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Long-term effects of saffron pollen on development, reproduction and predation capacity of *Neoseiulus cucumeris* (Acari: Phytoseiidae)

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Original research

ABSTRACT

Predatory phytoseiid mites as biocontrol agents are increasingly used in pest control programs across the globe, and Neoseiulus cucumeris (Oudemans) is one of them, which is a commercially available agent against various pests of greenhouse crops. This generalist predatory mite can feed on different foods and diets, including small arthropods and pollen. Feeding and reproducing on a pollen diet is a considerable characteristic of this predatory mite that facilitates the cost-effective production of large numbers of this predator socalled mass rearing. Therefore, the effects of saffron (Crocus sativus L.) pollen as an alternative food source on development and reproduction of N. cucumeris were determined over 20 successive generations. In addition, the predation ability of the nymphs and adults of N. cucumeris being switched to natural prey, Tetranychus urticae Koch after 10th and 20th generations was evaluated. The shortest and longest developmental time happened in the 1th and 5st generations, respectively. The predator in the 1st and 20th generations had a higher intrinsic rate of increase (r) (0.171 and 0.170 day⁻¹, respectively) than other generations, while the 5th generation had the lowest value of this parameter. The development and fecundity of N. cucumeris was not significantly different when this predator switched to a diet of T. urticae. The results of this study revealed that rearing of N. cucumeris for 20 generations on saffron pollen did not affect the performance of the predator negatively and this alternative food source can be used for the mass rearing of N. cucumeris.

Keywords mass rearing; predatory mite; life table; alternative food; Tetranychus urticae

Introduction

Release of the phytoseiid mites in integrated pest management (IPM) programs requires a sufficient population of these predatory mites, so it seems necessary to choose appropriate methods for their mass rearing. Since rearing large numbers of predatory mites on the main prey is time-consuming and expensive, it is very important to find suitable alternative food sources. Alternative foods can play an important role in mass rearing of these predators, improving their performance, and their success in pest control programs (Cloutier and Johnson 1993).

The generalist phytoseid mites can feed on nectar (van Rijn and Tanigoshi 1999), artificial diets (Nguyen *et al.* 2014), and pollen (Kishimoto *et al.* 2014; Soltaniyan *et al.* 2018). Feeding on pollen not only causes less cannibalism (Hoogerbrugge *et al.* 2008), but also supplies a great number of nutrients for phytoseiid mites (Khanamani *et al.* 2016). Since, the

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nutritional value of pollen varies with the type of plant species (Goleva and Zebitz 2013), it is crucial to know about the performance of various phytoseiid mites on different types of pollen. *Neoseiulus cucumeris* (Oudemans), as a generalist predatory mite, can feed on small pests such as phytophagous mites, thrips and whiteflies as well as pollen (McMurtry *et al.* 2013). This predatory mite has successful develop and reproduction when it was fed with pollen grains of castor bean, apple, sunflower, pear (van Rijn and Tanigoshi 1999), maize (Ranabhat *et al.* 2014; Sarwar 2016), and cattail (Gravandian *et al.* 2022).

Although, several studies have been conducted in the field of long-term rearing of *N. cucumris* on different pollens such as almond pollen (Yazdanpanah *et al.* 2021), castor bean and date palm pollens (Yazdanpanah *et al.* 2022a), and cattail pollen (Gravandian *et al.* 2022), no study has been carried out on saffron pollen as a food source of *N. cucumris*. Therefore, the current study aimed to discover the performance of *N. cucumeris* on saffron pollen during successive generations and determine its predation capacity on *Tetranychus urticae* Koch as a main prey after 10 and 20 generations of rearing on saffron pollen.

Materials and Methods

Pollen collection

Pollen of saffron (*Crocus sativus* L., Iridaceae) was collected from Khorasan Razavi province, Northeastern Iran. The pollen grains were dried at 25 °C for 36 h, and frozen at -20 °C for long-term storage or at 4 °C for daily use.

