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Functional response of two native species *Typhlodromus khosrovensis* and *Euseius amissibilis* (Acari: Phytoseiidae) feeding on eggs of *Tetranychus urticae* (Acari: Tetranychidae)

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Original research

ABSTRACT

Typhlodromus (Anthoseius) khosrovensis and *Euseius amissibilis* are two native phytoseiidae species in different parts of Tehran city, Iran, which were recorded from white mulberry trees, for the first time. The studies about the predator-prey dynamics of native phytoseiids are rare. Thus, this investigation aimed to determine the predatory efficiency of both species against eggs of *Tetranychus urticae*. Under constant conditions (27 °C, 65% RH, and a photoperiod of 16:8 h L/ D), six egg prey densities (2, 4, 8, 16, 32, and 64) were separately offered to each predator to record its predation rates for 24h. According to the results, *T. khosrovensis*, and *E. amissibilis* represented a type II functional response. In *T. khosrovensis*, the attack rate coefficient (α) was 0.0213 h⁻¹, and the handling time (T_h) was 2.8013 h. These values in *E. amissibilis* were recorded as 0.0204 h⁻¹ and 2.3018 h, respectively. The maximum attack rate (T/T_h) on eggs of *T. urticae* was estimated to be 8.57 h⁻¹ for *T. khosrovensis*, and 9.98 h⁻¹ for *E. amissibilis*. Therefore, both native predators illustrated a highly efficient biological control against eggs of *T. urticae*, although field studies are needed to show their ability as promising predators on mulberry trees in the future.


Keywords Phytoseiidae; predatory mite; indigenous species; mulberry trees

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Introduction

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most serious agricultural pests found in both field and greenhouse crops in various agroecosystems worldwide. This mite damages over 1,100 plant species from 140 families, with more than 150 of these plant species, having significant economic value (Takafuji 1994; Bolland *et al.* 1998; Migeon *et al.* 2011). Indeed, *T. urticae* poses a significant threat to various crops, including vegetables, large trees, horticultural plants, and deciduous fruit trees (Oliveira *et al.* 2007; Migeon *et al.* 2010).

Considering the evolution of pesticide resistance, the use of biological control agents is a necessary method against *T. urticae* all over the world (Gerson and Weintraub, 2007; Gerson, 2014; Amoah *et al.* 2016; Gigon *et al.* 2016). Predatory mites in the family Phytoseiidae are highly effective biological control agents. They play a critical role in controlling small-bodied

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pests such as spider mites, thrips, whiteflies, and nematodes in crops (Williams *et al.* 2004; Rahmani *et al.* 2009). Extensive research has been conducted on the distribution, ecology, biology, and behavior of this family in Iran (Hajiqanbar and Farazmand, 2021). Most studies have focused on the behavioral aspects and life table parameters of the phytoseiid species (Seiedy *et al.* 2012; Solymani *et al.* 2016; Fathipour *et al.* 2017; 2018; 2020; Dalir *et al.* 2021). However, there is limited information available about the biology and behavior of native phytoseiids (Ganjisaffar *et al.* 2011). Until now, the distribution of 76 native species in Iran has been recorded (Hajizadeh and Faraji 2016; Demite *et al.* 2024), but only four species, namely *Typhlodromus (Anthoseius) bagdasarjani* Wainstein and Arutunjan, *Phytoseius plumifer* (Canestrini & Fanzago), *Amblyseius herbicolus* Chant and *Neoseiulus barkeri* Hughes were considered in the biological and behavioral studies (Notghi Moghadam *et al.* 2010; Ganjisaffar *et al.* 2011; Jafari *et al.* 2012; Farazmand *et al.* 2012; Moghadasi *et al.* 2014; Riahi *et al.* 2016).

Functional response is an ecological parameter used to evaluate predatory mites' efficiency. It is often applied to estimate the effectiveness of natural enemies before releasing them (Xiao & Fadamiro, 2010). This parameter illustrates important information about the voracity of a biocontrol agent. It can be affected by abiotic factors such as temperature, experimental units, and applying pesticides, as well as biotic factors including prey species, prey stage, host plant, natural enemies' generation, and age (De Clercq *et al.* 2000; Mohaghegh *et al.* 2001; Skirvin and Fenlon 2001; Mahdian *et al.* 2006; Li *et al.* 2007). Holling (1959) was the first one who propose a functional response and classified it into three types. Most phytoseiids have shown functional response type II (Castagnoli and Simoni, 1999; Cedola *et al.* 2001; Reis *et al.* 2003; Ahn *et al.* 2010) in which the number of consumed prey approaches the asymptote hyperbolically as prey density increases. In type III, the number of consumed prey approaches the asymptote as a sigmoid function and the proportion of consumed prey exhibits a positive density dependence over some regions of prey density. Although functional response type III has also been recorded in different ages of some phytoseiid species (Fathipour *et al.* 2017; 2018).

