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# Can host plants affect egg predation of two-spotted spider mite by *Macrolophus pygmaeus* (Hemiptera: Miridae)?

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#### ABSTRACT

Different host plants can affect the performance of herbivores and their natural enemies. The life history of the mirid predator, *Macrolophus pygmaeus* Rambur, was studied after feeding on *Tetranychus urticae* Koch eggs reared on the solanaceous plants, including tomato (*Solanum lycopersicum* L. cv Nora) and sweet pepper (*Capsicum annuum* L. cv Kadia) under laboratory conditions. According to the age-stage, two-sex life table theory, developmental time, survival rate, and fecundity were analyzed. The results showed that premature developmental time of the predator was shorter when they were fed on tomato-reared spider mites than on sweet-pepper-reared spider mites. Some population parameters [Intrinsic rate of increase (*r*), Net reproductive rate ( $R_0$ ) and Mean generation time) of *M. pygmaeus* were significantly affected by the host plants. Mean number of *T. urticae* eggs consumed by different stages/sexes of *M. pygmaeus* was calculated based on the age-stage two-sex consumption rate model. The results indicated that the consumption rate increased from nymph to adult in both sexes. The net predation rate ( $C_0$ ) was 547.98 ± 24.00 and 381.6 ± 18.04 mites on tomato and sweet pepper, respectively. Transformation rate from prey population to predator offspring ( $Q_p$ ) was 7.11 ± 0.18 and 5.60 ± 0.24 on tomato and sweet pepper, respectively. The results showed that the survival and reproductive rate of *M. pygmaeus* was higher when they fed on *T. urticae* eggs laid by tomato-reared adults compared to sweet pepper-reared individuals. This study provides an opportunity to better understand the interactions of the three trophic levels through considering host plant importance in the success of biological control.

**KEY WORDS:** Intrinsic rate of increase; life history; predation rate; predatory bug; *Tetranychus urticae*.

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#### **INTRODUCTION**

Phytophagous mites cause serious damage to many vegetable crops, including greenhouse crops. Therefore, they are a significant aim of pesticides' application (Cranham and Helle 1985). Twospotted spider mite (*Tetranychus urticae* Koch; Acari: Tetranychidae) is a pest of numerous crops worldwide, including more than 200 plant species (Migeon and Dorkeld 2013). Despite their small size, they can cause severe damage very rapidly due to their great reproductive capacity (Liesering 1960; Tomczyk and Kropczynska 1985). The control of two-spotted spider mites have been mainly

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dependent on spraying of acaricides leading to the development of resistance in mites, which in turn causes environmental concerns (Escudero and Ferragut 2005; Khajehali *et al.* 2011; Attia *et al.* 2013). Integrated pest management (IPM) and primarily biological control are the best strategies to reduce problems related to insecticide usage (Casey *et al.* 2007; Holt *et al.* 2007). Deployment of biological control against phytophagous arthropods has been widely used under greenhouse conditions (Gigon *et al.* 2015).

Several species of natural enemies have been reported to prey on *T. urticae*. One of them is *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae), which originates from the Mediterranean region and often appears in greenhouses or tunnels, without long history of pesticide application (Alomar *et al.* 1994). This predatory bug is widely used commercially and is released against whiteflies and other pests of greenhouse crops (Perdikis and Lykouressis 1997). Earlier studies indicated that *M. pygmaeus* could thrive on whiteflies, aphids, thrips, leaf miners, mites, as well as eggs and larvae of lepidopteran pests feeding on tomato, eggplant, and pepper (Perdikis and Lykouressis 1997). This predator has shown a high capacity in biological control on solanaceous crops (Perdikis and Lykouressis 2000). In addition, *M. pygmaeus* is characterized for its omnivory, feeding on both plant and insect pests (Perdikis and Lykouressis 2000). In the absence of prey, *M. pygmaeus* sucks plant sap and exhibits Zoophytophagous habits that allow it to develop successfully on eggplant, tomato, cucumber, pepper, and beans with rare economic damage; it does not reproduce just by feeding on plant sap (Albajesm and Alomar 1999; Perdikis and Lykouressis 2004a).

