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INFLUENCE OF FEEDING WITH DIFFERENT PLANT POLLENS ON PREY- STAGE PREFERENCE AND PREDATION RATE OF *NEOSEIULUS CALIFORNICUS* (MCGREGOR) (ACARI: PHYTOSEIIDAE)

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ABSTRACT

Neoseiulus californicus (Acari: Phytoseiidae) is an effective predator in greenhouses which feeds on plant injurious mites. Searching capacity is one of importance in the effectiveness of this predatory mite. In this research, the population fed with corn pollen, walnut and date pollen and the spider mite (four strains) were investigated. The prey-stage preference of *N. californicus* was studied on different developmental stages of *Tetranychus urticae*. The predation rate of rearing population of *N. californicus* was determined; it was used eggs of *T. urticae*. Experiments were carried out on strawberry disc in Petri dish (6 cm diameter) under laboratory conditions ($27\pm 1^{\circ}\text{C}$ temperature, $70\pm 5\%$ RH and 16L: 8D photoperiod). After 24 hours, the total numbers of prey consumed were counted. The predatory mites reared on different pollens prefer eggs or nymphs to adults and the Preference Index of different strains was not different. Result of consumed of spider mites by female predatory mites indicated the predation rate of the predatory mites fed with corn pollen (9 ± 1.46), walnut pollen (8.19 ± 0.99) and Date pollen (8.28 ± 0.80) did not any significant difference, however when spider mites and the mentioned pollens were used, the predation rate of predatory mites when fed with *T. urticae* (14.74 ± 0.94), Walnut (15.24 ± 1.05) and date pollen (14.17 ± 1.04) show significant difference with the population of corn pollen (10.0 ± 0.95). The predation rate of four strains decreased at present of plant pollens. Use of the predatory mite fed with the different developmental stage of two-spotted spider mite and pollen in the biological control of the pests is useful.

Keywords: *Neoseiulus californicus*, Predation rate, Pollen.

INTRODUCTION

Neoseiulus californicus (McGregor) (Acari: Phytoseiidae) is an important spider mite predator that is active in habitats with high temperature and low humidity (Weintraub and Palevsky, 2008) such as Mediterranean area (De Moraes et al., 2004). This predator can successfully develop and reproduce on *Tetranychus urticae* Koch up to 28 generations per year. *Neoseiulus californicus* prefers tetranychid mites as food, but will also consume other mite species, small insects, such as thrips and even pollen when primary preys are unavailable (McMurtry and Scriven, 1966).

Some of the phytoseiid mites utilize pollens as a food

source, they develop and reproduce on a pollen diet as well (Nomikou et al., 2003). The nutritional value of pollen varies between plant species and thus the characterizes response of phytoseiid mites on different pollens can also be quite variable (Yue and Tsai, 1996). Pollens can be used as a food source for mass rearing or to improve predator efficacy in the field. The possibility of mass-rearing *N. californicus* on alternative foods such as pollens (Castagnoli and Simoni, 1999) increases the interest in this predator as a control agent and has consequently led us to evaluate the effects of nutritional history on its performance. Several studies were reported the influence of pollens on developmental and fecundity to identify indigenous pollens for mass rearing (Argov et al., 2006), for example using 17 pollens to investigate the effect of various alternative food

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substance on the biological parameter of this predator (Ragusa, 1991).

In this research, the predation rate and prey preference of the predatory mite *N. californicus* to eggs of *T. urticae* were studied. Four strains of the phytoseiid mites were compared for long-term feeding effect of the date pollen, walnut pollen and corn pollen and *T. urticae*. The prey preference and predation rate of *N. californicus* were assessed after three generations and compared with those reared on two-spotted spider mite.

MATERIALS AND METHODS

Colony: *Neoseiulus californicus* was obtained from "Koppert Biological Systems" and maintained on leaves of strawberry ("Gaviota" cultivar) which were infested with the spider mites. The stock culture of *N. californicus* was maintained in a growth chamber at 27± 1 °C, 70±5 RH and 16 L: 8 D hours conditions. The tested pollens were collected by hand (walnut pollen from Tabriz, corn pollen from Karaj and date pollen from Bam). Pollens were stored in the refrigerator during the experiments. The spider mites used in each experiment were obtained from a culture with strawberry leaves. The leaflets of strawberry (2×2 cm²) were placed upside down on water-saturated cotton and egg of *T. urticae* and date pollen, walnut pollen and corn pollen. The stock culture of *T. urticae* was maintained on common beans (*Phaseolus vulgaris* L.) in a control room at 27± 1 °C, 70±5% RH and 16 L: 8 D hours conditions.

