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How enriched diet of the second trophic level (*Tyrophagus putrescentiae*) affects the performance of the third trophic level (*Neoseiulus cucumeris*): the role of pollens and legumes

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Abstract

The generalist predatory mite, *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae) is one of the most effective biocontrol agents to control the pests of many crops in indoor cultivations. In this study, the effects of the enriched diets of the second trophic level, i.e. the stored-product mite, *Tyrophagus putrescentiae* (Schrank) on the performance of *N. cucumeris* as the third trophic level was determined in a tritrophic system. In the first step, different pollens including almond, maize, date palm, castor bean, saffron, and cattail or different legume flours including pinto bean, lentil, black-eyed pea, chickpea, mung bean and broad bean as enrichment additives were added to the basic diet, i.e. a mixture of wheat bran and flour as a basic diet of *T. putrescentiae*. In the second step, to reveal the effects of the mentioned additives on the performance of *N. cucumeris*, the demographic parameters of the predator were determined when it was fed with the prey enriched with the additives. Our results indicated that *N. cucumeris* had higher performance by feeding on the prey reared on diets enriched by either pollens or legumes compared with the basic diet. Overall, there was no significant difference between pollen grains and some legume flours when the predatory mite was fed with them through its prey. Since legumes are more available and cost-effective food sources than pollens, they can be affordable supplementary diets for the mass rearing of *N. cucumeris*.

Introduction

Mass rearing of natural enemies cost-effectively produces a large number of efficient predators in a short time (Nordlund, 1998), which is the foundation of augmentative release in biological control programmes. Since generalist phytoseiid predatory mites are increasingly used in integrated pest management (IPM) strategies for controlling phytophagous mites and insects in greenhouses (Yazdanpanah and Fathipour, 2023*a*) and in the open field (Vidrih *et al.*, 2021), mass-reared predators are needed more than past. The generalist phytoseiids can feed not only on their prey (different mites and insects), but also on non-prey food sources (nectar and pollen), which helps predators to survive in scarcity of prey (Tuovinen and Lindquist, 2010), as well as facilitate their mass-rearing for biocontrol purposes (McMurtry *et al.*, 2013; Yazdanpanah *et al.*, 2021).

Significant positive effects of various pollens on biological parameters of different phytoseiid mites have already been reported. Pollen supplies a great number of food elements that can increase the reproduction of phytoseiids (Sabelis and Van Rijn, 1997; Goleva and Zebitz, 2013). On the other hand, some generalist predatory mites can feed on factitious prey, including stored acarid mites as a solo diet (Yazdanpanah *et al.*, 2022*a*) or as part of a mixed diet (Pirayeshfar *et al.*, 2020; Yazdanpanah and Fathipour, 2023*b*).

The generalist predatory mite, *Neoseiulus cucumeris* (Oudemans) is an efficient biocontrol agent that has a considerable interest in its use because of its wide distribution, high mobility and adaptation to IPM programmes (Ranabhat *et al.*, 2014; Sarwar, 2019). This phytoseiid mite can feed and develop on some pests such as spider mites, whiteflies, broad mites and thrips (Weintraub *et al.*, 2003; Sarwar *et al.*, 2009; Zhang *et al.*, 2011), and on alternative food such as pollen and stored product mite (Yazdanpanah *et al.*, 2021, 2022*a*, 2022*b*). Cost-effective rearing of *N. cucumeris* on more economic food sources such as factitious prey would accelerate the mass production of this predator.

Although pollen grains of almond (Yazdanpanah *et al.*, 2021), cattail (Gravandian *et al.*, 2022), castor bean, date (Yazdanpanah *et al.*, 2022*b*) and saffron (Naqshbandi *et al.*, 2023), as well as the factitious prey *Tyrophagus putrescentiae* (Schrank) (Astigmatidae) (Yazdanpanah *et al.*, 2022*a*) alone, and mixed with these diets (Yazdanpanah and Fathipour, 2023*b*), seem to be promising candidates for mass rearing of *N. cucumeris*, the effects of different pollens through the second trophic level (prey) on the performance of this predatory mite are scarcely known. In addition, the effect of feeding (directly or via the second trophic level) on the other plant-based diets such as flour of legumes as a full protein diet on the biological parameters of *N. cucumeris* is an open question. Therefore, the main purpose of the current study is to determine the effects of the enriched diets of the second trophic level, i.e. *T. putrescentiae* on the performance of *N. cucumeris* as the third trophic level in a tritrophic system. The findings will provide the necessary information for improving our knowledge about optimizing the mass rearing of *N. cucumeris*.