Recognizing the host plants-*Macrolophus* interactions improves the biological control of *T*. *urticae* in greenhouses. The degree of compatibility between biological control and plant resistance is an important approach in pest control (Ode 2006). Our knowledge on the life history of *M*. *pygmaeus* could be used to integrate the host plant resistance and other nonchemical tactics for managing *T*. *urticae* populations. Therefore, this study aimed to compare the predatory capacity of *M*. *pygmaeus* on *T*. *urticae* eggs based on tomato, and sweet pepper host plants.

#### **MATERIAL AND METHODS**

#### Plants

Seeds of tomato, *Solanum lycopersicum* L. (cv Nora) and sweet pepper, *Capsicum annuum* L. (cv Kadia) were planted in 20-cm-diameter plastic pots filled with a mixture of field soil and compost (1:3) in a greenhouse located at the College of Agriculture, University of Tehran, Karaj, Iran. They were grown in a growth chamber at  $25 \pm 2$  °C,  $65 \pm 5\%$  RH, and a photoperiod of 16:8 h (L: D) without any fertilizer or pesticides. Fully expanded young leaves of host plants were used for the experiments when plants were four weeks old and did not have any flowers.

#### Prey and predators

The stock culture of *T. urticae* was originally collected from a colony established in acarology laboratory (Department of Plant Protection, College of Agriculture, University of Tehran, Karaj, Iran) on infested lima bean leaves. *Tetranychus urticae* was maintained on potted host plants (tomato and sweet pepper) under laboratory conditions. All experiments and the culture of *T. urticae* and *M. pygmaeus* were carried out and kept in a growth chamber  $(25 \pm 2 \, ^{\circ}C, 65 \pm 5\% \, RH$ , and a photoperiod of 16:8 h (L: D). To adapt *T. urticae* to the new host plant they were reared on each host plant for three generations in the growth chamber at the aforementioned conditions prior being used in the main experiments. *Macrolophus pygmaeus* used in the experiments was collected from tomato fields (Hashtguerd, Alborz province, Iran). Then the colony of *M. pygmaeus* was transferred to Petri dishes (15 cm diameter) containing leaves of each host plant (tomato or sweet pepper) separately. Before starting the trials, the offsprings were separately reared for four generations on *T. urticae* eggs fed on each host plant.

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### Life table and predation rate study

Host plant leaves were placed upside down on 2% agar layer inside ventilated plastic Petri dishes (15 cm diameter). To keep the leaves fresh, they were replaced daily. A total of 100 *M. pygmaeus* 24-h nymphs was selected randomly from the reared population in laboratory, as a cohort for each host plant and kept in a growth chamber under the same condition mentioned above. *Macrolophus pygmaeus* cultures were maintained on tomato and pepper plants for three generations before starting the experiments. Newly emerged 24-h nymphs were transferred into Petri dishes and reared individually on the host plant leaf. The leaves of each host plant (sweet paper and tomato) infested with 50 two spotted spider mite eggs were exposed to *M. pygmaeus* nymphs with daily charging. Nymphal mortality and development of *M. pygmaeus* were checked daily until adulthood. Adults were fed with the same food as the immature stages, because both adults and nymphs don't consume more than forty eggs daily. After the emergence of adults, males and females were paired and checked daily to record their survival and the number of eggs laid until females' death. Emerged pairs of *M. pygmaeus* adults were transferred to ventilated plastic cups (8 cm diameter and 10 cm height) containing 4-week old tomato and sweet pepper plants. For each plastic cap, total offspring produced by the females was recorded daily by counting nymphs.

