



Functional Response and Predation Interference of *Neoseiulus californicus* (Acari: Phytoseiidae) on *Tetranychus urticae* Nymphs: Influence of Plant Pollens as Supplemental Food Sources

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Abstract

Pollens provide important nutrients and help in the reproduction process for many predators. *Neoseiulus californicus* (McGregor) is one of the important and useful predators of the two-spotted spider mite. The response of three-day-old mated *N. californicus* female predatory mite fed by pollens (marshmallow, sunflower and tamarix) to different densities (۲, ۴, ۸, ۱۶, ۳۲, ۴۰ and ۶۴ numbers) of the prey (the second instar nymphs of two-spotted spider mite) and interference in different densities (۱, ۲, ۴, ۸, and ۱۶ numbers) of the predator and a fixed prey density (۴۰ number) were studied in ۱۰ replications on bean leaf discs. The experiment conditions included a temperature of 27 ± 1 °C, relative humidity of ۷۰%, and a photoperiod of ۱۶:۸ h (light: darkness). The type of functional response and its parameters were calculated using logistic and non-linear regression. According to the results of the logistic regression, the functional response was type II. The attack coefficient and handling time were calculated on marshmallow pollen (۰.۴۳ per h and ۴.۰۱ h), tamarix pollen (۰.۴۳ per h and ۴.۰۳ h) and sunflower pollen (۰.۴۲ per h and ۴.۰۲ h). The predatory ability in predation of the second instar nymph of the two-spotted spider mite on marshmallow and tamarix pollen was greater than that of sunflower pollen. Considering that the nutritional value of plant pollens is different, the relationship between the logarithm of predator density and the per capita attack coefficient was significant in the marshmallow and tamarix pollens. The predation rate of *N. californicus* increased with increasing density of the two-spotted spider mite. There was a significant relationship between the logarithm of predator density and the logarithm of per capita attack coefficient (calculated by the Nicholson equation). The predatory rate of *N. californicus* increases with increasing density of two-spotted spider mite nymphs and decreasing predator density. The predatory mite *N. californicus* reared on the mentioned plant pollens greatly controlled the two-spotted spider mite.

Keywords: Attack coefficient, marshmallow, tamarix, sunflower pollen, predator efficiency

Introduction

Pests cause significant losses to crops every year, so protecting these plants is necessary to improve food security and meet the food demand of the growing human population (Rodrigues et al. ۲۰۲۳). One common method of reducing these losses in different countries, especially developing countries like Iran, is the use of chemical pesticides. Only ۱% of pesticides used on crops reach the target pests and ۹۹% of these chemicals enter the environment and destroy non-target natural enemies (Fathipour and Maleknia ۲۰۱۶). The continuous and incorrect use of pesticides has caused the emergence of pests resistant to them, environmental pollution, and threats to human health (Rodrigues et al. ۲۰۲۳). Therefore, environmentally friendly pest management programs are a suitable strategy for food safety, fighting against pests, and reducing the harmful effects of chemical pesticides (Fathipour and Maleknia ۲۰۱۶). Biological control is an alternative to reduce pest resistance to pesticides and preserve natural resources and human health, and a useful tool for the sustainable development of the agricultural sector. By using parasitoids, predators, pathogens, antagonists, or competing microorganisms, this method reduces pest frequency and damage caused to plants by pests (Hajek and Eilenberg ۲۰۱۸).

Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a herbivorous and polyphagous pest and feeds on more than ۱۱۰۰ plant species, of which more than ۱۰۰ are important crops (Wakgari and Yigezu ۲۰۱۸). This pest has become a serious problem in greenhouse crops, because the widespread and continuous use of chemical acaricides has eliminated many natural enemies of mites. Also, this mite quickly becomes resistant to all kinds of acaricides due to its high reproduction rate and short generation length, even with a low frequency of spraying poison (Jeon and Lee ۲۰۱۴). Therefore, the use of biological control with the mass production of predatory mites has become a necessity to reduce the negative effects of chemical acaricides on the environment and decrease the population of two-spotted spider mites. Predatory mites belonging to the Phytoseiidae family are one of the most important natural enemies of this mite. Predatory mites are relatively small, do not spread over a wide area, and are characterized by some features such as easy mass rearing, the ability to control different types of pests, and the ability to release them mechanically (Momen et al. ۲۰۲۰). Predatory mite *Neoseiulus californicus* (McGregor) belongs to the Phytoseiidae family and is native to the Mediterranean region. This predator is a successful species in controlling mites in fields and greenhouses, which feeds on spider mites of Tetranychidae and Tarsonemidae families (Castagnoli ۱۹۹۹). In many studies, spider mites have been used as natural prey for rearing predatory mites, such as *N. californicus* around the world (Elmoghazy et al. ۲۰۱۱; Riahi et al. ۲۰۱۷). However, many predatory mites can feed on pollen, nectar, and artificial diets (Momen et al. ۲۰۲۰).

