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Orijinal araştırma **(*Original article*)**

The impact of daily limited prey density levels on some biological characteristics of *Phytoseiulus persimilis* Athis-Henriot at various temperature and humidity conditions

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Farklı sıcaklık ve nem koşullarında günlük sınırlı av yoğunluğu düzeylerinin *Phytoseiulus persimilis* Athis-Henriot'un bazı biyolojik özellikleri üzerine etkisi

Öz: Bu çalışmada, günlük sınırlı av düzeylerinin 25, 30 ve 27-35°C değişken sıcaklıklarda, %75±5 ve %90±5 bağıl nem koşullarında avcı akar *Phytoseiulus persimilis* Athias-Henriot'un bazı biyolojik özellikleri üzerine etkilerinin belirlenmesi amaçlanmıştır. Çalışmada av olarak avcı akar gruplarından ilkine her gün bir yumurta, bir larva ve bir *Tetranychus urticae* Koch (Kırmızı form) ergini (erkek); ikinci gruba iki yumurta, iki larva ve iki ergin erkek; üçüncü gruba üç yumurta, üç larva ve üç ergin erkek; dördüncü gruba ise dört yumurta, dört larva ve dört ergin erkek verilmiştir. Tüm *P. persimilis* larvaları 25 ve 30 °C'de (%75; %90 bağıl nem) beslenmeden protonimf dönemine ulaşmıştır. Ancak, her iki nem seviyesinde de 27-35 °C'lik değişken sıcaklık aralığında hiçbir yumurta açılmamıştır. En yüksek ortalama ömür, ergin öncesi protonimf döneminden itibaren toplam 10 gün ile 25 °C'de (%75 bağıl nem) ikinci av seviyesinde gözlenmiştir. En yüksek yumurta tüketimi, ortalama 27.4 adet ile dördüncü av seviyesinde 30 °C'de (%75 bağıl nem) gözlenmiştir. Buna karşılık, en yüksek larva ve ergin erkek tüketimleri, dördüncü av düzeyinde sırasıyla 26.80 larva ve 29 adet ergin erkek ortalamasıyla 25 °C'de (%90 bağıl nem) gerçekleşmiştir. İlk av düzeyinde, hiçbir sıcaklık ve nem düzeyinde üreme gerçekleşmemiştir. *P. persimilis*'in üreme gücü genel olarak 25 °C sıcaklıkta (%75 ve %90 bağıl nem) ikinci besin düzeyinden sonra av yoğunluğundaki artışa paralel olarak artmıştır. *P. persimilis*'in en yüksek üreme gücü 25 °C (%90 bağıl nem) ve dördüncü besin düzeyinde tüm ovipozisyon süresi için 1.9 adet yumurta olmuştur.

Anahtar sözcükler: *Phytoseiulus persimilis*, sıcaklık, nem, sınırlı av yoğunluğu

Abstract: This study aimed to determine the effects of daily limited prey levels on certain biological characteristics of the predatory mite *Phytoseiulus persimilis* Athias-Henriot at 25, 30, and 27-35 °C, with relative humidity conditions of 75±5% and 90±5%. In the study, four groups of predatory mites were established by providing varying densities of

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prey items daily. The first group received one egg, one larva, and one adult male of *Tetranychus urticae* Koch (Red form) each day; the second group received two eggs, two larvae, and two adult males; the third group received three eggs, three larvae, and three adult males; and the fourth group received four eggs, four larvae, and four adult males daily. All *Phytoseiulus persimilis* larvae successfully progressed to the protonymph stage without feeding at 25 and 30 °C (75% and 90% RH). However, no egg hatching was observed within the variable temperature range of 27-35 °C under both humidity conditions. The longest mean longevity was recorded at the second prey density level at 25 °C (75% RH), with an average duration of 10 days starting from the protonymph stage. The highest mean egg consumption was observed at 30 °C (75% RH) at the fourth prey level, with a mean of 27.4 eggs. In contrast, the highest consumption of larvae and adult males occurred at 25 °C (90% RH), with means of 26.8 larvae and 29 adult males, respectively, at the fourth prey level. At the first prey level, no oviposition occurred under either temperature or humidity condition. Oviposition of *P. persimilis* generally increased in tandem with the prey density, with a noticeable rise after the second prey level at 25 °C (75% and 90% RH). The highest oviposition rate, with a total of 1.9 eggs over the entire oviposition period, was observed at 25 °C (90% RH) at the fourth prey level.

