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RESEARCH REPORT

The effects of plant essential oils on the functional response of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) to its host

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Abstract

Habrobracon hebetor Say is an important ectoparasitoid wasp that can control Pyralidae and Noctuidae pests in agricultural crops. In this research, the effects of *Allium sativum* L., *Rosmarinus officinalis* L., *Piper nigrum* L., *Salvia officinalis* L. and *Glycyrrhiza glabra* L. essential oils were investigated on the functional response of *H. hebetor* to its host. The GC-MS analysis showed that tetracosamethyl cyclododeca siloxan, alpha-pinene, caryophyllene, beta-thujone and aristolene were major constituents of mentioned essential oils, respectively. In the experiments; the mated females of *H. hebetor* (under 24 h old) were exposed to sublethal concentrations (LC_{30}) of isolated essential oils for 24 h with fumigant exposure method. In the control, the treatment was performed by using distilled water. Then, six treated wasps were selected randomly to densities of 2, 4, 8, 16, 32 and 64 *Ephestia kuehniella* Zeller 5th instar larvae for 24 h under 25 ± 1 °C, 60 ± 5% RH and photoperiod of 16: 8 (L: D) h. Eight replicates were conducted for each host density in all treatments. The regression analysis based on Holling model (1959) indicated the functional response type II in the control, *P. nigrum*, *S. officinalis* and *G. glabra* and type III in *A. sativum* and *R. officinalis* essential oils. Also, *R. officinalis* essential oil and the control showed the longest (0.542 h) and shortest (0.411 h) handling times, respectively. The highest (0.047 h^{-1}) and lowest (0.033 h^{-1}) attack rates were also recorded in the control and *R. officinalis* essential oil, respectively. In addition, *R. officinalis* and *G. glabra* essential oils showed the maximum and minimum negative effects on the functional response type and it's parameters in *H. hebetor*, respectively. These results indicated that *G. glabra* essential oil can be recommended with *H. hebetor* in integrated pest management.

Key Words: *Ephestia kuehniella*; plant essential oils; functional response; *Glycyrrhiza glabra*; *Habrobracon hebetor*

Introduction

Habrobracon hebetor Say is an important ectoparasitoid wasp with special behavioral characteristics (idiobiont and gregarious) that has been applied successfully in many biological control programs in different regions over the world including Iran (Heimpel *et al*., 1997; Yu *et al*., 2002; Darwish *et al*., 2003; Salvador and Consoli, 2008; Abedi *et al*., 2012; Mahdavi and Saber, 2013). The mass rearing of *H. hebetor* are performed on the larval stage of flour moth (*Ephestia kuehniella* Zeller) as laboratory host in different commercial insectariums (Mudd and Corbet, 1982). This parasitoid wasp till now has been applied under inundative and inoculative release programs against *Helicoverpa armigera* (Hübner), *Sesamia cretica* (Lederer) and *Ostrinia nubilalis* (Hübner) (Hentz *et al*., 1998; Baker and Fabrick, 2000; Navaei *et al*., 2002).

One of the important behavioral features of natural enemies including parasitoids and predators is their functional responses. Holling (1959, 1961 and 1966) characterized three types of functional responses. The functional response type I has a linear shape (Hassell, 1978). In functional response type II, the numbers of hosts attacked by natural enemies reach to a fix rate. Most of the natural enemies show this type of functional response (Hassell, 1978; Luck, 1985; Yu *et al*., 2002; Abedi *et al.,* 2012; Mahdavi and Saber, 2013; Jarrahi and Safavi, 2015). The functional response type III also show sigmoid shape (Holling, 1959; Hassell, 1978).

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Combination of different pest management methods such as biological and chemical control has been recommended in IPM designs all over the world and the negative effects of different compounds on the biocontrol agents must be considered (Abedi *et al*., 2012). Abramson *et al*., (2006) studied the effects of citronella and alfazema essential oils on the fennel Aphids, *Hyadaphis foeniculi* Passerini (Hemiptera: Aphididae) and it's predator, *Cycloneda sanguinea* L. (Coleoptera: Coccinellidae) and concluded that citronella essential oil showed the most adverse effects on this predator. In addition, Poderoso *et al*., (2016) studied the effects of some plant extracts on developmental of the predator *Podisus nigrispinus* (Hemiptera: Pentatomidae) and concluded that the examined extracts caused relatively high mortality on the adults of this predator and must be used with care, because they can affect the life cycle of this important biocontrol agent. Therefore, natural enemies can be affecting by different botanical compounds that use against the insect pests. Therefore, estimation of the functional response types and their parameters under treatments of botanical compounds including essential oils and extracts are very important factors in IPM programs.

To date there have been no research conducted the effects of plant essential oils on *E. kuehniella* and functional response of *H. hebetor*; but, the researches about the lethal and sublethal effects of essential oils on this important biocontrol agent are available (Seyyedi *et al*., 2011; Hashemi *et al*., 2014; Ahmadpour, 2017). In addition, chemical pesticides can affect host-finding behavior and behavioral responses of *H. hebetor*. Rafiee-Dastjerdi *et al*., (2009b) showed that profenofos, thiodicarb, hexaflumuron and spinosad negatively changed the functional response of *H*. *hebetor*. Faal-Mohammad Ali *et al*., (2010) also concluded that chlorpyrifos and fenpropathrin changed the functional response of this parasitoid wasp to it's host that these negative effects of pesticides can lead to inefficiency of natural enemies and outbreak of plant pests. Therefore, the effects of different compounds must be investigated in assessment of natural enemies for biological control programs. The effects of azadirachtin, cypermethrin, methoxyfenozide and pyridalil also were studied by Abedi *et al*., (2012); who stated that cypermethrin had the highest negative effects on *H. hebetor*. Moreover, Mahdavi and Saber (2013) stated that malathion was compatible insecticide on the functional response of *H*. *hebetor* compared with diazinon in IPM programs. In addition, Jarrahi and Safavi (2015) concluded that proteus as a new formulated insecticide showed the highest negative effects on this parasitoid wasp compared with entomopathogenic fungus *Metarhizium anisopliae* Sensu lato and the control under laboratory conditions. Hence, the main objective of the present research was to investigate the effects of above mentioned essential oils isolated from some selective medicinal plants on the functional response of *H. hebetor* to evaluate the possibility of these botanical compounds to be integrated with this important ectoparasitoid wasp in IPM programs especially for the management of stored products pest.

