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Evaluation of different artificial diets for rearing the predatory mite *Neoseiulus californicus* (Acari: Phytoseiidae): diet-dependent life table studies

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ABSTRACT — The use of an artificial diet may represent a step toward more cost-effective rearing of generalist phytoseiid mites. Life table studies were performed to evaluate the nutritional value of ten different artificial diets as an alternative food source for rearing of *Neoseiulus californicus* McGregor. All experiments were carried out under laboratory conditions, at $25 \pm 1^\circ\text{C}$, $60 \pm 5\%$ RH and a photoperiod of 16:8 (L:D) h. Most enriched diets reduced the total developmental time of the predator compared to the basic artificial diet (AD1). All enriched artificial diets (except AD10 (diet enriched with multivitamin syrup) and AD5 (diet enriched with serum albumin protein)) increased the total fecundity of *N. californicus* compared with AD1, and the highest fecundity was observed on the diet supplemented with *Ephestia kuehniella* Zeller eggs (AD2). The highest intrinsic rate of increase (r) values were observed on the diets enriched with *E. kuehniella* eggs (AD2), *Artemia franciscana* Kellogg cysts (AD3) and maize pollen (AD6), whereas the diet enriched with serum albumin protein (AD5) had the lowest value of this parameter. In conclusion, the diets supplemented with arthropod components, as well as with bull sperm or maize pollen all enhanced survival, development and reproduction of *N. californicus*, and consequently its population growth parameters.

KEYWORDS — artificial diet; alternative diet; phytoseiid mites; life table

INTRODUCTION

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch is a major pest in many economically important crops (Helle and Sabelis, 1985; Luczynski *et al.*, 1990; Sedaratian *et al.*, 2011; Alipour *et al.*, 2016) and has a host range of more than 1,100 species of plants (Grbic *et al.*, 2011). Management of TSSM is extremely difficult, and a large quantity of pesticides are used for this purpose. Chemical pesticides can be harmful to humans, environment and nontarget organisms and may cause

secondary pest outbreaks and resistance (Debach, 1974). In contrast, biocontrol agents, including phytoseiid mites, are generally considered safe to humans and the environment and generally have no effect on other nontarget organisms (Fathipour and Maleknia, 2016).

In biological control programs accompanied with augmentation of biocontrol agents, providing the necessary facilities for mass production of these agents is the crucial subject. Mass production and release of biocontrol agents is the founda-

tion of augmentative biological control (King, 1993). Current mass-rearing systems are complex as they require multiple organisms, comprising the predator/parasitoid, the prey/host and the host plant. The necessity of maintaining three trophic levels in natural rearing systems may cause problems of discontinuity, and the high costs for rearing facilities and labor can lead to high market prices for the predator (De Clercq *et al.*, 2005). The availability of effective artificial diet will reduce the number of trophic levels, and the use of it may assist in making the mass production of a biocontrol agent more cost effective (Grenier and De Clercq, 2003).

Predatory mites of the family Phytoseiidae are economically important predators of phytophagous mites and insects in greenhouse crops (Greco *et al.*, 2005; Khodayari *et al.*, 2013). Several species of phytoseiid predatory mites rank among the most important biocontrol agents used in augmentative biological control against various pests (Cock *et al.*, 2010; Van Lenteren, 2012). *Neoseiulus californicus* McGregor (Acari: Phytoseiidae), one of the most important biocontrol agent of TSSM, can provide excellent biological control of spider mites over wide range of climatic and management conditions (Oatman *et al.*, 1977). The possibility of mass rearing of *N. californicus* on alternative and more economical diet such as pollen increases the interest in this predator as a control agent (Castagnoli and Simoni, 1999).

In the mass production of different generalist phytoseiid mites, astigmatid mites are being used as primary food sources instead of natural prey (Bolckmans and van Houten, 2006). However, this rearing system is often time-consuming and allergy problems can be generated using astigmatid mites (Fernandez-Caldas *et al.* 2007). The availability of effective artificial diet may assist in making the mass production of a phytoseiid mite more cost effective. Reducing costs of production could decrease the market price of phytoseiid mites and increase the number of growers using biological solutions for pest management.

To create an artificial diet for an arthropod, the feeding mechanism and its digestive system as well as its nutritional requirements and the biochemical

composition of its natural food should be known. However, much of the successes with artificial diets were based on a simple trial-and-error approach. According to the purity of components, artificial diets can be classified into three types: (1) holidic diet, is one of which the chemical structure of all ingredients is known; (2) meridic diet, has a holidic basis but at least one of the components has an unknown structure or purity; (3) oligidic diet, in which a few of the components are known chemically, and contain unpurified organic components, mainly raw organic materials (Dougherty, 1959). In addition, another classification according to the presence or absence of insect components (hemolymph, tissue homogenates or extracts, egg juice) has been proposed by Grenier and De Clercq (2003).

Whereas several artificial diets have been developed for predatory insects, relatively few attempts have been made for rearing of predatory mites on artificial diets (McMurtry and Scriven, 1966; Kennett and Hamai, 1980; Ogawa and Osakabe, 2008; Nguyen *et al.*, 2013, 2014a). Therefore, the main objective of this study was to develop a suitable artificial diet for mass rearing of phytoseiid predators in which *Neoseiulus californicus* (McGregor) was chosen as a phytoseiid predator to be studied in this research.