*Tetranychus urticae* females were placed on each experimental unit (a 15 cm diameter Petri dish) to lay eggs daily. Different numbers of *T. urticae* eggs were ad libitum to feed different stages of *M. pygmaeus*: 50 eggs for nymphs and 50 eggs for males and females. Experimental units were monitored daily and the number of consumed preys was recorded and replaced. After adult emergence and pairing females and males, each pair was provided by 50 eggs every day, and the number of consumed eggs was recorded until the death of predators. To estimate the predation rate of males and females specifically, the consumption rate of 20 single males was recorded under similar conditions separately. To determine the female daily consumption rate, the average male consumption rate was subtracted from the consumption of pairs (Farhadi *et al.* 2011).

#### Data analysis

Developmental time of all individuals, including males, females, and those dying before the adult stage, as well as the female daily fecundity, were analyzed according to the age-stage, two-sex life table theory (Chi and Liu 1985; Chi 1988) using the TWOSEX-MSChart program (Chi 2019a). For each cohort, the life table parameters were calculated accordingly:

Net reproductive rate  $(R_0)$ 

$$R_0 = \sum_{x=0}^{\infty} l_x \, m_x$$

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

Mean generation time (T)

$$T = \ln \frac{R_0}{r}$$

 $\lambda = e^r$ 

and finite rate of increase

The means and standard errors of different parameters were estimated by bootstrap procedure with 100.000 resamples. The paired bootstrap test was used to compare the treatments based on the confidence interval of the difference between treatments (Chi 2019a).

Raw data of daily predation of all individuals were analyzed according to Chi and Yang (2003) by CONSUME-MSChart software (Chi 2019b). The age-specific predation rate  $(k_x)$  is the mean

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number of prey consumed by the predator at age x and could be estimated by the following equation (Chi and Yang 2003):

$$k_x = \frac{\sum_{j=1}^{\beta} s_{xj} c_{xj}}{\sum_{j=1}^{\beta} s_{xj}}$$

The age-stage survival rate  $(s_{xi})$  means the probability that a newly laid egg will exist to age x and stage j. Where, b is the number of stages and  $c_{xj}$  is the age-stage specific consumption rate of individuals at age x and stage j. The age-specific net predation rate  $(q_x)$  considers the survival rate and is estimated as follows (Chi and Yang 2003).

$$q_x = l_x k_x$$

The net predation rate ( $C_0$ ) shows the mean number of prey consumed by a predator during its life span estimated as follows:

$$C_0 = \sum_{x=0}^{\infty} l_x k_x = \sum_{x=0}^{\infty} q_x$$

The ratio of the net predation rate to the net reproductive rate gives the transformation rate from prey population to predator offspring. Following Chi and Yang (2003), this ratio was defined as  $Q_p$ and estimated as follows:

$$Q_{p=\frac{Co}{Ro}}$$

This rate represents the mean number of prey needed for a predator to produce an egg. Data of the predation rate was analyzed using CONSUME-MSChart program (Chi 2019b). The variances and standard errors of predation parameters were calculated using the bootstrap resampling method. Figures were drawn by Sigma plot software (version 12).

#### RESULTS

Mean developmental times and adult longevities of female and male as well as reproductive attributes output are presented in Table 1. M. pygmaeus had a significant shorter development time (the duration from egg to adult) when fed on T. urticae reared on tomato. In other words, they reached the adult stage faster than those fed on the mites reared on sweet pepper (P < 0.05, Table 1).

**Table 1.** Developmental times and reproductive attributes (mean  $\pm$  SE) of *Macrolophus pygmaeus* when reared on *Tetranychus urticae* eggs fed on tomato and sweet pepper

Host plant	Egg (days)	Nymph (days)	Female (days)	Male (days)	APOP <sup>a</sup> (days)	TPOP <sup>b</sup> (days)	Fecundity (Nymphs/ females)
Tomato	$7.24\pm0.18b$	$25.88\pm0.24b$	$14.35\pm0.54a$	$14.35\pm0.54a$	$0.69\pm0.06a$	$0.34\pm33.81b$	$54.8\pm0.87a$
Sweet pepper	$9.35 \pm 0.24a$	$31.72\pm0.49a$	$11.81 \pm 0.7b$	$11.81 \pm 0.7b$	$0.63 \pm 0.7a$	0.85±41.34a	$46.0\pm0.61b$

Means in the same column followed by different letters are significantly different (Paired bootstrap test, P < 0.05).

