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Functional Response of *Eretmocerus delhiensis* (Hymenoptera: Aphelinidae) on *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) by Parasitism and Host Feeding

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Abstract

The parasitoid wasp, *Eretmocerus delhiensis* Mani is a thelytokous and syn-ovigenic parasitoid. To evaluate *E. delhiensis* as a biocontrol agent in greenhouse, the killing efficiency of this parasitoid by parasitism and host-feeding, were studied. Killing efficiency can be compared by estimation of functional response parameters. Laboratory experiments were performed in controllable conditions to evaluate the functional response of *E. delhiensis* at eight densities (2, 4, 8, 16, 32, 64, 100, and 120 third nymphal stage) of *Trialeurodes vaporariorum* Westwood on two hosts including; tomato and prickly lettuce. The maximum likelihood estimates from regression logistic analysis revealed type II functional response for two host plants and the type of functional response was not affected by host plant. Roger's model was used to fit the data. The attack rate (*a*) for *E. delhiensis* was 0.0286 and 0.0144 per hour on tomato and 0.0434 and 0.0170 per hour on prickly lettuce for parasitism and host feeding, respectively. Furthermore, estimated handling times (T_h) were 0.4911 and 1.4453 h on tomato and 0.5713 and 1.5001 h on prickly lettuce for parasitism and host feeding, respectively. Based on 95% confidence interval, functional response parameters were significantly different between the host plants solely in parasitism. Results of this study opens new insight in the host parasitoid interactions, subsequently needs further investigation before utilizing it for management and reduction of greenhouse whitefly.

Key words: *Eretmocerus delhiensis*, functional response, host density, host-feeding

The greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hem., Aleyrodidae) is a cosmopolitan, polyphagous, familiar, and key pest that attacks many crops and causes serious economic damage to crops throughout the tropical and subtropical areas and in greenhouses (Byrne 1990, Gerling 1990). The nymphs and adults of the greenhouse whitefly suck fluids and surplus sugars from plants are excreted as honeydew (Byrne 1990). Moreover, whiteflies are potential vectors of viruses (van der Linden and van der Staaij 2001). This pest have numerous wild and domestic host species from the families; Solanaceae and Asteraceae. For instance, prickly lettuce, *Lactuca serriola* (Asteraceae) is one of the most important wild hosts of the greenhouse whitefly (Roditakis 1990).

The *T. vaporariorum* populations can be attacked by some parasitoids from the family Aphelinidae. Among these parasitoid wasps, genera *Encarsia* and *Eretmocerus* have been received more attentions from entomologists (Urbaneja and Stansly 2004, Urbaneja et al. 2007, Liu et al. 2015). These genera are primary and solitary parasitoid for different nymphal stages of whiteflies (Zolnerowich and Rose 2008).

The genus *Eretmocerus* includes 85 nominal species which are very important in biological control and in integrated management of whiteflies (Noyes 2012). Potential of *Eretmocerus* sp. (Lopez and Botto 1997) and *E. eremicus* (Gamborena and van Lenteren 2004) as an agent for biological control were studies on greenhouse whitefly. Also, investigation on reproductive biology of *E. warrae* a thelytokous parasitoid of *T. vaporariorum* showed that this parasitoid can potentially contribute to biological control of greenhouse whitefly (Hanan 2012).

Some *Eretmocerus* parasitoid wasps can suppress host by parasitizing and feeding. By feeding on host haemolymph, a female parasitoid can increase her longevity and fecundity (Liu et al. 2015). Host-feeding by female parasitoids has been reported in many species of *Eretmocerus* and *Encarsia* (Zang and Liu 2008). Host-feeding is the consumption of host haemolymph coming from the wound, caused by the female ovipositor (Jervis and Kidd 1986).