Materials and Methods

The present research was carried out during 2016-2017, in the Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran.

Rearing of the parasitoid wasp

Parent population of *Habrobracon hebetor* wasps was provided by a private commercial insectarium (Kesht-Gostar Pishgam, Kermanshah Province, Iran), during 2016. Then, the parasitoid wasps were reared under laboratory conditions in growth chamber that was set at 25 ± 1 °C, 60 ± 5% RH and a photoperiod of 16: 8 (L: D) h, on the larvae of flour moth (*E. kuehniella*) as laboratory host for parasitism activities. Moreover, the honey solution (10%) was applied as food source for feeding of the adult parasitoids (Rafiee-Dastjerdi *et al*., 2008, 2009b). The ratio of parasitoid to host in our experiments was one female wasp to ten *E. kuehniella* larvae and the exposure interval of host to the parasitoid wasp was two days.

Isolation of essential oils

The selected medicinal plants including garlic, *Allium sativum*; rosemary, *Rosmarinus officinalis*; black pepper, *Piper nigrum*; sage, *Salvia officinalis* and liquorice, *Glycyrrhiza glabra* that was available in the Iranian flora and contained suitable amount of essential oil were collected from different regions of Islam-Abad Gharb city (34.11° N, 46.53° E) in Kermanshah Province, Iran, during May 2017. The collected plants were dried at room temperature (25 °C) under shade. Then, the parts of noted plants that contained the most insecticidal components including leaves of *R. officinalis, S. officinalis* and *G. glabra*, berries of *A. sativum* and seeds of *P. nigrum* were milled by electric grinder and 50 g of milled parts were added to 500 ml of distilled water and their essential oils were isolated by clevenger apparatus at 100 °C in 4h time for each plant (Shiva parsia and Valizadegan, 2015). The water of essential oils was removed by sodium sulfate and pure essential oils in special glasses were covered with aluminum coverage and stored in a refrigerator at 4 °C for using in experiments.

Chemical analysis of isolated essential oils

Chemical components of each essential oil were identified by using gas chromatography-mass spectroscopy (GC-MS/ Company: Agilent, Series: 7890 B, Manufacturer: USA). The comparative and original analyses are two common types of GC-MS analysis. The original analyses that apply about the essential oils and the other volatile compounds measure the peaks in relation to one another. In this method, the tallest peak is assigned 100% of the value and the other peaks being assigned proportionate values. The total mass of the unknown compounds is normally indicated by the parent peak. The value of this parent peak can be used to fit with a chemical formula containing the various elements which are believed to be in the compound (Hites, 2016).

Bioassays experiments

For investigation the fumigant toxicity of isolated essential oils on the young females of *H. hebetor* (under 24 h old); different concentrations of essential oils that lead to mortality between 20-80% put on filter paper (2×2 cm) in 60 ml glass Petri dishes as fumigant chambers by using a microaplicator. Distilled water was used in control treatments. Then, 20 adult females *H. hebetor* were released in each Petri dish without the presence of the host and the Petri dishes immediately were sealed with parafilm to prevent the exit of essential oils. Honey solution (10%) was used for the feeding of wasps on small pieces of paper. Each concentration of the essential oils was bioassayed in four replications and after 24 h of exposure; the number of dead wasps was recorded (Shiva parsia and Valizadegan, 2015).

Functional response experiments

In the functional response experiments, the LC_{30} of each essential oil was applied as the low lethal concentration. In first experimental setup, eighty mated females (under 24 h old) of *H. hebetor* that previously not in the presence of the host were exposed to LC_{30} of selected essential oils that were put on filter papers (2×2 cm) by using a microaplicator in 10 cm Petri dishes (volume 60 ml) for 24 h. All procedures were performed for the control treatments with distilled water. After 24 h, six treated wasps were selected randomly and transferred separately to the Petri dishes with the different densities (2, 4, 8, 16, 32, and 64) of *E. kuehniella* larvae (5^{th} instar) and were placed in growth chamber that was set at 25± 1°C, 60± 5% RH and a photoperiod of 16: 8 (L: D) h for 24 h. Ventilation in the Petri dishes were provided with pores in the lids of Petri dishes and honey solution (10%) was supplied as food source for the parasitoids. The functional response experiments were performed in eight replicates in all treatments and the numbers of parasitized host larvae by the parasitoid wasps were recorded after 24 h.

Used model

The model of Holling (1959) regarding the functional response of different natural enemies was used in this study as explained below:

$$N_{a} = aT_tN_0(1+aT_hN_0)$$

N_a = number of hosts attacked by *H. hebetor*
N_0 = different densities of host (2, 4, 8, 16, 32 and 64 5^{th} instar larvae of *E. kuehniella*)
T_t = total time of experiment (was 24 hour)
a = attack rate (area of host discoverage) by *H. hebetor*
T_h = handling time (time of handling) of *H. hebetor* to it's host
The other form of this equation is:

$$a= (d+bN_0)/(1+CN_0)$$

Here, "a" is host density and "b", "c" and "d" are estimated constants (Hassell *et al*., 1977; Juliano and Williams, 1987; Juliano, 1993).

