

# Performance of Five Species of Phytoseiid Mites (Acari: Phytoseiidae) on *Bactrocera zonata* Eggs (Diptera: Tephritidae) as a Factitious Food

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Development, survival and reproduction of the generalist predatory mites, *Amblyseius largoensis* (Muma), *Neoseiulus barkeri* (Hughes), *Typhlodromips swirskii* (Athias-Henriot), *Proprioseiopsis kadii* (El-Halawany and Abdel-Samad) and *Cydnosus negevi* (Swirski and Amitai) were assessed when fed on eggs of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) as a factitious food. For *N. barkeri* and *P. kadii*, the development was faster, while the reproduction was higher in *N. barkeri* and *A. largoensis* than for *P. kadii*. Survival of immatures of *T. swirskii* and *C. negevi* was low on eggs of *B. zonata* and all failed to develop beyond the protonymphal stage.

A total of 35.4, 31.2 and 19.6 eggs per female, respectively, were obtained when *N. barkeri*, *A. largoensis* and *P. kadii* were fed *B. zonata* eggs. A diet of the peach fruit fly eggs provided the longest female longevity and highest mean total fecundity, which resulted in the highest net reproductive rate ( $R_0 = 34.61$  and  $32.78$ ) and doubling time ( $DT = 1.53$  and  $1.60$ ) for *N. barkeri* and *A. largoensis*, respectively.

Keywords: factitious food, *Bactrocera zonata*, predatory phytoseiid mites, biology, demographic parameters.

The predatory mites, *Neoseiulus barkeri* (Hughes 1948), *Amblyseius largoensis* (Muma 1955), *Typhlodromips swirskii* (Athias-Henriot 1962), *Proprioseiopsis kadii* (El-Halawany and Abdel-Samad 1990) and *Cydnosus negevi* (Swirski and Amitai 1965) are an economically important biological control agents of several key pests in greenhouses and open fields in most parts of the world, such as whiteflies, thrips as well as eriophyid and tetranychid mites (Kamburov, 1971; Bonde, 1989; Nomikou et al., 2001; Messelink et al., 2008; Momen, 2009). All predatory mite species tested are of type III generalist predators, representing that they can feed on various types of food including insect and mite prey, pollen, and honeydew (McMurtry and Croft, 1997).

In recent years, more interest in the use of biological control to manage crop pests has led to an increase in companies producing natural enemies, including predators and

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parasitoids. Augmentative biological control is dependent upon the production of large numbers of natural enemies of high quality (Glenister and Hoffman, 1998; Nordlund, 1998).

Research by Collier and van Steenwyk (2004) indicated that augmentative releases are frequently more expensive than pesticides. Cost-effective rearing techniques are needed so that augmentation is more competitive strategy for managing arthropod pests (Nguyen et al., 2014). One way of facilitating this is reducing the costs associated with rearing natural enemies by using factitious (i.e. unnatural) foods (Riddick, 2009). Costs may be further reduced when natural enemies can be produced on unnatural or factitious hosts that are easier and less expensive to rear than the natural host (De Clercq, 2004).

Factitious hosts are organisms that are not normally attacked by the beneficial, mostly because they do not occur in its natural habitat, but do sustain its development. Factitious prey may comprise live, frozen, irradiated, or lyophilized insects, mites and crustaceans. Many lepidopterans can be reared effectively on diets that are devoid of living plant material (Riddick, 2009). Several species can be produced simply on relatively cheap food, such as wheat flour or grains.

Eggs of the Lepidopterans, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) are routinely used in the commercial production of various natural enemies (van Lenteren, 2003; De Clercq et al., 2005).

Generalist phytoseiids can be mass-produced on factitious food, i.e. a food source that they would not normally encounter in their natural habitat but on which they are able to feed and reproduced; these are primarily insect eggs and astigmatid mites. Different species of storage mites are routinely used as factitious prey in the mass production of phytoseiid mites (Bolckmans and van Houten, 2006; Fidgett and Stinson, 2008).