Plant pollens contain important nutritional compounds, such as proteins, carbohydrates, vitamins, amino acids, fats, flavonoids, and mineral elements, and are a natural, simple, and accessible food source for rearing predatory mites. Also, pollens of diverse plant species may be different in terms of nutritional value for predatory mites and therefore have different effects on life table parameters (Goleva and Zebitz ۲۰۱۳). The survival and reproduction of predatory mites belonging to the phytoseiid family increases by feeding on plant pollen. When these predators are faced with a lack of natural prey, they increase reproduction by feeding on pollen and therefore avoid the cannibalism phenomenon (Momen et al. ۲۰۲۰). Studying pollen's effect on the functional response of the predatory mite *Neoseiulus cucumeris* towards its prey (two-spotted spider mite) showed that pollen decreased the predatory rate and had a negative and significant effect on the attack speed and the time to reach the prey. Also, this predatory mite was able to be an efficient predator of the two-spotted spider mite, even when pollen was available (Li and Zhang ۲۰۲۰). Pollens of several plant species (e.g., almond, castor bean, date palm, maize, bitter orange, sunflower, and mixed bee pollen) can be used as a supplementary food source for the predatory mite *N. californicus* (Marafeli et al. ۲۰۱۴; Khanamani et al. ۲۰۱۷a).

Evaluating the efficiency of predatory mites as natural enemies is essential in the successful management of biological control. For this purpose, investigating the functional response (the effect of changes in prey density on predatory rate) and interference (the effect of changes in predatory density on its searching power) provides us with useful information about the predator's ability to control the prey population. Also, it is necessary to maintain the predator's potential to find and kill natural prey after long-term rearing on unnatural prey (Grenier and De Clercq ۲۰۰۳). In many researches, the functional response and interference of *N. californicus* mites have been studied, which show the relationship between predatory individuals with different prey densities and are necessary for a successful biological control program (Reis et al. ۲۰۰۳; Farazmand et al. ۲۰۱۲). Therefore, the functional response of reared predatory mite *N. californicus* in several plant pollens separately with feeding on *T. urticae*, and the interference of this predator in different densities and its effect on the predation rate of prey (the second instar nymphs of two-spotted spider mite) were investigated in this study.

Materials and Methods

Collecting Plant Pollen

Pollen from common mallow (*Malva sylvestris*), Tamarix (*Tamarix aphylla*), sunflower (*Helianthus annuus*) and river red gum (*Eucalyptus camaldulensis*) were collected from their natural habitats in Sistan and Baluchestan Province, Iran. Common mallow and sunflower pollens were gathered from flowering plants in rangelands and agricultural margins. Tamarix pollen was collected from mature trees in arid regions near Zabol, while eucalyptus pollen was harvested from urban and semi-urban plantations in Zahedan. The cleaned pollen was oven-dried at 37°C for 48 hours to reduce moisture content and prevent microbial contamination. For long-term storage, the pollen was frozen at -20°C. For short-term use, pollen was stored in airtight containers at 4°C for up to 2 weeks during the experiments. All pollen samples were labelled with species names and collection dates to ensure traceability.

Rearing Two-Spotted Mite:

Two-spotted spider mites, *Tetranychus urticae* Koch, were used as natural prey (control treatment) to feed the predatory mite. The specimens of *T. urticae* used in this study originated from an infested greenhouse at the Faculty of Agriculture, University of Zabol, Zabol, Iran. The mites were reared in the laboratory on detached bean leaves (*Phaseolus vulgaris* L. var. Khazar) placed on wet cotton wool in plastic containers. The containers were maintained in a growth chamber at 20 ± 2°C with a 16:8 (L: D) photoperiod and 60 ± 5% RH. Fresh bean leaves were provided three times per week to ensure a continuous and stable supply of healthy mites for experimental purposes.

Rearing of predatory mite

The initial colonies of *N. californicus* were obtained from Koppert Biological Systems and then reared on kidney bean leaves previously infested with *T. urticae*. The stock culture of *N. californicus* was maintained in a climate room at 25 ± 1°C, 70 ± 5% RH, and a photoperiod of 16:8 h (L:D). Laboratory colonies were transferred into rearing arenas (18 × 13 × 0.1 cm) and placed on water-saturated sponges inside plexiglass boxes (20 × 18 × 10 cm) that were half-filled with water. The edges of the arenas were covered with moist tissue paper to provide humidity and prevent predators from escaping. Bean leaves infested with *T. urticae* were added to the arena three times a week to ensure a continuous food supply (Hatherly and Walters 2005).