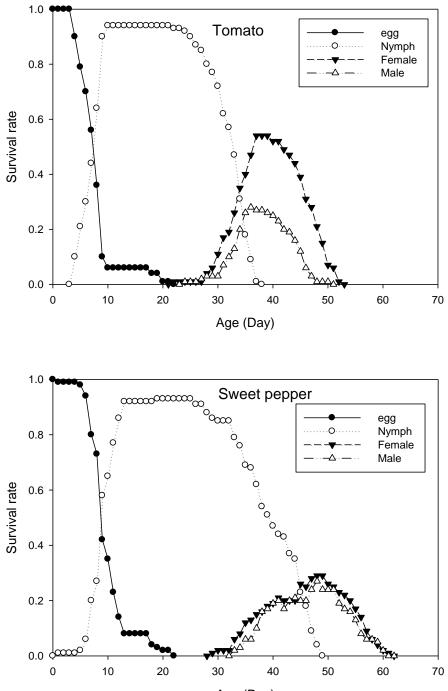
<sup>a</sup> APOP, adult pre-oviposition period; <sup>b</sup>, total pre-oviposition period.

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**Table 2.** Life table parameters (mean  $\pm$  SE) of *Macrolophus pygmaeus* when reared on *Tetranychus urticae* eggs fed on<br/>tomato and sweet pepper.

Host plant	$R_0$	r (day <sup>-1</sup> )	$\lambda$ (day <sup>-1</sup> )	T (day)
Tomato	$32.07\pm3.00a$	$0.087 \pm 0.002a$	$1.091 \pm 0.002a$	$39.41\pm0.34b$
Sweet pepper	$21.16\pm2.36b$	$0.066\pm0.001b$	$1.069\pm0.002b$	$45.73 \pm 0.8$ a

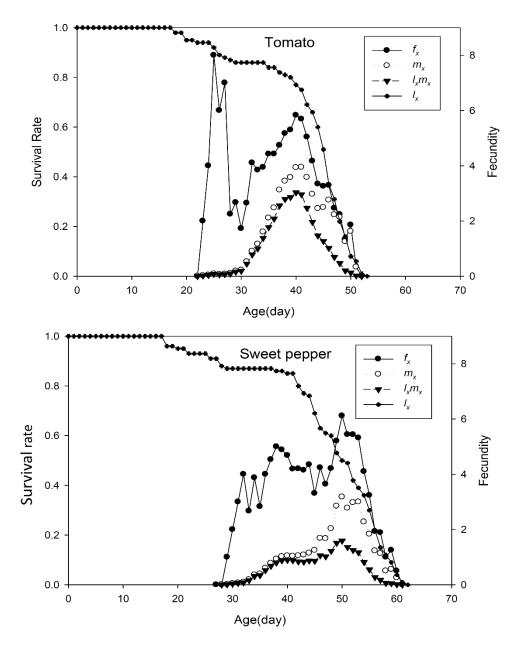
Means in the same column followed by different letters are significantly different (Paired bootstrap test, P < 0.05).



Age (Day)

Figure 1. The age-stage specific survival rate  $(s_{xj})$  of *Macrolophus pygmaeus* fed on *Tetranychus urticae* eggs reared on tomato and sweet pepper.

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**Figure 2**. Age-specific survivorship  $(l_x)$ , age-stage-specific fecundity  $(f_{xj})$ , age-specific fecundity of the total population  $(m_x)$ , and age-specific maternity  $(l_x m_x)$  of *Macrolophus pygmaeus* fed on *Tetranychus urticae* eggs reared on tomato plant and sweet pepper.

There was no significant difference for the adult pre-oviposition period (APOP) between the two treatments (P > 0.05). However, the total pre-oviposition period (TPOP) was significantly shorter when *T. urticae* reared on tomato offered as food. This reduction is due to the reduced length of immature stages of *M. pygmaeus* (P < 0.05) (Table 1).