MATERIALS AND METHODS

Predator stock culture

The culture of *N. californicus* at the laboratory was started with individuals obtained from Koppert Biological Systems. Laboratory colonies of *N. californicus* were reared in green plastic arenas (18×13×0.1 cm) on water-saturated sponge in a Plexiglas box (25×18×10 cm), which was half-filled with water. The edges of the arenas were covered with moist tissue paper to provide moisture and prevent predators from escaping (Walzer and Schausberger, 1999). Bean leaves (*Phaseolus vulgaris* var. Khomein) infested with TSSM were added to the arena every other day.

Providing dietary supplements

Pollen of maize (*Zea mays* L.) was collected from plants at the Faculty of Agriculture campus in Tarbiat Modares University, Tehran, Iran, in July 2014, then it was oven dried (at 37 °C for 48 h). Sterilized eggs (by UV rays) of *Ephestia kuehniella* Zeller (Lep.: Pyralidae) were provided by the Insectarium of Scientific and Industrial Research Organization of Iran, whereas the larvae of *E. kuehniella* were obtained from reared colonies on wheat flour at the Laboratory of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. *Artemia franciscana* Kellogg (Anostraca: Artemiidae) cysts were purchased from aquarium fish sale center in Isfahan, Iran, and decapsulated by washing in a hypochlorite solution (Van Stappen, 1996). Bull sperm was provided by livestock and poultry feed sale center in Kerman, Iran. Serum albumin protein (bovine serum albumin) was purchased from laboratory equipment center of Tarbiat Modares University, Tehran, Iran.

The specimens of *Plusia gamma* L. (Lep.: Noctuidae) were originally collected from infested corianders (*Coriandrum sativum* L.) at the Faculty of Agriculture greenhouse in Tarbiat Modares University, Tehran, Iran. *P. gamma* colony was reared on green bean pods (*Phaseolus vulgaris* L.) in a growth chamber at 25 ± 1 °C, relative humidity of 60 ± 5% and a photoperiod of 16:8 (L:D) h. The larval hemolymph was collected from live *P. gamma* larvae (last instar) according to Ghasemi *et al.* (2013).

All above-mentioned supplements were frozen at -20 °C for long-term storage or refrigerated at 4 °C for up to 2 weeks during the experiments. In addition, multivitamin capsule (each capsule contains: vitamin A 5000 IU, vitamin D 400 IU, vitamin E 30 IU, vitamin B₁ 1.5 mg, vitamin B₂ 1.7 mg, vitamin B₆ 2 mg, vitamin B₁₂ 6 mcg, vitamin C 60 mg, folic acid 0.4 mg, nicotinamide 20 mg, calcium 125 mg, iodine 150 mcg, iron 18 mg, magnesium 100 mg) and multivitamin syrup (each 5 ml contains: vitamin A 2500 IU, vitamin D 400 IU, vitamin E 15 IU, vitamin B₁ 1 mg, vitamin B₂ 2.1 mg, vitamin B₆ 1 mg, vitamin B₁₂ 5.4 mcg, vitamin B₅ 5.13 mg, and water in sufficient quantity) were prepared from pharmacy.

Preparation of artificial diets

The basic artificial diet (AD1) was prepared according to Nguyen *et al.* (2013), which consisted of 5 % honey, 5 % sucrose, 5 % tryptone, 5 % yeast extract, 10 % egg yolk, and 70 % distilled water (w/w). The other diets (AD2 to AD10) consisted of 80 % AD1 enriched with 20 % (w/w) of different supplements (Table 1). Liquid or soluble supplements such as bull sperm, serum albumin protein, haemolymph of *P. gamma*, the contents of multivitamin capsule, and multivitamin syrup, directly were mixed with the basic artificial diet (AD1) and then were centrifuged (at 12,000 rpm at 5 °C for 15 min). The other supplements (*E. kuehniella* eggs, decapsulated *A. franciscana* cysts, maize pollen, and *E. kuehniella* larvae) initially were ground in a ceramic mortar then mixed with the basic artificial diet (AD1) and then were centrifuged. The obtained diets were transferred to 2 ml micro tubes and frozen at -20 °C for long-term storage or refrigerated at 4 °C for up to 2 weeks during the experiments.

Experimental design

In order to evaluate the suitability of different artificial diets as alternative food sources for rearing of *N. californicus*, life table parameters were determined. At the beginning of the experiments, to obtain the synchronized eggs of *N. californicus*, 50 pairs of newly emerged *N. californicus* (male and female) were transferred from the conditioned colonies onto a bean leaf disc. After 24h, the deposited eggs were transferred individually to the experimental units (by using a small brush) up to 70 replicates per treatment. Experimental units were similar to those used for the predator culture, but in a smaller scale (Khanamani *et al.*, 2017).

These eggs (70 eggs for each treatment) were checked daily and the incubation period was recorded. After the emergence of larval predators, the respective test diet was offered as food. Artificial diets were absorbed on a small piece of filter paper (2 × 2 mm) (Nguyen *et al.*, 2013) which was placed in the corner of experimental unit sheet, and refreshed every two days. Duration and survival of different immature stages were monitored every 24

TABLE 1: Ingredients (%w/w) of different artificial diets.