Prey-stage preference: In this experiment, eggs, newly emerged protonymphs and 3-day-old females of *T. urticae* were used. Same-age eggs were reared separately to obtain 3-day old predatory females. The eggs were obtained from the laboratory population that was reared on different plant pollens, separately. Three-day-old mated females of *N. californicus* were used separately in prey-stage preference experiments. Each experiment had 10 replications. An equal number (20) of *T. urticae* eggs, newly emerged protonymphs and adults were offered to a 3-day-old mated female predator. The prey-stage preference was conducted in leaflets (strawberry leaves) arenas for each of the predatory strains. Leaflets of the strawberry (2×2 cm²) that were placed upside down on water saturated cotton balls. Each strain was tested separately from the others. After 24 hours, the numbers of prey consumed per predator were estimated. Preference Index was estimated based on formula described by Manly (1974).

$$\beta_1 = \frac{\log\left(\frac{e_1}{A_1}\right)}{\log\left(\frac{e_1}{A_1}\right) + \log\left(\frac{e_2}{A_2}\right)}$$

where, β_1 is the preference to prey 1 (egg, protonymph or adult *T. urticae*), e_1 and e_2 are the number of prey '1' and '2' remaining after the experiment, respectively, and A_1 and A_2 are the numbers of prey '1' and '2' presented to the predator. If the preference index is close to 1, the predator prefers prey '1', and if it is close to 0, it prefers prey '2'. An index close to 0.5 shows no preference, indicating that predation is random (Xu and Enkegaard, 2010).

Prey predation: Gravid females of the predatory mites were transferred from the main culture to strawberry leaves and left for 24 hours to oviposit. Only one egg remained on each leaflet and the mite and additional eggs were removed. The leaflet of each strawberry leaflet (2×2 cm²) was placed upside down on water-saturated cotton in a 6 cm diameter Petri dish surrounded by strips of wet cotton wool to prevent the mites from escaping. Experiments were carried out on strawberry disc in Petri dish (6 cm diameter) with 20 replicates under laboratory conditions (27±1°C temperature, 70±5% RH and 16L: 8D photoperiod). Four laboratory strains of predatory mites were prepared. Each strain was fed by different diets (date pollen, corn pollen, walnut pollen and *T. urticae* eggs). After 24 hours, the total number of prey (*T. urticae* eggs) consumed was counted. After that, the population fed with corn pollen, walnut and date pollen with the spider mite were investigated.

The present of pollens also were investigated, we compared the predation rate of each predatory strain in Petri dishes with 20 *T. urticae* eggs and Petri dishes with 20 *T. urticae* eggs and 5 gr. of plant pollens (date pollen, corn pollen and walnut pollen) separately. The prey consumed was counted after 24 hours.

Statistical analysis: Preference Index and Predation Rate were subjected to analysis of variance in SPSS. Data for prey- stage- preference and predation rate were analyzed using ANOVA (SPSS Inc, 2012).

RESULT

Prey-preference: Preference Index was different among the predatory mites reared on date pollen, corn pollen, walnut pollen and two-spotted spider mite. The Preference Index was higher on *T. urticae* eggs or nymphs compared to *T. urticae* adults, indicating that

the predatory mites prefer eggs or nymphs to adults (Table1), however the Preference Index of the different population of predatory mite on the prey eggs and

protonymph or adult was not different (ANOVA; on prey egg: $F_{3,36}=1.93$, $P=0.14$; on prey protonymph; $F_{3,36}=2.29$, $P=0.09$; on prey adult; $F_{3,36}=1.16$, $P=0.34$)(Table 2).

Table1. Prey- stage Preference Index of *Neoseiulus californicus* reared on corn pollen, date pollen, walnut pollen and two-spotted spider mite when offered eggs, nymph and adult of *Tetranychus urticae*, during 24 h in the laboratory.

Predatory mite reared on	Preference Index (β) on different growth stages of <i>T. urticae</i>			F	P
	Egg	Protonymph	Adult		
Corn pollen [†]	0.42±0.04 ^a	0.44±0.04 ^a	0.13±0.03 ^b	21.60	0.0001
Date pollen [†]	0.42±0.04 ^a	0.48±0.04 ^a	0.09±0.004 ^b	32.64	0.0002
Walnut pollen [†]	0.38±0.01 ^b	0.47±0.02 ^a	0.15±0.02 ^b	89.46	0.0001
Two- spotted spider mite [†]	0.42±0.01 ^b	0.55±0.02 ^a	0.11±0.02 ^b	93.13	0.00001

[†] Degrees of freedom for ANOVA=27.