Stock colony of N. cucumeris

The initial population to form stock colony was purchased from Bioplanet, Italy. To establish the colony of *N. cucumeris* in laboratory, the predatory mite individuals were transferred to a clear plastic box $(30 \times 16 \times 10 \text{ cm})$ half-filled with water, contains a water-soaked sponge and a green plastic sheet $(18 \times 12 \times 0.1 \text{ cm})$ on the top of the sponge. All four edges of the plastic sheet were covered by wet paper towels to prevent the mites from escaping (Walzer and Schausberger 1999) and also provide them water when they need. The stock colony of *N. cucumeris* was kept at 25 ± 1 °C, $65\pm5\%$ RH, and a photoperiod of 16L: 8D h. Fresh pollen was provided every 3 to 4 days. The predator was reared on the saffron pollen for 20 generations.

Experimental setup

The experimental units were similar to the stock culture, but their size was smaller. Experimental units consisted of green plastic sheets $(3 \times 3 \times 0.1 \text{ cm})$, plastic boxes $(8 \times 5 \times 4 \text{ cm})$, and watersoaked sponges. Some cotton was glued on the plastic sheet to provide a shelter for mites as well as a place to lay their eggs. To provide the moisture required by the mites, the edges of the plastic sheet were covered with paper towels and water was added daily to prevent the paper towels from drying out. More than 40 same-aged eggs (less than 24 h) were transferred individually to the experimental units. After hatching the eggs, ad lib pollen grains were offered as food. Developmental and survival of immature stages (about 40 replicates) were recorded daily until adult emergence. The females were coupled with the males individually using a fine brush. The adult longevity and oviposition period were recorded daily until the death of the last adult.

For the predation capacity experiments, the same-aged eggs (< 24 h old) laid by the females reared on prey (G1) and pollen (G10 and G20) were transferred to the experimental units individually (30–40 replicates). After larval emergence, about 30–40 and 50–60 protonymphs of *T. urticae* were provided daily for the nymphs and adults of the predator, respectively. The consumed prey and laid eggs were recorded daily.

All experiments were carried out in laboratory conditions at 25 ± 1 °C, $65\pm5\%$ RH, and a photoperiod of 16L: 8D h. Old saffron pollen grains were replaced with fresh ones every three days.

Body size of N. cucumeris

The body size of males and virgin females of the predator before rearing on saffron pollen as control (G1) and after 5, 10, and 20 generations of rearing on this pollen was measured. The mites were mounted on glass slides using Hoyer's medium. Body length was measured along the midline from the anterior to posterior margins of the idiosoma, and body width was denotes the maximum value of the idiosoma. The measurements were given in micrometers (μ m) using a calibrated ocular microscope (Olympus, Japan).

Data analysis

All life table parameters were calculated according to the age-stage, two-sex life table procedure (Chi and Liu 1985; Chi 1988; Chi *et al.* 2020). The values of all life table parameters and predation rate were calculated using the TWOSEX-MSChart (Chi 2022a) and Consume-MSChart software (Chi 2022b), respectively. The variances and standard errors of all life table parameters were determined using bootstrap with 100,000 samples. All parameters of different generations were compared using the paired bootstrap test. Body size data were analyzed using one-way ANOVA and Tukey's test for multiple comparisons.

Results

Life table parameters of N. cucumeris fed on saffron pollen

The average duration of the egg stage was from 2.31 to 2.67 days. There was no significant difference in the larval period in different generations (\sim 1 day). In the 5th generation, protonymphal and deutonymphal stages had the longest durations (2.57 and 2.47 days, respectively). The shortest and longest developmental time was in the first (7.51 days) and fifth (8.70 days) generations, respectively (Table 1).

Table 1 Duration of different life stages (days) and fecundity (eggs/female) (Mean±SE) of *Neoseiulus cucumeris* reared on saffron pollen for 20 generations.

