



Supplementation of natural prey with pollen grains exerts an influence on the life table parameters of *Neoseiulus californicus*

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Research Paper

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Abstract

Better performance of generalist predators, as well as an increase in their density, may be an incentive factor in the ability of the predators to exploit more than one food item or mixed diets. In this study, the effects of four pollen grains (cedar, pear, apricot, and pistachio) when provided to *Neoseiulus californicus* in mixed diets with prey, *Tetranychus urticae*, were evaluated. The result indicated that the fastest female developmental time was observed on pistachio pollen + *T. urticae*, together with apricot pollen + *T. urticae*. Females reared on the mixed diet comprising pistachio pollen reflected the longest total life span duration, while the shortest total life span was observed in those on the diet that included pear pollen. Furthermore, the lowest fecundity, as well as the shortest reproduction period, was determined on the diets that included pear pollen, while the highest fecundity and the longest reproduction period were observed in pistachio pollen + *T. urticae*. In addition, the intrinsic (r) and finite rate of increase (λ), net (R_0) and gross (GRR) reproductive rates were highest in pistachio pollen + *T. urticae*. These findings have important implications for developing a comprehensive biological control program of *T. urticae*, which will be discussed.

Introduction

Since strict regulations limit the availability of synthetic pesticides in greenhouses, new strategies are designed for the control of different phytophagous pests. Recent evidence has revealed that biological control methods can be used for various pests that are dangerous to different crops. After hymenopteran parasitoids, acarid predators are well known as the most effective and important group of organisms used for the biological control of phytophagous mites and insects on greenhouse crops (Buitenhuis *et al.*, 2015). For the explicit reason that phytoseiid predatory mites can feed on different food sources including spider mites, different insects, fungi, pollen, plant exudates, and nematodes (McMurtry *et al.*, 2013), they have been given much interest in the last decades. The preference of different food items including different pollen grains (Riahi *et al.*, 2016, 2017a; Khanamani *et al.*, 2017a), powdery mildew (Zemek and Prenerova, 1997; Duso *et al.*, 2003), factitious prey (Riahi *et al.*, 2017b), as well as artificial foods (Nguyen *et al.*, 2015; Riahi *et al.*, 2017c; Khanamani *et al.*, 2017c) to support the various phytoseiid mites' population has been frequently addressed.

It is generally accepted that generalist phytoseiid mites bear some advantageous traits ensuring the long-term control of greenhouse pests. Predator population can be sustained by feeding on either alternative food such as pollen and alternative prey or plant secretions in greenhouses and fields, especially in the absence of target pest species (van Rijn *et al.*, 2002). Bluntly put, the presence of pollen as an alternative or supplementary food source not only provides important nutrients including proteins, free amino acids, carbohydrates, lipids, vitamins, flavonoids, and minerals for predators, but also allows the predators to establish their population before the prey population (van Rijn *et al.*, 2002; Nomikou *et al.*, 2003). The role of pollen from different plants for various generalist predatory mites in both field and laboratory has been well documented (van Rijn *et al.*, 1999; Hoogerbrugge *et al.*, 2011; Riahi *et al.*, 2016; Khanamani *et al.*, 2017a).

Better performance of generalist predators, as well as an increase in their density, may be an incentive factor in the ability of the predators to exploit more than one food item or mixed diets (Momen and El-Borolossy, 2010). Hoogerbrugge *et al.* (2011) reported that the presence of pollen on strawberries accelerated the growth rate of *Amblyseius limonicus* Garman and McGregor, with increased *Trialeurodes vaporariorum* Westwood control as a consequence. In addition, it has been demonstrated that when pollen was supplemented on cucumber,

two species of predatory mites, *Iphiseius degenerans* Berlese and *A. limonicus*, developed faster and resulted in the suppression of thrips population (van Rijn et al., 1999). Furthermore, the effectiveness of *Neoseiulus californicus* (McGregor) in the control of *Tetranychus urticae* Koch was improved in the presence of maize pollen (Khanamani et al., 2017b). In another study, the presence of almond pollen augmented the control potential of *Amblyseius wirskii* (Athias-Henriot) against *T. urticae* (Riahi et al., 2017a).