El-Sawi and Momen (2005) revealed that, *T. swirskii* was able to develop and reproduce on eggs of *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) and *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) as factitious food under laboratory conditions. In addition, *Amblyseius zaheri* Yousef and El-Borolossy and *N. barkeri* were fed eggs of *E. kuehniella* (Momen and El-Laithy, 2007); *T. swirskii* succeeded to be mass reared on the astigmatid mites *Suidasia medanensis* (Oudemans) (Acari: Suidasiidae) (Midthassel et al., 2013), *Carpoglyphus lactis* L. (Acari: Carpocephalidae) (Bolckmans and van Houten, 2006), *E. kuehniella* (Nguyen et al., 2014). Research by Momen et al. (2013) verified that among eight phytoseiid mite species, only females of *N. barkeri*, *C. negevi* and *A. largoensis* were able to feed and sustain oviposition on eggs of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae).

No study has been published on the relationship between the diet *B. zonata* eggs and biological aspects of phytoseiid mites.

The objective of this study was to evaluate the relative nutritional value of *B. zonata* eggs (Tephritidae) as a factitious food source for the phytoseiid mites' *N. barkeri*, *C. negevi*, *P. kadii*, *A. largoensis* and *T. swirskii*. In particular, the reproduction potential and demographic parameters of each species were evaluated and compared under laboratory conditions using the eggs of *B. zonata* that possibly be used in their mass rearing.

## Materials and Methods

### *Host and phytoseiid predatory mite cultures*

The predatory mites, *N. barkeri* and *P. kadii* were collected from debris and litter of peach and guava cultivated in Fayoum Province, while *A. largoensis*, *T. swirskii* and *C. negevi* were found on leaves of guava at the same Province of Egypt. These mites were reared on kidney bean leaves heavily infested with two-spotted spider mites *Tetranychus urticae* Koch (Acari: Tetranychidae). The leaves were placed upside down on a layer of water-saturated cotton in a glass Petri dish, with an extra cotton layer on the leaf edges to provide free water and prevent the mites from escaping. A small piece of sewing thread was placed on the arenas to serve as an oviposition substrate. The newly deposited eggs of each species were used for the different test diet.

Predatory mites were cultured in a growth chamber set at  $28 \pm 1$  °C,  $75 \pm 5\%$  relative humidity (RH) and a 12:12 h (light:dark) photoperiod.

### *Stock colony of test diet Bactrocera zonata*

Infected fruits (peach and guava) with *B. zonata* were collected and inserted in plastic container its bottom covered with a thin layer of fine sand for pupation. Then the sand was sieved and the pupae were collected and introduced into the adult rearing cage. For collecting the eggs of *B. zonata*, a perforated plastic mandarin models provided with water, to prevent eggs from desiccation, at the 1/3 none perforated portion, were introduced into the adult rearing cage. The females of *B. zonata* lay their eggs inside these models by their ovipositors. The deposited eggs are collected each 3 days. By this method, one cage can provide 10 ml of eggs each 3 days (each cage can hold 3000–4000 adults). The obtained eggs are divided to two portions: one portion is used for feeding the predatory mites while the other portion is used for continuous rearing of *B. zonata*. The latter portion is scattered on the larval media, which consist of wheat bran, brewer's yeast, sodium benzoate, conc. Hcl sugar, and water (Shehata et al., 2008). The eggs hatch to larvae, which feed on this media until pupation. The produced pupae transferred to the rearing cage awaiting adult emergence and so on.

### *Experimental setup*

Eggs of each predatory species (less than 8-h old) were transferred singly to each rearing cell, and the newly hatched larvae were supplied with *B. zonata* eggs as food and refreshed daily. All handling of mites and moth eggs was performed with a very fine, moistened squirrel-hair brush.

Small translucent plastic cell were used as experimental units, the bottom of which was covered with a layer of 0.5 cm of a mixture of nine parts of gypsum and one part of activated charcoal; this layer was maintained humid by daily additions of water. The open end of each unit was sealed with a piece of transparent plastic film, to prevent organism from escaping.