Experimental unit:

Leaf discs were prepared according the methods described by Kostianen and Hoy (1994) with minor modifications. Fresh bean leaf discs were cut into sections of 1.5 × 1.5 cm and placed upside down in Petri dishes (7 cm in diameter). A 9-mm diameter hole was drilled in the centre of each dish. This prevented the mites from leaving the arenas and drowning. Cotton threads, arranged in circular barriers with a thickness of 0.2 cm to avoid predators from escaping and maintain humidity. The prepared Petri dishes were placed inside larger dishes with a diameter of 8 cm. Water was added daily to the larger dishes to keep the leaves fresh. The mixture of pollen and eggs was replaced daily during the mites' immature development and adult stages. All Petri dishes were kept at 20 ± 1°C, 75 ± 5% humidity, and a 16/8 light-dark cycle in a growth chamber

Functional response test

Leaf discs were placed in Petri dishes with a diameter of 7 cm 24 h before the experiment. To experiment, 3-day-old whole females of the predatory mite were used, which had previously become the same age on the colony corresponding to each separate pollen. Different numbers (2, 4, 8, 16, 32, 64, and 128) of the second-instar nymphs of two-spotted spider mites and pollen along with one adult predatory mite were released on leaf discs in Petri dishes. The number of hunted nymphs was recorded 24 h later. The predatory rate, the type of functional response, and its statistics were determined. The test was performed in 10 replications.

Data analysis was done based on Juliano's two-step method (Juliano 2001). The data were fitted to a polynomial function (relationship below) to determine the type of functional response.

$$\frac{N_a}{N_t} = \frac{\exp(P + P_1 N_t + P_2 N_t^2 + P_3 N_t^3)}{1 + \exp(P + P_1 N_t + P_2 N_t^2 + P_3 N_t^3)}$$

Statistics P , P_1 , P_2 , and P_3 were performed using SAS software and CATMOD procedure. The type of functional response was determined using the P_1 statistic sign. The positive and negative signs of P_1 statistics (linear part) indicate the type III and the type II of functional response, respectively (Juliano 2001).

After that, functional response statistics (attack coefficient and handling time) were calculated. For this purpose, non-linear regression was used in SAS software. Since the prey density was not constant during the experiment and the eaten

prey was not replaced, the appropriate model used to estimate the functional response statistics was the reduced Rogers model (Rogers ۱۹۷۲).

$$N_a = N \left[1 - \exp\left(\frac{-bN_0 T_h}{1 + bT_h N_0}\right) \right]$$

In this equation, N_a , N_0 , T , and T_h are the number of attacked host or prey, initial host number, exposure time (T h), and handling time, respectively and b is a fixed amount.

The following combined statistics D_b and D_{T_h} were used to compare the handling time and attack coefficient (Juliano ۲۰۰۱).

$$N_a = N \left\{ 1 - \exp\left[-(b + D_b(j))(T - (T_h + D_{T_h}(j))N)\right] \right\}$$

Interference or impact of different predator densities

This test was carried out to investigate the effect of different densities of predatory mites on the per capita attack coefficient and per capita predatory of *N. californicus* on the second instar nymphs of two-spotted spider mites. The densities (۱, ۲, ۴, ۸, and ۱۶ numbers) of predatory mite females were studied against the density of ۴۰ numbers of two-spotted spider mite nymphs (more density than the predatory mite needs) in a period of ۲۴ h (Rezaie et al. ۲۰۱۷). Bean leaves, which are suitable for the life of two-spotted spider mites and predatory mites, were provided to unmated ۳-۴-day-old adult female mites with different densities for ۲۴ h (Rezaie et al. ۲۰۱۷). Then, the number of eaten two-spotted spider mite nymphs was counted and recorded. The experiment was performed in ۲۰ replications. Using the following equation, the per capita attack coefficient (a) of the predator mite was calculated (Nicholson ۱۹۳۳).

$$a = \left(\frac{1}{P}\right) \ln \left[\frac{N_t}{(N_t - N_a)} \right]$$

In this equation, P , N_t , and N_a are the number of predators, the initial density of the host, and the number of eaten preys, respectively. Logarithms were taken from values P and a . The logarithm of the number of predators ($\log P$, as an independent variable) and the logarithm of the per capita attack coefficient ($\log a$, as a dependent variable) were shown on the x-axis and the y-axis, respectively. Linear regression was drawn using them. Then, the slope of the regression line was determined with the following equation (Hassell and Varley ۱۹۶۹).

$$\log a = \log Q - m \log P$$

In this equation, Q and m are search constant and interference coefficient, respectively. If there is a significant relationship between these two logarithms, the negative slope (m) of the regression line indicates that as predator density increases, the per capita attack coefficient decreases (Fathipour et al. ۲۰۰۲). Means were compared with Tukey's test.