Artificial diets	Ingredients
AD1	5 % honey, 5 % sucrose, 5 % tryptone, 5 % yeast extract, 10 % egg yolk, and 70 % distilled water
AD2	80 % AD1 + 20 % <i>Ephestia kuehniella</i> egg
AD3	80 % AD1 + 20 % decapsulated <i>Artemia franciscana</i> cyst
AD4	80 % AD1 + 20 % bull sperm
AD5	80 % AD1 + 20 % serum albumin protein (bovine serum albumin)
AD6	80 % AD1 + 20 % maize pollen
AD7	80 % AD1 + 20 % haemolymph of <i>Plusia gamma</i>
AD8	80 % AD1 + 20 % whole body tissue extracts of <i>Ephestia kuehniella</i> larvae
AD9	80 % AD1 + 20 % multivitamin capsule
AD10	80 % AD1 + 20 % multivitamin syrup

*AD, artificial diet; AD1, basic artificial diet.

h until the adult stage. When the adult stage was reached, females and males were coupled. The couples were kept together up to the end of the study, and survival and number of eggs produced by females were recorded daily. The experiment lasted until both adults died. All experiments were carried out under laboratory conditions, at $25 \pm 1^\circ\text{C}$, $60 \pm 5\%$ RH and a photoperiod of 16:8 (L:D) h.

The parameters recorded in these experiments were: duration of different life stages, immature survival, preoviposition and oviposition periods, fecundity, sex ratio ($\frac{\text{♀}}{\text{♂}+\text{♀}}$), female and male longevity, and lifespan.

Life table parameters estimation

Data obtained from all individuals on the ten artificial diets were subjected to the age-stage, two-sex life table procedure (Chi and Liu, 1985; Chi, 1988) to analyze the effect of diet on the bio-ecological parameters of *N. californicus*. The age-stage specific survival rate (s_{xj}) (x is the age and j is the stage); the age-specific survivorship (l_x); the age-specific fecundity (m_x); and the life table parameters (intrinsic rate of natural increase (r), finite rate of increase (λ), net reproduction rate (R_0), gross reproduction rate (GRR), and mean generation time (T)) were calculated accordingly. More details on two-sex life table parameters can be found in the relevant references (Huang and Chi, 2012; Khanamani *et al.*, 2013; Safuraie-Parizi *et al.*, 2014).

Statistical analysis

The life table parameters were calculated by using the TWOSEX-MSChart program (Chi, 2015). The standard errors of duration of different life stages, immature survival rate and life table parameters were estimated by using the bootstrap procedure (Efron and Tibshirani, 1993; Huang and Chi, 2013). Because the bootstrap method generated normally distributed estimates and smaller variances. To obtain stable estimates, we used 40,000 bootstraps. Standard error of the bootstrap is the standard deviation of the bootstrap replications (Efron and Tibshirani, 1993). The differences of bootstrap-values among the treatments were compared using the paired bootstrap test based on the confidence interval of difference (Akca *et al.*, 2015).

RESULTS

Life stages duration and survival

The life history parameters of *N. californicus* fed on ten different artificial diets are shown in Table 2. The egg incubation periods did not differ among diets. However, diet had a significant effect on the developmental time of larval and nymphal stages of *N. californicus*. The cohort reared on the artificial diet enriched with multivitamin capsule (AD9) could not complete their life cycle, and all of them died before adulthood in the larval and

protonymphal stages. Duration of total developmental time of the predator was significantly different among tested diets; this period was longer when the predator was fed on the basic artificial diet (AD1). All enriched diets (except AD10) reduced the duration of preadult developmental time,

A significant effect of diet was found for male and female longevity of adult *N. californicus*. On the diets enriched with multivitamin syrup (AD10) and *P. gamma* haemolymph (AD7), followed by AD2, *N. californicus* males and females were generally found to have the longest longevity, whereas the shortest longevity for both sexes was on the diet enriched with serum albumin protein (AD5). Furthermore, duration of total life span of *N. californicus* indicated significant differences among tested diets, and the longest period was related to those reared on the diets enriched with multivitamin syrup (AD10) and *P. gamma* haemolymph (AD7), and the shortest period was related to those on the diet enriched with serum albumin protein (AD5).

The age-stage specific survival rates (s_{xj}), and age specific survivorship (l_x) curves of *N. californicus* are shown in Figures 1 and 2, respectively. According to these results, the highest preadult mortality of *N. californicus* was on the diet enriched with multivitamin capsule (AD9), followed by AD5 (diet enriched with serum albumin protein), whereas no immature mortality was observed on the diet enriched with maize pollen; on the other diets immature mortality was intermediate.