Key words: *Phytoseiulus persimilis*, temperature, humidity, limited prey density

Introduction

Tetranychid mites reproduce rapidly and can attain significant population densities, particularly in unbalanced agricultural eco-systems (Van de Vrie et al. 1972). These mites disrupt chlorophyll synthesis by feeding on the foliage of host plants, which significantly impedes both plant growth and fruit development (Jeppson et al. 1975). While insecticides and acaricides may provide temporary mitigation of mite populations, their effectiveness is often compromised by challenges such as the development of resistance and the presence of undesirable pesticide residues (Burges 1974). In light of concerns related to environmental health and consumer safety, as well as the necessity to maintain mite populations below economical thresholds, biological control has emerged as a promising strategy. This approach has achieved considerable success in recent years (Vacante & Firullo 1983; Brodgaard 1989; Tiftikçi et al. 2020; 2022; Yaşar et al. 2024).

Although numerous natural enemies of phytophagous mites have been documented, predatory mites from the family Phytoseiidae within the subclass Acari are recognized as some of the most efficacious (Oatman et al. 1977; McMurtry et al. 2015). A substantial body of research has focused on the utilization of predatory phytoseiid mites as biological control agents against tetranychid mites (Havelka & Kindlmann 1984; Rasmy & Ellaithy 1988). Notably, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) has emerged as one of the most effective species within this family. This species is currently commercially reared in Europe (Anonymous 1977), Canada, and the United States (Steiner & Elliot 1987), and it has been successfully implemented as a biological control agent against tetranychid mites, particularly in greenhouse environments (Perdiki et al. 2008).

A considerable amount of research has been devoted to investigating the biological characteristics of *P. persimilis* under controlled laboratory conditions (Friese & Gilstrap 1982; Gillespie & Quiring 1994; Mutuku et al. 2024). In the majority of these studies, with the exception of those examining low prey densities (such as functional response experiments), prey availability was meticulously managed to circumvent any deleterious effects on the predator's consumption and reproductive capacity (Skirvin & Fenlon 2001). Consequently, these experiments have taken place in environments characterized by stable food availability. However, it is essential to acknowledge that the dynamics observed in laboratory settings may not accurately reflect those in natural environments (Havelka & Kindlmann 1984). Natural enemies typically thrive in habitats where prey or hosts are not consistently present at high densities, and these ecological interactions occur under variable temperature and humidity conditions (Nachman & Zemek 2002).

It is anticipated that a decrease in prey density will significantly adversely affect the biological characteristics of *P. persimilis*, akin to the effects observed in other predatory mite species (Park et al. 2021). Consequently, the population may show slower developmental rates, longer developmental periods, and reduced reproductive rates as prey density declines (Sentis et al. 2012; Nielsen et al. 2014). However, there is currently a paucity of information regarding the impact of restricted feeding conditions on the biological characteristics of predatory mites in general, and *P. persimilis* specifically. To address this knowledge gap, the present study aims to enhance understanding of the biological characteristics of *P. persimilis* under varying temperatures, different humidity levels, and conditions of limited prey availability.

Materials and Methods

Host plant and *Tetranychus urticae* rearings

Host plant rearing was conducted in an acclimatized climate room maintained at 25±1 °C, 60±10% relative humidity (16:8; L:D). *Phaseolus vulgaris* L. (common bean) served as the host plant for the rearing of *Tetranychus urticae* (red form) (Acari: Tetranychidae). For this purpose, bean seeds were germinated in plastic pots (10 x 15 cm sized), filled with a peat-sand mixture. Once the plants developed 3 to 4 true leaves, they were transferred to a separate climate room with identical conditions suitable for *T. urticae* rearing. Subsequently, these plants were inoculated with *T. urticae* using bean leaves previously infested with red spider mites.

Phytoseiulus persimilis rearing

Predatory mite rearing was carried out in a separate acclimatized climate room (25±1 °C; 70±5%; 16:8; L:D) throughout the study. Every other day, five bean plants exhibiting sufficient density of *T. urticae* were transferred to the *P. persimilis* rearing room. The *P. persimilis* obtained from predatory mite rearing was subsequently introduced onto these plants to facilitate the rearing of the predatory mites.