Materials and methods

Pollens

Pollen of castor bean (*Ricinus communis* L., Euphorbiaceae) was collected from the plants grown at the campus of the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran (51° 9′ 52″ N, 35° 44′ 32″ E, 1281 m a.s.l.). The pollen grain of maize (*Zea mays* L., Poaceae) was collected from the fields of Varamin, Tehran province, Central Iran (51° 39′ 57″ N, 35° 18′ 14″ E, 907 m a.s.l.). Pollen grains of almond (*Prunus amygdalus* Batsch, Rosaceae) and date (*Phoenix dactylifera* L., Arecaceae) were collected from trees planted in Shiraz, Fars province (52° 28′ 6″ N, 29° 39′ 43″ E, 1622 m a.s.l.), and in Bandar-Abbas, Hormozgan province, Southern Iran (56° 18′ 10″ N, 27° 11′ 17″ E, 11 m a.s.l.), respectively. Pollen of saffron (*Crocus sativus* L., Iridaceae) was collected from Khorasan Razavi province, Northeastern Iran (58° 21′ 14″ N, 35° 14′ 2″ E, 994 m a.s.l.). Cattail (*Typha latifolia* L., Typhaceae) pollen was collected from Dorud, Lorestan province, Western Iran (49° 3′ 40″ N, 33° 27′ 40″ E, 1500 m a.s.l.). The flower buds of almond and saffron were hand-picked and their pollen grains were removed by brush, while castor bean, maize, date and cattail pollen grains were collected by shaking the flowers on a try. The pollen grains were dried at 30°C for 24 h, and stored by frizzing at −20°C. These pollens were selected because all of them have already been known as suitable and affordable solo diets for *N. cucumeris* according to the findings of the previous works.

Legumes

The seeds of pinto bean (*Phaseolus vulgaris* L.), lentil (*Vicia lens* L.), black-eyed pea (*Vigna unguiculata* L.), chickpea (*Lathyrus aphaca* L.), mung bean (*Vigna radiata* L.) and broad bean (*Vicia faba* L.) all from the family Fabaceae were purchased from the Golestan company in Iran. They were sieved to remove any debris, washed in distilled water, dried at 35°C for 1 day, powdered and stored in refrigerator at 4°C. These legumes were selected because of their availability and reasonable price.

Stock culture of stored product mite, T. putrescentiae

The individuals of *T. putrescentiae* were originally collected from infested Petri dishes containing the fungus *Alternaria* sp. at the Plant Pathology Laboratory of the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. The prey was reared on wheat bran and flour as a basic diet (control). The other diets consisted of the aforementioned pollen grains as well as the flour of legumes, which were added separately to the wheat bran and flour in the ratio of 2 g wheat bran, 0.5 g wheat flour and 0.5 g pollen or legume flour. Each stock culture (Plexiglas container) containing 3 g of materials and prey was covered with a fine textile mesh and placed in an incubator at 27 ± 1°C, 75% ± 5 RH and a photoperiod of 16L:8D h. A mixture of wheat bran + wheat flour + pollen/legume flour was offered as food in 10-day intervals.

Stock culture of the phytoseiid predator, N. cucumeris

The stock culture of *N. cucumeris* was purchased from Bio-Planet, Italy. To establish the laboratory colony of *N. cucumeris*, the individuals were transferred onto a green plastic sheet (16 × 11 × 0.1 cm) on a water-saturated sponge in a Plexiglas container (20 × 13 × 10 cm). All plastic sheet edges were covered using moist tissue paper to provide moisture and prevent predators from escaping (Walzer and Schausberger, 1999). Some cotton fibres were added to the plastic sheet to provide a substrate for oviposition. The stock culture of *N. cucumeris* was maintained in a growth chamber at 25 ± 1°C, 60 ± 5% RH and a photoperiod of 16L:8D h. A mixture of different life stages of *T. putrescentiae* reared on wheat bran and flour (2000–3000 individuals) was offered as food twice a week.

Experimental setup

The experimental units were similar to those used for the predator culture but smaller, which consisted of green plastic sheets (3 × 3 × 0.1 cm), plastic trays (7 × 5 × 4 cm) and wet sponges, and some cotton fibres as shelter and oviposition site. To supply the moisture needed for the mites, the edges of the sheet were covered with moist tissue paper, and water was added daily to prevent the strips from drying out.

To obtain the same-aged eggs of *N. cucumeris*, more than 30 pairs of the predator were selected randomly from the stock culture and kept in a new experimental unit for less than 24 h. The newborn eggs of the predator were transferred to the experimental units. After larval emergence, they were fed individually with the prey reared on different diets. Immature development and survival were recorded daily until they reached adulthood. After adult emergence, the females were coupled with the males of the same treatment. Daily monitoring was continued until the adults' death. All the experiments were conducted at 25 ± 1° C, 60 ± 5% RH and a photoperiod of 16L:8D h. In all replicates (about 40), adequate different stages of the prey were offered once a week.