Numerous studies in Iran have dealt with this aspect of phytoseiid mites which are important commercially (Fathipour *et al.* 2017; 2018; 2020). On the other hand, studies held on native phytoseiids are rare (Kouhjeni Gorji *et al.* 2009; Farazmand *et al.* 2012; Jafari *et al.* 2012).

During a study on phytoseiids of Tehran city, three species were identified namely *Typhlodromus (Anthoseius) khosrovensis* Arutunjan, *Euseius amissibilis* Meshkov, and *T. bagdasarjani* (Afkhami *et al.* 2024). The functional response of *T. bagdasarjani*, as well as biology, behavioral characteristics, and its interaction with other predators have already been studied (Ganjisaffar *et al.* 2011; Farazmand *et al.* 2012, 2015; Moghadasi *et al.* 2014; Jafarian *et al.* 2022). van Houten *et al.* (2016) reported that *E. amissibilis* has the potential to control whiteflies and thrips. However, there are no studies about the biology and behavioral/ecological features of *E. amissibilis* and *T. khosrovensis* while feeding on *T. urticae*.

In this study, two phytoseiid mites *T. khosrovensis* and *E. amissibilis* were collected from mulberry trees in Tehran city, Iran to evaluate their functional response when preying on *T. urticae* eggs. Therefore, the potential of both phytoseiid species as a native biological control agent could be explored.

Materials and methods

Stock prey colony

The colony of *Tetranychus urticae* used in this study was obtained from the Department of Agricultural Research Zoology, Iranian Research Institute of Plant Protection, Tehran, Iran. The mites were reared on lima bean (*Phaseolus vulgaris*) at 25 ± 1 °C, 60–70% RH, and a photoperiod of 16:8 h (L: D).

Collecting and rearing predators

Typhlodromus khosrovensis, and *E. amissibilis* were collected from mulberry tree leaves in Chitgar Forest Park and Shad Park, respectively in Tehran, Iran, in 2023. For rearing both predator species, they were transferred into an arena consisting of a plastic sheet putting on water-saturated foam in a Plexiglas box (26 × 18 × 10 cm), which was half filled with water. The edges of the plastic sheet were covered with moist tissue paper to prevent predators from escaping (Walzer and Schausberger 1999). Bean leaves fully infested with *T. urticae* were put on the arena three times a week. The phytoseiid colony was kept in a growth chamber in constant condition (25 ± 1 °C, 60± 70% RH, and a photoperiod of 16:8 h L: D). The age of the adult females used for *T. khosrovensis* and *E. amissibilis* was 24 hours for the functional response experiments.

Functional response

The experimental unit was a 3.5 cm diameter mulberry leaf disc placed upside down on a thin layer of cotton in a petri dish with a 6 cm diameter, and 1 cm height. Six densities (2, 4, 8, 16, 32, and 64) of *T. urticae* eggs were used in functional response experiments. 44 adult females of *T. urticae* from the stock culture were transferred onto the leaf discs and allowed to oviposit for 24 hours, then they were removed. The eggs deposited were counted, and only the specified number was kept in the experimental unit, and the remaining eggs were removed. After that, the adult female predatory mites were starved for 6 hours and transferred to the leaf discs to feed on the prey for 24 hours. To prevent the escape of predatory mites, the petri dishes' openings were sealed with parafilm, and to provide ventilation, about 10 tiny holes were made in parafilm. Each experiment was replicated 10 times at temperature of 25 ± 1 °C, 60–70% RH, and a photoperiod of 16:8 h (L: D). After 24 h, the number of intact eggs remaining was counted. The data on functional response were analyzed in two steps (Juliano, 2001). First, the type of functional response was determined by logistic regression of the proportion of prey consumed (N_a / N_0) as a function of prey density (N_0):

$$\frac{N_e}{N_o} = \frac{\exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3 + P_4N_0^4)}{1 + \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3 + P_4N_0^4)} \quad (1)$$

Where P_0 , P_1 , P_2 , and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively, which were estimated using the method of maximum likelihood. The sign of the P_1 from equation (1) can be used to distinguish the type of the functional response curve. If $P_1 < 0$, it describes a type II functional response. If $P_1 > 0$ and $P_2 < 0$, it shows a type III functional response (Juliano, 2001). After determining the type of functional response, the next step is to estimate the handling time and attack rate coefficients. In this study, we used an explicit deterministic model for type II functional response (Royama, 1971; Rogers, 1972):

$$N_a = N_t[1 - \exp(a(ThNa - T))](2)$$

Where N_a is the number of prey killed, N_t is the initial number of prey, T is the total time available for the predator, a is the attack rate, and T_h is the handling time. For a type III response, the attack rate is assumed to increase with host density according to the equation $a = (d + bN_0) / (1 + cN_0)$ (Hassell *et al.* 1977). In cases where both d and c are not significantly different from 0, this leads to $a = bN_0$ which can be inserted into equation (2). Then, for each host density, the attack coefficient (a) can be determined as $a = bN$.