Effects of daily limited prey density levels on *Phytoseiulus persimilis*

The effects of daily limited prey densities on *P. persimilis* under varying temperature and humidity conditions were determined using cells with a diameter of 3 cm and a height of 1 cm, affixed to plexiglass plates measuring 7 x 4 cm and 0.5 cm in thickness. Then, a certain number of *T. urticae* eggs, larvae, and adult males were placed in each cell as daily prey. Following the introduction of newly hatched predatory mite larvae into the same arena, the tops of the cells were encircled with a specialized adhesive to prevent the escape of individuals from the cells.

Cells containing prey and predators were subsequently placed in transparent plexiglass boxes measuring 27 x 30 x 10 cm, which were covered with glass. Ambient relative humidity was maintained using saturated salt solutions (Winston & Bates, 1960). Specifically, pre-prepared solutions were positioned at the bottom of the boxes to achieve the desired humidity levels at the specified temperatures. Sodium chloride (NaCl) and Barium chloride ($BaCl_2$) were employed to maintain relative humidity levels of 75±5% and 90±5%, respectively. The experiments were conducted in climate cabinets set to a constant temperature of 25±1 and 30±1 °C, as well as a daily fluctuating temperature of 27 °C (16h) and 35 °C (8h) within a photoperiod of 16:8, L:D.

Four groups of predatory mites were established by providing varying numbers of prey items daily. The first group received one egg, one larva, and one adult male of *T. urticae* each day; the second group received two eggs, two larvae, and two adult males; the third group received three eggs, three larvae, and three adult males; and the fourth group received four eggs, four larvae, and four adult males daily. Male predatory mites were introduced into the cells for a period of time to allow adult female predatory mites to mate. Daily counts were conducted until the adult mites died, during the observations the number of prey consumed and the eggs laid by the females were counted and subsequently removed from the arena. No prey was given to control treatments in the experiments. All stages of the prey were transferred to the cells using a fine-tipped brush.

Experiments were conducted with 15 replicates for each daily prey density level across all the temperature and humidity combinations. Since the larvae passed to the protonymph stage without feeding, the impact of daily limited prey densities on the longevity of *P. persimilis* was evaluated as the sum of the protonymph, deutonymph and adult life spans of female individuals. A one-way analysis of variance was applied to the data and Duncan multiple comparision test was used to determine the difference between means (P<0.05). However, analysis of variance was not applied to the oviposition data due to the very low egg-laying capacity of the predator at both temperature and humidity levels.

Result and Discussion

Effects of daily limited prey density levels on total longevity

All *P. persimilis* larvae successfully developed to the protonymph stage without feeding at 25 and 30 °C (75% and 90% RH). However, no egg hatching occurred within the variable temperature range of 27-35 °C across both humidity conditions. In all treatments, the overall mean longevity of *P. persimilis* was significantly different from the control, with the exception of the first prey density level at 30 °C (90% RH) (Table 1).

The highest mean longevity was recorded at the second prey density level at 25 °C (75% RH), with an average duration of 10 days starting from the protonymph stage (Table 1). This mean was statistically different from the total mean longevity observed in the control and at the first prey density level at both 25 °C (75% and 90% RH) and at 30 °C (75% RH), as well as at all prey density levels (first, second, third, and fourth) at 30 °C (90% RH) (Table 1). In control, all predatory mites died of starvation before reaching the deutonymphal stage, resulting in no significant differences in total mean longevity among the individuals. In the control, the mean duration of the protonymph stage was 0.97, 1.17, 1.11, and 0.95 days at 25 and 30 °C (75% and 90% RH), respectively (Table 1).

Table 1. Total mean longevity of *Phytoseiulus persimilis* from the protonymph stage, based on daily limited prey density levels at two different temperature and humidity levels (day)*

Temperature	**25 ºC**		**30 ºC**	
Humidity (%)	**75±5**	**90±5**	**75±5**	**90±5**
Control	0.97 f	1.17 f	1.11 f	0.93 f
Prey level 1	4.66 cde	6.22 bcd	3.31 e	2.02 f
Prey level 2	10.00 a	8.51 ab	7.28 abc	4.15 de
Prey level 3	9.24 ab	8.73 ab	7.68 abc	6.64 bcd
Prey level 4	9.20 ab	9.48 ab	9.06 ab	4.97 cde

*Means with different letters are significantly different according to Duncan multiple comparison test (P <0.05).

