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# Non-choice vs. Free-choice: when and how *Cydnoseius negevi* (Swirski and Amitai) female attack its prey?

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## Abstract

**Background** *Cydnoseius negevi* (Swirski and Amitai) (Acari: Phytoseiidae) is a promising bio-agent in the Mediterranean region. It attacks a wide range of pest species. Two fundamental questions have been raised when *C. negevi* was a promising candidate for biological control applications in a hydroponic system. This study was carried out to answer the raised questions: (1) how long a predatory female takes to attack its prey in a non-choice biological control application? and (2) what do the predatory females prefer when offered a multiple choice (free-choice) of pest preys?, to understand the potentialities of *C. negevi* attacking behaviour.

**Results** Two sets of experiments were conducted for 240 min under laboratory conditions of  $27 \pm 2$  °C,  $60 \pm 5\%$  RH, and 18:6h L:D photoperiod. The tested prey species were *Tetranychus urticae* Koch, *Bemisia tabaci* (Gennadius), *Frankliniella occidentalis* (Pergande), *Parlatoria ziziphi* (Lucas), and *Icerya aegyptiaca* (Douglas). Non-choice experiments tested the time (min) needed to the 24 h starved female to attack its prey. The free-choice experiments tested the female's choices and the time recorded for each female during the full experimental time. Significant differences were recorded in both sets at confidence level of 95%. The spider mite *T. urticae* recorded the shortest predatory female's response in both non- and free-choice sets. On the contrary, the insect *I. aegyptiaca* had the least female's choice in both sets of experiment.

**Conclusion** The present study discussed the behavioural categories of *C. negevi*. The female responses showed a list of priorities to the tested herbivore species. Such behavioural simulation is considerable for the future pest control applications to predict the generalist predator's preferences among a wide range of multiple herbivore species.

**Keywords** Biological control, Hydroponic, *Cydnoseius negevi*, Phytoseiidae, Predator–herbivore interactions, Prey choice

## Background

Prey choice behaviour is considered as one of the theoretical keys that support the knowledge of predator–prey interaction (Abrams 2000). Predators act in a way that maximises their energy intake by preferentially preying

on the most beneficial resources (Samaras et al. 2021). The nutritional value of the prey and how it is easy to be consumed are two attributes of the prey that affect a predator's preference (Zhang et al. 2022). Predation on prey of the highest nutritive value boosts the predator's fitness (increased survival and reproduction rates in a short time) (Dicke et al. 1990).

*Cydnoseius negevi* (Swirski and Amitai) is a generalist predator of the subtype III-e (McMurtry et al. 2013) where it has been originally found in the low-growing grasses associated with date palm trees in the Mediterranean basin and the Middle East (Zain El Abidin et al.

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2024). Its potentialities have been assessed on a wide range of serious herbivore species, e.g. the two-spotted spider mite, *Tetranychus urticae* Koch (Rasmy et al. 2014), date palm spider mite, *Oligonychus afrasiaticus* (McGregor) (Alatawi et al. 2018), and the mango red spider mite, *O. mangiferus* (Rahman and Sapra) (Acari: Tetranychidae) (Fahim and Momen 2022), in addition to the onion thrips, *Thrips tabaci* Lindeman (El-Laithy et al. 2021), the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thripidae: Thysanoptera) (Sanad and Hassan 2019), and the silver leaf whitefly, *Bemisia tabaci* (Gennadius) (Aleyrodidae: Hemiptera) (Elkholy et al. 2022). Besides, suitability of pollen grain sizes, shapes, and surfaces has been proved to significantly affect its life table parameters either for a single generation (Roshdy et al. 2023) or for multiple generations (Lamlom et al. 2024). Furthermore, the phytoseiid mite *C. negevi* potentialities have been experimented on lepidopteran insects (Roshdy et al. 2023) as well as the dipteran species (Momen et al. 2016). A little is known about the prey choice behaviour of *C. negevi*, especially for scale insects and mealybugs species (Hemiptera: Coccoidea). However, some phytoseiid species have recorded positive and/or negative responses, e.g. *Amblyseius largoensis* (Muma) (Carrillo et al. 2010), *A. swirskii* Athias-Henriot (Abou-Elella et al. 2013), *Euseius scutalis* (Athias-Henriot) (Abou-Elella et al. 2013), *E. finlandicus* Oudemans, *Typhlodromus pyri* Schuten, *Kampimodromus aberrans* Oudemans (Schausberger 1998), and *Neoseiulus zaheri* (El-Borolossy) (Rasmy et al. 2003).

Prey selection does not happen randomly among available species (Ishii and Shimada 2010). The tendency of a predator to choose a given prey type over another may change as the relative frequencies of the prey species, sizes, ages, or sex classes in the predator's environment change (Whitney et al. 2018). Rahmani et al. (2016) concluded that in the first and second nymphal stages of *Neoseiulus californicus* (McGregor), when given the option to choose between the first larval instar of *T. tabaci* and immature of *T. urticae*, they clearly preferred the second option; in the presence of *T. urticae* females, *N. californicus* stages directly switched their preference to *T. tabaci*.