Neoseiulus californicus, a type II selective predator, is one of the most important biological control agents used in augmentative biological control against various pests (McMurtry et al., 2013). While it is true that this predator is mostly active on tetranychid mites, especially tetranychids producing heavy webbing, the fact still remains that they can exploit other mite species, small insects, such as thrips, and even pollen (McMurtry and Croft, 1997). Many new publications that deal with different aspects of biology, ecology, and practical use of *N. californicus* against tetranychid mites are attributable to the wide efficiency of this predator (Barber et al., 2003; Khanamani et al., 2017a, 2017b). Interestingly, despite this content of researches, the knowledge on alternative food spectrum accepted by this predator, as well as the effects of mixing natural prey with pollen on its efficacy, still remains insufficient.

Considering our previous study (Soltaniyan et al., unpublished data), which reflected the nutritional value of four different pollen grains compared with main prey (*T. urticae*), it was revealed that both development and reproduction of *N. californicus* were conditioned by the type of pollen. The fact remains that the presence of different pollens as a supplementary food source can affect the performance, together with the life table parameters of *N. californicus* for the control of *T. urticae*. Therefore, this study describes the effects of the addition of four pollen grains in a mixed diet with main prey on the development, reproduction, and growth rate of *N. californicus*.

Materials and methods

Stock culture of mites

The initial population of *T. urticae* was collected from infested greenhouses in Pakdasht region (South-Eastern part of Tehran). The mite colony was established on bean plants (*Phaseolus vulgaris* var. Khomein) at $25 \pm 5^\circ\text{C}$, under natural humidity and photoperiod of greenhouse.

The population of *N. californicus* was obtained from Koppert Giah-Bazr-Alvand Company (Tehran, Iran). The individuals were reared on experimental rearing arena at $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH, and a photoperiod of (16:8) L:D hours. Each experimental rearing arena consisted of a plastic sheet ($23 \times 14 \times 0.3$ cm) put on a water-saturated sponge placed inside a plexiglas box ($23 \times 14 \times 9$ cm) half-filled with water. In order to not only provide a water source, but also prevent mites from escaping, small layers of towel were used for covering all margins of plastic sheet. Bean leaves heavily infested with *T. urticae* were added to each arena as a food source every 2 days.

Pollen sampling

Pollen of cedar (*Cupressus sempervirens*), pear (*Pyrus communis*), apricot (*Prunus armeniaca*) and pistachio (*Pistachio vera*) were collected from aforementioned trees not treated with pesticides

in East Azarbaijan province, Iran, during March 2016. The blooms of each plant were gathered separately by hand during the spring season. After separating the flags, they were oven dried (at 37°C for 48 h), and then severely taken on a newspaper to the laboratory. Pollen was aggregated into a glass vial and stored in a refrigerator (at 4°C) awaiting the experiments.

Experimental unit

The experimental units comprised of a bean leaf disc (3 cm diameter) placed upside down on water-saturated cotton located on a fiber in a plastic saucer ($17 \times 8 \times 8$ cm). The leaf margins were covered with moist tissue paper which served as a water supply source, as well as prevented predators from escaping. Some of the cotton strings, together with a piece of fiberglass, were placed in the center of the bean leaf disc as a shelter and oviposition site for the predatory mites.

Diets

The treatments tested were as follows:

- About 0.05 mg of apricot pollen + either 15 (during immature stages) or 40 (during adult stage) *T. urticae* nymphs
- About 0.05 mg of pistachio pollen + either 15 (during immature stages) or 40 (during adult stage) *T. urticae* nymphs

Experimental design

To assess the effects of mixed diet on the development and reproduction of *N. californicus*, mites were reared on the experimental units and fed with one of the four treatments. After two generations, the eggs of adults were used for initiating the experiments. By considering the same rearing history, obtained eggs were transferred individually to the experimental unit, and from the larval stage onward, the mites were provided with the respective diet. The foods were replenished every day. Experimental units were daily checked by recording the development as well as the survival of individuals by removing the exuviae of the moulted stages. Upon adult emergence, females and males were coupled and then transferred to a new experimental unit and immediately fed with the same diet with which they were reared on formerly. In addition to adult survival and longevity, the number of eggs produced by females was recorded daily until the death of all individuals. All experiments were conducted in a growth chamber at $25 \pm 1^\circ\text{C}$, $65 \pm 10\%$ RH, and a photoperiod of 16:8 (L:D) hours.