Means within a row followed by the different letter are significantly different at the 5% confidence level according to ANOVA test.

Table 2. The comparison of Prey-stage Preference Index of *Neoseiulus californicus* reared on corn pollen, date pollen, walnut pollen and two-spotted spider mite when offered eggs, nymph and adult of *Tetranychus urticae*, during 24 h in the laboratory.

Predatory mite reared on		Corn pollen	Date pollen	Walnut pollen	Two-spotted spider mite	F	P
Preference Index (β) on different growth stages of <i>T. urticae</i>	Egg [†]	0.42±0.04	0.44±0.04	0.38±0.01	0.42±0.01	1.93	0.14
	Protonymph [†]	0.44±0.04	0.48±0.04	0.47±0.02	0.55±0.05	2.29	0.09
	Adult [†]	0.13±0.03	0.09±0.04	0.15±0.02	0.11±0.02	1.16	0.34

[†] Degrees of freedom for ANOVA=36.

Predation rate: The predation rate of *N. californicus* on different growth stages of *T. urticae* was different among the predatory reared on corn pollen, walnut pollen, date pollen and *T. urticae* eggs (ANOVA, for egg prey $F_{3,40}=5.42$, $P=0.001$, for nymph prey $F_{3,40}=2.80$,

$P=0.001$, for adult prey, $F_{3,40}=5.96$, $P=0.001$). The highest predation rate of *T. urticae* egg, nymph and adult was observed for predatory mite reared on date pollen. The average daily number of prey consumed by *N. californicus* gravid females is exhibited in Table 3.

Table 3. Mean (+SE) number of *Tetranychus urticae* eggs, nymphs and adults consumed by *Neoseiulus californicus* reared on corn pollen, date pollen, walnut pollen and two-spotted spider mite when offered eggs, nymph and adult of *Tetranychus urticae*, during 24 h in the laboratory.

Predatory mite reared on		Corn pollen	Date pollen	Walnut pollen	Two-spotted spider mite	F	P
Predation rate on different growth stages of <i>T. urticae</i>	Egg [†]	7.75±0.52 ^b	10.18±1.00 ^a	7.58±0.19 ^b	6.44±0.74 ^c	5.42	0.0001
	Protonymph [†]	10.41±0.99 ^a	11.00±0.72 ^a	9.00±0.00 ^b	8.67±0.17 ^b	2.80	0.0001
	Adult [†]	2.50±0.31 ^b	3.00±0.00 ^a	3.75±0.46 ^a	1.78±0.32 ^b	5.96	0.0001

[†] Degrees of freedom for ANOVA=40.

Means within a row followed by the different letter are significantly different at the 5% confidence level according to ANOVA test.

Result of consumed of eggs of spider mite by female predatory mites that fed on plant pollen (corn pollen, walnut pollen and date pollen) indicated the predation

rate of the predatory mite fed with corn pollen (9±1.46), walnut pollen (8.19±0.99) and Date pollen (8.28±0.80) did not any significant difference, however when the

predatory mites fed on pollen with *T. urticae*, the predation rate show significant difference and the predatory mites when fed with *T. urticae* (14.74±0.94), Walnut (15.24±1.05) and date pollen (14.17±1.04) show significant difference with the population on corn pollen (Table 4). The present of pollens were affected on the

predation rate the predatory mite (Table 5). The presence of plant pollens (corn pollen, walnut pollen and date pollen) in Petri dishes decrease the predation rate (for the predator fed on corn pollen $t_{41}=3.38$, $p=0.01$; for the predator fed on date pollen $t_{43}=4.39$, $p=0.04$; for the predator fed on walnut pollen $t_{51}=4.73$, $p=0.03$).

Table 4. Predation rate of *Neoseiulus californicus* on egg *Tetranychus urticae* when fed on plant pollens (corn pollen, date pollen and walnut pollen) and plant pollen with two-spotted spider mite.

		Predatory populations				F	df	p
		Corn pollen	Walnut pollen	Date pollen	Two-spotted spider mite			
Predation Rate	Fed on pollen	9.00±1.46	8.19±0.99	8.28±0.80		0.15	70	0.86
	Fed on pollen with Two-spotted spider mite	10.0±0.95 ^b	14.17±1.04 ^a	15.24±1.05 ^a	14.74±0.94 ^a	0.0036	84	4.24

Means within a row followed by the different letter are significantly different at the 5% confidence level according to ANOVA test.