Parameter	Generation					
	1	5	10	20		
Egg	2.31 ± 0.082^{b}	2.67 ± 0.086^{a}	2.65 ± 0.078^{a}	2.40±0.127 ^{ab}		
Larva	$1.05{\pm}0.035^{a}$	$1.00{\pm}0.000^{a}$	$1.03{\pm}0.027^{a}$	$1.00{\pm}0.000^{a}$		
Protonymph 1.97±0.084		2.57 ± 0.147^{a} 2.32 ± 0.094^{a}		2.28±0.106 ^a		
Deutonymph 2.18±0.08		2.47 ± 0.123^{a} 2.11 ± 0.100^{b}		2.08 ± 0.112^{b}		
Pre-adult 7.51±0.114		8.70 ± 0.177^{a}	8.11 ± 0.141^{b}	7.76±0.172 ^{bc}		
Male longevity 79.66±14.911 ^{at}		45.53±8.241 ^c 68.40±8.113 ^b		86.27±4.148		
Female longevity 90.21±4.787 ^a		55.78±3.473 ^c	54.96±4.698°	69.38±3.265		
Total lifespan	95.29 ± 4.988^{a}	60.72±3.684 ^c	$68.52 \pm 4.392^{\circ}$	81.87±2.979		
APOP	$3.10{\pm}0.087^{b}$	$3.53{\pm}0.138^{a}$	3.00 ± 0.145^{b}	2.78±0.169 ^b		
ГРОР 10.70±0.126 ^b		12.00 ± 0.226^{a}	11.18 ± 0.221^{b}	10.56±0.288		
Oviposition days	$35.49{\pm}1.933^{a}$	$32.53{\pm}1.865^{ab}$	$26.67 \pm 1.818^{\circ}$	^c 28.12±2.977 ^{bc}		
Fecundity 52.68±2.764 ^a		48.64±3.011 ^{ab}	44.12 ± 3.111^{b}	42.95 ± 3.959^{10}		

Male longevity was significantly longer in the first (79.66 days) and 20th (86.27 days) generations than others, and the first generation had the longest female longevity (90.21 days). The total lifespan was significantly longer in the first generation (95.29 days) than other generations. The longest adult pre-oviposition period (APOP), and total pre-oviposition period (TPOP) were recorded in the fifth generation. The days of oviposition was varied from 26.67 to 35.49 days. Fecundity in the first generation was significantly higher than the 10th and 20th generations (Table 1).

Figure 1 shows the age-stage-specific survival rate (s_{xy}) , the beginning and end of all life stages by feeding on saffron pollen for 20 generations. In the first and 20th generations, the first protonymph appeared on the second day, and in 5th and 10th generations, the first protonymph appeared on the third day. The first deutonymph in the first and 20th generations appeared after four days, while the first deutonymph in 5th and 10th generations appeared on the fifth day. The emergence of adult stages was different among generations (between 6 and 8 days). The females and males of the first generation lived more days (133^{\circ} to 139^{\circ} days), while females and males of the 20th generation died after 99 and 108 days, respectively (Fig. 1).

Based on the fecundity curves, the first egg laid in 1, 5, 10, and 20 generations were observed at the ages of 12, 11, 9, and 8 days, respectively; oviposition stopped at the ages of 87, 60, 54, and 72 days, respectively. The highest fecundity was recorded in the 10th generation (1.95 egg/female) at the age of 17 days (Fig. 2).

In the 1st to 20th generations, there were significant differences between population growth parameters. No significant difference was recorded in any of the gross reproductive rate (*GRR*), net reproductive rate (R_0), intrinsic rate of increase (r), and finite rate of increase (λ) parameters between the 1st and 20th generations. The mean generation time (T) in 20th generation (20.09 days) was significantly shorter than the 1st and 5th generations; but there was no significant difference with 10th generation (Table 2).