The life table parameters were calculated based on the data of the entire cohort, i.e., both sexes and the variable developmental rates among individuals. Calculated parameters and standard errors of the intrinsic rate of increase (*r*), net reproductive rate ( $R_0$ ), the finite rate of increase ( $\lambda$ ), and mean generation time (*T*) are shown in Table 2. Statistical analysis indicated significant differences between *r*,  $R_0$  and *T* of two population of *M. pygmaeus* using 100000 bootstrap (P < 0.05). Also, the mean generation time of *M. pygmaeus* fed on *T. urticae* reared on sweet pepper was longer than those on tomato (P < 0.05, Table 2).

Table 3. Mean daily consumption rate (± SE) of Tetranychus urticae eggs eaten by different stages/sexes of Macrolophus	
<i>pygmaeus</i> on tomato and sweet pepper.	

Host plant	Nymph	Female	Male	
Tomato	$7.11 \pm 0.18a$	$34.34 \pm 0.41a$	$25.63\pm0.23a$	
Sweet pepper	$5.60\pm0.24b$	$25.29\pm0.38b$	$22.74\pm0.21b$	

Means in the same column followed by different letters are significantly different (Paired bootstrap test, P < 0.05).

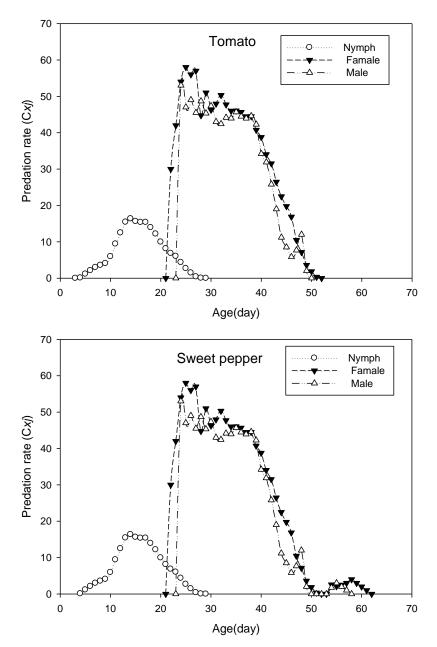


Figure 3. Age-stage predation rate  $(c_{xj})$  of *Macrolophus pygmaeus* fed on *Tetranychus urticae* eggs reared on tomato and sweet pepper.

The age-stage survival rate  $(s_{xj})$  shows the probability that a newborn egg survives to age x and stage j. As shown in Figure 1, there was considerable overlapping among different stages due to the variable developmental rates among individuals. Females' longevity (53 and 62 days on tomato and sweet pepper, respectively) was more than males' longevity (51 and 61 days on tomato and sweet

pepper, respectively). Moreover, the survival rate of female and male adults on tomato was considerably higher than those reared on sweet pepper (Fig. 1).

 Table 4. Predation rates (mean ± SE) of Macrolophus pygmaeus on Tetranychus urticae eggs reared on tomato and sweet pepper

Host plant	Net predation rate (C <sub>0</sub> )	Transformation rate (Qp)	Finite predation rate ( $\omega$ )
Tomato	$381.6\pm18.04b$	$5.60 \pm 0.24b$	$3.85\pm0.08b$
Sweet pepper	$547.98 \pm 24.00a$	$7.11\pm0.18a$	$5.6 \pm 0.18a$

Means in the same column followed by different letters are significantly different (Paired bootstrap, P < 0.05).