### *Effect of diet on the development, consumption, reproduction and demographic parameters*

The developmental periods and consumption rates of the different stages of the predatory mites were recorded every 12 h. The developmental stage of each individual was determined based on the presence of exuviae in the cells. Newly emerged females were allowed to copulate with males within 24 h, and they were confined individually on rearing cell. The oviposition and longevity were observed daily. Progeny from females of the same age were transferred to new cells and fed on the same diet as their parents in order to determine the sex ratio of the offspring for each predator. Mites that died due to unnatural causes were excluded from data analysis.

Twenty-two eggs of each predatory mite species were used for testing the prey insect. All of the experiments were conducted under laboratory conditions of  $28 \pm 1$  °C,  $75 \pm 5\%$  RH and a 12 L:12 D h photoperiod.

### *Statistical analysis and life table parameters*

A total of 18 individuals (replicates) of each species per *B. zonata* eggs were analyzed using 1-way ANOVA; data were fitted with the assumption of normality, not transformed, and the treatment means were compared by Tukey HSD at a 5% probability level. A fertility life table (Birch, 1948) was constructed for predators reared on *B. zonata*. Net reproductive rate ( $R_0$ ), intrinsic rate of population increase ( $r_m$ ), finite rate of population increase ( $\lambda$ ), mean generation time ( $T$ ) and standard errors were calculated using Jack-knife procedure and compared by Students test using Life Table SAS in the software SAS System (Maia et al., 2000).

## Results

### *Effect of diet on biological aspects*

The predatory mites, *N. barkeri*, *P. kadii* and *A. largoensis* successfully developed and reproduced on eggs of *B. zonata* as a factitious food (Table 1). In contrast, immatures of *C. negevi* and *T. swirskii* failed to develop beyond the protonymphal stage. The mean developmental period from egg to adult, (life cycle) was significantly affected by the predatory species tested. Larvae of all tested phytoseiid species moult to protonymphal stage without feeding. The consumption rate increased through the developmental stages respectively (Table 2). The immature stages of *A. largoensis* consumed more *B. zonata* eggs than *N. barkeri* and *P. kadii*.

The longest mean oviposition period (25.72 days) was recorded on *A. largoensis*, whereas *P. kadii* resulted in the shortest mean oviposition duration (19.72 days), respectively. A similar trend was observed for female longevity (duration of the adult stage) (Table 1). During the oviposition period, *N. barkeri* and *A. largoensis* consumed nearly twice more amount of prey eggs than *P. kadii* (Table 2). The mean total number of con-

**Table 1**

Mean developmental period (days) (mean  $\pm$  SE) of *Neoseiulus barkeri*, *Amblyseius largoensis* and *Proprioseiopsis kadii* females fed eggs of *Bactrocera zonata* as a factitious food

Developmental stages	Predatory mite species			Calculated (F) value
	<i>Neoseiulus barkeri</i> (18) <sup>z</sup>	<i>Amblyseius largoensis</i> (18)	<i>Proprioseiopsis kadii</i> (18)	
Egg	1.17 $\pm$ 0.09	1.39 $\pm$ 0.11	1.44 $\pm$ 0.12	1.767 <sup>Ns</sup>
Larva	1.00 $\pm$ 0.00 b <sup>y</sup>	1.17 $\pm$ 0.09 a	1.00 $\pm$ 0.00 b	3.400*
Protonymph	2.00 $\pm$ 0.00 b	2.78 $\pm$ 0.10 a	2.00 $\pm$ 0.00 b	59.50**
Deutonymph	3.78 $\pm$ 0.10 a	3.05 $\pm$ 0.15 b	3.00 $\pm$ 0.00 b	17.19**
Life cycle	7.94 $\pm$ 0.09 ab	8.44 $\pm$ 0.24 a	7.44 $\pm$ 0.12 b	8.88**
Pre-oviposition	2.44 $\pm$ 0.12 b	4.33 $\pm$ 0.11 a	1.00 $\pm$ 0.00 c	303.76**
Oviposition	22.50 $\pm$ 0.39 b	25.72 $\pm$ 0.93 a	19.72 $\pm$ 0.36 c	23.40**
Post-oviposition	4.22 $\pm$ 0.19	3.78 $\pm$ 0.17	3.83 $\pm$ 0.23	1.47 <sup>Ns</sup>
Adult longevity	29.17 $\pm$ 0.35 b	33.83 $\pm$ 0.94 a	24.55 $\pm$ 0.45 c	53.18**
Life span	37.11 $\pm$ 0.34 b	42.22 $\pm$ 0.94 a	32.00 $\pm$ 0.48 c	63.29**