Results

The functional response of both phytoseiid species showed a type II on eggs of *T. urticae* (Figure 1). The linear coefficient of Equation (1) in both cases was negative and significantly different from 0 ($P < 0.01$), indicating that the consumption rate of *T. urticae* eggs declined as

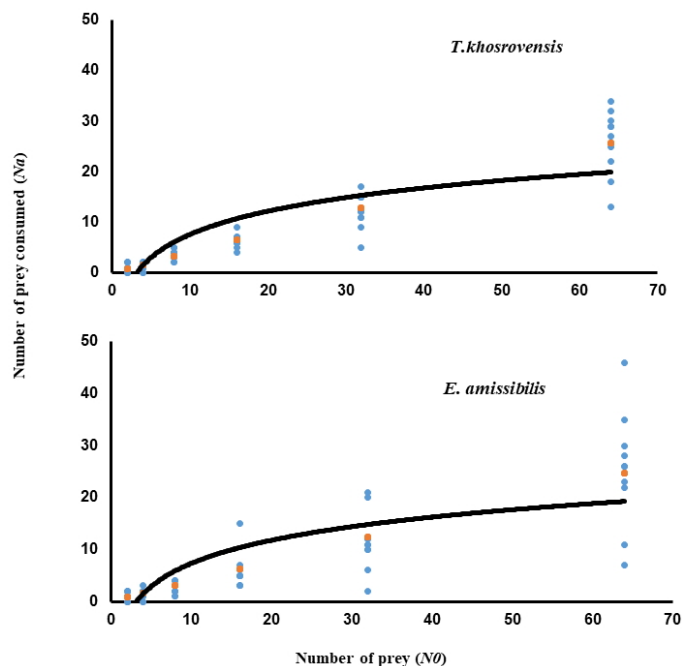


Figure 1 The functional response of *Euseius amissibilis* and *Typhlodromus khosrovensis* feeding on eggs of *Tetranychus urticae*.

prey density increased (Table 1). The highest attack rate, and the lowest handling time were recorded for *E. amissibilis* on the eggs of *T. urticae* (Table 2). *Euseius amissibilis* consumed higher numbers of eggs compared to *T. khosrovensis*, particularly at higher prey densities (Table 2). The maximum attack rate (T / T_h) on eggs of *T. urticae* was estimated to be 9.98 for *E. amissibilis*, and 8.57 for *T. khosrovensis*, respectively (Table 2).

Discussion

In this study, the functional response of both phytoseiids, *T. khosrovensis*, and *E. amissibilis* feeding eggs of *T. urticae* showed a type II, where the predation rate decreases as prey density increases. This type of functional response has been reported in phytoseiid mites in previous studies (Ahn *et al.* 2010; Castagnoli *et al.* 1999; Farazmand *et al.* 2012; Jafari *et al.* 2011) and other species of the genus *Typhlodromus* and *Euseius* showed the same type of functional response on eggs and immature stages of *T. urticae* (Badii *et al.*, 2004; Farazmand *et al.*, 2012).

According to the lifestyle classification of Phytoseiidae family, *T. khosrovensis*, and *E.*

Table 1 Maximum-likelihood estimates from logistic regression of the proportion of *Tetranychus urticae* eggs consumed by *Euseius amissibilis* and *Typhlodromus khosrovensis* adults as a function of initial prey density.

Prey stage	Parameters	<i>Euseius amissibilis</i>		<i>Typhlodromus khosrovensis</i>			
		Estimate (\pm SE)	X2	P	Estimate (\pm SE)	c^2	P
Egg	P_0	-0.2223 \pm 0.3953	0.32	0.5738	-0.6342 \pm 0.3884	2.67	0.1025
	P_1	-0.1222 \pm 0.0621	3.87	0.0491	0.0204 \pm 0.0594	0.12	0.0305
	P_2	0.00482 \pm 0.00236	4.16	0.0414	-0.00158 \pm 0.00225	0.49	0.4837
	P_3	-0.00005 \pm 0.00002	3.93	0.0474	0.000020 \pm 0.000022	0.84	0.36