Data analysis

The values of all the life table parameters, including the net reproductive rate (R_0), gross reproductive rate (GRR), finite rate of increase (λ), intrinsic rate of increase (r) and mean generation time (T), as well as age-stage-specific survival rate (s_{xj}), age-specific survival rate (l_x) (the probability that a newborn will survive to age x, calculated by pooling all of the surviving individuals of different stages), age-stage-specific fecundity (f_{xj}) and age-specific fecundity (m_x), were calculated according to the age-stage, two-sex life table procedure (Chi and Liu, 1985; Chi, 1988) by using the TWOSEX-MSChart software (Chi, 2023). The variances and standard errors of the life table parameters were estimated by the bootstrap procedure (100,000 samples) (Huang and Chi, 2012). The differences in life table bootstrap values among the treatments were determined using the paired bootstrap test (Reddy and Chi, 2015; Bahari *et al*., 2018).

Results

Life table parameters of N. cucumeris *directly fed on different legumes*

The predator, *N. cucumeris* did not feed on the different legume flours, and none of the individuals reached adulthood. The age-stage-specific survival rates (s_{xj}) indicate the initiation and termination of all immature stages (fig. 1). The predatory mite fed on chickpea flour lived more days than other treatments (about 21 days), while the individuals reared on broad bean flour lived less than others (about 11 days).

Life table parameters of N. cucumeris *fed on* T. putrescentiae *reared on different legumes and pollens*

Total pre-adult period of the predator in all treatments was significantly shorter than the basic diet (control), and there was no significant difference between the total immature durations on the pollen treatments. The adult longevity of the predator on the maize and saffron pollens, as well as the total lifespan (from birth to death) on the almond, maize and saffron pollens were significantly longer than the other pollen treatments. However, no significant difference in these parameters was observed among legume flour treatments, except for the diet containing the black-eyed pea flour. The adult longevity, total lifespan and oviposition days of *N. cucumeris* fed on the prey reared on lentil, mung bean, pinto bean and black-eyed pea flours were shorter than the control, and the shortest ones were observed on the treatment of black-eyed pea flour, while the durations of the mentioned parameters in the other tested treatments had no significant difference with the control. The predatory mite that was fed with the prey reared on the black-eyed pea flour had the shortest adult longevity, total life span and oviposition days among all treatments. In all treatments, the TPOP (total preoviposition period) was significantly shorter than the control. The fecundity of *N. cucumeris* in the treatments of broad bean flour, date pollen and maize pollen was significantly more than the control and other legumes tested, while the lowest value of this parameter was observed in the treatments of mung bean flowed by black-eyed pea flours (table 1).

The first adult appeared on day 5 feeding on the prey reared on a supplementary diet of almond and saffron pollen grains, mung bean and chickpea flours. This stage started on day 7 when the predatory mite was fed with *T. putrescentiae* reared on a supplementary diet of pinto bean flour and control diet, and it started on day 6 in other diets. Females fed on the prey reared on a diet of