Statistical analysis

The logistic and non-linear regression models were applied to determine the types of functional response and for the estimation of the parasitoid attack rate and handling time under different essential oils treatments and the control, respectively, using SAS V 9.1 software (SAS Institute, 2002).

Results

Chemical analysis of isolated essential oils

The GC-MS analyses results of isolated essential oils are shown in Tables 1 to 5. Eleven major compounds from *A. sativum* essential oil, fourty-three compounds from *S. officinalis* and *P. nigrum* essential oils and fourty-four compounds from *S. officinalis* and *G. glabra* essential oils were detected. Tetracosamethyl cyclododeca siloxan (15.82%) from *A. sativum,* alpha-pinene (9.99%) from *R. officinalis*, caryophyllene (36.03%) from *P. nigrum*, beta-thujone (25.63%) from *S. officinalis* and aristolene (20.14%) from *G. glabra* were detected as major constituents of each mentioned essential oil.

Table 1 Chemical constitutents of *Allium sativum* L. essential oil

Peak	Material	Retention Time (RT)	% of Total
1	5- 2', 6', 6'-Trimethyl-Cyclohexene	36.49	2.16
2	Octasiloxane	36.87	3.64
3	5, 6, 8, 9-Tetramethoxy-2-Methylpep	37.38	5.99
4	N-Methyl-1-Adamantaneacetamide	37.50	7.97
5	Silicone grease, Siliconfett	37.56	8.80
6	1, 3-Xylyl-15-crown-4, 2, 3-Pinan	37.59	8.63
7	4-Methoxy-3-(3-Methoxyphenyl)	37.80	10.20
8	1, 4-Cyclohexadiene,1, 3, 6-tris	37.92	10.57
9	1-Amino-1-ortho-Chlorophenyl	38.02	12.87
10	Anhydro 5-Hydroxy-3-Piperonyl	38.14	13.34
11	Tetracosamethyl cyclododeca siloxan	38.27	15.82

Table 2 Chemical constitutents of *Rosmarinus officinalis* L. essential oil

Peak	Material	Retention Time (RT)	% of Total
1	Tricyclene	5.21	0.27
2	Alpha-Pinene	5.45	9.99
3	Camphene	5.70	4.25
4	Bicyclo [3.1.0] Hex-3-en-2-ol	5.79	0.42
5	Bicyclo [3.1.1] Heptane, 6, 6-Dimet	6.21	1.19
6	3-Octanone	6.36	1.31
7	Beta-Myrcene	6.44	1.73
8	(+)-4-Carene	6.94	0.37
9	Benzene,1-Methyl	7.10	0.96
10	dl-Limonene	7.18	2.88
11	1, 8-Cineole	7.26	6.11
12	Gamma-Terpinene	7.76	0.51
13	Alpha-Terpinolene	8.39	0.52
14	Linalool L	8.63	2.17
15	Chrysanthenone	9.29	0.55
16	Bicyclo [2.2.1] Heptan-2-one	9.85	7.78
17	Bicyclo [3.1.1] Heptan-3-one	10.23	0.57
18	Borneol L	10.41	5.02
19	Bicyclo [3.1.1] Heptan-3-one	10.64	1.07
20	3-Cyclohexen-1-ol, 4-Methyl	10.70	1.01
21	Alpha Terpineol	11.10	1.67
22	Estragole	11.29	0.91
23	Bicyclo [3.1.1] Hept-3-en-2-one	11.77	7.24
24	Bicyclo [2.2.1] Heptan-2-ol	14.50	5.71
25	Caryophyllene	19.37	3.81
26	Alpha-Humulene	20.41	0.66
27	Heptasiloxane, Hexadeca Methyl	36.26	4.78
28	3-(4-Chlorophenyl)-4, 6-Dimethoxy	36.35	1.21
29	1, 3-Xylyl-15-crown-4, 2, 3-Pinan	36.38	2.25
30	Cyclononasiloxane, OctadecaMethyl	36.55	1.70
31	Acetamide, 2-(Adamantan-1-yl)	36.68	3.01
32	Bistri, Methylsilyl n-Acetyl Eicos	36.83	5.14
33	1, 1, 1, 5, 7, 7, 7-HeptaMethyl	37.22	0.77
34	6-Phenyl-3, 5-Dithioxo	37.33	1.09
35	Cyclodecasiloxane, EicosaMethyl	37.46	1.50
36	Octadeca Methyl Cyclononasiloxane	37.56	1.26
37	1-Amino-1-Ortho-Chlorophenyl	37.60	0.64
38	Cyclodecasiloxane, Eicosa Methyl	37.69	2.71
39	5, 6, 8, 9-Tethramethoxy-2-Methylpep	37.82	2.25
40	1, 1, 5, 7, 7, 7- Heptamethyl-3	37.89	0.69
41	Cyclononasiloxane, octadecamethyl	38.91	0.41
42	Benzene, 2, 3-dimethyl	38.12	1.10
43	Iron, Monocarbonyl	38.23	0.82