Effects of daily limited prey density levels on prey consumption

The total mean numbers of eggs, larvae, and adults consumed by *P. persimilis* during its immature and adult stages at both temperature and humidity levels are shown in Table 2. The highest mean egg consumption was observed at 30 °C (75% RH) at prey density level four, with a mean of 27.4 eggs. In contrast, the highest mean consumption of larvae and adult males occurred at 25 °C (90% RH), with means of 26.80 larvae and 29 adults, respectively at fourth prey density level. Overall, for all three prey types, consumption at 25 °C (75% RH) for the first and second prey density levels, as well as the fourth prey density level at both 25 °C and 30 °C (90% RH), and fourth prey level at 30 °C (75% RH) were significantly different from consumption at other temperature, humidity and prey density levels (Table 2).

Table 2. Total mean number of *Tetranychus urticae* consumed by *Phytoseiulus persimilis* from the protonymph stage, based on daily limited prey density levels at two different temperature and humidity levels*.

Temp.	**RH.**	**Prey level**	**Egg**	**Larvae**	**Adult**
25 ºC	75%	1	3.80 fg	3.20 ef	3.06 fg
		2	16.20 cd	15.40 bc	15.90 cd
		3	19.20 abcd	19.10 ab	21.70 abcd
		4	25.60 abc	24.60 a	23.80 ab
25 ºC	90%	1	5.26 f	5.06 fe	5.00 fg
		2	13.80 de	10.80 cd	12.50 de
		3	17.80 bcd	17.10 bc	17.50 bcd
		4	25.80 abc	26.80 a	29.00 a
30 ºC	75%	1	2.53 g	1.93 f	2.66 g
		2	12.50 de	11.00 bcd	12.20 de
		3	16.20 cd	14.50 bc	16.90 cd
		4	27.40 a	26.10 a	26.80 ab
30 ºC	90%	1	1.66 h	1.40 f	1.20 h
		2	7.06 ef	5.06 de	6.40 ef
		3	15.20 d	14.20 bc	13.80 de
		4	13.20 de	11.80 bc	14.00 cd

*****Means with different letters in the same column are significantly different according to Duncan multiple comparison test ($P < 0.05$).

Oviposition related to daily limited prey density levels

The total mean number of eggs laid by *P. persimilis* females during the oviposition period at the limited prey dnesity levels is shown in Figure 1. No oviposition was observed at the first prey density level under both temperature and humidity conditions. Oviposition of the *P. persimilis* increased in parallel with the increase in prey density after the prey level two at 25 °C (75% and 90% RH). The highest oviposition of *P. persimilis* was 1.9 eggs for the whole oviposition period at 25 °C (90% RH) and prey level four. At 30 °C (90% RH), reproduction occurred only at the prey level four (Figure 1).

Begljarow (1967) and Böhm (1970) reported low survival rates of *P. persimilis* eggs at temperatures above 30 °C, while McClanahan (1968) found significant mortality of *P. persimilis* eggs even at 30 °C. Similarly, Pruszynski & Cone (1973) observed 100% mortality of *P. persimilis* eggs at 35°C, and Tanigoshi et al. (1975) noted 100% mortality of *Galendromus occidentalis* (Nesbitt) (Acari: Phytoseiidae) eggs at 38.4 °C. In the present study, an 8-hour exposure to 35 °C resulted in 100% mortality of *P. persimilis* eggs under both humidity conditions, despite temperature variability. The egg stage of predatory mites is particularly sensitive to environmental conditions, especially humidity. Unlike the mobile life stages, which can meet their moisture requirements through feeding, the egg stage lacks this ability. Consequently, fluctuations in ambient and egg temperatures can lead to rapid moisture loss and significantly increased mortality within a short period of time (Ghazy et al. 2016).