Eretmocerus delhiensis Mani (1941) has recently been reported on sugarcane whitefly, *Neomaskellia andropogonis* Corbett from

Iran (Khadempour et al. 2014b). The biology of this parasitoid has been studied on *N. andropogonis* and *T. vaporarioirum* (Khadempour 2013, Ebrahimifar et al. 2016). Results showed that the increase rate of natural increase of *E. delhiensis* on *N. andropogonis* was higher (0.24) than *T. vaporarioirum* (0.17). The development time from egg to adult and adult longevity of *E. delhiensis* on greenhouse whitefly were 15.5 and 5.7 d, respectively (Ebrahimifar et al. 2016). However, Viggiani (1985) has been reported male and female for *E. delhiensis*, it was reported only female from Iran (Ebrahimifar et al. 2016). Therefore the *E. delhiensis* shows a thelytokous reproduction behavior in Iran. This parasitoid is a destructive syn-ovigenic parasitoid (Ebrahimifar et al. 2016). Destructive syn-ovigenic parasitoids fed on their hosts which lead to death of the hosts (Jervis 2007).

Differences in killing efficiency can be compared by estimation and comparison of functional response parameters (Livdahl and Stiven 1983, Juliano 2001). The key factor for a predator is assessment of potential and predation rate on different host densities (Yu et al. 2013). The functional response is the behavioral reaction of a natural enemy to host density which means numbers parasitized or eaten versus the initial numbers. There are many evidences that show type and parameters of a functional response are affected by different abiotic and biotic factors including temperature, host species, natural enemy, physical conditions in laboratory, host plant and the age of the parasitoid (Mohaghegh et al. 2001, Allahyari et al. 2004, Reay-Jones et al. 2006, Moezipour et al. 2008, Jamshidnia et al. 2010, Jamshidnia and Sadeghi 2014). Among the criteria utilized for evaluating the potential of natural enemies, are attack rate and handling time that measured by functional response of natural enemies (parasitoids or predators) to increasing host density. Different factors may influence the type of functional response or parameters values by change in searching pattern (Holling 1959).

The objectives of the current study were to determine the type of functional response, its parameters and evaluation of host-feeding and parasitism of parasitoid wasp, *E. delhiensis* on *T. vaporariorum* on two plant hosts, tomato and prickly lettuce at different densities.

The results from this study will help to our knowledge about, interaction between parasitoid and host density to improve it use in biological control program.

Materials and Methods

Rearing of *T. vaporariorum* and *E. delhiensis*
Population of parasitoid wasp *E. delhiensis* was collected from origin colony on sugarcane whitefly, *N. andropogonis* (Hem., Aleyrodidae) from Khuzestan Province, Iran (Latitude: 31° 20′ N, Longitude: 48° 40′ E). The greenhouse whiteflies were collected from tomato greenhouses in Tehran. Parasitoid population was reared on *T. vaporariorum* colony on tomato plant (*Solanum lycopersicum* L. cultivar super-chief) and its wild host, prickly lettuce, *Lactuca serriola* L. (Asteraceae) in greenhouse conditions (25 ± 3 °C, 60 ± 10% RH) at College of Aburaihan, University of Tehran, Iran. Two distinct colonies of hosts and parasitoids on host plants were reared for three generations and then used in experiments.

Functional Response Experiments Design
In order to determine the functional response of *E. delhiensis*, individual parasitoids were exposed to eight densities (2, 4, 8, 16, 32, 64, 100 and 120) third nymphal stages of greenhouse whitefly. This parasitoid preferred third nymphal instars for parasitism

(unpublished data). The leaves with whitefly nymphs of tomato and prickly lettuce plants were used. For densities of 2, 4, 8, 12 replicates were used while for other densities, 10 replicates were utilized for each host plants. Each leaf was fixed on moist filter paper (to prevent desiccation) in a Petri dish (10 cm diameter). A few drops of water were added to the filter paper to keep them moist during the experiments. A ventilation hole (1 cm diameter) was created in the lid of each Petri dishe, which was covered with net cloth. One female parasitoid (<24 h old) was introduced into the experimental arena. After 24 h, the parasitoid wasps were removed. Host mortality by parasitism and host-feeding was determined 7–8 d later. In parasitized hosts the mycetome displacement was visible, while hosts killed by host-feeding were flattened and desiccated (Yan and Wan 2011). All experiments were performed in controlled conditions at temperature of 25 ± 1 °C, 65 ± 5% RH and a photoperiod of 16: 8 h (L: D) in growth chamber.