Table 3 Chemical constitutents of *Piper nigrum* L. essential oil

Peak	Material	Retention Time (RT)	% of Total
1	Bicyclo [3.1.0] Hexane, 4-methyl	5.28	0.67
2	R-Alpha-Pinene	5.42	3.05
3	Camphene	5.69	0.18
4	Sabinene	6.15	3.96
5	2-Beta-Pinene	6.22	4.34
6	Beta-Myrcene	6.43	0.73
7	1-Phellandrene	6.71	1.34
8	3-Carene	6.84	5.17
9	Alpha Terpinene	6.94	0.16
10	Benzene,1-Methyl	7.09	0.58
11	l-Limonene	7.19	7.02
12	1, 8-Cineole	7.24	0.11
13	1, 4-Cyclohexadiene,1-Methyl	7.75	0.27
14	Bicyclo [3.1.0] Hexan-2-ol	7.93	0.30
15	Alpha-Terpinolene	8.33	0.10
16	(+)-4-Carene	8.39	0.35
17	1, 6-Octadien-3-ol, 3, 7-Dimethyl	8.61	1.07
18	3-Cyclohexen-1-ol, 4-Methyl	10.67	0.64
19	Beta Fenchyl Alchol	11.05	0.21
20	Estragole	11.29	2.72
21	Alpha-Terpinene	16.50	2.41
22	Alpha-Cubebene	16.93	0.29
23	Alpha-Copaene	17.90	3.84
24	Beta Elemene	18.46	1.69
25	Caryophyllene	19.51	36.03
26	Azulene	19.96	0.38
27	Alpha-Humulene	20.43	2.94
28	Trans-beta-Farnesene	20.52	0.29
29	1, 6-Cyclodecadiene	21.25	0.45
30	Beta-Selinene	21.42	2.70
31	Aalpha-Selinene	21.67	2.40
32	Naphthalene	21.82	0.57
33	Cyclohexene, 1-methyl	22.08	5.13
34	1-Naphthalenol	22.26	0.36
35	Delta-Cadinene	22.49	2.52
36	Cadina-1, 4-Diene	22.70	0.21
37	Cyclohexane methanol, 4-Ethenyl	23.17	0.59
38	1, 6, 10-Dodecatrien	23.55	0.58
39	(-)-Caryophyllene Oxide	24.09	1.45
40	Pentalene, Octahydro	25.24	0.79
41	Bicyclo [4.4.0] dec-1-ene	25.56	0.24
42	Copaene	25.67	1.09
43	4H-1, 3, 5-Thiadiazin-4-one	32.52	0.09

Table 4 Chemical constitutents of *Salvia officinalis* L. essential oil

Peak	Material	Retention Time (RT)	% of Total
1	Cis-Salvene	4.00	0.15
2	Tricyclo [2.2.1.0 (2, 6)] Heptane	5.20	0.13
3	1S-Alpha-Pinene	5.41	3.73
4	Camphene	5.69	3.36
5	Bicyclo [3.1.1] Heptane	6.20	0.84
6	beta-Myrcene	6.43	0.35
7	Benzene,1-Methyl	7.09	0.82
8	dl-Limonene	7.17	0.80
9	1, 8-Cineole	7.25	10.56
10	1, 6-Octadien-3-ol, 3, 7-dimethyl	8.70	0.27
11	Beta-Thujone	8.90	25.63
12	Thujone	9.11	6.54
13	Bicyclo [2.2.1] heptan-2-one	9.87	16.46
14	Borneol L	10.37	1.69
15	3-Cyclohexen-1-ol, 4-Methyl-1	10.68	0.52
16	P-Menth-1-en-8-ol	11.06	0.23
17	2 (3H)-Furanone	11.27	0.23
18	Benzene, 1-Methoxy-4-(1-Propenyl)	14.42	0.52
19	Phenol, 2-Methyl-5-(1-Methylethyl)	15.06	0.27
20	Caryophyllene	19.34	1.68
21	1H-Cycloprop[e] Azulene	19.96	0.77
22	Alpha-Humulene	20.42	2.81
23	Ledene	21.66	0.47
24	1H-Cycloprop[e] Azulene	23.94	0.52
25	(-)-Caryophyllene Oxide	24.08	0.86
26	Veridiflorol	24.33	4.71
27	1, 2-Dihydropyridine	24.50	0.35
28	12-Oxabicyclo [9.1.0] Dodeca	24.77	1.37
29	trans-Z-alpha-Bisabolene Epoxide	25.34	0.71
30	Cyclopentan,1-Methylen-2-Vinyl	25.97	0.27
31	Bicyclo [6.1.0] Nonane	30.89	0.21
32	Cembrene	31.50	0.13
33	1-Naphthalenepropanol	31.79	4.27
34	5, 7-Dimethoxy-1-Naphthol	31.96	0.16
35	4-Methoxy-3-(3-Methoxyphenyl)	36.83	0.18
36	1, 3-Xylyl-15-crown-4, 2, 3-Pinan	37.17	0.39
37	Methoxy-3-(3-Methoxyphenyl)	37.39	0.82
38	Cyclononasiloxane, OctadecaMethyl	37.45	0.23
39	Etracosamethyl cyclododeca siloxan	37.56	1.16
40	Hexasiloxane, Tetradecamethyl	37.65	0.63
41	Iron, Monocarbonyl	37.00	1.76
42	Silicone Grease, Siliconfett	83.10	0.73
43	1-Naphthalene Ethanol	37.89	1.20
44	5, 6, 8, 9-Tetramethoxy-2-Methylpep	38.04	0.55