Results and discussion

Functional response

The functional response of the predatory mite *N. californicus* on different densities of *T. urticae* second-instar nymphs by feeding pollens (marshmallow, tamarix, and sunflower) is shown in Figure ۱. Fitting the functional response data of the predatory mite on the *T. urticae* second-instar nymph by feeding marshmallow, tamarix, and sunflower pollens showed that in all three pollens, the linear part of the relationship is negative, so the functional response is type II (Table ۱). In this type of functional response, the number of preys eaten per predator increases with increasing initial density, then gradually decreases, and extends parallel to the horizontal asymptote line of the curve. Using the Rogers model, functional response statistics (attack coefficient and handling time) were calculated as described in Table ۲. Accordingly, handling time was longer in predatory mites reared on tamarix pollen (Table ۲). In order to compare the functional response statistics, the amount of D_a and D_{T_h} statistics for the predatory mite fed with pollens (marshmallow, tamarix, and sunflower) was estimated based on the data in Table ۳. Since the amount of D_{T_h} statistic in all pollens shows a significant difference with the zero number, the handling time of the predator mite in hunting the second instar nymph of the two-spotted spider mite

on these pollens had a significant difference.

Table ۱- Logistic regression analysis of the proportion of prey eaten (second-instar nymph of two-spotted spider mite) by the predatory mite *Neoseiulus californicus* (N_e/N_0) fed with different pollens to determine the type of functional response.

Pollen	Coefficient	Estimate	SE	Chi-squared value	P value
Marshmallow	Constant	۲۸.۰۶۷	۰.۵۰۵۴	۳۰.۸۴	<.۰۰۰۱
	Linear	-۰.۰۰۸۰۰	۰.۰۵۳۵	۲.۲۳	۰.۱۳۵۳
	Quadratic	-۰.۰۰۰۴۸	۰.۰۰۱۶۳	۰.۰۹	۰.۷۶۹۰
Tamarix	Constant	۲.۷۸۹۱	۰.۵۳۱۱	۲۸.۶۴	<.۰۰۰۱
	Linear	-۰.۰۴۱۳	۰.۰۵۴۲	۰.۵۸	۰.۴۴۶۲
	Quadratic	-۰.۰۰۱۸۲	۰.۰۰۱۶۳	۱.۲۶	۰.۲۶۲۵
Sunflower	Constant	۱.۷۹۰۳	۰.۳۸۰۲	۲۲.۱۷	<.۰۰۰۱
	Linear	-۰.۰۶۰۱	۰.۰۴۳۶	۱.۹۰	۰.۱۶۸۰
	Quadratic	۰.۰۰۰۰۳۳	۰.۰۰۱۳۷	۰.۰۰	۰.۹۸۱۰