Life table parameters of N. cucumeris fed on T. urticae

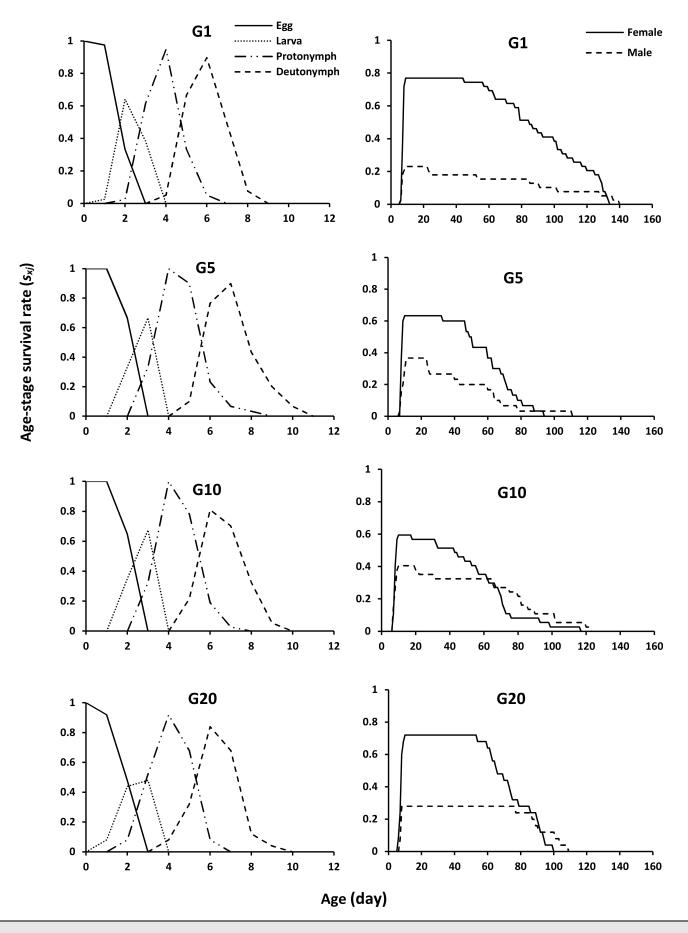
After 10 and 20 generations of rearing *N. cucumeris* mites on saffron pollen, they were transferred to feed on *T. urticae* as a main prey and the predator's life table parameters were determined. The developmental time in the 10th and 20th generations was 8.67 and 8.44 days, respectively. In addition, the male and female longevities were 40.97, 46.31 days, and 30.45, 36.46 days, respectively, in the 10th and 20th generations. The total lifespan in the 10th and 20th generations was 41.95 and 46.80 days, respectively (Table 3).

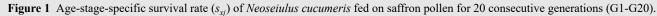
The adult pre-oviposition period (APOP) was the only parameter in which a significant difference was recorded between the 10^{th} (3.40 days) and 20^{th} (2.46 days) generations, but the total pre-oviposition period (TPOP) and other life table parameters had no significant differences in the mentioned generations. The days of oviposition was 19.09 and 22.72 days in the 10^{th} and 20^{th} generations, respectively; and fecundity in the 10^{th} and 20^{th} generations had no significant difference (Table 3).

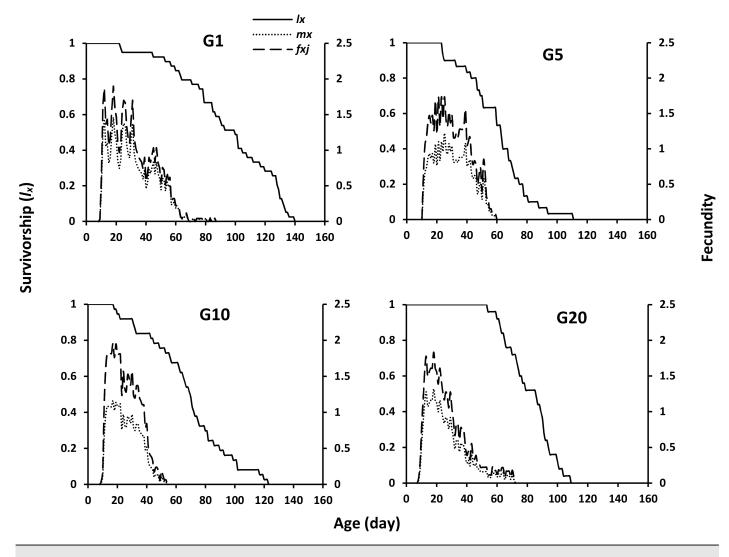
The age-stage-specific survival rate (s_{xj}) on *T. urticae* showed that in the 10th and 20th generations, the first protonymphs appeared at the age of 3 and 2 days, respectively; and the

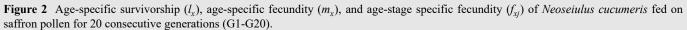
 Table 2
 The life table parameters (Mean±SE) of *Neoseiulus cucumeris* reared on saffron pollen for 20 generations.