Reproductive parameters, sex ratio and fecundity curves

Reproductive periods (adult pre-ovipositional period (APOP), total pre-ovipositional period (TPOP), and oviposition days) and fecundity of *N. californicus* fed on different artificial diets are shown in Table 2. Diet significantly influenced the reproductive periods and fecundity of *N. californicus*. All enriched artificial diets (except AD10 and AD7) reduced the duration of pre-ovipositional periods (APOP and TOPO) compared with basic artificial diet (AD1), and the shortest pre-ovipositional pe-

and the enriched diet with serum albumin protein (AD5), followed by AD7, had the fastest development. However, despite short development time, immature mortality was high on the enriched diet with serum albumin protein (AD5).

riod was on the artificial diet enriched with *A. franciscana* cysts (AD3).

The highest number of oviposition days was obtained on the artificial diet enriched with *E. kuehniella* eggs (AD2), followed by AD3 (enriched with *A. franciscana* cysts), whereas the lowest number was on the diet enriched with serum albumin protein (AD5). All enriched artificial diets (except AD10, diet enriched with multivitamin syrup; and AD5, diet enriched with serum albumin protein) increased the total fecundity of *N. californicus*, and the highest fecundity was observed on the diet enriched with *E. kuehniella* eggs (AD2). Furthermore, offspring sex ratio of *N. californicus* on all artificial diets (except AD6) was female-biased.

Age specific fecundity (m_x) of *N. californicus* fed on different artificial diets is shown in Figure 2. Fecundity curve was not drawn for the diet enriched with multivitamin capsule (AD9), because it did not allow individuals to reach adulthood. The fecundity curves showed that *N. californicus* had the highest peak of oviposition on the diet enriched with *E. kuehniella* eggs (AD2), followed by the diets enriched with *A. franciscana* cysts (AD3) and maize pollen (AD6). The lowest peak of oviposition was observed on the diet enriched with serum albumin protein (AD5).

Population growth parameters

Population growth (life table) parameters of *N. californicus* fed on different artificial diets are shown in Table 3. Population growth parameters of the predator were significantly different among tested artificial diets. All enriched artificial diets (except AD10, diet enriched with multivitamin syrup; and AD5, diet enriched with serum albumin protein) increased the intrinsic rate of increase (r) of *N. californicus* compared with the basic artificial diet

TABLE 2: Ingredients (%w/w) of different artificial diets.

Parameters	Artificial diets									
	AD1	AD2	AD3	AD4	AD5	AD6	AD7	AD8	AD9	AD10
Egg (d)	2.2±0.1 ^a	2.3±0.1 ^a	2.3±0.1 ^a	2.3±0.2 ^a	2.3±0.1 ^a	2.2±0.2 ^a	2.2±0.1 ^a	2.2±0.2 ^a	2.4±0.2 ^a	2.3±0.2 ^a
Larva (d)	1.1±0.0 ^b	1.0±0.0 ^c	1.2±0.1 ^{ab}	1.0±0.0 ^c	1.3±0.1 ^a	1.2±0.1 ^{ab}	1.1±0.0 ^b	1.1±0.0 ^b	1.2±0.1 ^{ab}	1.1±0.0 ^b
Protonymph (d)	2.8±0.1 ^b	2.4±0.1 ^{de}	2.5±0.1 ^{cde}	2.4±0.1 ^{de}	2.1±0.1 ^f	2.1±0.1 ^f	2.6±0.1 ^{bc}	2.3±0.1 ^e	8.3±0.2 ^a	2.6±0.1 ^{bc}
Deutonymph (d)	3.5±0.2 ^a	2.3±0.1 ^d	2.8±0.1 ^b	2.9±0.2 ^b	1.8±0.1 ^f	2.5±0.1 ^{cd}	2.7±0.1 ^{bc}	2.0±0.1 ^e	3.5±0.2 ^a
Preadult (d)	9.7±0.2 ^a	8.1±0.1 ^d	8.9±0.2 ^{bc}	8.5±0.1 ^c	7.5±0.2 ^e	8.5±0.1 ^c	8.7±0.1 ^c	7.8±0.1 ^{de}	9.5±0.3 ^{ab}
Male (d)	32.7±2.6 ^{cd}	58.6±6.8 ^{ab}	41.9±5.5 ^{bc}	50.8±2.4 ^b	23.2±4.8 ^d	47.1±2.1 ^b	62.8±3.1 ^a	39.7±2.7 ^c	71.8±6.6 ^a
Female (d)	42.9±3.6 ^{de}	61.0±4.5 ^b	56.9±5.7 ^{bc}	55.2±5.5 ^{bcd}	21.7±2.1 ^f	46.6±3.1 ^{cde}	74.2±2.9 ^a	40.7±3.3 ^c	68.1±8.9 ^{ab}
Life span (d)	48.4±2.5 ^d	68.3±3.8 ^{ab}	59.6±4.2 ^{bc}	62.3±3.7 ^{bc}	29.3±2.9 ^e	56.0±1.8 ^{cd}	78.3±2.3 ^a	48.5±2.7 ^d	79.4±5.4 ^a
APOP (d)	13.0±1.1 ^a	8.1±0.8 ^c	5.1±0.2 ^e	9.2±0.5 ^b	7.0±0.0 ^{cd}	5.8±0.6 ^{de}	11.5±1.1 ^{ab}	7.1±0.9 ^{cd}	10.8±0.8 ^{ab}
TPOP (d)	22.7±1.2 ^a	16.3±0.8 ^{cd}	14.0±0.1 ^e	17.8±0.6 ^{bc}	14.0±0.0 ^e	14.3±0.7 ^{de}	20.3±1.1 ^{ab}	15.1±0.9 ^{de}	21.0±0.6 ^a
Oviposition days	6.0±0.9 ^d	24.4±2.3 ^a	18.5±2.3 ^{ab}	10.7±1.6 ^c	1.0±0.0 ^e	16.7±1.6 ^b	14.6±1.1 ^b	9.7±1.3 ^c	10.3±1.2 ^c
Fecundity (egg)	5.1±0.9 ^d	25.2±2.3 ^a	18.5±2.3 ^b	9.6±1.6 ^c	0.1±0.06 ^e	17.1±1.8 ^b	13.7±1.2 ^b	9.0±1.3 ^c	8.0±1.4 ^{cd}
Sex ratio	0.58	0.60	0.61	0.66	0.69	0.48	0.59	0.63	0.56
Immature Survival (%)	0.80±0.05 ^c	0.87±0.04 ^{bc}	0.90±0.04 ^{bc}	0.78±0.06 ^c	0.56±0.07 ^d	1.00±0.00 ^a	0.92±0.03 ^b	0.88±0.04 ^{bc}	0.00±0.00 ^c	0.76±0.06 ^c