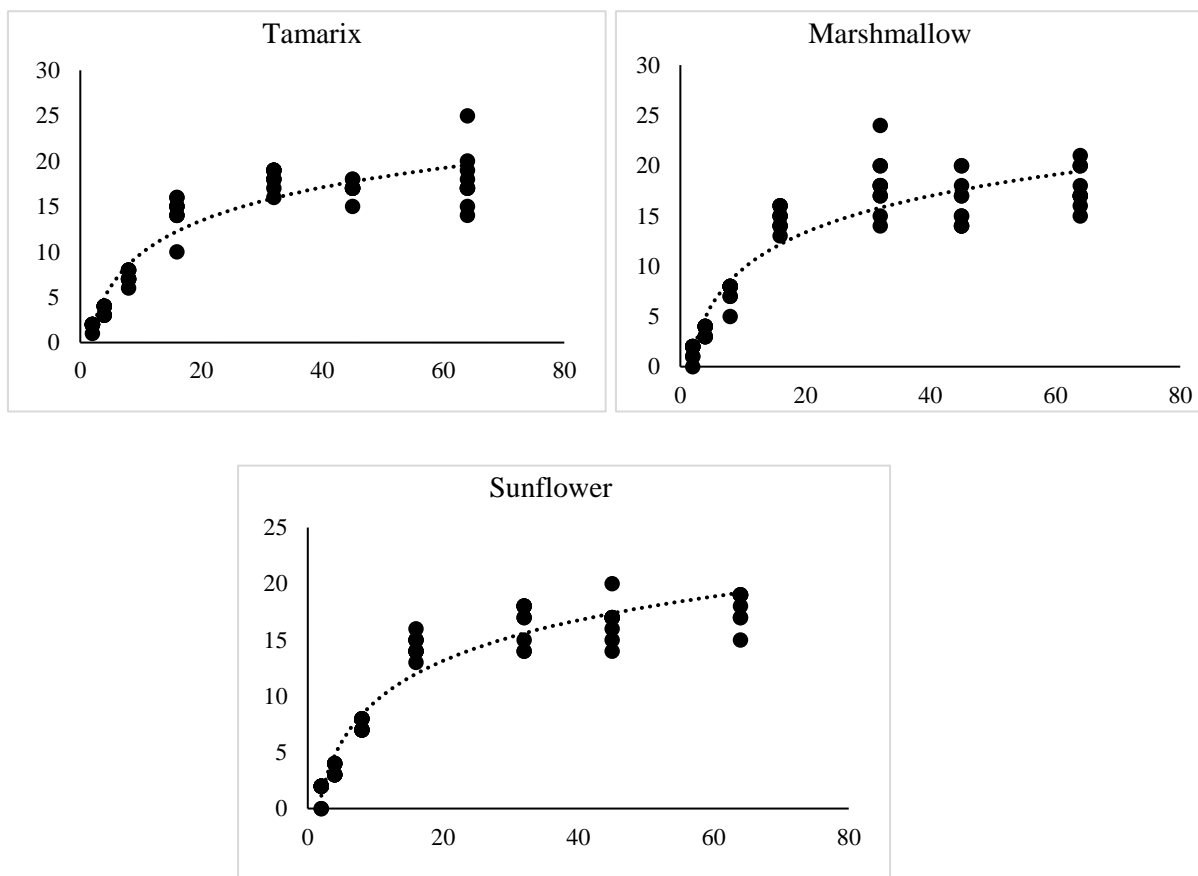


Figure ۱- Functional response (type II) of predatory mite *Neoseiulus californicus* fed with different pollens (marshmallow, tamarix, and sunflower) on different densities of second instar nymphs of two-spotted spider mite.

Table ۲- Estimated values of attack coefficient and handling time by Rogers model for predatory mite *Neoseiulus californicus* on the second instar nymph of two-spotted spider mite in case of feeding on marshmallow, tamarix, and sunflower pollens.

Pollen	Attack coefficient (<i>a</i>) (h ⁻¹)	Handling time (<i>T_h</i>) (h)
	Approximate (lower- upper)	Approximate (lower- upper)
Marshmallow	۰.۴۳±۰.۰۳ (-۰.۱۹-۱.۰۴)	۴.۰۱±۰.۲۷ (۳.۴۸-۴.۵۵)
Tamarix	۰.۴۳±۰.۰۹ (-۰.۷۲-۱.۶۰)	۴.۰۳±۰.۳۲ (۳.۴۱-۴.۶۵)
Sunflower	۰.۴۲±۰.۵۷ (-۰.۷۱-۱.۵۶)	۴.۰۲±۰.۳۱ (۳.۴۱-۴.۶۴)

Table ۳- Calculated values of *D_a*, *D_{th}* statistics to compare the attack coefficient and handling time of predatory mite *Neoseiulus californicus* in case of feeding on marshmallow, tamarix, and sunflower pollens.

Pollen	Coefficient	Estimate	SE	Approximate (۹۵٪)	
				lower	Upper
Sunflower-Tamarix	<i>D_a</i>	۱.۰۹۹۴	-۰.۳۷۰۹	۰.۳۷۱۷	۰.۳۶۴۲
	<i>D_{Th}</i>	۰.۱۰۱۲	-۰.۳۳۱۴	۰.۱۰۹۴	-۰.۱۱۵۱
Sunflower-Marshmallow	<i>D_a</i>	۱.۴۷۶۶	-۰.۷۲۹۶	۰.۵۵۷۸	۰.۳۷۳۵
	<i>D_{Th}</i>	۰.۹۴۷۹	-۰.۹۶۶۴	۰.۴۸۴۰	-۰.۰۰۹۲۶
Marshmallow-Tamarix	<i>D_a</i>	-۰.۰۲۰۵	-۰.۳۵۵۷	۰.۰۸۴۸	-۰.۱۸۸۱
	<i>D_{Th}</i>	-۰.۸۳۶	۰.۴۱۱	-۱.۶۵۰	-۰.۰۲۲

Holling (۱۹۵۹) divided the functional response into three types, including I, II, and III types. The functional response of the type II is the most common type, in which the proportion of killed prey decreases in a parabolic shape with the increase in density. In mites of the Phytoseiidae family, the functional response is usually of the type II. Seiedy et al. (۲۰۱۲) reported that the functional response of the predatory mite *Phytoseiulus persimilis* on the two-spotted spider mite on the leaf disc of cucumber is of the type II. Researchers investigated the functional response of *Amabseius andsoni* and *Neoseiulus neoreticuloides* (Acari: phytoseiidae) to *Aceria pallida* (Acari: Eriophyoidae) and stated that