Table 2 Estimate (\pm SE) of instantaneous attack rate and handling time of *Euseius amissibilis* and *Typhlodromuskhosrovensis* on eggs of *Tetranychus urticae*

Predator species	Prey stage	Functional response parameters			
		$a \pm SE$	$T_h \pm SE$	T/T_h	R^2
<i>E. amissibilis</i>	Egg	0.0204 \pm 0.0014 (0.0174 – 0.0234)	2.3018 \pm 0.0943 (2.2156 – 2.5919)	9.98	0.84
<i>T. khosrovensis</i>		0.0213 \pm 0.0009 (0.0195 – 0.0231)	2.8013 \pm 0.0791 (2.2134 – 2.9592)	8.57	0.88

The values in parentheses represent 95% confidence intervals. a : attack rate (h^{-1}); T_h , handling time (h); T/T_h , maximum attack rate.

amissibilis are categorized in type III and IV, respectively (McMurtry *et al.* 2013). The type IV predators are generally pollen feeders. They have their highest reproductive capacity when feeding on pollen, and populations in the field often increase significantly when the crop or the surrounding vegetation is flowering. Also, type III phytoseiids feed on pollen, though they prefer, or show better performance on prey such as pest species in Acaridae, Eriophyidae, Tetranychidae, Tenuipalpidae, Tydeidae, as well as thrips, whiteflies, and mealybugs (McMurtry *et al.* 2013). *Euseius amissibilis* as a phytoseiid type IV prefer to feed on pollen grains (Badii *et al.* 2004). However, some reports are showing the pollen-feeding generalist predators in the genus *Euseius* can also prey on some small arthropods (de Moraes and Lima, 1983; Grout *et al.* 1992; Stathakis *et al.* 2021). Several studies have indicated that species type IV are effective biocontrol agents of spider mites on some crops (McMurtry *et al.* 2013).

In this study, only eggs of the two-spotted spider mites were used for the evaluations of the functional response. According to Badii *et al.* (2004), *T. urticae* consumption as a prey was inversely related to its size; thus, eggs were the most preferred stage that the predators could consume. On the other hand, ingestion of the deutonymphs or adults was very rare.

The maximum handling time, which shows the time required for capturing and feeding on prey was estimated as 2.30 (h), and 2.80 (h) for *T. khosrovensis* and *E. amissibilis*, respectively, for 24 h. Based on the attack rate, and handling time, both phytoseiids in this study performed lower ability compared to the other native phytoseiid mite *T. bagdasarjani* when preyed on eggs of *T. urticae* ($a = 0.089 h^{-1}$ and $T_h=1.80$), although *T. bagdasarjani* was still a weaker predator in comparison to *Neoseiulus californicus* ($a=0.09 h^{-1}$ and $T_h=1.61$) (Farazmand *et al.* 2012). In addition, the ability of *T. khosrovensis* and *E. amissibilis* to catch the spider mites' eggs was estimated lower in comparison to *P. plumifer* (Kouhjeni Gorji *et al.* 2009), and *E. hibisci* in the study conducted by Badii *et al.* (2004).

Most functional response studies have been conducted on the specialist phytoseiid, *Phytoseiulus persimilis*, as well as selective and generalist phytoseiids, *N. californicus* and *Amblyseius swirskii* Athias-Henriot (Fathipour *et al.* 2017, 2018, Ahn *et al.* 2010), which are recommended for biological control of two-spotted spider mites, thrips, and whiteflies (Xiao and Fdamiro, 2010, Castagnoli & Simoni 1999). On the other hand, phytoseiid mites within the type III and IV lifestyles which are generally pollen feeders, have a moderate and partial impact on spider mite eggs and immatures. However, these potential predators can be considered as an auxiliary biocontrol agent. In augmentative release programs, they could play a supporting role in achieving biological control of *T. urticae* when used in combination with specialized imported predatory mites, which appear to be more effective.

In conclusion, neither *E. amissibilis* nor *T. khosrovensis* are specialist predators of *T. urticae*, but they have the potential to improve the control of this mite on mulberry trees existing in urban green spaces. However, extra studies are necessary to obtain the knowledge about behavior of these predators in their natural environment. Their ecological attributes are somewhat different and they seem to act quite independently.

In this study, only the eggs of *T. urticae* were provided to the phytoseiids, but in the field, the predators must overcome the dense web of the two-spotted spider mites to catch the prey. Spider mites, particularly species of the genus *Tetranychus* create complicated webs to hinder the movement of the phytoseiids categorized as ecological types III, and IV. Also, more studies about the other ecological aspects of predatory mites including prey preference, switching behavior, and intra-specific interaction, help us to obtain much more information to use them as an auxiliary and potential biocontrol agent to prevent applying extra pesticides on urban pests.

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