Also, Alatawi et al. (2018) reported that *C. negevi*, when reared on date palm pollen, preferred and consumed juveniles of *O. afrasiaticus* more than *T. urticae*. However, the field race of *C. negevi* did not show significant feeding preference between both species of spider mites in the choice test. The observed and expected rates of predation in mixed food species varied significantly, and the observed differences cannot be attributed to behavioural changes in the prey species as a result of being together. Consequently, it likely appears that the predator's behaviour in selecting prey differed depending on

whether the prey species was presented in groups or alone (Alatawi et al. 2018). Switching among preys is an important aspect in defining a predator's individual fitness since it ultimately impacts hunting success and a predator's ability to gain and keep body reserves (Jaworski et al. 2013). Indeed, through prey choice, predators can alter the distribution of life history features over generations and impact the evolution of their prey.

It has been hypothetically asked: When several prey species are presented together, will the predator's preference change? Therefore, this study was targeted to answer two raised questions: (1) how long a predatory female takes to attack its prey in non-choice biological control application? and (2) what do predatory females prefer when offered a multiple choice (free-choice) of pest preys?, in order to be a prospective candidate for biological control applications in the hydroponic system.

## Methods

### Plant locality

Lettuce (green Batavia), *Lactuca sativa* var. *capitata* L. (Asteraceae), and lacianto kale, *Brassica oleracea* var. *palmifolia* L. (Brassicaceae), have been grown in a hydroponic system. The hydroponic farm is a private sector company (small-scale producers) in total land area of about 14,000 m<sup>2</sup> (1.3hec) at Gamalet Ahmed Orabi, Al Obour, Al Qalyubia Governorate (30°14'06.8'' N 31°31'29.0'' E), Egypt.

### Predatory species collection

*Cydnoseius negevi* individuals were extracted from low-growing weeds associated with the hydroponic farm at Gamalet Ahmed Orabi. Five females were collected after extraction and mounted on Hoyer's medium for microscopic identification according to the most recent keys of the Egyptian phytoseiid mites by Abo-Shnaf and de Moraes (2014). Fifteen specimens were randomly collected from the stock colony and have been taxonomically confirmed by Dr. Ismail Döker, Çukurova University, Turkey.

### Stock colonies

Fifty gravid *C. negevi* females and 20–25 active males were transferred by fine brush into five arenas/colonies of 12 cm in diameter (cm Ø) upside-down acalypha, *Acalypha marginata* L. (Euphorbiaceae) in 15 cm Ø Petri dish with wet-saturated cotton pad at the Acarology laboratory, National Research Centre (NRC, Egypt). Colonies of *C. negevi* were reared on fresh pollen grains of castor bean, *Ricinus communis* L. (Euphorbiaceae), provided every 3 days. Culture maintenance, cleaning, and changing were carried out weekly on fresh leaves of acalypha. Laboratory conditions were 27° ± 2 °C, 60 ± 5% RH, and

16:8 photoperiod. Cultures were established for five weeks before starting the experimental trials.

### Pest species

Active stages of the two-spotted spider mite *T. urticae* were collected from heavily infested *L. sativa* var. *capitata* and *B. oleracea* var. *palmifolia* leaves. Eggs and crawlers of the tomato whitefly, *B. tabaci*, infested on *B. oleracea* var. *palmifolia* leaves. The first larval stage of the western flower thrips, *F. occidentalis*, infested on leaves of *L. sativa* var. *capitata* and *B. oleracea* var. *palmifolia*. Eggs and crawlers of the black parlatoria scale insect *Parlatoria ziziphi* (Lucas) (Insecta: Hemiptera: Diaspididae) infested on lemon leaves, *Citrus limon* (L.) (Rutaceae). Also, egg clusters of the Egyptian fluted scale, *Icerya aegyptiaca* (Douglas) (Insecta: Hemiptera: Monophlebidae), were collected from heavily infested lemon seedlings and hibiscus shrubs, *Hibiscus rosa-sinensis* L. (Malvaceae), found in the associated biodiversity around the same hydroponic location.

### Experimental procedures

Trial sets were conducted to estimate the females' attacking time and aggressiveness when offered (1) non-choice and (2) free-choice natural preys. The methodologies of Bazgir et al. (2020) were followed with some modifications. Lettuce, kale seedlings and leaves of lemon and hibiscus were sampled twice weekly to collect the selected pest stages. For *T. urticae*, 20 gravid females were transferred by a fine brush onto fresh lettuce leaves and kept under laboratory conditions for egg depositing. After egg hatching, larvae were kept for feeding and moulting. The newly emerged protonymphs were transferred individually onto experimental arenas (3 cm Ø).

The 1st larval instar of *F. occidentalis* was directly collected from infested lettuce and kale seedlings and set as prey in the experimental trials. Eggs and crawlers of *P. ziziphi* were removed from lemon leaves by a fine needle. A disc of ½ cm<sup>2</sup> of *B. oleracea* var. *palmifolia* leaves containing circle-like cluster of *B. tabaci* eggs and crawlers was cut with a sharp cutter and placed onto experimental arenas. The egg clusters of *I. aegyptiaca* were removed softly by a fine brush from lemon leaves, then separated individually in wet filter paper, and transferred into the experimental arenas.