Parameter	Generation				
	1	5	10	20	
GRR (eggs/ individual)	$42.49{\pm}4.046^{a}$	$34.54{\pm}4.758^{ab}$	28.49 ± 4.168^{b}	$31.17{\pm}4.849^{ab}$	
<i>R</i> ₀ (eggs/ individual)	$40.54{\pm}4.151^{a}$	$30.82{\pm}4.659^{ab}$	26.22 ± 3.996^{b}	$30.92{\pm}4.779^{ab}$	
$r (day^{-1})$	$0.171 {\pm} 0.007^{a}$	$0.146{\pm}0.008^{b}$	$0.157{\pm}0.009^{ab}$	$0.170{\pm}0.009^{a}$	
$\lambda (day^{-1})$	$1.186{\pm}0.008^{a}$	$1.157{\pm}0.010^{b}$	$1.170{\pm}0.010^{ab}$	$1.186{\pm}0.011^{a}$	
T (day)	21.68 ± 0.471^{b}	23.46±0.646 ^a	$20.73 {\pm} 0.433^{bc}$	20.09±0.617 ^c	









first deutonymph appeared at the age of 5 days for both generations. The emergence of adult stages in both generations occurred at the ages of 7 and 8 days, respectively. The total lifespan of adult mites in the 20th generation was slightly longer (64 to 68 days) than the total lifespan of adults in the 10th generation (57 to 62 days) (Fig. 3).

In the fecundity curves, the first egg laid in 10 and 20 generations were observed at the ages of 13 and 12 days, respectively; oviposition stopped at the ages of 51 and 47 days, respectively. The highest fecundity in the 10th and 20th generations was recorded at the ages of 17 (2.22 eggs/female) and 18 (2.09 eggs/female) days, respectively (Fig. 4).

There was no significant difference between the 10th and 20th generations in terms of population growth parameters such as *GRR*, R_0 , r, λ , and T (Table 4).

Predation capacity of *N. cucumeris* after long-term feeding on saffron pollen

The predatory mites did not show any activity in the larval stage (Fig. 5). The amount of prey consumed had increasing trend from protonymphal stage to the adult stage. The female predation rate was higher during the oviposition period than in pre-oviposition and

post-oviposition periods (Fig. 5). In the 10th and 20th generations, the females consumed 19.6 and 18.7 prey, respectively; while males consumed 12 and 11.9 prey, respectively.

The age-specific predation rate (k_x) is the mean number of prey eaten by each predator at the age of x, and the age-specific net predation rate (q_x) can be calculated by considering the survivorship (Fig. 6). No significant difference was observed between the 10th and 20th generation in any of the predation rate parameters (Table 5).

Body size of the predator during long-term rearing on saffron pollen

The length of the dorsal shield of males and females of the 1st generation was smaller than other generations (261.25 and 346.75 μ m, respectively). As the generation progressed, the length of the dorsal shield of males and females and the width of the dorsal shield of males increased; while the width of the dorsal shield of females increased from early generations to later ones and then, in a way that no significant difference was observed between the 1st and the 20th generations (Table 6).

Discussion

Alternative foods can help reduce the costs of long-term rearing and maintain the efficiency of biocontrol agents. Since alternative foods may negatively affect biocontrol agents during the long-term rearing (Bellutti 2011), it is important to evaluate the quality of these agents dealing with natural prey, before releasing them. We investigated the effects of saffron pollen as an alternative diet for *N. cucumeris* over the long-term (20 generations) and this predatory

 Table 3
 Duration of different life stages (days) and fecundity (eggs/female) (Mean±SE) of Neoseiulus cucumeris reared on Tetranychus urticae after 10 and 20 generations of feeding on saffron pollen.