Data analysis

The age-stage, two-sex life table procedure was chosen for data analysis to allow considering both variable developmental rate among individuals and stage differentiation (Chi and Liu, 1985). All parameters including the age-stage-specific survival rate (s_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), as well as all population growth parameters [the intrinsic rate of increase (r), the finite rate of increase (λ), the gross reproductive rate (GRR), and the net reproductive rate (R_0)] were calculated according to the method of Chi and Liu (1985) and Chi (1988) using TWOSEX-MS Chart (Chi, 2016). Multiple comparisons of all estimated parameters including developmental duration of the different life stages, fecundity, reproduction periods, as well as population growth parameters were performed by a

Table 1. Comparative duration (day, mean \pm SE) of immature stages, longevity, and total life span of *Neoseiulus californicus* reared on four different mixed diets

Parameter	Cedar + <i>T. urticae</i>	Pear + <i>T. urticae</i>	Apricot + <i>T. urticae</i>	Pistachio + <i>T. urticae</i>
Male				
Egg	2.09 \pm 0.21 a	2.08 \pm 0.19 a	2.38 \pm 0.14 a	2.42 \pm 0.15 a
Larva	1.18 \pm 0.12 a	1.00 \pm 0.00 a	1.15 \pm 0.10 a	1.08 \pm 0.15 a
Protonymph	4.09 \pm 0.21 a	2.42 \pm 0.23 b	2.46 \pm 0.14 b	2.50 \pm 0.23 b
Deutonymph	3.18 \pm 0.23 a	2.58 \pm 0.15 b	2.08 \pm 0.08 b	2.08 \pm 0.00 b
Pre-adult	10.55 \pm 0.31 a	8.08 \pm 0.29 b	8.08 \pm 0.24 b	8.08 \pm 0.15 b
Adult longevity	26.36 \pm 0.61 b	23.25 \pm 0.66 c	31.85 \pm 0.58 ab	35.25 \pm 0.15 a
Total life span	36.91 \pm 0.61 c	31.33 \pm 0.66 d	39.92 \pm 0.70 b	43.33 \pm 0.78 a
Female				
Egg	2.72 \pm 0.06 a	2.22 \pm 0.11 b	2.52 \pm 0.07 ab	2.48 \pm 0.07 b
Larva	1.11 \pm 0.04 a	1.20 \pm 0.06 a	1.11 \pm 0.04 a	1.13 \pm 0.05 a
Protonymph	4.39 \pm 0.07 a	2.43 \pm 0.08 b	2.37 \pm 0.07 b	2.20 \pm 0.07 b
Deutonymph	3.20 \pm 0.06 a	2.65 \pm 0.08 b	1.76 \pm 0.08 c	1.69 \pm 0.08 c
Pre-adult	11.43 \pm 0.12 a	8.50 \pm 0.18 b	7.76 \pm 0.14 c	7.50 \pm 0.13 c
Adult longevity	26.76 \pm 0.23 b	23.20 \pm 0.30 c	29.61 \pm 0.24 b	35.65 \pm 0.37 a
Total life span	38.19 \pm 0.25 b	31.70 \pm 0.32 c	37.37 \pm 0.26 b	43.15 \pm 0.37 a

The standard errors were calculated using the bootstrap procedure with 100,000 bootstraps. The means followed by different letters in the same row are significantly different between diets using the paired bootstrap test ($P < 0.05$).

Table 2. Comparative duration of reproductive periods and fecundity (Mean \pm SE) of *Neoseiulus californicus* reared on four different mixed diets.

Parameter	Cedar + <i>T. urticae</i>	Pear + <i>T. urticae</i>	Apricot + <i>T. urticae</i>	Pistachio + <i>T. urticae</i>
APOP (day)	4.74 \pm 0.11 b	3.80 \pm 0.14 c	5.19 \pm 0.09 a	3.46 \pm 0.10 c
TPOP (day)	16.17 \pm 0.16 a	12.30 \pm 0.22 b	12.94 \pm 0.10 b	10.96 \pm 0.17 c
Oviposition period (day)	12.02 \pm 0.23 c	10.83 \pm 0.20 d	17.15 \pm 0.22 b	24.67 \pm 0.27 a
Fecundity (eggs/female)	12.02 \pm 0.23 c	12.07 \pm 0.21 c	18.15 \pm 0.25 b	32.35 \pm 0.16 a

APOP, adult pre-ovipositional period; TPOP, total pre-ovipositional period.