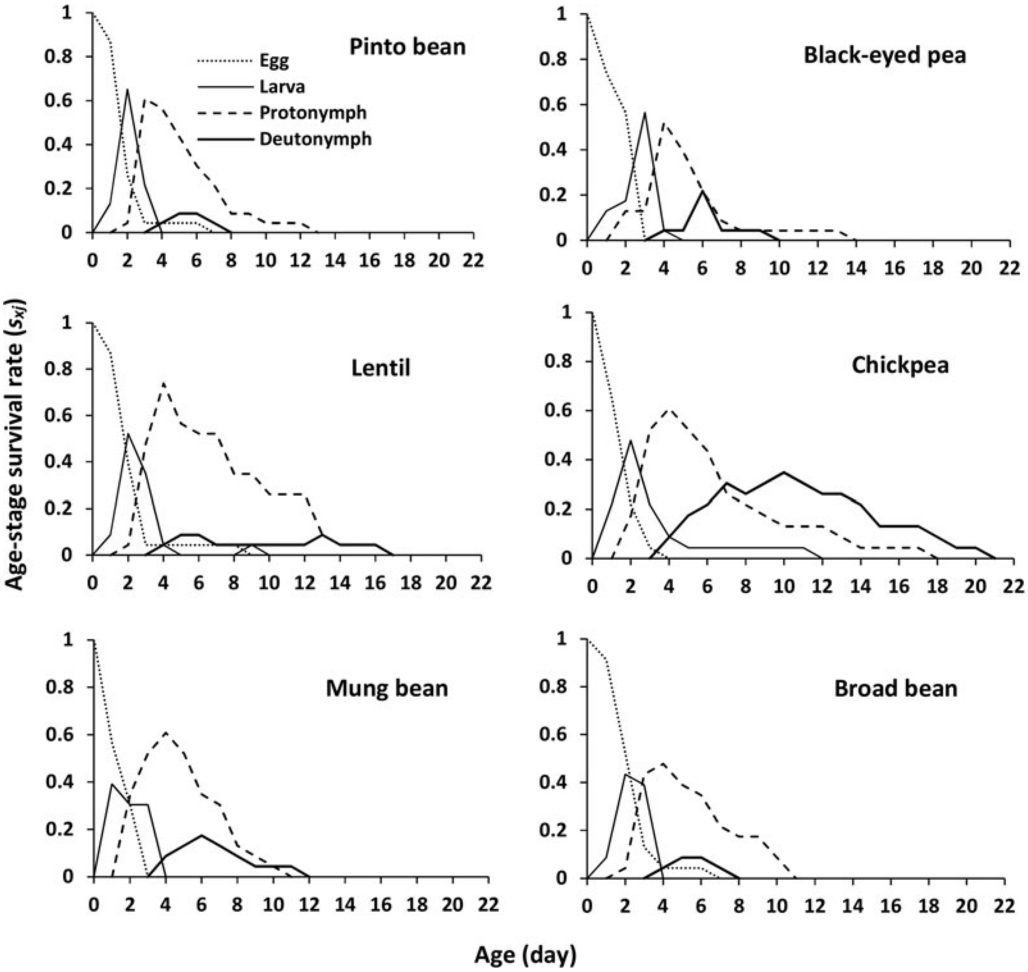

Figure 1. Age-stage survival rate (s_{xj}) of *Neoseiulus cucumeris* directly fed on the flour of pinto bean, lentil, black-eyed pea, chickpea, mung bean and broad bean.

Table 1. Duration of different life stages (days), and fecundity (eggs per female) (mean ± SE) of *Neoseiulus cucumeris* fed on *Tyrophagus putrescentiae* reared on the pollen of date, castor bean, almond, cattail, maize and saffron, and the flour of pinto bean, lentil, black-eyed pea, chickpea, mung bean and broad bean

Diet/parameter	Total pre-adult (day)	Adult longevity (day)	Total life span (day)	APOP (day)	TPOP (day)	Oviposition days	Fecundity (eggs per female)
Castor bean pollen	7.41 ± 0.150bc	39.31 ± 4.033bcd	46.72 ± 4.072bcd	2.17 ± 0.119d	9.50 ± 0.228cdef	19.40 ± 1.619b	34.69 ± 2.934abc
Almond pollen	7.44 ± 0.306bcde	47.75 ± 4.139ab	55.19 ± 4.090a	2.08 ± 0.082d	9.42 ± 0.427defg	18.91 ± 1.484b	33.46 ± 3.706abcd
Cattail pollen	7.05 ± 0.198bcde	41.51 ± 4.955bcd	48.55 ± 5.039bcd	2.67 ± 0.220bc	9.67 ± 0.278cdef	16.66 ± 2.781b	27.38 ± 5.180bcde
Date pollen	7.26 ± 0.186bcde	39.52 ± 1.427b	46.78 ± 1.391bc	2.08 ± 0.082d	9.50 ± 0.394cdefg	23.66 ± 1.246a	41.06 ± 3.916a
Maize pollen	7.48 ± 0.125b	56.71 ± 3.850a	64.20 ± 3.849a	2.94 ± 0.148b	10.44 ± 0.198b	21.67 ± 1.389ab	41.34 ± 2.727a
Saffron pollen	7.07 ± 0.181bcde	58.50 ± 4.813a	65.58 ± 4.808a	2.25 ± 0.128cd	9.17 ± 0.237fg	19.50 ± 1.785b	32.84 ± 4.854abcde
Pinto bean flour	7.16 ± 0.066c	28.49 ± 4.479de	35.65 ± 4.489de	2.28 ± 0.134cd	9.44 ± 0.164def	11.67 ± 0.935de	23.12 ± 1.866e
Lentil flour	7.41 ± 0.218bcd	27.27 ± 4.156e	34.67 ± 4.175e	2.24 ± 0.158cd	9.53 ± 0.317cdef	12.82 ± 1.549de	25.24 ± 3.025de
Black-eyed pea flour	7.00 ± 0.000de	11.06 ± 0.355f	18.06 ± 0.355f	4.00 ± 0.759ab	11.00 ± 0.759bcd	2.00 ± 0.000f	2.47 ± 0.908g
Chickpea flour	7.00 ± 0.170cde	35.09 ± 5.117bcde	42.09 ± 5.130cde	2.15 ± 0.102d	9.23 ± 0.318fg	14.69 ± 1.308cd	30.23 ± 3.039cd
Mung bean flour	6.79 ± 0.171e	23.73 ± 4.122e	30.51 ± 4.200e	2.33 ± 0.103cd	8.86 ± 0.123g	9.18 ± 1.691e	15.51 ± 3.119f
Broad bean flour	7.28 ± 0.200bcde	32.04 ± 2.247cde	39.32 ± 2.249de	2.59 ± 0.121bc	9.59 ± 0.304cdef	21.12 ± 1.413ab	38.82 ± 2.780ab
Basic diet (control)	10.71 ± 0.409a	43.90 ± 5.874abc	54.62 ± 5.719abc	4.00 ± 0.287a	14.60 ± 0.540a	19.53 ± 2.730abc	27.27 ± 3.872cde