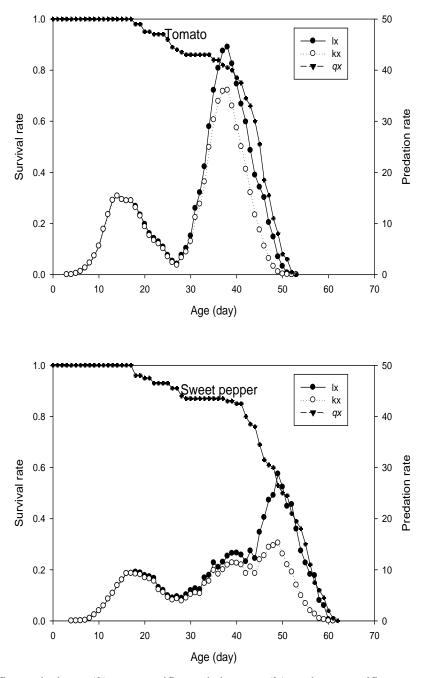


Figure 4. Age-specific survival rate  $(l_x)$ , age-specific predation rate  $(k_x)$ , and age-specific net predation rate  $(q_x)$  of *Macrolphus pygmaeus* fed on *Tetranychus urticae* eggs reared on tomato and sweet pepper.

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The age-specific survival rate of the total cohort  $(l_x)$  is a simplified form of  $s_{xj}$  and shows how survivorship declines with time. In addition to the  $l_x$ , female age-stage specific fecundity  $(f_{xj})$ , agespecific fecundity of the total population  $(m_x)$ , and age specific maternity  $(l_xm_x)$  are illustrated in Figure 2. The first highest peak of age-specific fecundity of the total population  $(m_x)$  occurred at the day 41 and 50 in tomato and sweet pepper, respectively.  $f_{xj}$  and  $l_xm_x$  showed similar trends (Fig. 2).

The mean daily predation rate of *T. urticae* eggs consumed by different stages and sexes of *M. pygmaeus* are shown in Table 3, indicating the increasing of consumption rates from nymph to adult in both sexes. The mean number of eggs consumed during juvenile development were  $197.12 \pm 4.13$  and  $185.26 \pm 10.63$  on tomato and sweet pepper, respectively. The total eggs consumed by the females were more than males on both host plants (P < 0.05).

The age-stage predation rate  $(c_{xj})$  of *M. pygmaeus* on *T. urticae* eggs is shown in Figure 3, illustrating the mean number of consumed prey by a predator at age *x* and stage *j*. On both tomato and sweet pepper, the maximum daily predation rate of *M. pygmaeus* adults was higher than nymphs (Fig. 3).

The net predation rate ( $C_0$ ), finite predation rate ( $\omega$ ), and transformation rate ( $Q_p$ ) of *M. pygmaeus* fed on *T. urticae* reared on sweet pepper were significantly lower than those consumed *T. urticae* reared on tomato (Table 4). The net predation rate of the predators fed on *T. urticae* reared on tomato (547.98 ± 24.00 eggs) was significantly higher than those fed on *T. urticae* reared on sweet pepper (381.6 ± 18.004 eggs). The *Qp* value indicates that *M. pygmaeus* requires 7.11 ± 0.18 eggs to produce one egg on tomato, which is higher than the required eggs on sweet pepper (5.60 ± 0.24 eggs).

The age-specific predation rate  $(k_x)$  is the mean number of mite eggs consumed per *M. pygmaeus* of age *x* (Fig. 4), while the age-specific net predation rate  $(q_x)$  is the weighted number of prey consumed by a predator of age *x*. Both  $k_x$  and  $q_x$  were calculated by considering sex and age differentiation, so their values on tomato are higher than that of *M. pygmaeus* reared on sweet pepper. Population and predation parameters showed that the *M. pygmaeus* cohort reared on the tomato could increase their population faster and kill more prey than *M. pygmaeus* on sweet pepper.

#### **DISCUSSION**

Biological control in greenhouses is a proper alternative to pesticides from environmental and economic perspectives (van Lenteren 2000, 2012). This implies that biological control agents (predators, parasitoids, or entomopathogens) suppress pest populations to such levels that crop damage is prevented or minimized. It is imperative to consider that host plants may affect the biological and physiological parameters of the pests. On the other hand, plants, through pests, affect the biological or reproductive characteristics of the natural enemies. In fact, as the first level of food chain, host plants may have significant effects on the biology of natural enemies as the third level in a tritrophic context (Kalushkov and Hodek 2004).