### Predatory female

Fifty deutonymph individuals of the predatory mites were collected from mite colony and transferred to 7 cm Ø upside-down lettuce, *L. sativa* var. *capitata*, leaf discs in 10 cm Petri dish with wet-saturated cotton pad with excessive pollen of *R. communis* under the previous mentioned laboratory conditions. After maturity, predatory

females were mated with newly emerged males. Twenty 5-day-old gravid females were transferred individually into the experimental arenas (3 cm Ø lettuce leaf discs) and starved for 24 h. After starvation period, two sets of experiments have been conducted:

- (i) *Non-choice test*: Each pest species was introduced individually to the 24h starved predatory female ( $n=20$  females  $\times$  5 pest species).
- (ii) *Free-choice test*: five individuals from each tested pest species were introduced to 24 h starved females ( $n=20$ ).

Both sets were kept for 240 min. The experimental time was divided into 8 phases/intervals: 0–15 min, 16–30 min, 31–45 min, 46–60 min, 61–90 min, 91–120 min, 121–180 min, and 181–240 min. The first phase started after loading the prey into the experimental arena. In the free-choice trials, the time whenever *C. negevi* female took to attack and consume its first choice and the other later choices during the experimental phases were recorded (Fig. 1).

### Statistical analysis

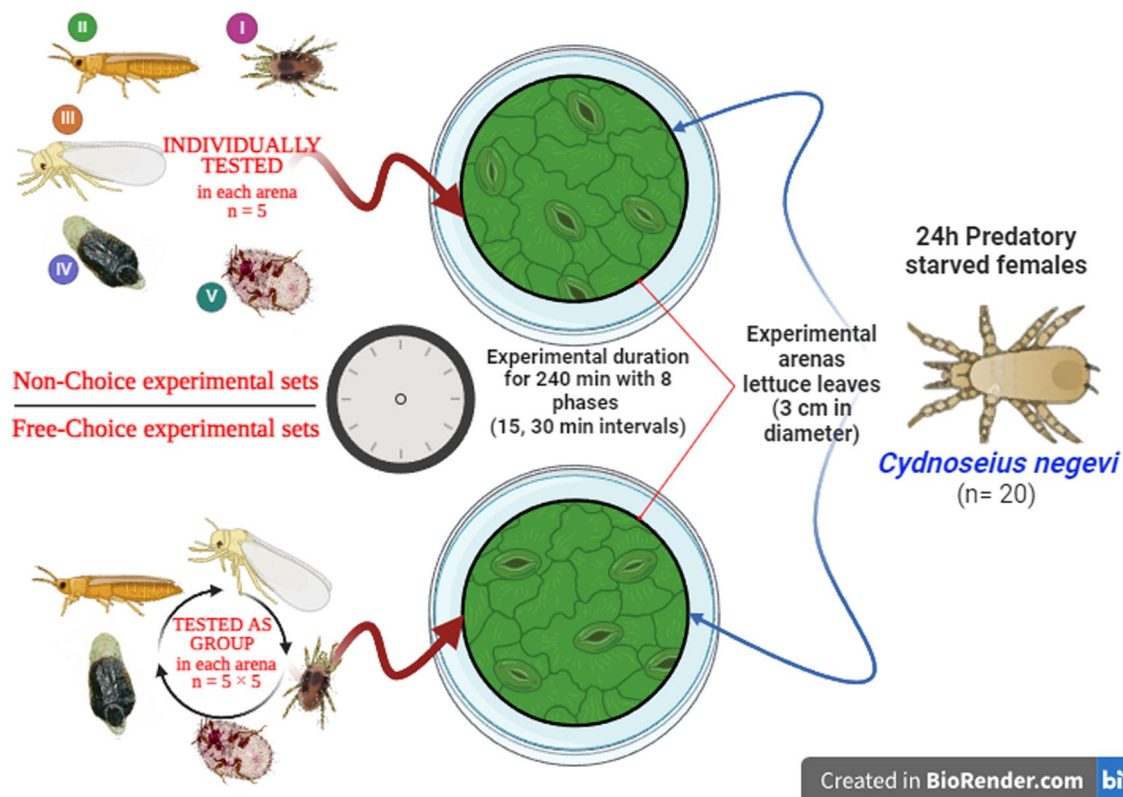
Data normal distribution was tested using Kolmogorov–Smirnov and Shapiro–Wilk at 95% confidence level. Preliminary statistical tests were performed to experiment the normal distribution using Leven's test using an IBM SPSS computer program v.26 (SPSS 2019). Data were arranged and grouped using Microsoft office Excel ver. 2019 for each single prey species in both non- and free-choice tests (attacking time/min). Data were not transformed. Figures and data visualisation were performed using SPSS (SPSS 2019). Mean comparison of predatory females data in both non-choice (attacking time of prey/min) and free-choice (attacking time of x preys/min) tests was made and analysed using one-way analysis of variance (ANOVA). Mean differences were compared for all experiments with Duncan's test at 95% confidence level using SPSS (SPSS 2019).