Table 5 Chemical constitutents of *Glycyrrhiza glabra* L. essential oil

Peak	Material	Retention Time (RT)	% of Total
1	1S-Alpha-Pinene	5.40	0.38
2	Linalool	8.63	1.02
3	Alpha Terpineol	11.07	0.61
4	Lavandulyl Acetate	18.29	6.26
5	Bicyclo [3.1.1] Hept-2-ene	19.23	0.97
6	Caryophyllene	19.36	0.51
7	Endo-2, 6-Dimethyl-6-(4-Methyl)	19.89	1.81
8	1H-Cycloprop Azulene	20.93	1.33
9	Geranyl Propionate	21.14	3.31
10	Benzene,1-(1,5-Dimethyl-4-Hexen)	21.35	0.76
11	Trans-Beta-Farnesene	21.40	1.16
12	3-Buten-2-ol, Benzoate	21.53	0.97
13	Cyclohexene, 1-Methyl-4-(5-Methyl)	22.06	0.42
14	Propanoic Acid, 2-Methyl	22.24	2.19
15	Beta-Sesquiphellandrene	22.49	0.48
16	Butanoic Acid, 3, 7-Dimethyl	23.59	7.34
17	Nerolidol	23.74	4.11
18	3-Hexen-1-ol, Benzoate	23.89	4.37
19	Linalool L	23.93	0.71
20	Caryophyllene Oxide	24.15	1.90
21	3-Cyclohexene-1-Ethanol	24.26	0.61
22	2, 6-Octadien	24.70	8.54
23	(E)-2-Formyl-6-Methyl	24.81	2.68
24	1, 6, 10-Dodecatrien	25.09	2.52
25	Naphthalene	25.34	2.55
26	Aristolene	25.58	20.14
27	Hinesol	25.67	1.90
28	Beta-Eudesmol	25.90	1.73
29	2-Naphthalene methanol	25.96	2.21
30	Beta-Bisabolol	26.33	0.48
31	Alpha-Bisabolol	26.61	0.20
32	Geranyl tiglate	27.04	3.79
33	Acetic acid, 1-Methylcyclopentyl	27.22	0.71
34	Neryl Propionate	28.19	1.67
35	Geranyl Acetate	28.31	0.84
36	Benzyl benzoate	28.48	2.72
37	Neryl 2-Methylpropanoate	29.47	0.76
38	2-Hexadecen	29.82	0.34
39	2-Pentadecanone	29.92	0.58
40	Neophytadiene	30.35	0.37
41	Neryl Acetate	31.01	0.51
42	Hexadecanoic Acid	31.10	0.40
43	Geranyl Benzoate	31.15	2.88
44	Cyclohexene,1-Methyl-5-(1-Methyl)	31.49	0.30

Table 6 The acute toxicity of selected essential oils on the adult females of *H. hebetor*

Treatment	n	Slope ± E	LC_{30} µl/liter air (95% CL)	LC_{50} µl/liter air (95% CL)	LC_{90} µl/liter air (95% CL)	χ^2
A. sativum	480	1.41±0.20	2.22 (1.14 - 3.31)	5.22 (3.57 - 6.76)	42.05 (29.59 - 74.68)	9.85
R. officinalis	480	2.35±0.30	2.48 (1.60 - 3.28)	4.15 (3.11 - 5.04)	14.51 (12.09 - 18.88)	15.72
P. nigrum	480	1.39±0.20	5.41 (2.84 - 7.93)	12.88 (9.06 - 16.42)	107.33 (73.00 - 205.55)	7.82
S. officinalis	480	1.12±0.15	6.30 (3.21 - 9.62)	18.36 (12.69 - 24.30)	250.47 (154.06 - 551.65)	6.19
G. glabra	480	1.08±0.12	8.72 (4.81 - 13.07)	26.51 (18.68 - 35.38)	401.83 (274.39 - 837.72)	15.22

CL: Confident limit, χ2: Chi-Square value.

Table 7 The logistic regression analysis of *E. kuehniella* larvae parasitized by *H. hebetor.*

Treatments	Coefficient	Estimate	SE	χ^2	P-value
Control	P_0 (constant)	2.1769	0.6643	10.74	0.0011
	P_1 (linear)	-0.0116	0.0964	0.01	0.9044
	P_2 (quadratic)	-0.0011	0.0036	0.09	0.7603
	P_3 (cubic)	0.00001	0.00003	0.11	0.7350
A. sativum	P_0 (constant)	0.0261	0.4235	0.42	0.9508
	P_1 (linear)	0.0868	0.0653	1.76	0.1841
	P_2 (quadratic)	-0.0033	0.0025	1.81	0.1782
	P_3 (cubic)	0.00003	0.00002	1.41	0.2348
R. officinalis	P_0 (constant)	0.1075	0.4190	0.07	0.7975
	P_1 (linear)	0.0351	0.0640	0.30	0.5837
	P_2 (quadratic)	-0.0012	0.0024	0.26	0.6075
	P_3 (cubic)	0.00001	0.00002	0.12	0.7306
P. nigrum	P_0 (constant)	1.5420	0.5193	8.82	0.0030
	P_1 (linear)	-0.0229	0.0762	0.09	0.7635
	P_2 (quadratic)	-0.0007	0.0028	0.07	0.7927
	P_3 (cubic)	0.00001	0.00003	0.14	0.7096
S. officinalis	P_0 (constant)	1.6186	0.5090	10.11	0.0015
	P_1 (linear)	-0.0694	0.0744	0.87	0.3509
	P_2 (quadratic)	0.0016	0.0027	0.32	0.5727
	P_3 (cubic)	-0.00002	0.00003	0.31	0.5764
G. glabra	P_0 (constant)	2.1836	0.6279	12.09	0.0005
	P_1 (linear)	-0.0469	0.0891	0.28	0.5986
	P_2 (quadratic)	-0.0004	0.0032	0.02	0.8990
	P_3 (cubic)	0.00001	0.00003	0.08	0.7764