* d, day; Male, male longevity; Female, female longevity; APOP, adult pre-ovipositional period; TPOP, total pre-ovipositional period (from egg to first oviposition). AD1=basic artificial diet, AD2 to AD10 = basic artificial diet enriched with *Ephestia kuehniella* eggs (AD2); *Artemia franciscana* cysts (AD3); bull sperm (AD4); Bovine serum albumin protein (AD5); maize pollen (AD6); *Plusia gamma* haemolymph (AD7); *E. kuehniella* larvae (AD8); multivitamin capsule (AD9); and multivitamin syrup (AD10). The means followed by different letters in the same row are significantly different ($P < 0.05$, paired bootstrap test).

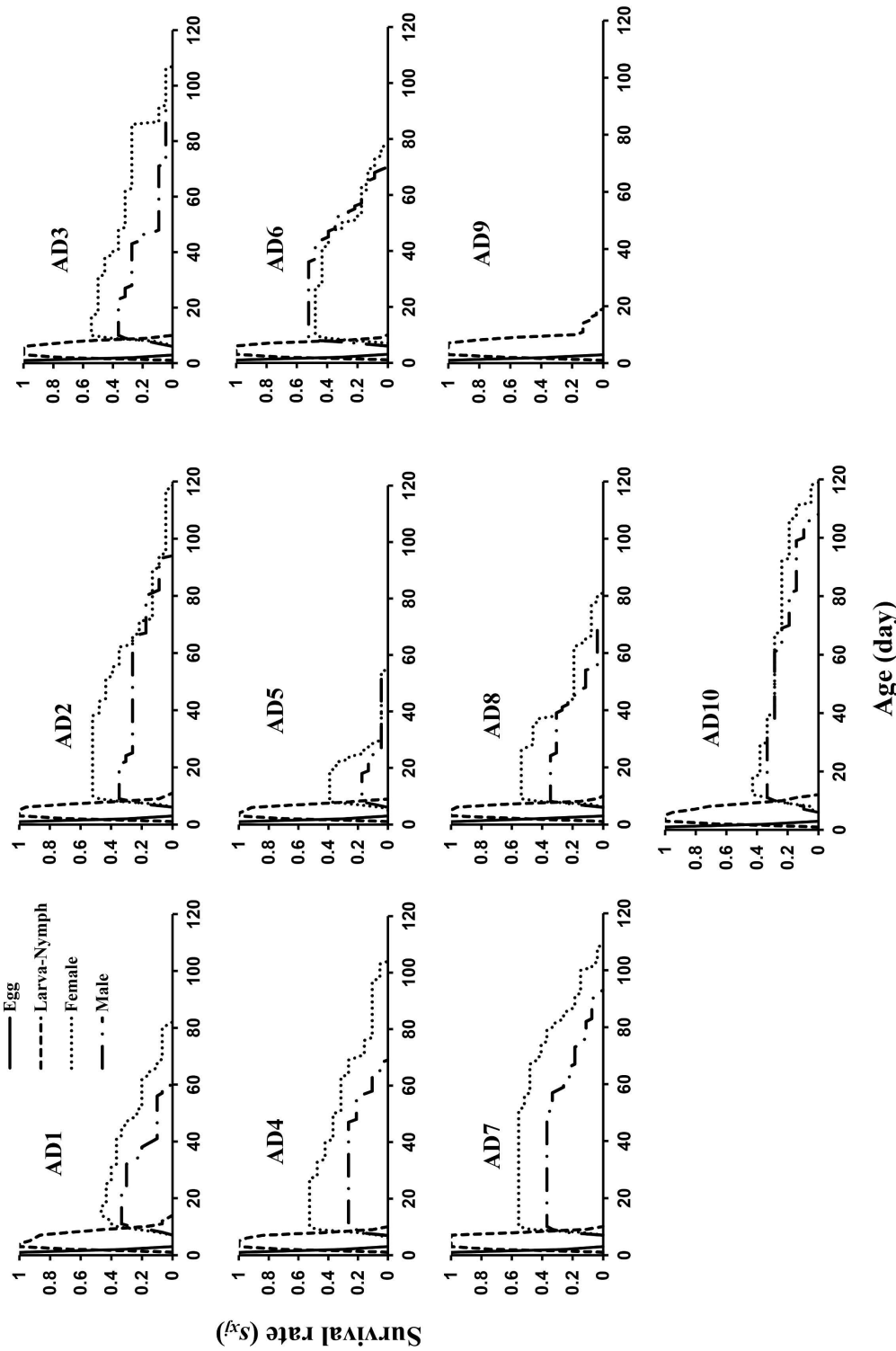


FIGURE 1: Age-stage survival rate (s_{xj}) of *Neoseiulus californicus* on different artificial diets. AD1= basic artificial diet, AD2 to AD10 = basic artificial diet enriched with *Ephesia kuehniella* eggs (AD2); *Artemia franciscana* cysts (AD3); bull sperm (AD4); Bovine serum albumin protein (AD5); maize pollen (AD6); *Plusia gamma* haemolymph (AD7); *E. kuehniella* larvae (AD8); multivitamin capsule (AD9); and multivitamin syrup (AD10).

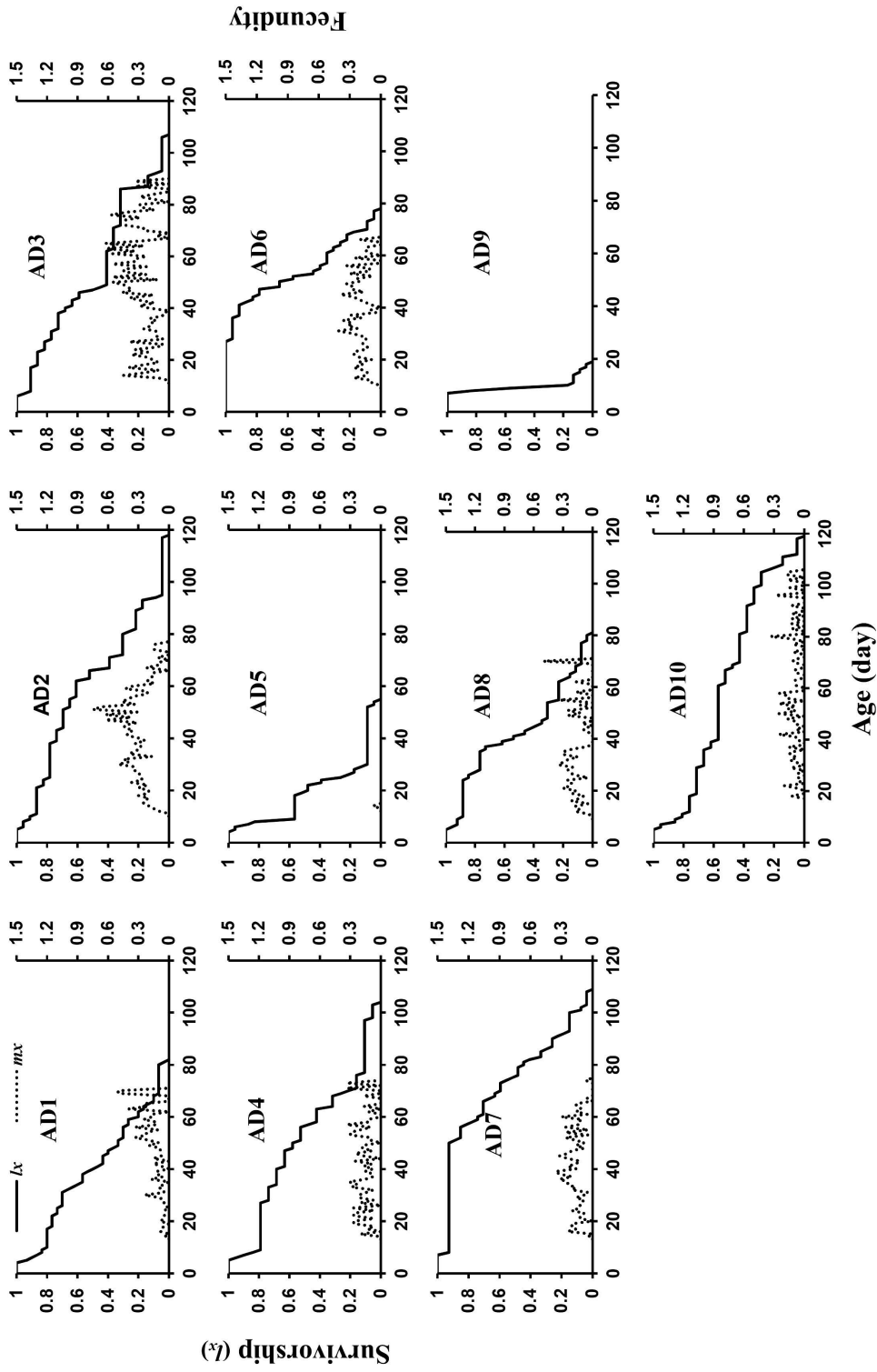


FIGURE 2: Age-specific survivorship (l_x), and age-specific fecundity (m_x) of *Neosaiulus californicus* on different artificial diets.