the functional response was the type II and the predation of both species increased with increasing prey density (Xiaotong et al. ۲۰۲۱). The predator, *N. californicus* (a type II generalist) that prefers spider mites to other food sources, showed a type II response to the eggs, larvae, and nymphs of spider mites (Li and Zhang ۲۰۲۰). The functional response of *N. californicus* mite feeding on the eggs and nymphs of the two-spotted spider mite was the type II (Farazmand et al. ۲۰۱۲). Song et al. (۲۰۱۶) reported the type II of functional response of *N. californicus* on *T. urticae* and by providing different densities of prey for the predator, showed that most of the prey was eaten at high densities and the interaction between predator *N. californicus* and prey density was significant. The study of the functional response of *N. californicus* on western flower thrips larvae in different strawberry cultivars showed that this response was the type II (Rezaie et al. ۲۰۱۷). The functional response type of a predator can change with factors, like prey size, prey life stages, host plant species, and other environmental factors, such as temperature (Li and Zhang ۲۰۲۰). The developmental stage of the prey is an effective factor in the access rate of the predator to the prey. The difference in the functional response of predators depends on the defence level of the prey individuals. If the defence level of prey is raised, the handling time of the predator increases, and subsequently the predator efficiency reduces and the amount of prey eaten also decreases (Reis et al. ۲۰۰۳). In the present study, the prey is the second instar nymph of two-spotted spider mites, which is mobile, and the predatory mite may be disturbed in high densities of prey. This can be the reason for the difference in the type of functional response compared to the researches in which the prey is resident (egg stage). On the other hand, Nwilene and Nachman (۱۹۹۶) stated that functional responses in different experiments are comparable when laboratory conditions, temperature, humidity, and the type of prey and predator are constant.