Parameter	Generation			
	10	20		
Egg	2.47±0.128 ^a	2.32±0.169 ^a		
Larva	$1.00{\pm}0.000^{a}$	1.12±0.082 ^a		
Protonymph	2.60±0.126 ^a	2.63±0.194 ^a		
Deutonymph	$2.60{\pm}0.228^{a}$	2.37±0.213 ^a		
Pre-adult	8.67±0.322 ^a	8.44±0.373 ^a		
Male longevity	40.97 ± 5.616^{a}	46.31±6.896 ^a		
Female longevity	30.45±4.541 ^a	36.46±3.916 ^a		
Total lifespan	41.95±3.707 ^a	46.80±3.649ª		
APOP	$3.40{\pm}0.360^{a}$	$2.64{\pm}0.148^{b}$		
TPOP	11.90±0.668 ^a	11.09 ± 0.480^{a}		
Oviposition days	19.09 ± 2.727^{a}	22.72±1.963ª		
Fecundity	30.64±3.111 ^a	33.85±6.214 ^a		

 Table 4
 Standard life table parameters (Mean±SE) of Neoseiulus cucumeris reared on Tetranychus urticae after 10 and 20 generations of feeding on saffron pollen.

Parameter		Generation
	10	20
GRR (eggs/ individual)	26.24±5.651a	31.16±5.180a
R ₀ (eggs/ individual)	22.37±5.045a	27.85±5.202a
r (day ⁻¹)	0.160±0.014a	0.173±0.012a
$\lambda (day^{-1})$	1.174±0.016a	1.189±0.014a
T (day)	19.25±0.494a	19.16±0.657a

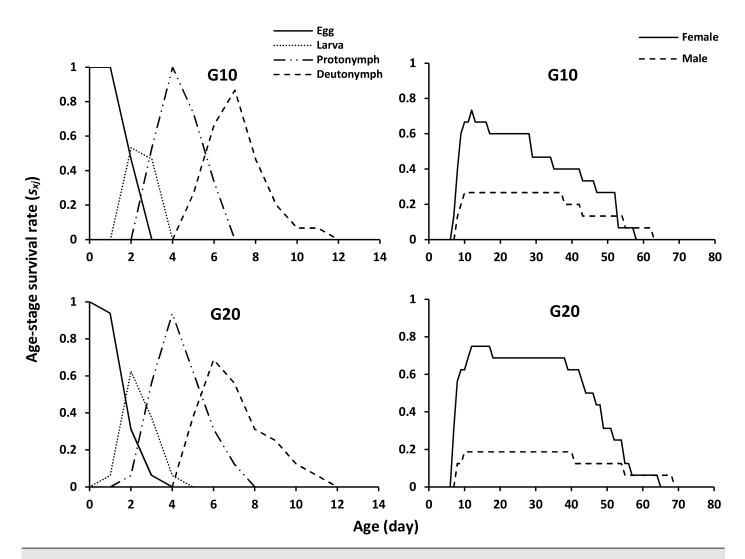


Figure 3 Age-stage-specific survival rate (s_{xj}) of *Neoseiulus cucumeris* when fed on saffron pollen for 10 and 20 generations (G10, G20) then switched to *Tetranychus urticae*.

Table 5 Mean (\pm SE) predation rates of *Neoseiulus cucumeris* reared on *Tetranychus urticae* after 10and 20 generations of feeding on saffron pollen.

Parameter	Generation			
	10	20		
Net predation rate (C_0) (prey)	427.29±48.633a	525.29±51.348a		
Transformation rate (Qp) (prey per viable predator egg)	19.83±3.902a	19.31±2.804a		
Stable predation rate (ψ) (prey/day)	5.07±0.285b	5.09±0.298b		
Finite predation rate (ω) (prey/day)	5.96±0.410b	6.06±0.418b		

mite was able to maintain its development and reproduction for 20 consecutive generations on saffron pollen.