TABLE 3: The mean (\pm SE) life table parameters of *Neoseiulus californicus* on different artificial diets.

Artificial diets	Parameters				
	r (day ⁻¹)	λ (day ⁻¹)	GRR (offspring)	R_0 (offspring)	T (day)
AD1	0.0213 \pm 0.0056 ^d	1.0216 \pm 0.0058 ^d	6.83 \pm 1.27 ^b	2.39 \pm 0.53 ^e	39.69 \pm 2.11 ^b
AD2	0.0782 \pm 0.0067 ^a	1.0814 \pm 0.0072 ^a	18.49 \pm 2.89 ^a	13.14 \pm 2.22 ^a	32.79 \pm 1.18 ^d
AD3	0.0685 \pm 0.0061 ^{ab}	1.0709 \pm 0.0065 ^{ab}	20.77 \pm 2.68 ^a	10.10 \pm 1.87 ^{ab}	33.52 \pm 1.23 ^{cd}
AD4	0.0478 \pm 0.0070 ^c	1.0489 \pm 0.0073 ^c	8.32 \pm 1.73 ^b	5.05 \pm 1.13 ^{cd}	33.36 \pm 1.09 ^{cd}
AD5	-0.2089 \pm 0.0355 ^e	0.8119 \pm 0.0288 ^e	0.09 \pm 0.04 ^c	0.05 \pm 0.02 ^f	15.00 \pm 0.00 ^g
AD6	0.0726 \pm 0.0073 ^{ab}	1.0754 \pm 0.0079 ^{ab}	10.12 \pm 1.90 ^b	8.17 \pm 1.53 ^{abc}	28.70 \pm 0.95 ^e
AD7	0.0558 \pm 0.0049 ^{bc}	1.0574 \pm 0.0052 ^{bc}	8.6 \pm 1.20 ^b	7.60 \pm 1.11 ^{bc}	36.16 \pm 1.01 ^{bc}
AD8	0.0601 \pm 0.0083 ^{abc}	1.0620 \pm 0.0088 ^{abc}	7.43 \pm 1.21 ^b	4.85 \pm 0.93 ^{cd}	25.99 \pm 0.64 ^f
AD10	0.0243 \pm 0.0056 ^d	1.0245 \pm 0.0057 ^d	6.6 \pm 1.37 ^b	3.43 \pm 0.857 ^{de}	49.49 \pm 2.42 ^a

*The means followed by different letters in the same column are significantly different among treatments using the paired bootstrap test at 5% significance level.

(AD1), and the highest r values were observed on the diets enriched with *E. kuehniella* eggs (AD2), *A. franciscana* cysts (AD3) and maize pollen (AD6), whereas the diet enriched with serum albumin protein (AD5) had the lowest r value. The highest and lowest values of the finite rate of increase (λ) were obtained on the diets enriched with *E. kuehniella* eggs (AD2) and serum albumin protein (AD5), respectively. The cohort reared on the diets enriched with *E. kuehniella* eggs (AD2), followed by the diets enriched with *A. franciscana* cysts (AD3) and maize pollen (AD6), had the highest net reproductive rate (R_0), and those on the diet enriched with serum albumin protein (AD5) had the lowest R_0 . The highest gross reproductive rate (GRR) was observed on the diets enriched with *E. kuehniella* eggs (AD2) and *A. franciscana* cysts (AD3). In addition, the longest and shortest mean generation time (T) of *N. californicus* were obtained on the artificial diets enriched with multivitamin syrup (AD10) and serum albumin protein (AD5), respectively.

DISCUSSION

The use of an artificial diet may represent a step toward more cost-effective rearing of generalist phytoseiid mites and other biocontrol agents (Abou-Awad *et al.*, 1992). Artificial diets can be an alter-

native to natural and factitious prey for the mass production of biocontrol agents, reducing costs and facilitating automation of the production (Kennett and Hamai, 1980). The main purpose of this study was to develop a suitable artificial diet for mass rearing of the two-spotted mite predator, *N. californicus*.

Life tables are descriptions of survival potential at different ages or stages, and the life table parameters is a powerful tool for analyzing and understanding the impact of an external factor (such as diet) on the development rate, survival rate, reproduction rate and rate of population increase of an arthropod population (Momen, 2001). The most important advantage of a life table is that it summarizes multiple life history parameters like immature survival, development time, adult fecundity, and longevity by a single value defined as r (Carey, 1993). Thus, r can be used as the most important criterion in evaluating the suitability of diets for rearing of arthropods.