APOP, adult pre-ovipositional period; TPOP, total pre-ovipositional period (from egg to first oviposition).
The means followed by different letters in the same column are significantly different ($P < 0.05$, paired-bootstrap).

saffron pollen lived more days, with the last female dying after 110 days, while males fed on the prey reared on lentil flour, mung bean, chickpea, pinto bean and black-eyed pea, and the pollen of cattail and almond lived more days compared with the females (fig. 2). Based on the fecundity curves, the highest daily fecundity was observed in the diets, which consisted of flours of chickpea and pinto bean (2.38 eggs per female) at the age of 12 and 13 days, respectively, while the lowest was in the treatments of mung bean and control (1.66 and 1. 9 eggs per female, respectively) (fig. 3).

The lowest values of *GRR*, R_0, r and λ were observed in the black-eyed pea treatment. There was no significant difference between all treatments and control in terms of R_0 except black-eyed pea. The values of the most important parameters r and λ in all treatments except black-eyed pea (as mentioned above) were significantly higher than the values of these parameters in the control. In all treatments, mean generation time (T) was significantly shorter than the control (table 2).

Discussion

Alternative prey has already been used to develop successful rearing systems for phytoseiid mites. Diets for mass-rearing process could be expanded to include a much wider range of food sources by combining specific nutrients. A predator's performance is generally affected by the nutritional quality of prey, which is primarily influenced by the prey's diet. The enriched basic diet of factitious prey resulted in increasing the fecundity of phytoseiid mites. For example, the larval stages of the prey mite, *T. putrescentiae*, reared on a protein-rich and fat-rich diet (dog food) resulted in a high oviposition rate of *A. swirskii* (Pirayeshfar *et al.*, 2020). Since some pollen have already been recorded as promising diets for long-term rearing of *N. cucumeris* (Gravandian *et al.*, 2022; Yazdanpanah *et al.*, 2022*a*, 2022*b*; Naqshbandi *et al.*, 2023), in the current study, the effects of enriched rearing diet of *T. putrescentiae* as its alternative prey by different high-quality pollen grains and legume flours were determined on the performance of *N. cucumeris* in a tri-trophic system.

Legumes are the third largest family of angiosperms that belong to Fabaceae/Leguminosae (Gepts *et al.*, 2005), which provide a range of essential nutrients including protein, low glycaemic index carbohydrates, dietary fibre, minerals and vitamins (Kouris-Blazos and Belski, 2016). Although most species of stored-product insects are unable to develop in the legume seeds (Sinha and Watters, 1985) because of containing a wide range of toxic compounds (Hou and Fields, 2003), the stored-product mite *T. putrescentiae* was successfully reared on different legume flours. However, we found that these flours as solo diets are not suitable food sources for *N. cucumeris* due to long pre-adult duration, high immature mortality and lack of fecundity that can be related to antifeedant factors, and the imbalance in the specific nutrients needed for immature development. On the other hand, the feeding ability of phytoseiid mites depends on matching their mouthpart morphology with food morphology, and digestive metabolism in the mites, which requires further investigation (Flechtmann and McMurtry, 1992).

Based on the current results, total pre-adult period in all treatments was significantly shorter than the control. Short pre-adult period is a good feature for biocontrol agents by leading to shortening generation duration and consequently increasing the population growth potential of the predator. From a biocontrol point of view, predation rate and ultimately control effect on the pest may