## Results

### Responses of *C. negevi* female to the non-choice prey preference

Only *T. urticae* recorded non-significant *F*-value due to *C. negevi*'s rapid response which was recorded in the 1st phase, where 100% of the females attacked *T. urticae* protonymphs in less than 5 min. During the 1st phase, 45% of *C. negevi* tested females attacked *B. tabaci* as a second choice, then 15% attacked *F. occidentalis*, while 10% were attacked both *I. aegyptiaca* and *P. ziziphi* (Table 1).

In the second phase (16–30 min), 30% of *C. negevi* females choose *P. ziziphi* as the first choice, 25% choose *B. tabaci*, then *F. occidentalis* (10%), and the last choice



**Fig. 1** Experimental procedure graphical diagram: (i) *Tetranychus urticae* (ii) *Frankliniella occidentalis*, (iii) *Bemisia tabaci*, (iv) *Parlatoria ziziphi*, and (v) *Icerya aegyptiaca*. The illustration was created with [www.biorender.com](http://www.biorender.com)

**Table 1** Mean time recorded (min ± SE) of *C. negevi* females when offered non-choice prey species during the experimental duration of 240 min

| Non-choice prey species | 0–15 min                                | 16–30 min                            | 31–45 min                            | 46–60 min                             | 61–90 min                              | 91–120 min                             | 121–180 min     | $F_{(df)}$                               | Sig   |
|-------------------------|-----------------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|----------------------------------------|----------------------------------------|-----------------|------------------------------------------|-------|
| <i>T. urticae</i>       | 3.30 ± 0.52 <sup>c</sup>                | –                                    | –                                    | –                                     | –                                      | –                                      | –               | –                                        | –     |
| <i>F. occidentalis</i>  | 14.50 ± 0.50 <sup>d</sup> D             | 29.00 ± 0.00 <sup>d</sup> C          | 40.00 ± 0.00 <sup>d</sup> C          | –                                     | 79.13 ± 3.93 <sup>d</sup> C            | 110.00 ± 2.10 <sup>d</sup> B           | 141.00 ± 0.00 A | 66.02 <sup>***</sup> <sub>(5, 18)</sub>  | 0.000 |
| <i>B. tabaci</i>        | 6.77 ± 1.71 <sup>b</sup> C              | 20.60 ± 1.44 <sup>d</sup> B          | 34.00 ± 1.00 <sup>d</sup> A          | –                                     | –                                      | –                                      | –               | 38.14 <sup>***</sup> <sub>(2, 15)</sub>  | 0.000 |
| <i>P. zizyphi</i>       | 6.50 ± 3.50 <sup>bc</sup> D             | 25.67 ± 1.56 <sup>d</sup> C          | 41.00 ± 4.00 <sup>d</sup> B          | 50.00 ± 2.52 <sup>d</sup> B           | 78.25 ± 1.18 <sup>d</sup> AB           | 92.00 ± 0.00 <sup>b</sup> A            | –               | 155.29 <sup>***</sup> <sub>(5, 17)</sub> | 0.000 |
| <i>I. aegyptiaca</i>    | 10.00 ± 0.00 <sup>b</sup> D             | 27.00 ± 0.00 <sup>d</sup> C          | 41.00 ± 0.00 <sup>d</sup> C          | 52.00 ± 1.78 <sup>d</sup> C           | 76.80 ± 4.10 <sup>d</sup> B            | 118.33 ± 1.67 <sup>d</sup> A           | –               | 91.83 <sup>***</sup> <sub>(5, 15)</sub>  | 0.000 |
| $F_{(df)}$              | 16.35 <sup>***</sup> <sub>(4, 34)</sub> | 3.81 <sup>*</sup> <sub>(3, 13)</sub> | 1.87 <sup>ns</sup> <sub>(3, 6)</sub> | 0.451 <sup>ns</sup> <sub>(1, 6)</sub> | 0.096 <sup>ns</sup> <sub>(2, 16)</sub> | 19.16 <sup>***</sup> <sub>(2, 7)</sub> | –               |                                          |       |
| Sig                     | 0.000                                   | 0.047                                | 0.311                                | 0.532                                 | 0.909                                  | 0.005                                  | –               |                                          |       |

Within columns, small bold italic similar letters are not significant at  $P \leq 0.05$  (Duncan's test of homogeneous subsets). Within rows, similar capital letters are not significant difference at confidence level of 95% (Duncan's test of homogeneous subsets). ANOVA \* significant, \*\*\* highly significant, and <sup>ns</sup> non-significant at confidence level of 95% ( $P \leq 0.05$ )

was *I. aegyptiaca* (5%) (Table 1). Then, in the third phase (31–45 min), 10% of tested females equally attacked *F. occidentalis*, *P. zizyphi*, and *B. tabaci* and *I. aegyptiaca* was in the later choice with 5% (Table 1).