Our study revealed distinct effects of different artificial diets on bioecological parameters of the predatory mite *N. californicus*. Our results show that *N. californicus* can feed and develop to the adult stage on all the tested diets (except on AD9, diet enriched with multivitamin capsule); however, preadult duration was different among tested diets. All

enriched diets (except AD10) reduced the duration of immature developmental time. This can be explained by deficiency of primary essential nutrients for growth and development in the basic artificial diet (AD1) and this deficiency could be compensated by adding supplements (especially protein and insect components). The developmental time of *N. californicus* has been reported to be 5.87 and 6.16 days on almond and date palm pollens, respectively (Khanamani *et al.*, 2017) which is shorter than the development time on any of the artificial diets studied here. Therefore, these pollens can be considered to be favorite food for the immature stages of *N. californicus* in mass-production system (Khanamani *et al.*, 2017). The suitability of pollen grains has also been proved for other phytoseiid species (e.g., Riahi *et al.*, 2016)

In addition to differences in immature development time, there were also significant differences in juvenile survival and fecundity among the different diets. All individuals of the cohort reared on the artificial diet enriched with multivitamin capsule (AD9) died in the larval and protonymphal stages, and after that individuals reared on AD5 (enriched with serum albumin protein) had the highest preadult mortality (approximately 50%) and lowest fecundity. Therefore, the use of these two supplements is not recommended in the preparation of artificial diet for *N. californicus*. Adding insect components to artificial diets enhanced their suitability and improved their nutritional quality for entomophagous insects (Grenier and De Clercq, 2003; De Clercq, 2004). The supplemented diets with maize pollen (AD6), insect components (AD2, AD7, and AD8) and artemia cysts (AD3) reduced the immature mortality and increased fecundity of *N. californicus*. Although these values of fecundity were lower than those reported on *T. urticae* (38.31 eggs), almond pollen (46.87 eggs), and maize pollen (34.89 eggs) (Khanamani *et al.*, 2017), these values exceed the values reported on date palm (2.18 eggs), bee (2.55 eggs), bitter orange (10.01 eggs) and sunflower (12.80 eggs) pollens (Khanamani *et al.*, 2017).

Differences in survival, developmental and reproductive characteristics were reflected in the life table parameters especially in the *r* value. The high-

est *r* value in our study were observed when *N. californicus* was fed with the diets enriched with *E. kuehniella* eggs (AD2), *A. franciscana* cysts (AD3) and maize pollen (AD6). These growth rates exceed the values reported on date palm (0.010 day⁻¹) and bee-collected (0.005 day⁻¹) pollens (Khanamani *et al.*, 2017), and *Thrips tabaci* Lindeman (0.041 day⁻¹) (Rahmani *et al.*, 2009). However, these values were lower than those reported for *N. californicus* when fed on *T. urticae* (0.154 day⁻¹), almond pollen (0.231 day⁻¹), maize pollen (0.179 day⁻¹) (Khanamani *et al.*, 2017), *Lepidoglyphus destructor* (Schrank) (0.245 day⁻¹) and *Acarus siro* L. (0.104 day⁻¹) (Simoni *et al.*, 2006). Thus, the almond pollen and *L. destructor* followed by the maize pollen and *A. siro* are more suitable diets for mass-rearing of *N. californicus*. In addition, interesting results were obtained with the alternative prey *Petrobia harti* (Ewing) and pollens of *Carpobrotus edulis* (L.) and *Scrophularia peregrina* L. (Ragusa *et al.*, 2009).

Although the reproductive performance of the predator on most of the prepared artificial diet was relatively low, because of their suitability for preservation of the adult predator they can be used as a food source supplied during the delivery of commercially mass-produced phytoseiid mites or for maintenance of the population in times of demand shortage. In addition, their application can also support the predator population in times of prey scarcity in the crop. In times of prey scarcity, long-term preservation of phytoseiid mites was more important than increasing their capacity for egg production, because an over-abundance of the predators can lead to an over-consumption of the food resource and may cause an increase in the frequency of cannibalism (Schausberger, 2003). It should be mentioned that solid artificial diets have several advantages than liquid ones, including more convenient application and storage. In addition, solid artificial diets are believed to have better potential for use as supplemental foods to sustain predatory mite populations in the crop after release (Nguyen *et al.*, 2014b).

The artificial diet developed by Ogawa and Osakabe (2008) supported preadult development and survival of the predatory mite *N. californicus* but its

oviposition rate on this diet was negligible compared with a diet of *T. urticae*. In the present study, the basic artificial diet (AD1) was prepared according to Nguyen *et al.* (2013), however, the oviposition rate and ultimately the obtained *r* values of *Amblyseius swirskii* (Athias-Henriot) on AD1 (Nguyen *et al.* 2013) and AD1 enriched with extracts of decapsulated *A. franciscana* cysts (Nguyen *et al.* 2014a) were higher than those obtained in the present study for *N. californicus*. The higher performance of *A. swirskii* on artificial diets may be due to the generalist feeding habits (Type III) of this predator, whereas, *N. californicus* is a selective predator (type II) of tetranychid mite (McMurtry *et al.*, 2013).

In conclusion, the diets supplemented with arthropod components, as well as with bull sperm or maize pollen all enhanced survival, development and reproduction of *N. californicus*, and consequently its population growth parameters. However, further experiments are needed to develop a suitable artificial diet for providing higher reproductive performance for phytoseiid mites. In addition, further work will be needed to develop a diet in a practical form for mass production and/or as a supplementary diet in the crop. Liquid diets are not practical. The diets should be as simple as possible but support a good population growth rate, i.e. unnecessary components of the diets should be removed.

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