Data Analysis
Data analysis for functional response includes two steps. In the first step, shape (type) of functional response must be determined by determining if the data fit a type II or III functional response. Logistic regression of the proportion of parasitized hosts versus the initial number of host is the most effective way of determining this (De Clercq et al. 2000, Juliano 2001, Allahyari et al. 2004, Jamshidnia et al. 2010). In the first step, we fitted a polynomial function (Juliano 2001).

$$\frac{N_a}{N_0} = \frac{\exp\ (P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp\ (P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)} \tag{1}$$

Where, P_0, P_1, P_2, and P_3 are parameters intercept, linear, quadratic and cubic coefficients, respectively, that are to be estimated. N_a is the number of parasitized or attacked nymphs and N_0 is the initial host density. These parameters were estimated using the CATMOD procedure in SAS software (SAS Institute 2011). The sign of P_1 and P_2 can be utilized to distinguish the shape of the curves. A positive linear parameter (P_1) indicates that functional response is type III while the functional response is type II when the linear parameter is negative (Juliano 2001). After determining the type of functional response, parameters of handling time (T_h) and attack rate (*a*)were estimated (Juliano 2001). We used nonlinear least square regression (NLIN procedure with DUD method in SAS) to estimate the parameters of the Rogers (1972) random parasitoid equation (2) and random predator equation (3).

$$N_a = N_0 \left[1 - \exp \left(-\frac{aT_1}{1 + aT_h N_0} \right) \right] \tag{2}$$

$$N_a = N_0 \{ 1 - \exp[a(T_h N_a - T_t)] \} \tag{3}$$

Where, N_a= Host attacked, N_0= Host density, T_t= Time of exposure to parasitoid, *a*= Instantaneous searching rate and T_h= Handling time.

Results and Discussion

Functional response curves of parasitoid wasp, *E. delhiensis* are shown in Fig. 1. The average number of hosts fed on and parasitized by increasing the host densities at first increased, and then approached a constant level. The results of logistic regression analysis (Table 1) indicated the linear coefficient (P_1) was negative for parasitism and host feeding on two host plants. These results indicate a type II functional response for *E. delhiensis*. Thus, type of functional response was not affected by host plant in both cases of parasitism
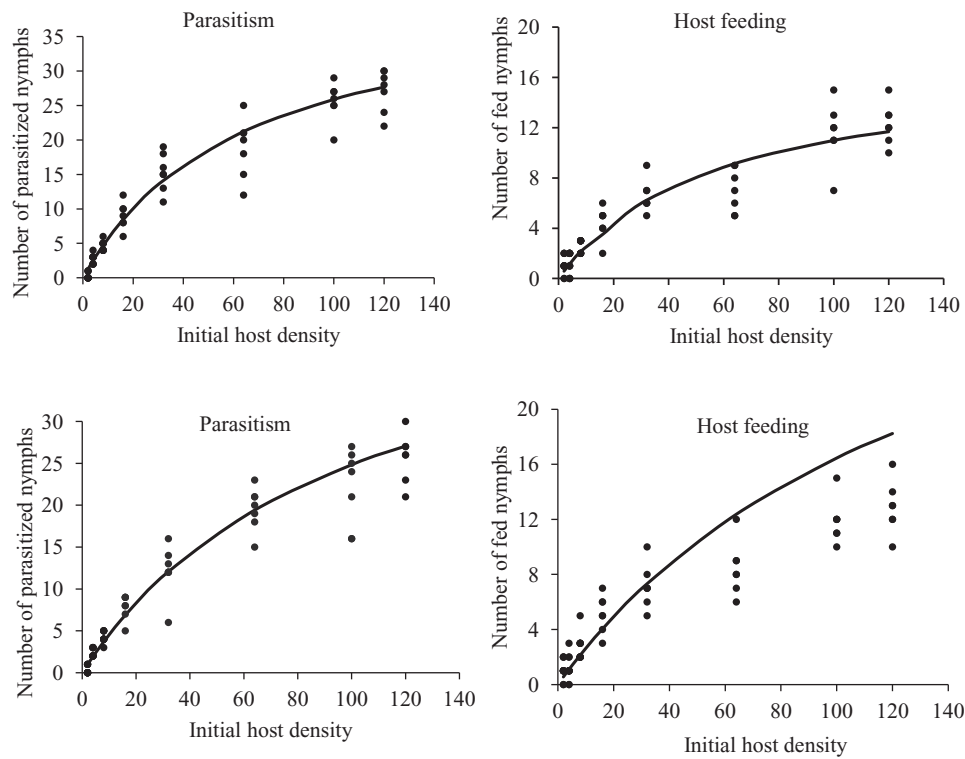
Fig. 1. Functional response of *E. delhiensis* on tomato (upper) and on prickly lettuce (lower) to different densities of *T. vaporariorum* (symbols: observed, line: predicted by model).