SE: Standard Error, χ^2: Chi-square value

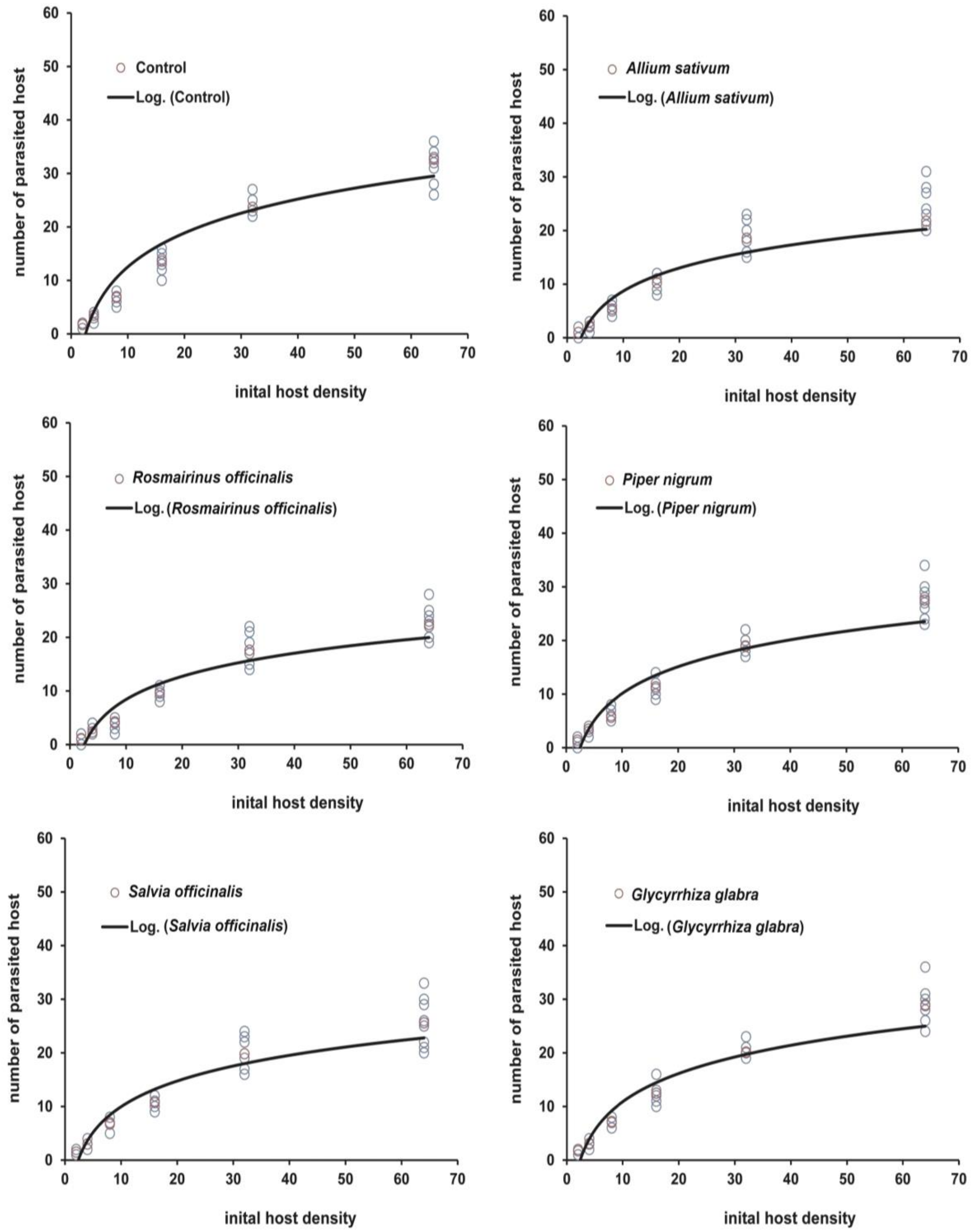

Fig. 1 Functional response curve of *H hebetor* previously exposed to LC_{30} of selected essential oils and the control to different densities of *E. keuhniella* larvae

Bioassay

The LC_{30} and LC_{50} values for *A. sativum*, *R. officinalis*, *P. nigrum*, *S. officinalis* and *G. glabra* essential oils against the females of *H. hebetor* are shown in Table 6. The adult bioassays indicated that acute toxicity of *R. officinalis* essential oil on the female wasps of *H. hebetor* was higher than the others. Also, *G. glabra* essential oil showed the lowest acute toxicity in this research.

Functional response type

Logistic regression model with linear and non-linear parameters indicated the functional response types in the control and essential oils treatments (Table 7). According to the results, the functional response type II ($P_1 < 0$) were determined in the control and *P. nigrum*, *S. officinalis* and *G. glabra* and type III ($P_1 \geq 0$) in *A. sativum* and *R. officinalis* essential oils, respectively (Figs 1 and 2).