<sup>z</sup> Numbers between parentheses represent the number of replicates

<sup>y</sup> Within rows, means followed by a common letter do not differ significantly (Tukey HSD,  $\alpha=0.05$ )

\*\* Highly significant at  $P=0.01$ ; \* Significant; Ns = not significant

**Table 2**

Mean consumption rate (mean  $\pm$  SE) of *Neoseiulus barkeri*, *Amblyseius largoensis* and *Amblyseius kadii* females fed eggs of *Bactrocera zonata* as a factitious food

Developmental stages	Predatory mite species			Calculated (F) value
	<i>Neoseiulus barkeri</i>	<i>Amblyseius largoensis</i>	<i>Proprioseiopsis kadii</i>	
Larva	Non feeding	Non feeding	Non feeding	
Protonymph	1.00 $\pm$ 0.00 c	2.00 $\pm$ 0.00 a	1.39 $\pm$ 0.12 b	54.532**
Deutonymph	6.00 $\pm$ 0.24 a	5.89 $\pm$ 0.36 a	4.22 $\pm$ 0.15 b	14.031**
Total feeding instars	7.00 $\pm$ 0.24 b	7.89 $\pm$ 0.36 a	5.61 $\pm$ 0.21 c	16.790**
Pre-oviposition period	8.39 $\pm$ 0.44 b	13.00 $\pm$ 0.72 a	2.00 $\pm$ 0.00 c	127.163**
Oviposition period	56.39 $\pm$ 1.13 a	58.72 $\pm$ 1.76 a	32.11 $\pm$ 0.59 b	137.332**
Post-oviposition period	5.67 $\pm$ 0.55	6.11 $\pm$ 0.36	4.78 $\pm$ 0.37	2.392 <sup>Ns</sup>
Adult longevity	70.44 $\pm$ 0.83 b	77.83 $\pm$ 1.73 a	38.89 $\pm$ 0.82 c	293.380**
Total no. eggs consumed / ♀	77.44 $\pm$ 0.70 b	85.72 $\pm$ 1.77 a	44.50 $\pm$ 0.75 c	339.300**
Daily no. eggs consumed / ♀	2.08 $\pm$ 0.02 a	2.03 $\pm$ 0.03 a	1.39 $\pm$ 0.01 b	317.632**

\*\* Highly significant; Ns: not significant

**Table 3**

Demographic parameters of *Neoseiulus barkeri*, *Amblyseius largoensis* and *Amblyseius kadii* females fed eggs of *Bactrocera zonata* as a factitious food

Life table parameters	Predatory mite species		
	<i>Neoseiulus barkeri</i> (18) <sup>z</sup>	<i>Amblyseius largoensis</i> (18)	<i>Proprioseiopsis kadii</i> (18)
Mean total fecundity (eggs / ♀)	34.39 ± 0.57 a <sup>y</sup>	32.17 ± 1.13 a	19.61 ± 0.39 b
Daily no. of eggs / ♀	1.52 ± 0.02 a	1.25 ± 0.012 b	0.99 ± 0.00 c
Net reproductive rate (R <sub>0</sub> )	34.61 ± 0.57 a	32.78 ± 1.31 b	19.89 ± 0.45 c
Mean generation time (T)	16.77 ± 0.14 a	17.81 ± 0.33 b	16.11 ± 0.18 a
Intrinsic rate of increase (r <sub>m</sub> )	0.211 ± 0.001 a	0.196 ± 0.003 b	0.186 ± 0.002 c
Finite rate of increase (λ)	1.234 ± 0.002 a	1.215 ± 0.0041 a	1.203 ± 0.002 b
Sex ratio (females/ total)	0.6	0.68	1
♀♀: ♂♂	209 : 135	242 : 110	270 : 0