Equally in the fourth (46–60 min) and fifth (61–90 min) phases, 40% of the reached females attacked the 1st larval instars of *F. occidentalis*, then 20% attacked *I. aegyptiaca*, and 15% attacked *P. zizyphi*. After that, in the



sixth (90–120 min) phase, *C. negevi* females showed high significantly attacking rates against *F. occidentalis* (20%), then *I. aegyptiaca* (15%), and *P. ziziphi* (5%). By the seventh phase (121–180 min), only 5% of tested females have attacked *F. occidentalis* (Table 1).

Figure 2 illustrates the significant responses of *C. negevi* females when kept with each pest species individually during the non-choice experimental duration (240 min). The first choice was *T. urticae* with an average time about 3.30 min, the second choice was *B. tabaci* (average of 11.60 min), the third choice was *P. ziziphi* (average of 40.20 min), the fourth choice was *I. aegyptiaca* (average of 51.75 min), while the last choice was *F. occidentalis* (average of 70.30 min) all at  $P \leq 0.05$ .

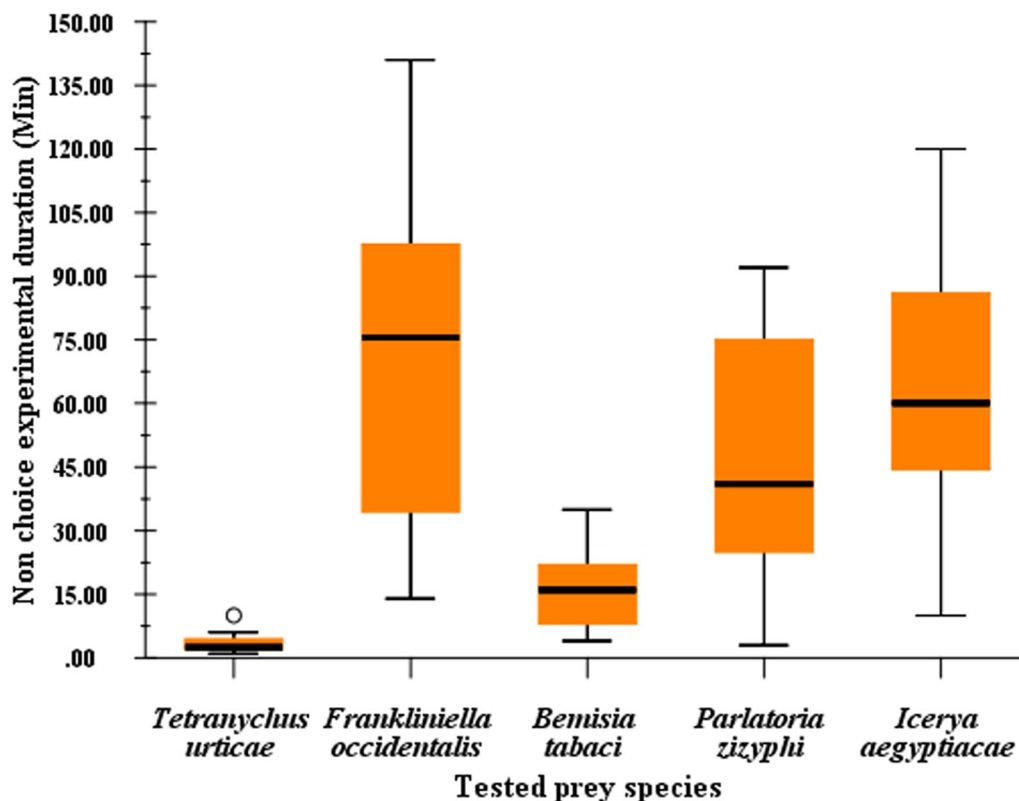
**Responses of *C. negevi* female to the free-choice prey preference**

The experimented *C. negevi* females recorded high significant differences at  $P \leq 0.05$ . It was recorded that the fastest and the most targeted prey was the 1st nymph of *T. urticae* ( $F_{(7, 64)} = 363.87, P = 0.000$ ). Eggs and crawlers of *B. tabaci* were the second choice ( $F_{(7, 22)} = 87.85, P = 0.000$ ) as well as the eggs and crawlers of *P. ziziphi* ( $F_{(7, 27)} = 149.83, P = 0.000$ ). The third choice was the 1st larval stage of *F. occidentalis* ( $F_{(7, 22)} = 96.68, P = 0.000$ )

and the eggs of *I. aegyptiaca* have recorded the last choice for *C. negevi* female ( $F_{(5, 9)} = 373.51, P = 0.000$ ) (Table 2).

Figure 3 illustrates the relationship between the experimental time and *C. negevi* female’s free-choice preferences at  $P \leq 0.05$ . In the 1st phase (0–15 min), *C. negevi*’s first choice was *T. urticae*, then *B. tabaci* and *P. ziziphi* were equally the second choice, *F. occidentalis* was the third choice, and the last one was *I. aegyptiaca* (Fig. 3a). In the 2nd phase (16–30 min), the first choice was *F. occidentalis*, then *I. aegyptiaca* was the second choice, *B. tabaci* was the third, *P. ziziphi* was the fourth, and *T. urticae* was the fifth choice (Fig. 3b). In the 3rd phase (31–45 min), *C. negevi*’s first choice was *T. urticae*, the second was *F. occidentalis*, while *B. tabaci* and *P. ziziphi* were equally the third choice, and the fourth choice was *I. aegyptiaca* (Fig. 3c).