The standard errors were calculated using the bootstrap procedure with 100,000 bootstraps. The means followed by different letters in the same row are significantly different between diets using the paired bootstrap test ($P < 0.05$).

paired bootstrap test using TWOSEX-MS Chart with 100,000 samples.

Results

Table 1 provides the effects of four different mixed diets on the development and reproduction of *N. californicus*. It is apparent from this table that although male incubation period differences were not significant among different diets, it was significantly different for females (table 1). Paired bootstrap test showed that the completion of both protonymphal and deutonymphal development was slowest on a diet of cedar pollen + *T. urticae* for males, while there was not any significant difference among other diets (table 1). The longest female deutonymphal duration was obtained on cedar pollen + *T. urticae*, followed by pear pollen + *T. urticae*, apricot pollen + *T. urticae*, and pistachio pollen + *T. urticae* (table 1). As it is clear from table 1, female individuals fed on pistachio pollen + *T. urticae*, along with apricot pollen + *T. urticae*, reached the adulthood more rapidly than those fed on pear pollen + *T. urticae* than those reared on cedar pollen + *T. urticae*.

The longest and shortest female, together with male adult longevity, were obtained by feeding on pistachio pollen + *T. urticae* and pear pollen + *T. urticae*, respectively. Males reared on pistachio pollen + *T. urticae* survived significantly more days than those fed on apricot pollen + *T. urticae*, than on other diets. Females reared on the mixed diet that comprised pistachio pollen reflected the longest total life span duration, while the shortest was on the diet that included pear pollen (table 1).

Feeding on different diets caused differences in the reproduction periods and fecundity of *N. californicus* (table 2). The lowest fecundity, as well as the shortest reproduction period, was determined on the diets that included pear pollen, while the highest fecundity and the longest reproduction period were observed on pistachio pollen + *T. urticae*. The total pre-oviposition period (TPOP) on pistachio pollen + *T. urticae* was significantly shorter than that of the other three diets (table 2). The presence of pistachio pollen interestingly reduced the duration of adult pre-oviposition period in contrast to apricot and cedar pollen (table 2).

Fig. 1 presents the age-stage-specific survival rate (s_{xj}) representing the probability that a newly born individual will survive