Table 1. Maximum likelihood estimates from regression logistic analysis of the proportion of *T. vaporariorum* parasitized and fed by *E. delhiensis* on two hosts

Host	Behavior	Coefficient	Estimate	SE	χ^2 value	*P* value
Tomato	Parasitism	*Constant*	−0.00578	0.2024	0.00	0.9772
		Linear	−0.0215	0.0142	2.31	0.1284
		Quadratic	0.000193	0.000252	0.59	0.4443
		Cubic	−8.42E-7	1.263E-6	0.45	0.5046
	Host feeding	*Constant*	−0.1239	0.2127	0.34	0.5601
		Linear	−0.0650	0.0167	15.10	0.0001
		Quadratic	0.000733	0.00031	5.58	0.0182
		Cubic	−2.77E-6	1.584E-6	3.05	0.0809
Prickly lettuce	Parasitism	*Constant*	0.4100	0.1978	4.30	0.0382
		Linear	−0.0214	0.0138	2.40	0.1212
		Quadratic	0.000059	0.000247	0.06	0.8126
		Cubic	−7.497E-8	1.28E-6	0.00	0.9518
	Host feeding	*Constant*	−0.3064	0.2129	2.07	0.1502
		Linear	−0.0427	0.0165	6.72	0.0095
		Quadratic	0.000336	0.000305	1.21	0.2713
		Cubic	−8.79E-7	1.563E-6	0.32	0.5738

and host feeding. Type II functional response was previously recorded in a number of species *Eretmocerus* (Sohani et al. 2008, Shao et al. 2010, Xu et al. 2014).

Different studies on functional response of insect parasitoids show that more than three-quarters are type II functional response while less than one-fifth have type III functional response (Fernández-Arhex and Corley 2003). The functional response of parasitoids can be affected by density of parasitoid and its host (Mills and Lacan 2004). The type II functional response of *Eretmocerus mundus* on *Bemesia tabaci* and *E. hayati* on *B. tabaci* biotype B and Q has been reported (Sohani et al. 2008,

Shao et al. 2010). Furthermore, functional response of *Encarsia formosa* on *T. vaporariorum* and *Aphelinus thomsoni* on the aphid, *Drepanosiphum platanoidis* was type II (Collins et al. 1981, Fransen and Montfort 1987). The type II functional response of *E. delhiensis* on sugarcane whitefly *N. andropogonis* was reported by Khadempour et al. (2014a). Based on our results and the findings of other researchers (Collins et al. 1981, Sohani et al. 2008, Shao et al. 2010, Xu et al. 2014) it seems that the type II functional response is common in aphelinid wasps.

