools for studying the responses of different insects to any synthetic or natural compounds (Forbes and Calow 1999). The sublethal results showed that application of the isolated essential oils negatively have changed the biological parameters of *H. hebetor* including the adult longevity, daily fecundity and daily fertility. Similar observations were reported by Seyyedi (2011), Hashemi et al. (2014) Ahmadpour (2017), Asadi et al. (2018a) and Razmjou et al. (2018).

In our study, the sublethal concentration of *A. sativum* essential oil caused the most significant negative effects on the above-mentioned parameters; while, *G. glabra* essential oil induced the less negative effects. The obtained values from reproductive parameters in the treated females of *H. hebetor* including the net and gross fecundity

and fertility rates were in agreement with the results reported earlier. According to the results of Ahmadpour (2017) and Razmjou et al. (2018), *F. vulgare* and *C. carvi* essential oils showed the most and *A. millefolium* and *H. persicum* essential oils caused the lowest adverse effects on these parameters, respectively. In another study, Asadi et al. (2018a) found that concerning these parameters *R. officinalis* and *S. officinalis* showed the highest and lowest negative effects, respectively.

In our study there were significant differences on the stable population parameters in the treated wasps of *H. hebetor*. The lowest female fecundity in *A. sativum* essential oil caused the lowest gross (GRR) and net reproductive ( $R_0$ ) rates among three examined essential oils. The net reproductive rate in all treatments was significantly lower than the gross reproductive rate indicating that the survival rate ( $l_x$ ) was negatively affected by the isolated essential oils. The longest mean generation time was observed after *A. sativum* essential oil treatment. Hashemi et al. (2014), Ahmadpour (2017); Asadi et al. (2018a) and Razmjou et al. (2018) concluded that *F. assafoetida*, *F. vulgare*, *R. officinalis* and *C. carvi* essential oils showed the substantially negative effects on the gross and net reproductive rates, finite rate of increase, mean generation and doubling time of *H. hebetor*. In our investigation *G. glabra* essential oil caused the lowest adverse effects on these parameters.

This research showed that the tested essential oils had negative sublethal effects on *H. hebetor*. Among the different demographic parameters, the intrinsic rate of increase ( $r_m$ ) is the most important parameter for evaluating the total effect of different compounds on insect populations. Hashemi et al. (2014), Ahmadpour (2017); Asadi et al. (2018a) and Razmjou et al. (2018) stated that  $r_m$  value of *H. hebetor* under all examined essential oils decreased compared with the control. At the same time, our findings indicated that *G. glabra* essential oil has the less negative effects and is relatively safe on *H. hebetor*. It means that this essential oil can be recommended as a suitable plant-derived compound on *H. hebetor* containing IPM approaches.

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