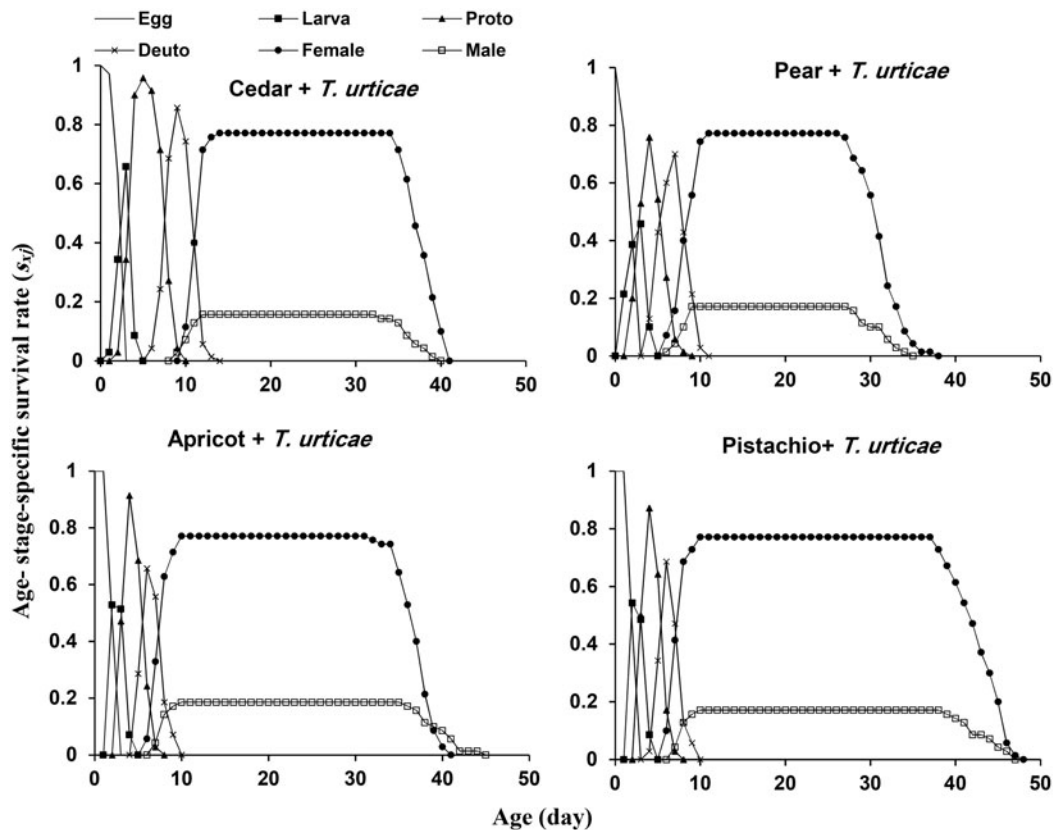


Figure 1. Age-stage-specific survival rate (s_{xj}) of *Neoseiulus californicus* fed on four different diets.

Table 3. The mean (\pm SE) of population growth parameters of *Neoseiulus californicus* reared on four different mixed diets.

Parameter	Cedar + <i>T. urticae</i>	Pear + <i>T. urticae</i>	Apricot + <i>T. urticae</i>	Pistachio + <i>T. urticae</i>
R_0 (offspring/individual)	9.27 \pm 0.62 c	9.31 \pm 0.62 c	14.00 \pm 0.93 b	24.96 \pm 1.65 a
r (day ⁻¹)	0.1010 \pm 0.0032 c	0.1272 \pm 0.0043 b	0.1288 \pm 0.0036 b	0.1540 \pm 0.0039 a
λ (day ⁻¹)	1.1062 \pm 0.0035 c	1.1357 \pm 0.0049 b	1.1375 \pm 0.0041 b	1.665 \pm 0.0045 a
GRR (offspring/individual)	9.98 \pm 0.59 c	9.88 \pm 0.59 c	14.63 \pm 0.09 b	26.48 \pm 1.57 a
T (day)	22.04 \pm 0.16 a	17.53 \pm 0.23 c	20.48 \pm 0.16 b	20.88 \pm 0.20 b

The standard errors were calculated using the bootstrap procedure with 100,000 bootstraps. The means followed by different letters in the same row are significantly different between diets using the paired bootstrap test ($P < 0.05$).

up to each age-stage unit, on four different diets. The highest value of s_{xj} for adult stage on all diets was 0.771 for females and 0.171, 0.157, 0.171, and 0.186 for males on the mixture of *T. urticae* with pistachio, cedar, pear, and apricot pollen, respectively. The interesting thing about this figure is that besides the survivorship, stage differentiation can be easily seen. What is more, it is by virtue of variable developmental rate among individuals that overlapping between curves has occurred (fig. 1).

Table 3 presents the means, together with the standard errors of the population growth parameters estimated using the bootstrap procedure. The intrinsic rate of increase (r) and finite rate of increase (λ) were highest on pistachio pollen + *T. urticae*, followed by the mixture of either apricot, pear, or cedar pollen with immature *T. urticae*. Net as well as gross reproductive rate was significantly conditioned by the type of diet; as their highest values were obtained in the presence of pistachio pollen, cedar

and pear pollen resulted in the lowest values of the mentioned parameters (table 3).

The age-specific fecundity (m_x), mean number of offspring produced by individuals at age x , as well as age-specific survivorship (l_x), and the probability that a new born individual will survive to age x , of *N. californicus* on different combinations of diet, have been depicted in fig. 2. The highest daily oviposition was 0.83, 1.05, 1.06, and 1.76 eggs on the combination of *T. urticae* with different pollen including cedar, pear, apricot, and pistachio, respectively, which occurred at the age of 19, 18, 21, and 23 days, respectively (fig. 2).