z Numbers between parentheses represent the number of replicates

y Within rows, values followed by a common letter do not differ statistically. Total and daily no. of eggs: F = 108.937\*\*, 286.130\*\*, df 2.53; P = 0.000; \*\* highly significant

sumed eggs / female of *N. barkeri* and *A. largoensis* was the highest on eggs of *B. zonata* (Table 3). In the contrary, the lowest number of deposited eggs was recorded on *P. kadii*. A similar trend was observed for the mean daily number of eggs / female.

#### *Effect of diet on demographic parameters*

The highest value of the net reproductive rate (R<sub>0</sub>) was 34.61 expectant females / female of *N. barkeri*, whereas the lowest value was 19.89 expectant females / female of *P. kadii*. A similar trend was observed with the intrinsic rate of natural increase (r<sub>m</sub>) and, subsequently, the finite rates of increase (λ) were the highest (0.21 and 1.23), respectively, when individuals of *N. barkeri* preyed on *B. zonata* eggs. The shortest mean generation time (T, 16.11 and 16.77 days) of *P. kadii* and *N. barkeri* was found compared to the longest mean per period (17.81 days) on *A. largoensis* (Table 3).

The age-specific survival rate L<sub>x</sub> (percent of surviving females at the instant x) and the age-specific fecundity rate m<sub>x</sub> (number of female eggs laid per female per day) for *P. kadii*, *N. barkeri* and *A. largoensis* reared on *B. zonata* eggs are shown in Fig. 1.

## Discussion

It is well known that members of the family Phytoseiidae show considerable variation in their feeding habits, including pollen grains, scale insects, moth eggs as well as phytophagous mites in their diet (McMurtry and Croft, 1997).