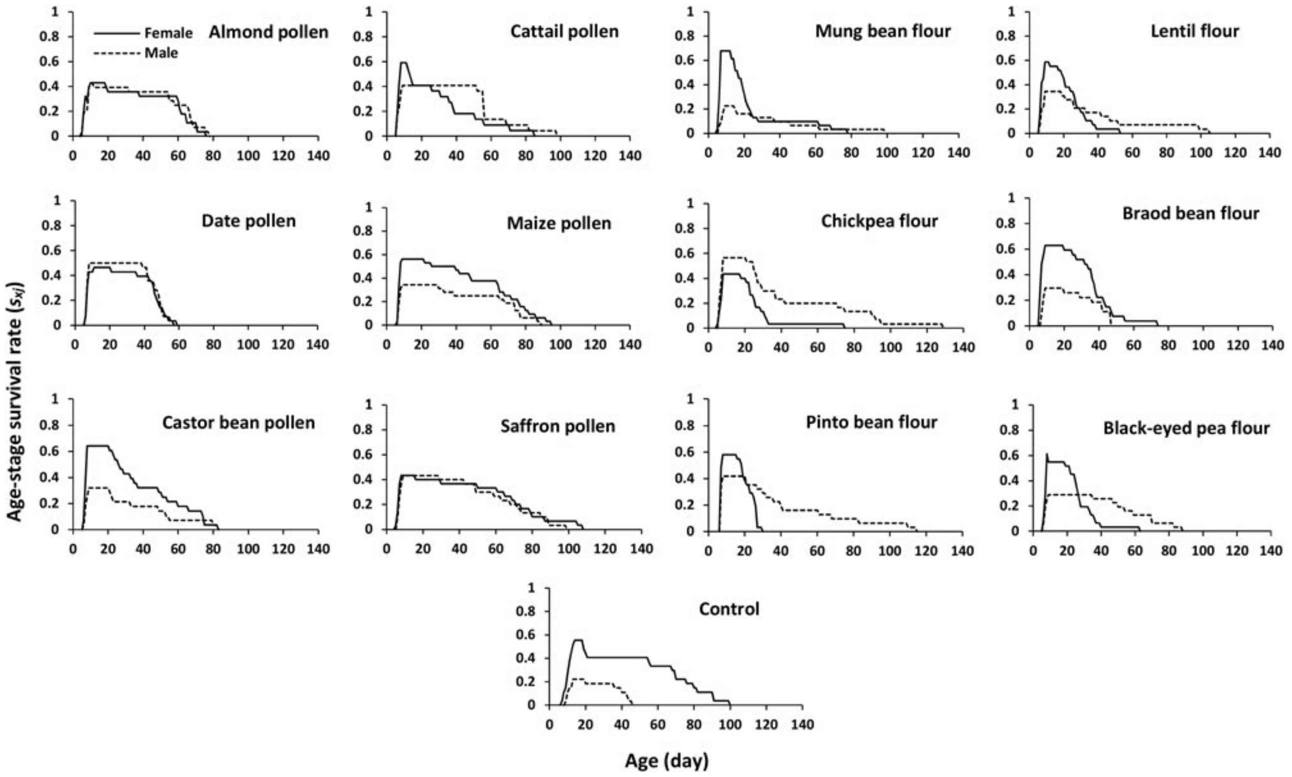

Figure 2. Age-stage survival rate (s_{xj}) of *Neoseiulus cucumeris* fed on *Tyrophagus putrescentiae* reared on the pollen of date, castor bean, almond, cattail, maize and saffron, and the flour of pinto bean, lentil, black-eyed pea, chick pea, mung bean and broad bean.