There are few studies on the effect of plant pollen substitute food on the functional response of predatory mites. Plant pollen can affect the functional response of predators due to its different nutritional value. In this research, pollens did not change the type of functional response of *N. californicus*, which was consistent with other studies (Li and Zhang ۲۰۲۰). Badii et al. (۲۰۰۴) indicated that pollen prolonged the handling time, which was partially in line with our study about the tamarix pollen. This does not mean that it would lead to failure in mite control. This may result from the predator's speed and the time spent subduing individual prey, which is an important factor affecting handling time (Hassell and Southwood ۱۹۷۸). According to our results, when prey is scarce, the pollens of marshmallow and tamarix can serve as supplementary food sources to sustain the population of *N. californicus*. It has been documented in some research that supplementary food (e.g., plant pollens) can increase pest control in the long run by enhancing the population of predators with additional nutrients and holding them onto the host plant to prohibit the resurgence of pests (Khanamani et al. ۲۰۱۷b; Hatt and Osawa ۲۰۱۹). The predator *Amblyseius swirskii* Athias-Henriot consumed more prey (*T. urticae*) in the presence of bee pollens than in the presence of almond and date pollens (Riahi et al. ۲۰۱۷). The maximum consumption of predatory mites (*Neoseiulus bicaudus*) was higher when pollen was supplied, with the highest amount obtained in the presence of *Borago officinalis* L. pollen (Han et al. ۲۰۲۴). In this study, the predatory ability to prey the second instar nymph of the two-spotted spider mite on sunflower pollen was low. In this regard, it has been reported that *Typha orientalis* pollen caused a decrease in the predation rate of *N. cucumeris* in the short-term test (Li and Zhang ۲۰۲۰). It has been reported that feeding on pistachio and thorn apple pollen causes the best performance of *N. californicus* and poor growth of this mite in unsuitable plant pollens has been attributed to long and sharp spines or a thick impenetrable exine layer (Eini et al. ۲۰۲۲).

Interference

The results showed that in conditions of feeding from all three pollens, the relationship between the logarithm of the density of female individuals of predatory mites and the logarithm of their per capita search power is significant in the density of ۴ second instar nymphs of two-spotted spider mites. For marshmallow pollen, the regression line relationship is $\log a = ۰.۱۸۷۹ \log P - ۰.۷۰۳۹$ and the correlation coefficient (r^2) is ۰.۲۰۱. For sunflower pollen, the regression line relationship is $\log a = ۰.۰۲۸۲ \log P - ۰.۷۹۸۸$ and the correlation coefficient (r^2) is ۰.۰۰۵۲. For tamarix pollen, the regression line relationship is $\log a = ۰.۰۰۶۳ \log P - ۰.۶۵۱۶$ and the correlation coefficient (r^2) is ۰.۰۰۰۲ (Figure ۲). Considering the negative slope of the regression line, it can be concluded that the average per capita searching efficiency decreases per each predatory mite at higher density of predatory mite. This indicates the presence of interference as a density-dependent factor (Figure ۲). In the conditions of rearing predatory mites by feeding on tamarix pollen, the highest per capita searching efficiency was obtained at a density of ۴ predatory mites (Table ۴). In the conditions of rearing predatory mites by feeding on marshmallow pollen, there was no significant difference between predatory mite densities in terms of per capita searching efficiency (Table ۵). In the conditions of rearing predatory mites by feeding on sunflower pollen, per capita searching efficiency decreased sharply in the predatory mite density of ۱۶ (Table ۶). An increase in the density of predatory mites caused an increase in the total predation rate and a decrease in the predation rate per individual and per capita searching efficiency (Tables ۴, ۵, and ۶). The decrease in per capita predatory at the highest density is clearly evident because, without interference, the predation rate should have been more than this. The obtained results were in line with the results of other researchers who stated that the per capita searching efficiency and predation rate per individual decreases with increasing predatory density (Nachman ۲۰۰۶; Farazmand et al. ۲۰۱۲; Farhadi et al. ۲۰۱۵; Rezaie et al. ۲۰۱۷). When the predatory mite density was at the highest number (۱۶), the predatory mite's per capita searching efficiency in rearing conditions was greater on tamarix pollen than on marshmallow pollen, and in both cases, it was greater than on sunflower pollen (Tables ۴, ۵, and ۶). One factor affecting predatory efficiency is the difference in the density of the prey-predator. The difference in the interference and functional response of the predatory mite feeding on different pollens on the second instar nymph of two-spotted spider mite can be due to secondary chemical compounds. Also, the difference in the functional response of predators depends on the defence level of the prey individuals. If the defence level of prey is raised, the handling time of the predator increases, and subsequently the predator efficiency reduces and the amount of prey eaten also decreases (Reis et al., ۲۰۰۳). One of the important feeding behaviours of predators is interference, which occurs in the interaction between several predators of the same species and causes a decrease in attack coefficient (searching power). When in nature a predator is not alone and in contact with its fellows, this interaction changes the amount of feeding per individual (Hassel and Varly ۱۹۶۹). Li and Zhang (۲۰۲۰), reported that the predation rate decreased at high density and attributed it to the interference of the prey (*T. urticae*) on the predation capacity and the rising satiation of the predator (*N. cucumeris*).