Discussion

Over the past decade, there has been a dramatic increase in the use of age-stage, two-sex life table procedure to investigate the

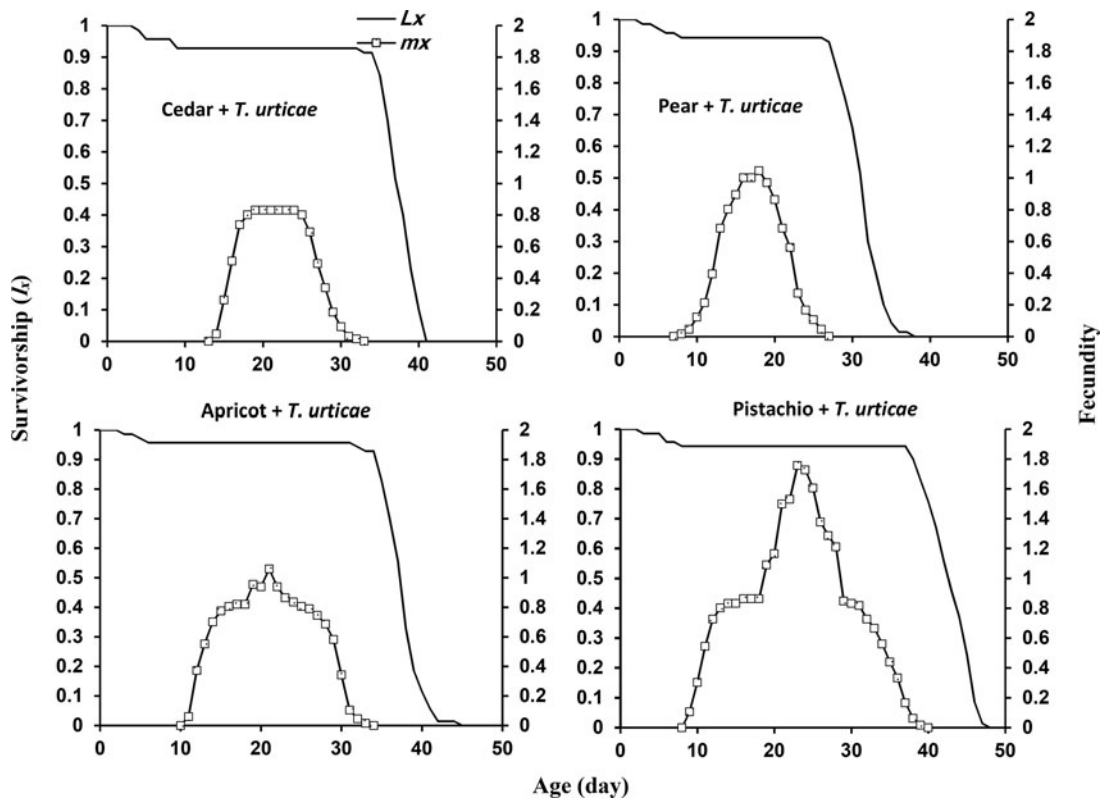


Figure 2. Age-specific survivorship (l_x) and age-specific fecundity (m_x) of *Neoseiulus californicus* fed on four different diets.

effects of different variable factors on various organisms. In contrast to the traditional female age-specific life table, not only the emergence of each stage, but also the overlaps between stages could be completely characterized, as a result of considering stage differentiation. In addition, ignoring stage differentiation has a serious effect on l_x and m_x curves, as a consequence of considering adult age (Huang and Chi, 2012). Akkoprui *et al.* (2015) published a paper in which the difference between the standard statistical procedure and the bootstrap technique was described. These two procedures are different from each other in using the data of all individuals or the means of all resampling for the estimation of SE. It is true that estimated SE is almost the same, but what is surprising is that a normal frequency distribution, which is essential for the following analysis and comparison, is only produced by bootstrapping (Akkoprui *et al.*, 2015). This is why in this study, age-stage, two-sex life table procedure was used for data analysis and bootstrapping was used for calculating the standard errors (SE) of all parameters including stage duration, developmental time, longevity, fecundity, as well as population growth parameters.