In the present study, tomato and sweet pepper had different effects on the growth and development of *M. pygmaeus* and feeding on *T. urticae* eggs reared on tomato led to better results. Also, the higher *r* value of *M. pygmaeus* fed on *T. urticae* eggs reared on tomato indicated its lower developmental time and the earlier oviposition compared to feeding on *T. urticae* eggs reared on sweet pepper. Furthermore, predation parameters of *M. pygmaeus* were in accordance with population growth parameters. For example, feeding on tomato-reared *T. urticae* led to higher numbers of consumed preys by an individual predator during its entire life span ( $C_0$ ), which is the combination of growth rate ( $\lambda$ ), age-stage predation rate ( $c_{xj}$ ) and stable age-stage structure ( $a_{xj}$ ). Likely, these outcomes might be due to different factors, such as trichome density, plant allelochemicals or plant chemical composition, which could reduce the overall quality of the plant food, affect the quality of the prey (*T. urticae*) and, finally, interfere with the foraging behavior of the predator (De Clercq *et al.* 2000; Francis *et al.* 2001; Madadi *et al.* 2007). The fitness of predators such as *M. pygmaeus* might depend not only on the type of prey food but also on the host plant of the prey (Carletto *et al.* 2009).

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Our results corroborate previous studies indicating that eggs of mites reared on tomato, in general, are suitable prey for *M. pygmaeus* (Pappas *et al.* 2015). This study showed that *T. urticae*, as a food source, increases *M. pygmaeus* longevity and reproduction rate, specifically when these mites were reared on tomato plants. Thus, the nutritional value of mites is probably linked to host plant quality or mites' adaptation. The low quality of the sweet pepper probably increased the developmental time and life table parameters of *M. pygmaeus*.

An earlier study showed that female *M. pygmaeus* did not prefer pepper plants for egg laying even in the absence of prey (Perdikis and Lykouressis 2004a). In another study, Perdikis and Lykouressis (2004b), showed the different effects of two prey host plants, including eggplant and tomato on nymphal development and adult survival of *M. pygmaeus*, due to differences in their nutritional value or the presence of allelochemicals. Also, in accordance with the results obtained here, Lykouressis *et al.* (2014) reported that despite the higher suitability of peppers for development or reproduction of *M. pygmaeus* compared to *Solanum nigrum*, the predation rate of *M. pygmaeus* was significantly lower on leaves of pepper than on those of *S. nigrum*.

Integrated pest management (IPM) strategies are being increasingly used in open field and greenhouse crops. In the last three decades, invasive pests such as the mite, T. urticae, have posed a major threat to the continuous production of vegetable crops (Jaworski 2015). Nowadays, these pests are fully integrated in agro-ecosystems and are successfully controlled by IPM programs based on the use of natural enemies, particularly generalist predators (Nogia and Meghwal 2014). Our results showed that T. urticae, as an important pest of tomato crops, is a high-quality food source for M. pygmaeus. Specifically, Sylla et al. (2016) stated that the fitness of predators such as M. pygmaeus might depend not only on the type of prey as food but also on the host plant of the prey. The considerable variation among the tested host plants in respect to their effects on the biological characteristics might be related to the differences in leaf morphology (Tazerouni et al. 2016; Madahi et al. 2019), plant quality, in terms of the nutrients required for prey growth (Zarghami et al. 2010; Nogia and Meghwal 2014; Madahi et al. 2019), or the existence of a primary and secondary chemical compound with anti-nutritional and/or antibiotic characteristics, consequently affecting pest growth (Baldin et al. 2009). This study provides an opportunity to better understand the interactions of the three trophic levels through considering host plant importance in the success of biological control. However, further field and laboratory experiments are needed to identify the phytochemicals of these two host plants and the possible effects of these compounds on T. urticae and M. pygmaeus performance.

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# آیا گیاهان میزبان می توانند شکار تخمهای کنهٔ تارتن دو لکهای را توسط Macrolophus pygmaeus (Hemiptera: Miridae) تحت تاثیر قرار دهند؟

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## چکیدہ

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