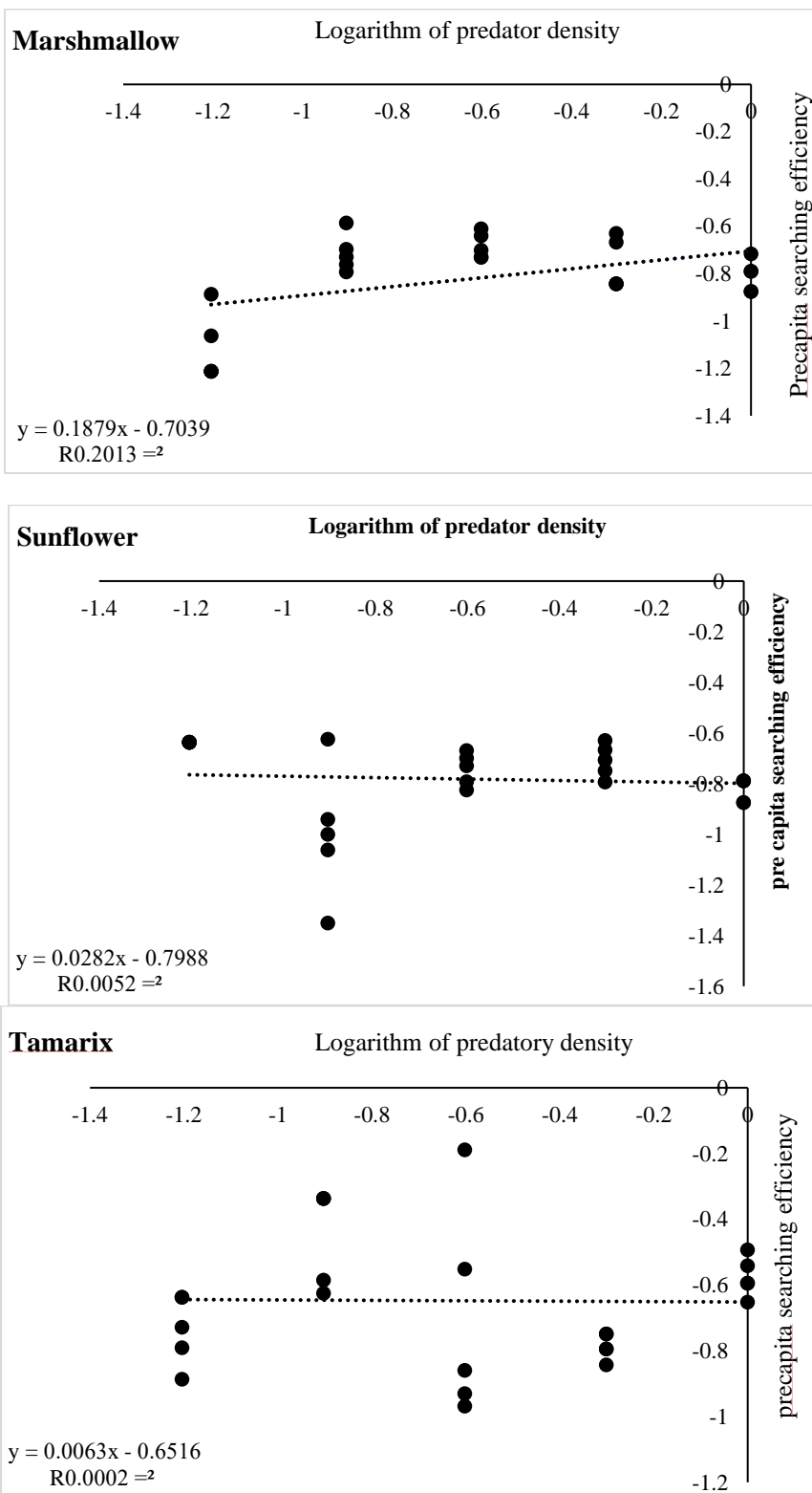


Figure ۲- Regression line between the logarithm of per capita searching efficiency and the logarithm of the density of predatory mite *Neoseiulus californicus* fed with marshmallow, tamarix, and sunflower pollens at a density of ۴۰ preys (second instar nymphs of two-spotted spider mite).

Table 4- Mean (\pm standard error) of total predation rate, predation rate per individual, and per capita searching efficiency of *Neoseiulus californicus* mite reared on tamarix pollen at a density of 4 preys (second instar nymphs of two-spotted spider mite)

Density of predator	Total predation rate	Predation rate per individual	Per capita searching efficiency
1	9.4 \pm 0.5 ^d	9.4 \pm 0.5 ^a	0.3 \pm 0.1 ^c
2	11.2 \pm 0.3 ^c	5.6 \pm 0.1 ^b	0.1 \pm 0.1 ^c
4	22.4 \pm 0.4 ^b	5.6 \pm 1.1 ^{bc}	0.16 \pm 0.1 ^a
8	37.2 \pm 1.1 ^a	4.65 \pm 0.1 ^c	0.10 \pm 0.05 ^b
16	37.6 \pm 0.7 ^a	2.35 \pm 0.05 ^d	0.3 \pm 0.03 ^c

Means within a row followed by same letter were not significantly different based on Tukey test ($\alpha=5\%$).

Table 5- Mean (\pm standard error) of total predation rate, predation rate per individual, and per capita searching efficiency of *Neoseiulus californicus* mite reared on marshmallow pollen at a density of 4 preys (second instar nymphs of two-spotted spider mite)

Density of predator	Total predation rate	Predation rate per individual	Per capita searching efficiency
1	5.8 \pm 0.3 ^c	5.8 \pm 0.3 ^a	0.2 \pm 0.1 ^a
2	11.18 \pm 1.1 ^b	5.9 \pm 0.5 ^a	0.4 \pm 0.03 ^a
4	22.6 \pm 0.8 ^b	5.65 \pm 0.2 ^a	0.2 \pm 0.1 ^a
8	31.4 \pm 1.0 ^a	3.92 \pm 0.12 ^b	0.2 \pm 0.03 ^a
16	28.4 \pm 0.4 ^a	1.75 \pm 0.13 ^c	0.2 \pm 0.1 ^a

Means within a row followed by same letter were not significantly different based on Tukey test ($\alpha=5\%$).

Table 6- Mean (\pm standard error) of total predation rate, predation rate per individual, and per capita searching efficiency of *Neoseiulus californicus* mite reared on sunflower pollen at a density of 4 preys (second instar nymphs of two-spotted spider mite)

Density of predator	Total predation rate	Predation rate per individual	Per capita searching efficiency
1	5.6 \pm 0.24 ^d	5.6 \pm 0.24 ^a	0.1 \pm 0.1 ^a
2	13 \pm 0.7 ^c	6.5 \pm 0.35 ^a	0.2 \pm 0.1 ^a
4	20.6 \pm 0.93 ^b	5.15 \pm 0.23 ^a	0.2 \pm 0.1 ^a
8	22.4 \pm 3.5 ^b	2.8 \pm 0.44 ^b	0.4 \pm 0.03 ^a
16	39 \pm 0 ^a	2.43 \pm 0 ^b	0.1 \pm 0 ^b

Means within a row followed by same letter were not significantly different based on Tukey test ($\alpha=5\%$).

Conclusion

In this study, the functional response of the *N. californicus* on the second instar nymphs of two-spotted spider mite was of the second type. Predation rate per individual and per capita searching efficiency decreased with the increase of predator's density from 1 to 16. At the higher density of predatory mite (16), the highest per capita searching efficiency (the least interference) was observed in the rearing conditions on tamarix pollen. In general, the predatory mite *N. californicus* reared on the mentioned plant pollens had a great ability to control the second instar nymphs of two-spotted spider mite. Therefore, we recommend marshmallow and tamarix pollens to ensure a plentiful population of predatory mites. The obtained results have considerable implications for using the pollens of plants in eco-friendly pest control strategies within agricultural sectors. However, in the current study, functional response and interference were tested only in a laboratory and a closed environment, so more studies are needed in field conditions.

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