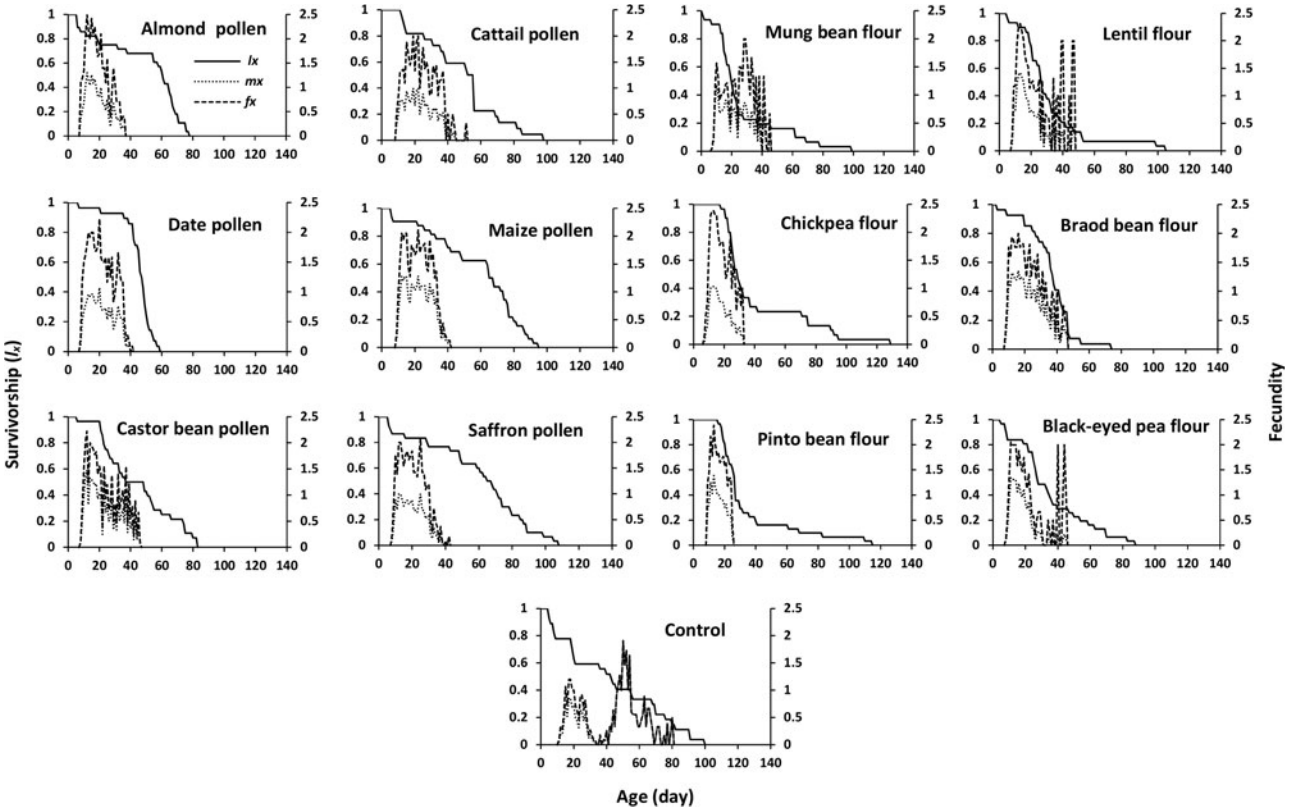

Figure 3. Age-specific survivorship (l_x), age-specific fecundity (m_x) and age-stage-specific fecundity (f_{xj}) of *Neoseiulus cucumeris* fed on *Tyrophagus putrescentiae* reared on the pollen of date, castor bean, almond, cattail, maize and saffron, and the flour of pinto bean, lentil, black-eyed pea, chick pea, mung bean and broad bean.

Table 2. Population growth (life table) parameters (mean ± SE) of *Neoseiulus cucumeris* fed on *Tyrophagus putrescentiae* reared on the pollen of date, castor bean, almond, cattail, maize and saffron, and the flour of pinto bean, lentil, black-eyed pea, chickpea, mung bean and broad bean

Diet/Parameter	*GRR* (eggs per individual)	R_0 (eggs per individuals)	*r* (day^{-1})	λ (day^{-1})	*T* (day)
Castor bean pollen	28.15 ± 4.335ab	22.31 ± 3.650ab	0.178 ± 0.011a	1.195 ± 0.013a	17.40 ± 0.473cd
Almond pollen	19.62 ± 4.239bc	15.55 ± 3.587ac	0.163 ± 0.017a	1.177 ± 0.020a	16.72 ± 0.534de
Cattail pollen	20.02 ± 5.154bc	16.18 ± 4.167ac	0.144 ± 0.015a	1.155 ± 0.018a	19.06 ± 0.728bc
Date pollen	20.15 ± 4.483bc	19.05 ± 4.268ac	0.160 ± 0.015a	1.174 ± 0.018a	18.28 ± 0.526bc
Maize pollen	26.76 ± 4.187ab	23.25 ± 3.917a	0.165 ± 0.011a	1.179 ± 0.012a	19.02 ± 0.430b
Saffron pollen	18.37 ± 4.233bc	15.35 ± 3.724ac	0.153 ± 0.016a	1.165 ± 0.019a	17.73 ± 0.537bcd
Pinto bean flour	14.72 ± 2.606c	13.42 ± 2.313c	0.171 ± 0.012a	1.187 ± 0.014a	15.07 ± 0.222f
Lentil flour	20.86 ± 5.119abc	14.80 ± 2.908ac	0.171 ± 0.013a	1.187 ± 0.016a	15.61 ± 0.402ef
Black-eyed pea flour	1.70 ± 0.737d	0.347 ± 0.185d	−0.092 ± 0.040c	0.913 ± 0.037c	13.08 ± 0.770g
Chickpea flour	14.57 ± 3.611c	13.11 ± 3.022bc	0.162 ± 0.016a	1.176 ± 0.019a	15.71 ± 0.421ef
Mung bean flour	21.91 ± 6.619abc	10.51 ± 2.461c	0.152 ± 0.012a	1.164 ± 0.014a	15.33 ± 0.896efg
Broad bean flour	30.58 ± 4.775ab	24.44 ± 4.000a	0.180 ± 0.011a	1.197 ± 0.013a	17.73 ± 0.499bcd
Basic diet (control)	32.82 ± 3.377a	15.16 ± 3.361ac	0.096 ± 0.010b	1.100 ± 0.011b	28.24 ± 1.388a

The means followed by different letters in the same column are significantly different ($P < 0.05$, paired-bootstrap).

increase with quick emergence of the adults that have higher predation rate than immatures. Therefore, the diets enriched by pollen or legume flours are more favourable foods for pre-adult stages of *N. cucumeris*. On the other hand, the results showed no significant difference in the total immature durations across the pollen treatments. Although it has been the shortest pre-adult period for this predator when it was fed with castor bean or date palm pollen directly (Yazdanpanah *et al.*, 2021), it seems that their tri-trophic effects via the second trophic level (*T. putrescentiae*) were different.