According to the results, male longevity was longer than female longevity in G10 and G20 by feeding either on saffron pollen or *T. urticae*. Long longevity of *N. cucumeris*'s males has already been recorded when it was fed with almond pollen (Yazdanpanah *et al.* 2021), date and castor bean pollen (Yazdanpanah *et al.* 2022a), and *T. urticae* (Yazdanpanah *et al.* 2022b). In addition, other phytoseiid mites such as *Amblyseius swirskii* (Athias-Henriot) reared on

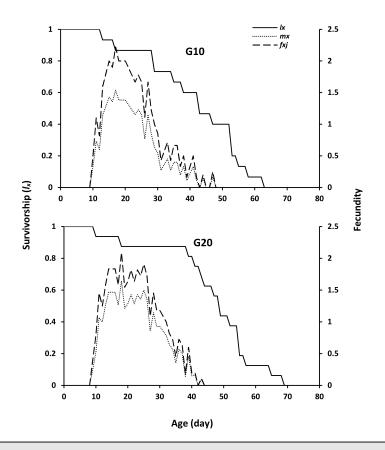


Figure 4 Age-specific survivorship (l_x) , age-specific fecundity (m_x) , and age-stage specific fecundity (f_{xj}) of *Neoseiulus cucumeris* when fed on saffron pollen for 10 and 20 generations (G10, G20) then switched to *Tetranychus urticae*.

almond pollen (Ansari-Shiri *et al.* 2022) and cattail pollen (Hadadi *et al.* 2022), and *Neoseiulus californicus* (McGregor) fed on pollen of blooming wild almond, walnut, saffron, pomegranate as well as *T. urticae* (Eini *et al.* 2022) had longer male longevity than females. It seems that wasting energy for oviposition prosses caused shorter longevity for the females compared with the males.

Comparing two *Neoseiulus* species namely *N. californicus* and *N. cucumeris* shows that the pre-adult period, oviposition days, total lifespan and fecundity of *N. cucumeris* is longer than *N. californicus* when fed on saffron pollen as a diet for the first time. However, *N. californicus* had shorter TPOP and higher intrinsic rate of increase than *N. cucumeris* by feeding on the same diet (Eini *et al.* 2022). It can be concluded that the saffron pollen is a suitable diet for *Neoseiulus* spp.

Table 6 Body length and width (μ m) (Mean ± SE) of sequential generations (1 to 20) of *Neoseiulus cucumeris* reared on saffron pollen.

Sex	Parameter			Generation				
		G1	G5	G10	G20	F	df	Р
Female	Idiosoma length	$346.75 \pm 2.74b$	362.92±3.10a	369.60±2.01a	370.45±3.31a	8.17	3,30	0.001>
	Idiosoma width	230.38±2.37ab	215.08±4.77ab	202.81±5.73b	231.69±7.35a	4.91	3,30	0.007
Male	Idiosoma length	261.25±4.75b	289.17±2.04a	288.34±1.84a	286.65±2.15a	14.77	3,16	0.001>
	Idiosoma width	156.75±4.75b	167.37±6.62b	190.53±2.56a	182.10±3.20ab	8.53	3,16	0.001

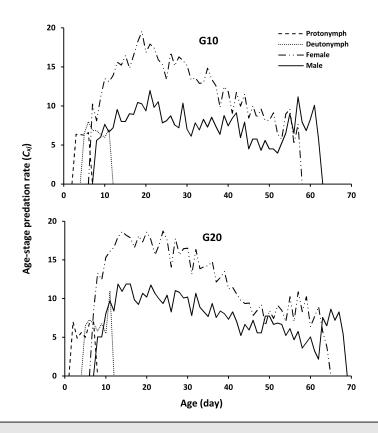


Figure 5 The age-stage specific consumption rate (C_{xy}) of *Neoseiulus cucumeris* when fed on saffron pollen for 10 and 20 generations (G10, G20) then switched to *Tetranychus urticae*.