The type II functional response indicate an inverse density dependent relationship between the proportion of parasitism (or host

Table 2. Estimated functional response parameters by Rogers model for *E. delhiensis* to different densities of *T. vaporariorum* on two hosts

Host	Behavior	Type	Parameter	Estimate	Asymptotic SE	Asymptotic 95% CI	
						Lower	Upper
Tomato	Parasitism	II	A	0.0286	0.00161	0.0254	0.0318
			T_h	0.4911	0.0227	0.4457	0.5365
	Host feeding	II	A	0.0144	0.00153	0.0113	0.0174
			T_h	1.4453	0.1042	1.2375	1.5632
Prickly lettuce	Parasitism	II	A	0.0434	0.0221	0.0390	0.0478
			T_h	0.5713	0.0149	0.5416	0.6010
	Host feeding	II	A	0.0170	0.00149	0.0141	0.0200
			T_h	1.5001	0.0772	1.3461	1.6542

CI, confidence interval, a (h^{-1}), T_h (h).

feeding) and host density (Holling 1959). Therefore, *E. delhiensis* can be more efficient in control of greenhouse whitefly at low density.

The random parasite and predator equations fit the experimental data well for *E. delhiensis* in parasitism and host feeding, respectively. Rogers random model is more suitable than Holling disq equation for describing functional response when host depletion occurs (Juliano 2001). Results of NLIN regression indicated that parameters *a* (searching rate) and T_h (handling time) were both significant (Table 2). Estimated searching rate values (*a*) for *E. delhiensis* on tomato were 0.0286 and 0.0144 h^{-1} and on prickly lettuce were 0.0434 and 0.0170 h^{-1} for parasitism and host feeding, respectively. The values of T_h for this parasitoid on tomato were 0.4911 and 1.4453 h and on prickly lettuce were 0.5713 and 1.5001 h for parasitism and host feeding, respectively. Based on 95% confidence intervals, for the values of T_h and *a* for parasitism, the observed difference was statistically significant on both hosts because there was no overlapping between them but in host feeding, the observed difference was not statistically significant. The tomato cultivar (super-chief) was used in this study has more pubescent and trichome than prickly lettuce, consequently *a* values were reduced in tomato. Presumably, pubescent and trichome of plant host affected the parasitism and host feeding of parasitoids. Furthermore, it seems that hairy leaf of tomato may be slowing the parasitoid movement than in prickly lettuce leaf. The values of *a* and T_h for *E. delhiensis* on *N. andropogonis* on sugarcane were reported 0.0594 h^{-1} and 0.766 h (Khadempour et al. 2014a). Handling time for *E. mundus* on *B. tabaci* was reported 0.343 h by Sohani et al. (2008).

Differences observed in parameters values of the current study compared with other studies may be due to the difference of host and parasitoid species. On the other hand, parasitoids after parasitizing and/or feeding on their hosts maybe spent different times to clean their body which lead to changes in the values handling time and attack rate.

The results of the functional response study can be used to preselect the candidates of biological control (van Lenteren et al. 2016). The results of this study in both parasitism and host-feeding showed the functional response of *E. delhiensis* was type II. van Lenteren et al. (2016) suggested that natural enemies with type II functional response could be used in inundative biological control. Clearly, this parasitoid kill their hosts not only by parasitism but also by host-feeding. Hence, it can be a promising candidate for biological control of greenhouse whitefly.

Although functional response is an important tool for evaluating natural enemies but success and failure of a natural enemy in biological control cannot be only attributed to this factor. In addition, different factors such as; biotic and abiotic factors, host traits, may influence the discovery and parasitism efficiency of natural enemies. Thus, further studies should be performed to evaluate the efficiency of *E. delhiensis* as a biological control agent of greenhouse whitefly.

Acknowledgments

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