Based on our findings, the total lifespan of *N. cucumeris* on the prey that was fed with almond, maize and saffron pollens was significantly longer than the other pollen treatments; it seems that the tri-trophic effects of both almond and maize pollens on the lifespan of the predatory mite were better than when it was fed with them directly (Yazdanpanah *et al.*, 2021). Since the adult longevity, total lifespan and oviposition days of *N. cucumeris* fed on the prey reared on lentil, mung bean, pinto bean and black-eyed pea flours were shorter than the control, it can be concluded that protein alone cannot be a key indicator to reveal the diet quality for *N. cucumeris*. In other words, the quality of prey as a diet depends on other essential nutrients, which enhance survival and reproduction in *N. cucumeris*.

The higher fecundity of *N. cucumeris* in treatments of date and maize pollen compared with other treatments and control showed that although legumes are rich in protein content, which is required for reproduction in the phytoseiid mites (Lundgren, 2009), pollen grains had higher nutritional value because they have sterols and lipids in addition to proteins and carbohydrates that make them more nutritious for the predator's reproduction (Sarwar, 2016). It seems that the predatory mite in addition to high protein sources needs a combination of different types of micro and macro elements for more fecundity, which was provided by pollen grains. Pollens provide important food elements, including proteins, free amino acids, carbohydrates, lipids, vitamins, flavonoids and minerals. In addition, pollens from different plant species may differ in their nutritional value for the predatory mites; however, phytoseiid mite species differ in ability to utilise pollen.

The quality of alternative or supplementary diets for mass rearing of predators is usually determined by their biological parameters (Callebaut *et al.*, 2004). Since the fecundity, development and survival rate are reflected in the intrinsic rate of increase (*r*), this parameter can be used as a reliable criterion to indicate the suitability of diets used for rearing of the phytoseiid generalist predators. According to demographic theory, when *r* is greater than zero, the food is suitable for population growth (Chen *et al.*, 2017). The results showed that, *r* values of *N. cucumeris* on almost all diets were higher than the basic diet (control), which indicated that the prey reared on enriched diets was more suitable than that reared on the basic diet for the performance of the predatory mite. Accordingly, it has been reported that the reproduction of *Amblyseius swirskii* Athias-Henriot was influenced by both the stage and the food substrate used for the rearing of *T. putrescentiae*, and the predatory mite's potential was higher when it was fed with the prey reared on the enriched diet (Pirayeshfar *et al.*, 2020). By contrast, when the predatory mite *Neoseiulus pseudolongispinosus* (Xin, Liang & Ke) was fed with *T. putrescentiae* reared on the basic diet (wheat flour), the performance of the predator was higher than that was fed with the prey reared on the diet enriched by soybean flour or maize pollen (Sarwar *et al.*, 2010).

The range of *r* value in the present study was from 0.144 to 0.180 day^{-1} (except the treatments of control and black-eyed pea) by feeding on pollen grains and legume flours via the second trophic level, while the value of this parameter has been reported to be lower than our findings when *N. cucumeris* was fed on almond pollen (0.129 day^{-1}) (Yazdanpanah *et al.*, 2021) or cattail pollen (0.120 day^{-1}) (Gravandian *et al.*, 2022) directly. It seems that at least the above-mentioned pollen grains are more suitable diets when they are offered to the predator via the second trophic level, i.e. *T. putrescentiae*. However, *N. cucumeris* reared on a diet combination of *T. putrescentiae* and pollen including castor bean ($r = 0.203$ day^{-1}), date ($r = 0.159$ day^{-1}), almond ($r = 0.181$ day^{-1}), cattail ($r = 0.147$ day^{-1}) and saffron ($r = 0.170$ day^{-1}) showed population growth potential similar to our findings (Yazdanpanah and Fathipour, 2023*b*). It should be noted that in these experiments, the predatory mite had the chance to feed on pollen directly or through its prey or both.

The performance of *N. cucumeris* was enhanced when it was fed with *T. putrescentiae* reared on the diets enriched by either pollen or legume flours. The results indicated that there was no significant difference between pollen grains and some of the legume flours when the predatory mite was fed with them through its prey. Since legumes are more readily available and cost-effective food sources than pollens, they can be considered as an affordable supplementary diet for the mass rearing of *N. cucumeris*.

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