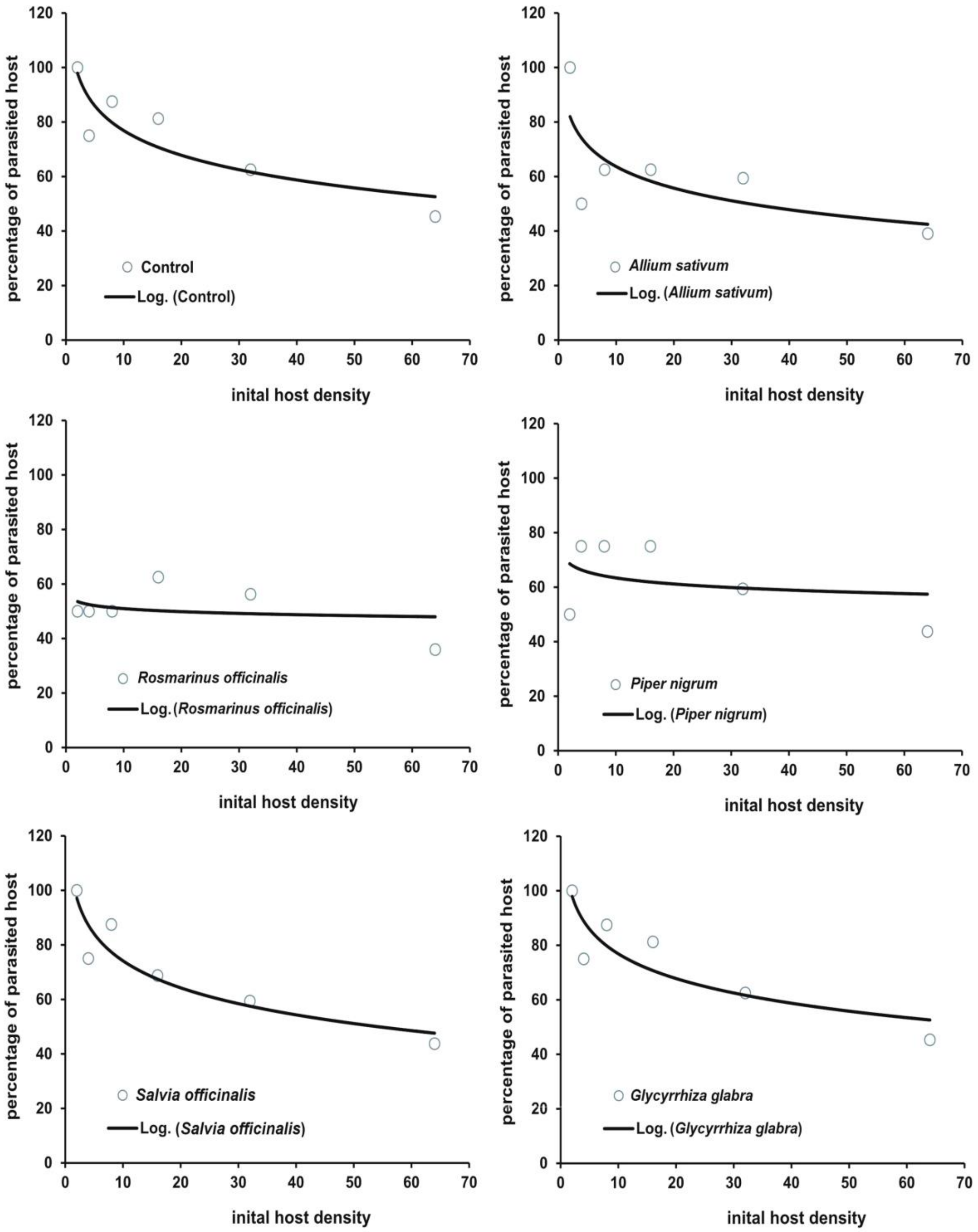

Fig. 2 The percentage curve of parasitized larvae by *H. hebetor* previously exposed to LC_{30} of tested essential oils and the control

Functional response parameters

The estimation results of handling time, attack rate and theoretical maximum attack rate values from treated wasps of *H. hebetor* are shown in Table 8. Accordingly, the control and *R. officinalis* essential oil treatments showed the shortest (0.411± 0.028 h) and longest (0.542± 0.058 h) values of handling time, respectively. Also, the highest and lowest attack rate values were recorded in the control (0.047± 0.003 h^{-1}) and *R. officinalis* essential oil (0.033± 0.003 h^{-1}) treatment, respectively. In addition, the highest value of the theoretical maximum attack rate base on T/T_h was obtained in the control (58.35) and the lowest being in *R. officinalis* essential oil (44.28) treatment; however, the difference between *R. officinalis* and *S. officinalis* wasn't significant.

Table 8 Functional response parameters in *H. hebetor* previously exposed to LC_{30} of essential oils

Treatment	Type of functional response	Attack rate (h) a ± SE (Lower-Upper)	Handling time (h^{-1}) T_h ± SE (Lower-Upper)	Theoretical maximum attack rate (T/T_h)	R^2
Control	II	0.047 ± 0.003 (0.042 - 0.053)	0.411 ± 0.028 (0.355 - 0.469)	58.35	0.93
A. sativum	III	0.036 ± 0.003 (0.023 - 0.042)	0.515 ± 0.055 (0.405 - 0.626)	46.57	0.86
R. officinalis	III	0.033 ± 0.003 (0.027 - 0.038)	0.542 ± 0.058 (0.425 - 0.659)	44.28	0.85
P. nigrum	II	0.039 ± 0.002 (0.034 - 0.043)	0.462 ± 0.036 (0.389 - 0.534)	51.99	0.85
S. officinalis	II	0.041 ± 0.004 (0.034 - 0.048)	0.530 ± 0.052 (0.426 - 0.635)	45.27	0.86
G. glabra	II	0.042 ± 0.002 (0.037 - 0.047)	0.444 ± 0.031 (0.381 - 0.506)	54.09	0.86

a: Attack rate, T_h: Time of handling (Handling time),R^2: Coefficient of specification, SE: Standard error.

Discussion

Studies about the effects of different plant compounds such as essential oils on the functional response of *H. hebetor* can be useful tool for forecasting *H. hebetor* success in IPM programs, especially in the management of stored pests. The essential oils are safe compounds for human and environment programs and many of them showed high toxic effects due to aromatic and biologically active vapours (Yildirim *et al*., 2011). There is no study about the effects of selected essential oils on the other natural enemies; but, the effects of these essential oils were investigated on different insect pests; such as *A. sativum* essential oils on *Tribolium castaneum* (Herbst), *R. officinalis* essential oil on larvae of *Pseudaletia unipuncta* (Haworth) and *Trichoplusia ni* (Hübner), *P. nigrum* essential oil on rice weevil, *Sitophilus oryzae* L. and rice moth, *Corcyra cephalonica* (St.), *S. officinalis* essential oils on *Drosophila melanogaster* Meigen and *Bactrocera oleae* (Rossi) and *G. glabra* essential oil on potato tuber moth *Phthorimaea operculella* (Zeller); and showed suitable effects on control of mentioned insects (Yildirim *et al*., 2011; Yazdgerdian *et al*., 2015).