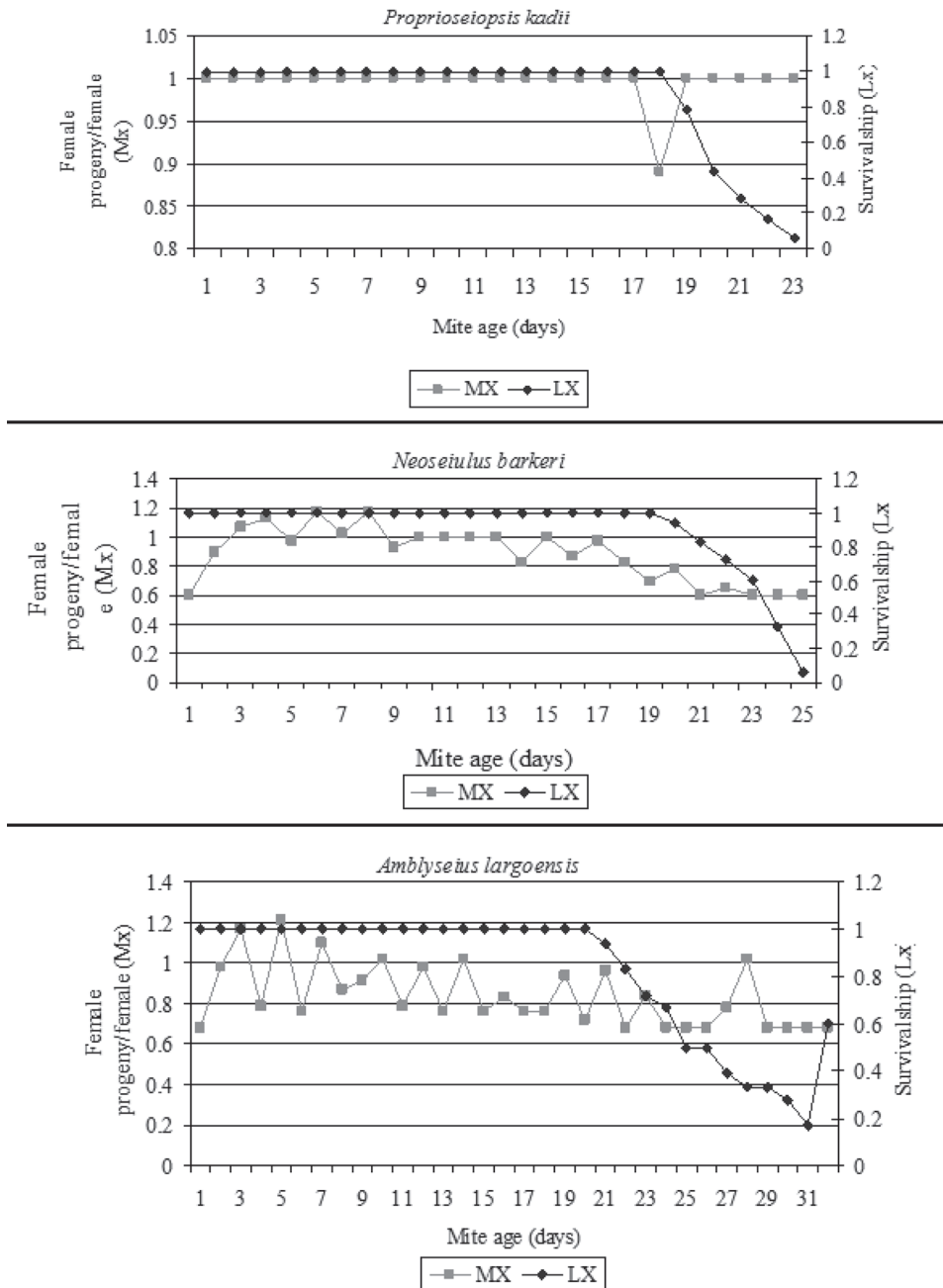


Fig. 1. Life table parameters of predatory mites, *Proprioiseiopsis kadii*, *Neoseiulus barkeri* and *Amblyseius largoensis* fed on eggs of *Bactrocera zonata*

Few studies have been carried out on the life history of phytoseiid mites being fed on factitious food. In the present study, *B. zonata* eggs provided commensurate nutritional effects on survivorship, longevity and fecundity of *N. barkeri*, *A. largoensis* and *P. kadii* females. In contrast, *T. swirskii* and *C. negevi* were not able to develop beyond the protonymphal stage when offered the diet eggs.

When *N. barkeri* and *A. largoensis* were fed on natural / main host such as, *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae), nymphs of *Tetranychus urticae* Koch, *Tetranychus gloveri* Banks and *Eutetranychus orientalis* (Klein) (all Acari: Tetranychidae), *Raoiella indica* Hirst (Acari: Tenuipalpidae), the developmental period of both species was considerably shorter than on eggs of *B. zonata* (Bonde, 1989; Momen, 1995; Momen and El-Borolossy, 1999; Carrillo et al., 2010). When *P. kadii* was preying on *E. orientalis* the developmental period was close to present data (unpublished data).

The nutritional value of a diet for predatory mites could be measured by estimation of life table parameters. More favorable diet conditions will result in better population growth in predatory mites: *N. barkeri* on *T. tabaci* (Bonde, 1989), *T. swirskii* on eriophyid mite (Momen, 2009), *Euseius scutalis* (Athias Henriot) on pollen grains and mites (Momen and Abdel-Khalek, 2008), *C. negevi* on pollen grains (Momen et al., 2009) and *Proprioseiopsis cabonus* on tydeoid mites (Momen, 2011). Our study is also consistent with these studies in that the higher quality food sources resulted in higher values in net reproductive rate ( $R_0$ ), intrinsic rate of increase ( $r_m$ ) and finite rate of increase ( $\lambda$ ).