By the 4th phase (46–60 min), *C. negevi* first choice was *F. occidentalis*, then *T. urticae* and *P. ziziphi* were equally the second choice, while *B. tabaci* was the third, and the last choice was *I. aegyptiaca* (Fig. 3d). After that, in the 5th phase (61–90 min), the female choices were *T. urticae*, *P. ziziphi*, *B. tabaci* and *F. occidentalis*, respectively, while no response recorded towards *I. aegyptiaca* (Fig. 3e).

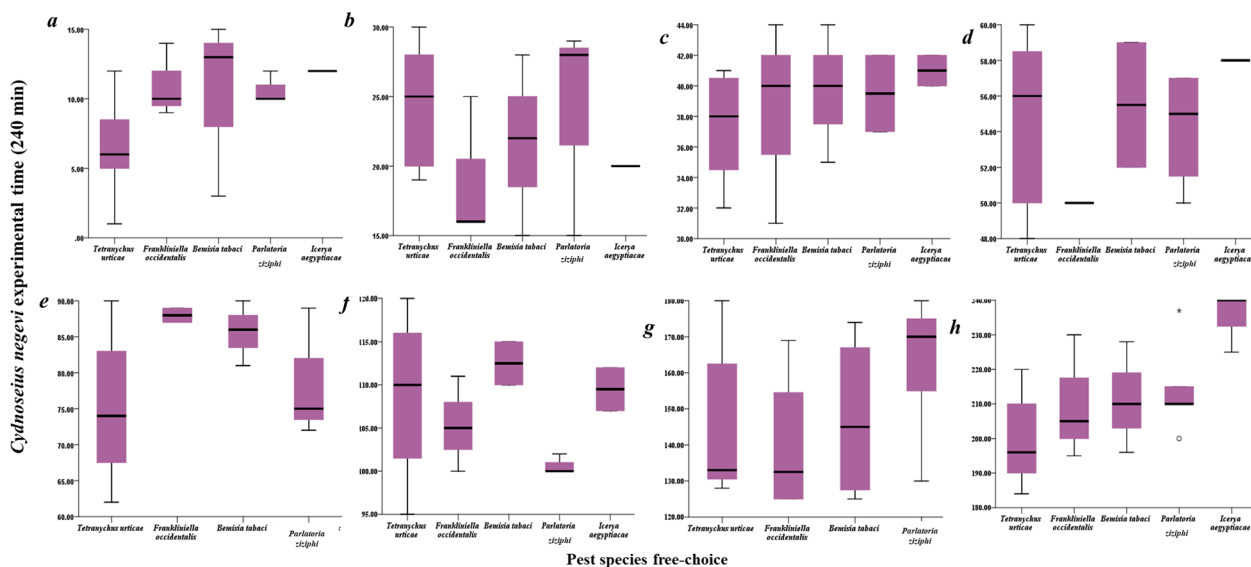


**Fig. 2** Significance duration (min) of the predatory mite *Cydnoseius negevi* response when offered individually non-choice prey species ( $P \leq 0.05$ )

**Table 2** Mean time recorded (min ± SE) of *C. negevi* females when offered free-choice prey species during the experimental duration of 240 min

| Free-Choice prey species            | 0–15 min                  | 16–30 min                  | 31–45 min                  | 46–60 min                  | 61–90 min                 | 91–120 min                 | 121–180 min                 | 181–240 min                | F (df)         | P     |
|-------------------------------------|---------------------------|----------------------------|----------------------------|----------------------------|---------------------------|----------------------------|-----------------------------|----------------------------|----------------|-------|
| <i>T. urticae</i>                   | 6.73 ± 0.81 <sup>b</sup>  | 24.38 ± 1.51 <sup>g</sup>  | 37.29 ± 1.48 <sup>f</sup>  | 54.43 ± 1.88 <sup>e</sup>  | 75.13 ± 3.46 <sup>d</sup> | 108.57 ± 3.63 <sup>c</sup> | 146.71 ± 8.00 <sup>b</sup>  | 199.33 ± 5.53 <sup>a</sup> | 363.87 (7, 64) | 0.000 |
| <i>F. occidentalis</i> <sup>a</sup> | 11.00 ± 1.53              | 19.00 ± 3.00               | 38.33 ± 3.84               | 50.00 ± 0.00               | 88.00 ± 1.00              | 105.25 ± 2.25              | 139.75 ± 10.37              | 210.00 ± 10.41             | 96.68 (7, 22)  | 0.000 |
| <i>B. tabaci</i>                    | 10.33 ± 3.72 <sup>g</sup> | 21.66 ± 3.76 <sup>fg</sup> | 39.67 ± 2.60 <sup>f</sup>  | 55.50 ± 3.50 <sup>ef</sup> | 85.67 ± 2.60 <sup>d</sup> | 112.50 ± 2.50 <sup>c</sup> | 147.20 ± 11.80 <sup>b</sup> | 211.33 ± 9.26 <sup>a</sup> | 87.85 (7, 22)  | 0.000 |
| <i>P. ziziphi</i>                   | 10.67 ± 0.67 <sup>g</sup> | 24.00 ± 4.51 <sup>fg</sup> | 39.00 ± 2.50 <sup>ef</sup> | 54.25 ± 1.70 <sup>e</sup>  | 78.67 ± 5.24 <sup>d</sup> | 100.67 ± 0.67 <sup>c</sup> | 162.00 ± 9.03 <sup>b</sup>  | 214.40 ± 6.15 <sup>a</sup> | 149.83 (7, 27) | 0.000 |
| <i>I. aegyptiaca</i> <sup>a</sup>   | 12.00 ± 0.00              | 20.00 ± 0.00               | 41.00 ± 1.00               | 58.00 ± 0.00               | –                         | 109.50 ± 2.50              | –                           | 235.00 ± 5.00              | 373.51 (5, 9)  | 0.000 |
| <i>F</i> (df)                       | 2.15 (4, 24)              | 0.66 (4, 17)               | 0.37 (4, 16)               | 0.42 (4, 14)               | 1.89 (3, 15)              | 1.13 (4, 17)               | 0.91 (3, 19)                | 3.25 (4, 19)               |                |       |
| <i>P</i>                            | 0.112                     | 0.630                      | 0.827                      | 0.791                      | 0.185                     | 0.386                      | 0.460                       | 0.042                      |                |       |