With the comparison of four diets, this study aimed to investigate the efficacy and performance of *N. californicus* in controlling *T. urticae* in the presence of different pollen grains. The single most striking observation that emerged from the data comparison was that the presence of pistachio pollen resulted in reduced pre-adult development and increased oviposition rate, as well as population growth rates of *N. californicus*. These results could be attributed to the desirable nutritional quality of pistachio pollen for *N. californicus* compared with other pollen tested. Mixing cedar pollen with natural prey resulted in the drastic reduction in oviposition rate and prolonged pre-adult development

compared with the other three pollens. Inferior nutritional quality of cedar pollen for *N. californicus* is the simple reason for this result. In another study, the effects of the combination of *T. urticae* with three pollen grains on the performance of *N. californicus* were compared (Khanamani *et al.*, 2017b). On the basis of the mentioned study, the composite of maize pollen caused the best efficiency ($r=0.1409$), which is lower than the highest growth rate in the current study ($r=0.1540$). There are grounds for suggesting that pollen grain size does not have an important effect on its quality as a food source for the mites (van Rijn and Tanigoshi, 1999); therefore, other pollen features, such as exine thickness and structure, as well as nutritional composition are the contributory factors in these differences.

It is generally accepted that the nutritional value of a diet for predatory mites can be compared by the estimation of the life table parameters. Among them, intrinsic rate of increase is the most important parameter. In the present study, this parameter varied from 0.1010 to 0.1540 day^{-1} . Variable growth rates of *N. californicus* as a consequence of different diets including natural prey such as *Panonychus ulmi* (Koch) (0.25 day^{-1} ; El Taj and Chuleui 2012), *T. urticae* (0.1545 day^{-1} ; Khanamani *et al.*, 2017a), and *Thrips tabaci* Lindeman (0.041 day^{-1} ; Rahmani *et al.*, 2009); artificial diets (−0.2089 to 0.0782 day^{-1} ; Khanamani *et al.*, 2017c); and different pollen grains (0.0057–0.2318 day^{-1} ; Khanamani *et al.*, 2017a) have been reported. Such variation may be attributed not only to different food items offered to predators, but also to different experimental conditions, genetic variation among predator populations, and/or different data analysis procedures.

The presence of pollen beside natural prey and its effects must be considered in both positive and negative aspects. On one hand,

pollen grains may change the predator's behavior into feeding on pollen, consequently resulting in the reduced control of target pests in the short term (van Rijn et al., 2002; Nomikou et al., 2010). On the other hand, pollen grains may give rise not only to an increase in the predator's offspring production, but also eliminate their negative effects on the predator's functional response which will result in the reduction in pest population over a long period of time (van Rijn et al., 2002; Nomikou et al., 2010). In addition, it is adequate to say that during periods where natural prey are scarce or absent, predators can maintain their population on pollen (Riahi et al., 2017a; Khanamani et al., 2017b). It could be reasonably argued that supplementation of natural prey with pollen grains may lead to complicated effects in its nature for the simple reason that it depends on the feeding preference of predator. While it is true that reduction in predation rate by virtue of the presence of pollen besides prey has been reported for different phytoseiid mites (Nomikou et al., 2002; Riahi et al., 2017a), the fact still remains that this was compensated for by increasing the predator's egg production, together with growth rate (Nomikou et al., 2002; Hoogerbrugge et al., 2011; Pappas et al., 2013; Khanamani et al., 2017b; Riahi et al., 2017a). With the results of Khanamani et al. (2017b) in mind, the present results indicated that different pollen species showed large differences in changing the quality of *T. urticae* as a food source for *N. californicus*. By way of comparison, it is difficult to elude the obvious conclusion that the performance of *N. californicus* fed on the diet comprising both pistachio pollen and *T. urticae* was better than the diets that included solely *T. urticae* (Khanamani et al., 2017b). Pre-adult development as well as TPOP was shorter, while the values of R_0 , r , and λ were higher on the mixture diet than *T. urticae* solely. A possible explanation for this might be that pistachio pollen is full of nutrients which are in accordance with the need of this species.

In conclusion, from the control point of view, the presence of pistachio pollen in the field or greenhouses infested with *T. urticae* can enhance both the development and growth rate of *N. californicus*, especially during the periods of prey scarcity. This event will help the predators to build up a population before the pest mites reach outbreak levels and consequently will control the pest population while they are still at non-damage levels. On the other hand, this phenomenon will allow predators to sustain and increase their population. Therefore, in order to achieve the positive effects of pistachio pollen in the efficacy of *N. californicus* to control *T. urticae*, it is suggested to plant pistachio in and around croplands and orchards, or spray the crops in greenhouses with stored pollen. In particular, uncertainty still prevails as to whether a reduction in predation rate due to feeding on pollen is compensated for by a greater amount of predation due to an increased predator population during the season after *T. urticae* starts to increase. This is an important issue for future research.

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