The quality of alternative or supplementary diets for mass rearing of predators is usually determine by biological parameters (Grenier and De Clercq 2003; Callebaut *et al.* 2004); among them, the intrinsic rate of increase (r) is more important. The maximum r value in the present research in the 1st generation was 0.171 day⁻¹ in which it is higher than the values reported for *N. cucumeris* reared on different pollens ranging from 0.101 to 0.156 day⁻¹ (Ranabhat *et al.* 2014; Yazdanpanah *et al.* 2021; Gravandian *et al.* 2022); on insect egg diets from 0.082 to 0.126 day⁻¹ (Al-Shemmary 2018); on artificial diet (0.090 day⁻¹) (Nguyen *et al.* 2015); and on the natural prey (0131 day⁻¹) (Al-Azzazy *et al.* 2018). As mentioned, in the present study, the maximum r was recorded in the first generation, while *N. cucumeris* fed on almond pollen had the highest r in the fifth generation (0.169 day⁻¹) (Yazdanpanah *et al.* 2021) and in the study on cattail pollen, this predator in the tenth generation had the highest r (0.175 day⁻¹) (Gravandian *et al.* 2022).

Since some factors such as body size and searching efficiency have a direct impact on predation ability of predators and their distribution, we need to measure such factors to reveal the quality of predators (van Lenteren 2003). In our study, the body size of male and female mites increased up to the 5th generation and then remained constant, which means that the predator has not increased its size after being adapted to saffron pollen and has reached its maximum size compared with the predators of the stock culture from Bioplanet compony (G1). In mites of the family Phytoseiidae, females are larger and heavier than males, and sexual dimorphism is evident among these mites (Beard 2001); our results confirmed this size difference between males and females on saffron pollen diet.

Switching *N. cucumeris* mites from saffron pollen to natural prey (*T. urticae*) after 10 and 20 generations showed that the values of the population growth parameters such as *GRR*, R_0 , and

r did not decrease over the generations; N. *cucumeris* after 10 and 20 generations on almond pollen (Yazdanpanah *et al.* 2023) and cattail pollen (Gravandian *et al.* 2022) had similar results. However, when N. *cucumeris* fed on the natural prey, T. *urticae*, the total lifespan decreased significantly; mostly because of decreasing in adult longevity. Since, reproduction and population growth potential of N. *cucumeris* remained constant after switching to T. *urticae*, decreasing adult longevity and subsequently total lifespan may be due to spending a lot of energy by the predator for chasing and attacking the prey and processing animal nutrients.

A predator must maintain the ability to locate, capture and kill prey after a long-term rearing on alternative food (Grenier and De Clercq 2003). Our study revealed that saffron pollen did not affect negatively the predation capacity of *N. cucumeris* after 20 generations. Similarly, *N. cucumeris* maintained its predation potential after 20 generations of rearing on cattail pollen (Gravandian *et al.* 2022), and after 30 generations of feeding on almond pollen (Yazdanpanah *et al.* 2023).

Conclusion

In conclusion, *N. cucumeris* was able to breed successfully for at least 20 consecutive generations and produce fertile offspring when was fed with the saffron pollen. The quality of the individuals produced during these 20 generations was not affected by the diet, and even after switching this predator to *T. urticae*, it was able to feed on the prey and had a high potential of predation. In addition, mass rearing of this predatory mite on pollen would be more cost-effective than using main prey as a food source.

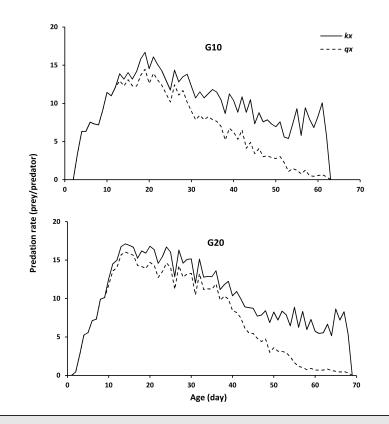


Figure 6 The age-specific predation rate (k_x) , and age-specific net predation rate (q_x) of *Neoseiulus cucumeris* when fed on saffron pollen for 10 and 20 generations (G10, G20) then switched to *Tetrany-chus urticae*.

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