<sup>a</sup> Post hoc tests are not performed for *F. occidentalis* and *I. aegyptiaca* because at least one group has fewer than two cases at 95% confidence interval for means. Within rows, similar small letters following means are not significant difference. The group sizes are unequal using Duncan's test of homogeneous subsets ANOVA at  $P \leq 0.05$  where *F*-values and degrees of freedom were varied



**Fig. 3** Relationship between the phases of the experimental time: **a** 1st phase, 0–15 min, **b** 2nd phase, 16–30 min, **c** 3rd phase, 31–45 min, **d** 4th phase, 46–60 min, **e** 5th phase, 61–90 min, **f** 6th phase, 91–120 min, **g** 7th phase, 121–180 min, and **h** 8th phase, 181–240 min and *C. negevi* female’s free-choice preferences when offered *T. urticae*, *F. occidentalis*, *B. tabaci*, *P. ziziphi*, and *I. aegyptiaca* at  $P \leq 0.05$

When *C. negevi* reached the 6th phase (91–120 min), *P. ziziphi* was the first choice, then *F. occidentalis* was the second, then *T. urticae* and *I. aegyptiaca* were equally the third and fourth choices, respectively, and the last choice recorded was *B. tabaci* (Fig. 3f). By the 7th phase (121–180 min), tested *C. negevi* females’ choices were *F. occidentalis*, *T. urticae*, *B. tabaci* and *P. ziziphi*, respectively, with no response to *I. aegyptiaca* (Fig. 3g). In the 8th phase (181–240 min), *C. negevi* choices were *T. urticae*, *F. occidentalis*, *B. tabaci*, *P. ziziphi*, and *I. aegyptiaca*, respectively (Fig. 3h).

**Discussion**

The present results showed that *C. negevi* attacking behaviour has been categorised in seven stages: (1) inspection, (2) patrol, (3) invasion, (4) prey penetration, (5) searching, (6) resting, and (7) grooming (Mirza et al. 2018). Accordingly, *C. negevi* female showed an aggressive behaviour towards the spider mite *T. urticae* protonymphs in both non- and free-choice experiments. It has taken few minutes ( $3.30 \pm 0.52$  in non-choice and  $6.73 \pm 0.81$  in free-choice sets) to inspect its prey. During this period, predatory females went in circles patrolling inside the experimental arena before prey invasion and penetration. Functionally, *C. negevi*’s handling time ( $T_h$ ) and attacking rate ( $\alpha$ ) were clarified to different stages of *T. urticae* (Rasmy et al. 2014) and *O. afasiaticus* (Alatawi et al. 2018). Concerning  $T_h$  analysis, which is the time (in hours) for inspecting an individual prey, investigating and invading the

prey as well as the time spent in penetrating the prey body. Besides,  $\alpha$  describes the predator’s response distance (patrolling behaviour), the predator–prey chasing and escaping rapidity, and the quantity of predatory female’s successful attacks (Sulek et al. 2023). Together, these functional values are covering the response categories of the predatory female. Consequently, *T. urticae* protonymphs were significantly preferable than other choices in the free-choice experiments. Due to its fragile bodies, and having less anti-predator behaviour than the adult stages (Dittmann 2015). In addition, Fagan et al. (2002) stated that the body softness of preys was a recommended option by the predatory female, as an easy source for nutrients and water supply, which helped retaining the needed resource after 24h starvation either for survival or egg production (Sulek et al. 2023). Moreover, data showed there was no much difference in *C. negevi*’s selection during the 1st experimental phase (0–15 min). *B. tabaci* and *P. ziziphi* recorded the second female’s choice in the non- and/or free-choice experiments. Accordingly, *C. negevi* fed on eggs of *B. tabaci*, its immature stages could successfully survive, and females recorded high significant life table parameters (Elkholy et al. 2022). Additionally, *C. negevi* females recorded higher preference to *B. tabaci* eggs (as an extra-guild prey) than the offered intra-guild preys of *C. negevi* larvae and/or *A. swirskii* eggs (Momen et al. 2013). The recent results hypothesised that learning plays an important role in the genetic recognition of suitable preys (Ishii and Shimada 2010).