Both the ( $R_0$ ) and ( $\lambda$ ) of *N. barkeri* and *A. largoensis* reared on *E. kuehniella*, *Aleuroglyphus ovatus* Toupeau (Acari: Acaridae), *R. indica* and *T. gloveri* were lower than our results with *B. zonata* eggs (Momen and El-Laithy, 2007; Carrillo et al., 2010).

For *N. barkeri*, *B. zonata* egg diet resulted in a total oviposition lower than those of females fed with nymphs of *T. tabaci*, *T. urticae*, *Aceria dioscoridis* (Soliman and Abou-Awad) (Acari: Eriophyidae) (main food) / eggs of *E. kuehniella* and *T. absoluta* (factitious food) (Bonde, 1989; Momen, 1995; Momen and El-Laithy, 2007; Momen et al., 2013), while a comparable total number of eggs laid was reported on a diet of *A. ovatus* (Xia et al., 2012). For *A. largoensis*, our results clearly show that eggs of *B. zonata* are an acceptable food resource, promoting oviposition greater than that obtained with diets of tenuipalpid and tetranychid mites and pollen grains (Carrillo et al., 2010).

Unlike the obligatory predators, generalist phytoseiid mites tested here are distinguished by whether they survive by preying on insect and mites in addition to feed on pollen grains and factitious food.

In the present study, the predatory mite *P. kadii* was consumed the lowest number of diet eggs and exhibited the lowest mean total fecundity as well as the net reproductive rate. The low number of eggs attacked by *P. kadii* may be attributed to an apparent avoidance reaction by the predator to egg encountered after attacking the first egg and possible reason for the relative low fecundity observed, include the inherent characteristics of or inadequacy of *B. zonata* eggs as a food source for oviposition.

Pre-imaginal mortality of *C. negevi* and *T. swirskii* mainly occurred in the protonymphal stage and that may be due to that eggs of *B. zonata* are being unfavorable for these phytoseiids. Negative results with above 2 tested phytoseiid predators on developmental, mortality and egg production can be attributed to the following reasons: (i) may



be due to resistance of egg chorion (ii)- it could be caused by the presence of an antifeedant factor in the eggs (iii)- may be due to inadequacies of level of certain nutrients of *B. zonata* eggs if compared with the high nutritional value of other tested factitious food.

By contrary to our results with *T. swirskii*, the predator succeeded to complete its development and reproduced when offered eggs of *P. operculella* and *S. littoralis* (El-Sawi and Momen, 2005), a diet of *Suidasia medanensis* (Oudemans) (Acari: Suidasidae) (Midthassel et al., 2013), *E. kuehniella* and decapsulated dry cysts of the brine shrimp *Artemia franciscana* Kellogg (Anostraca: Artemiidae) (Nguyen et al., 2014) as factitious food.

Since no males were known for *P. kadii* (unpublished data), this species producing parthenogenesis. All the progeny are all females (Thelytoky): this behavior in mites of the Mesostigmata has been reported in the family Phytoseiidae (Wysoki, 1985).

It is worthy to mention that the components used in rearing both adult and larvae of *B. zonata* are substantially cheap and easy to obtain. For example, the cost needed for producing 285,000 eggs of *B. zonata* is approximately 0.25 USD where, we need ¼-kilogram sugar and 20 ml Bominal for feeding the *B. zonata* female to produce the previous huge number of eggs. This cost is so cheap that it can be ignored.

An effective factitious food should satisfy the nutritional requirements of a predator and ensure the continuous production of progeny of high quality (Cohen, 2003). As nutrient imbalances in a diet may be expressed only after several generations of rearing (De Clercq et al., 2005), so further studies should also be made to culture the predators for several consecutive generations on selected food and assess its fitness in the subsequent generation.

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