In addition, there are few investigations about the effects of selected plant essential oils on the important ectoparasitoid wasp, *H. hebetor* (Seyyedi *et al*., 2011; Hashemi *et al*., 2014; Ahmadpour, 2017). In our study, the tested essential oils showed different acute toxicity on the adult females of *H. hebetor* that are in agreement with the findings reported by Seyyedi *et al*., (2011), who studied the impacts of isolated essential oil from *Ferula gummosa* L. on the female wasps of *H. hebetor* and concluded that mortality of *H. hebetor* was increased after 24 h of exposure (LC_{50}= 9.16 µl/liter air). Moreover, Hashemi *et al*., (2014) concluded that *Ferula assafoetida* L. essential oil had high toxicity on *H. hebetor*.

Tetracosamethyl cyclododeca siloxan, alpha-pinene, caryophyllene, beta-thujone and aristolene as major components in *A. sativum*, *R. officinalis*, *P. nigrum*, *S. officinalis* and *G. glabra* essential oil are volatile and aromatic compounds that showed high toxicity against *H. hebetor* in our research. These compounds contain active molecules that have fumigant, contact, antifeedants and repellent mode of actions and can be considered as efficient insecticides against different insect pests especially in enclosed environments (Yazdgerdian *et al*., 2015). Accordingly, the sublethal concentrations of different essential oils and the other botanical compounds can have negative effects on natural enemies especially on the functional response parameters (Croft, 1990; Mahdavi and Saber, 2013; Jarrahi and Safavi, 2015). The results of this research showed that the attack rate values in treated wasps of *H. hebetor* with sublethal concentrations of studied essential oils was lower than control; but, the handling time values were higher than control; this shows that these essential oils have changed the searching behavior and the other parasitism activities of *H. hebetor*; because, when handling time increase therefore attack rate decrease and this is a negative effect of a compound on a biocontrol agent.

There is no research about the effects of plant essential oils on the functional response of *H. hebetor*; but, the researches on the insecticides effects in this case are available. Mahdavi (2011) studied the effects of abamectin, carbaryl, chlorpyrifos and spinosad and reported functional response type III in the control and all insecticides treatments that his results are in agreement with our results about *A. sativum* and *R. officinalis* essential oil. Mahdavi and Saber (2013) also concluded that malathion had lower negative effects on the functional response of *H. hebetor* compared with diazinon in IPM programs; but, our results indicated that *G. glabra* essential oil was compatible compound with *H. hebetor*. Because, this essential oil showed the lowest adverse effects on the functional response type and it's parameters in this parasitoid wasp. According to the result of Rafiee-Dastjerdi *et al.*, (2013) and Nazeefullah *et al*., (2014); *G. glabra* also showed low toxic effects on against potato tuber moth *Phthorimaea operculella* (Zeller) and *Tribolium castaneum* (Herbst), respectively.

Rafiee-Dastjerdi *et al*., (2009b) also reported that the functional response of *H. hebetor* under hexaflumuron, profenofos, spinosad and thiodicarb and control treatments was type II, and their results are in agreement with our results about the control and *P. nigrum*, *S. officinalis* and *G. glabra* essential oils treatments. Faal-Mohammad Ali *et al*., (2010) stated that the functional response type in *H. hebetor* under larval and pupal treatments with chlorpyrifos and fenpropathrin and in the control was type III that their results are in disagreement with our results about the control, *P. nigrum*, *S. officinalis* and *G. glabra* due to differences of treatments, growth stage of parasitoid wasps and it's response type to different densities of host. Moreover, Abedi *et al*., (2012) studied the sublethal effects of azadirachtin, cypermethrin, methoxyfenozide and pyridalil on the functional response of *H. hebetor* and concluded that among them based on obtained handling time values, cypermethrin showed the highest adverse effect on the host-finding behavior of this parasitoid wasp; but, in our study *R. officinalis* essential oil showed the highest effects on this important characteristic of this parasitoid wasp. In addition, Jarrahi and Safavi (2015) concluded that proteus as a new formulated insecticide (based on combination of thiacloprid and deltamethrin) in pupal stage treatment of *H. hebetor* showed the highest handling time and the lowest attack rate compared with *Metarhizium anisopliae* and the control; because the results are same, in our study *R. officinalis* showed the highest handling time and the lowest attack rate values on *H. hebetor* and therefore is an incompatible essential oil with this parasitoid wasp. The theoretical maximum attack rate also in all examined treatments was different and the highest value of this parameter was recorded for the control that this is in agreement with the results obtained by Rafiee-Dastjerdi *et al*., (2009b); Abedi *et al*., (2012) and Mahdavi and Saber, (2013).

Functional response studies under laboratory conditions may have low similarity to the results that obtained in the field conditions (Munyaneza and Obrycki, 1997). Houck and Strauss (1985) and Darwish *et al*., (2003) concluded that laboratory functional response has important role in understanding of the relations between different natural enemies and their hosts in biological control programs. Such studies can provide valuable informations for developers of biological control programs and release of natural enemies in agricultural crops. In conclusion, this research showed that isolated essential oils affected the functional response and quality control of this parasitoid wasp. This study indicated that there isn't significant difference between *G. glabra* compared with the control and *G. glabra* essential oil hadn't negative effects on the functional response of *H. hebetor* and it's parameters including attack rate, handling time and theoretical maximum attack rate. Therefore, *G. glabra* essential oil can be recommended as a suitable botanical compound in Integration with *H. hebetor* in IPM programs. In this research, we investigated the effects of selected essential oils against the ectoparasitoid wasp *H. hebetor* for the first time. This study shows the potential of essential oils as effective and natural compounds on different insects. The authors recommend more researches about the effects of essential oils on the other natural enemies and also application of these compounds for management of insect pests especially in enclosed environments.

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