There were no many data about the biological potentialities and life table parameters of *C. negevi* when offered coccoid insects, as these species considered a minor importance for phytoseiid predators (Gerson et al. 1990). However, some indigenous species such as *E. scutalis* (El-Sharabasy et al. 2017) and *N. zaheri* (Rasmy et al. 2003) were reported as successfully survive, reproduce, and consume *P. ziziphi* eggs (and/or eggs + crawlers). Additionally, it was proved that mealybugs have low nutritional values, which had negatively affected the predatory mite *A. swirskii* potentialities (Ragusa and Swirskii 1977). On the contrary, *E. scutalis* recorded high life table parameters when it offered preys such as *I. purchase* as mentioned by Yousef and El-Halawany (1982) and *I. aegyptiaca* by Momen and Abdel-Khalek (2008).

It is an assumption that after the post-penetration phases of resting and grooming, *C. negevi* females were went to make another round to get fullness. It has been observed that *C. negevi* females aggressively scanned the arena during the inspection and patrolling phases. In order to build a chemo-physical response of the targeted prey, the predatory mite uses its fore legs before making a decision of attack and penetration. For searching, female mite used its chemoreceptors on its body (Su et al. 2021). The present observations confirm Mirza et al. (2018), who mentioned *C. negevi* behaviour during resting and grooming: when predatory females were cleaning their tarsus using chelicerae and palps, it can be stated that this action may occur as a learning behaviour of the outer habitat. Hypothetically, it may be proposed that *C. negevi* performs searching images to the spatial properties of prey species while searching, resting, and grooming phases (Ishii and Shimada 2010). In this regard, it has been recorded the priorities of *C. negevi* female: when offered non- and/or free-choice prey, it put *I. aegyptiaca* in the 'less important' image. Due to its lower nutritional value (Gerson et al. 1990), additionally, the 1st larval body has very long legs and antennae (Mani and Shiv-araju 2016).

Nevertheless, *F. occidentalis* larvae forced the predatory female to get backward to the grooming phase and retrying the process after a while. However, *F. occidentalis* was a targeted prey for *C. negevi* in biological control applications in field (Sanad and Hassan 2019). Unfortunately, there were no data available on *C. negevi*'s functional and/or numerical responses to thrips pests. However, the current observations showed an anti-predator behaviour of *F. occidentalis* 1st larval instars when kept together with a starved female of *C. negevi* in both experimental sets. The larva of the prey physically defended itself by swinging its body ends aggressively, when being attacked to hit the attacking predatory female. This physical counter-attack

behaviour was reported with *F. occidentalis* and *T. tabaci* and was clarified by Beretta et al. (2024). The counter-attack of *F. occidentalis* larvae forced the predatory female get backward to the grooming phase and retrying the process after a while. As a result, escape predation extends the inspection and patrolling durations. Therefore, to delay the invasion and penetration phases up to an average of 70 min (in non-choice) and ~80–85 min in free-choice sets (current study), the behaviour aspects of *C. negevi* need more investigations to completely understand its potentialities as a prospective bio-agent in the biological control application. Additionally, being a common species in the Mediterranean area makes *C. negevi* as a prospective candidate in different ecosystems such as the hydroponic system.

## Conclusion

The phytoseiid species *C. negevi* recorded significant positive responses to both non- and free-choice tests. The 24 h starved female arranged its choices as images of priorities, as follows: (1) *T. urticae*, (2) *B. tabaci* + *P. ziziphi*, (3) *F. occidentalis*, and (4) *I. aegyptiaca*. This list of prey images indicated the aggressiveness of *C. negevi* as a generalist predator, in the meantime, recommending it as a promising bio-agent in biological control programme on a wide scale. Further behavioural studies need to be performed to fully understand its responses to several stresses.

## Acknowledgements

We gratefully thank Dr. Abd El-Hameed Moussa, Dr. Huda El-Behery, and Dr. Samar Ibrahim, Pests & Plant Protection Dept., NRC, for their valuable comments on the first drafts of the manuscript. We sincerely thank Mr. Khaled A. Dawoud and Mr. Saad A. Tawfik, technicians, Pests & Plant Protection Dept., for their assistance during this work.

## Author contributions

The authors have contributed equally in this work. All authors have read, revised, and approved the final manuscript for submission.

## Funding

The study was supported by the National Research Centre (NRC) internal project No. 13050119 (2022–2024).

## Availability of data and materials

The datasets generated and/or analysed during the current study are available upon reasonable request.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare there is no conflict of interest.



Received: 18 March 2024 Accepted: 18 July 2024  
Published online: 30 July 2024

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