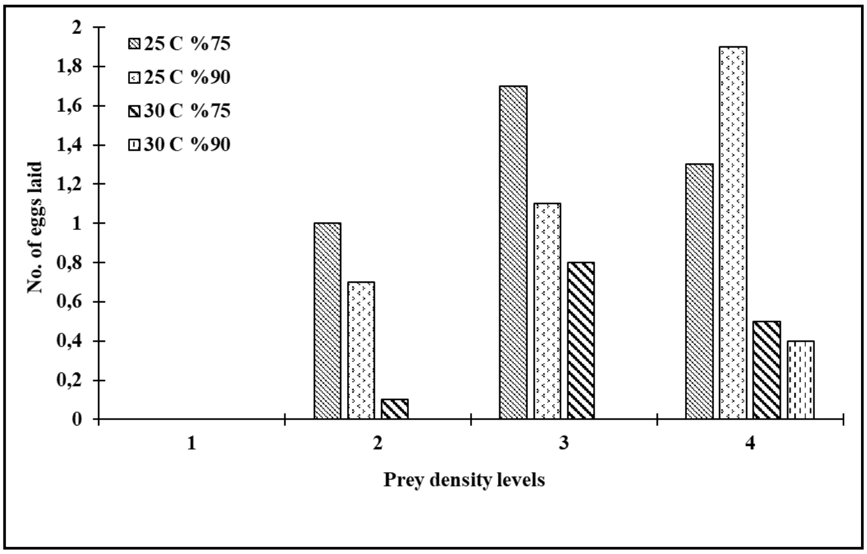

Figure 1. Total mean number of eggs laid by *Phytoseiulus persimilis* throughout its oviposition period, based on daily limited prey density levels

The total mean longevity of *P. persimilis*, including immature stages, was 10 days at 25 °C (75% RH) at prey density level two, while no difference was observed in the longevity of predators at prey density levels three and four at the same temperature and humidity, despite the increase in prey densities. It was found that the increase in prey density did not have a significant effect on the longevity of *P. persimilis* at 30 °C (90% RH). This result is thought to be due to increased reproduction associated with increased prey availability, with some of the energy gained directed toward reproductive activities. When *P. persimilis* was fed 1, 2, 4, and 20 *Tetrancyhus pacificus* (McGregor) (Acari: Tetranychidae) protonymphs daily as prey, the total developmental time of the predatory mite was 8.7, 3.7, 3.2, and 3.2 days, respectively (Takafuji & Chant 1976). Although the total developmental time from the egg stage was not determined in this study, it was observed that this period varied between 3 and 5 days on average. Kazak (2008) reported that the developmental time of *P. persimilis* fed on *T. cinnabarinus* at 25 and 30 ºC (75±10%) varied between 5.79 and 3.88 days.

Based on these reports, the adult longevity of *P. persimilis* at 25 °C varied between 4 and 5 days depending on increasing prey density under both humidity conditions. However, at 30 °C, this duration was shorter except at 75% RH and prey level four. These results suggest that elevated temperatures increase the energy requirements of the predatory mite, while the daily provision of four eggs, larvae and adults at the highest prey density is insufficient to meet these requirements. Furthermore, Zhang et al. (2014) reported that reactive oxygen accumulation in *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae) exposed to extreme temperatures caused antioxidant system deficiency and death.

Depending on the prey levels used in the study, the highest total consumption was 84.1 eggs, larvae and adults occurred at the prey density level four at 25 °C (90% RH) with a total mean longevity was 9.48 days. When *P. persimilis* was provided with one egg, larva and adult per day, no reproduction was observed at any temperature and humidity level. The lowest oviposition was recorded at 30 °C (75% RH) at the second prey density level, with a total mean of only 0.3 eggs per female. In contrast, the highest reproduction was observed at 25 °C (90% RH) at the fourth prey density level, where the total number of eggs per female reached two eggs over the lifetime.

These findings suggest that *P. persimilis* needs to consume more than one egg, one larva, and one adult prey daily to optimize its egg production. However, the lack of reproduction at certain food levels indicates that surrounding temperature and humidity significantly influence reproductive success at lower prey densities. Lababidi (1988) reported that at 25 °C (60% RH), *P. persimilis* must consume 7.8 *T. urticae* eggs to lay a single egg. Furthermore, it was observed that other predatory mites, such as *A. cucumeris* and *G. occidentalis*, require 8.4 and 8.8 *T. urticae* eggs, respectively, to lay one egg. Despite differences in reproductive capacity among predatory mites, egg laying across all assessed limited food levels was generally low.

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