Table 5. Effect of the present of pollen (corn pollen, date pollen and walnut pollen) and *Tetranychus urticae* on Predation rate of *Neoseiulus californicus*

		<i>Tetranychus urticae</i> eggs	<i>Tetranychus urticae</i> eggs with pollen	t	df	P
Predation Rate	Fed on corn pollen	16.8±0.95 ^a	8.00±1.22 ^b	3.38	41	0.010
	Fed on date pollen	19.3±1.14 ^a	9.28±0.40 ^b	4.39	43	0.037
	Fed on walnut pollen	17.24±1.01 ^a	8.19±0.49 ^b	4.73	51	0.035

Means within a row followed by the different letter are significantly different at the 5% confidence level according to ANOVA test.

DISCUSSIONS

Pollen has been shown to be critical for biological control in some cases. The ability to food from plant origin increases their survival during periods when prey is locally sparse. The quality tests for investigation of date pollen, corn pollen and walnut pollen for mass rearing *N. californicus* was studied in this study. Four strains of the predatory mites had a higher predation rate on egg or nymph of spider mite than adults. Rezaie et al. (2018) reported Female *N. californicus* had a higher predation rate on active stages of spider mites. Blackwood et al. (2001) reported adult female has no prey-stage preference between *T. urticae* eggs and nymphs. Phytoseiid mites often prefer feeding on immature stages and eggs (Sabelis, 1985). A gravid *N. californicus* female consumed more eggs, larvae and nymphs than adult male or female of *T. urticae* (Canlas et al., 2006). In our study, female predatory mite preferred *T. urticae* eggs and nymphs to adults. The

preference Index of strains in this study did not differ and the reared predator on plant pollens preferred *T. urticae* same as the reared predator on *T. urticae*. Castagnoli and Simoni (1999) investigated the different nutritional (*T. urticae*, *Dermatophagoides Farinae* pollen, *Quercus* pollen) histories affected the response of *N. californicus* on tetranychus.on the whole; the wild strain gave better performance to *T. urticae*.

In this study, the maximum number of prey consumed by laboratory strains *N. californicus* when fed just on plant pollens did not different and they consumed egg *T. urticae* between 8.19 to 9.00, however, when the predatory fed on plant pollens and two-spotted spider mite, the predation rate was different and maximum number of prey consumed by *N. californicus* fed on walnut pollen, date pollen and Two-spotted spider mite ranged between (14.17 to 15.24) and it was higher than the prey consumed by *N. californicus* fed on corn pollen (10.10). The maximum number of *T. urticae* egg and

protonymph consumed by the predatory mite in 24 hours, on different cultivars, were 22.3 to 31.9 and 16.0 to 22.7, respectively (Rezaie et al., 2018). These estimates were higher than estimates of Canlas et al. (2006) and Ahn et al. (2010). Canlas et al. (2006) reported the maximum number of *T. urticae* eggs and protonymphs consumed by *N. californicus* were 20.91 and 12.57, respectively while, Ahn et al. (2010) reported that the maximum number of *T. urticae* eggs and protonymphs consumed by *N. californicus* were 17.14 and 11.81, respectively. Gotoh et al. (2004) reported the average daily consumption rate of adult female *N. californicus*, during the first 20 days after emergence, at 25 °C, was 13.4 eggs of *T. urticae*. However, Marafeli et al. (2011) reported a maximum number of *T. urticae* consumed protonymphs was 60 individuals. The highest number of adult male and female prey consumed by a female predator was 9.22 and 2.51 respectively (Canlas et al., 2006). The difference between the estimates of different studies may be due to laboratory conditions or host plants and predatory strains.

We estimated the predation rate of *N. californicus* when plant pollen was present as well. The present plant pollen decreased the predation rate. Wei and Walde (1997) found *Typha latifolia* significantly reduced the predation rate of *Typhlodromus pyri* on *Panonychus ulmi* but that the magnitude of the effect was not large. Using pollen simultaneously with prey larvae decreased the consumption of the latter over the fall range of prey densities (Badii et al., 2004). Sabelis and Van Rijn (1997) suggested the presence of pollen can sometimes be detrimental to control, particularly if the pollen is also used by the pest.

Ragusa (1991) reported pollen of *Carpobrotus edulis* (L.) And *Scrophularia peregrine* L. are the suitable diet for *N. californicus*. Argov et al. (2006) reported six species pollen (*Zea mays*, *Quercus ithaburensis*, *Pistacia atlantica*, *P.vera*, *P. lentiscus* were found to be suitable for mass rearing of *N. californicus*. In this study, date pollen, corn pollen and walnut pollen are recommended for mass-rearing of *N. californicus*. Use of the predatory mite fed with different developmental stages of two-spotted spider mite and pollen in the biological control of the pests is useful. A supply of alternative food is generally thought to enhance the effectiveness of a biological control agent.

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