ORIGINAL ARTICLE

Developing an artificial diet for rearing *Orius albidipennis* Reuter (Het., Anthocoridae)

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Abstract

The use of suitable mass rearing methods is crucial to establish successful inundative or inoculative biological control programs. The development of an artificial diet considerably reduces costs of mass rearing. In this study, the efficacy of a new meridic artificial diet for rearing the predatory bug, *Orius albidipennis* (Het., Anthocoridae), was studied. The artificial diet was composed of some natural materials including lamb liver, hen yolk, whey protein, honey, royal jelly and some specific vitamins. To determine the artificial diet efficacy life table parameters of the bugs, using the two-sex life table method, fed artificial and factitious diets, *Ephestia kuehniella* egg + date palm pollen, were compared. Results showed that *O. albidipennis* could complete its life stages and reproduce when reared on the recommended artificial diet. However, its fecundity and survival rate when fed the artificial diet was lower than the controls. Overall, due to lower production costs the artificial diet can be recommended for mass rearing of *O. albidipennis* despite the lower fecundity and survival rate.

Keywords: anthocorid bugs, biological control, mass rearing, meridic diet, *Orius albidipennis*

Introduction

Minute pirate bugs (Het., Anthocoridae) are recognized as potential biocontrol agents against many arthropod pests in different parts of the world (Omkar 2016). The bugs have economic importance due to their high colonization rates, mobility, prey consumption efficacy and fecundity. Among various genera of the family *Orius* Wolff is the most important genus which has worldwide distribution. These predatory bugs can attack many soft body insects including thrips (Tommasini *et al.* 2004; Rajabpour *et al.* 2011), mites (Hassanzadeh *et al.* 2015), aphids (Akramovskaya 1978), eggs of pentatomids (Woodroffe 1973), eggs and larvae of lepidopteran pests (Salehi *et al.* 2016) and whiteflies (Stansly 2010; Banihasemi *et al.* 2017). *Orius albidipennis* Reuter is a dominant species in many regions of Iran (Hassanzadeh *et al.* 2013). Some characteristics of the species, including its ability to tolerate high temperatures and lack of photoperiod induced diapauses, make the predator an ideal candidate for mass rearing and augmentative releases against many field and greenhouse pests in subtropical and tropical regions (Sobhy *et al.* 2010).

Usually, the success of inoculative or inundative releases of natural enemies (NEs) depends on economic and efficient mass-rearing programs (Bueno *et al.* 2006). Developing new methods for mass-rearing a natural enemy reduces biological control costs (Bueno *et al.* 2006; Safaei *et al.* 2015). Mass rearing methods for *Orius* continue to be improved by decreasing costs and increasing efficiency of production as primary goals (Shapiro and Ferkovich 2006). In the current mass rearing methods for *O. albidipennis* and many other *Orius* bugs, lepidopteran eggs, especially *Ephestia kuehniella* Zeller (Lep., Pyralidae) eggs, and pollens are used as a factitious diet (Bonte and De Clercq 2010; Safaei *et al.* 2015). The diet has many limitations since it is relatively expensive and is available seasonally (Arijs and De Clercq 2004). Therefore, the availability of a cheap and adequate artificial diet could lead to a more economic large-scale rearing of the predators (Arijs and De Clercq 2004). Some artificial diets were previously reported for rearing some *Orius* predatory bugs including *O. laevigatus* Fieber (Arijs and De Clercq 2004; Bonte and De Clercq 2010), *O. strigicollis* Poppius (Lee and Lee 2004), *O. sausteri* Poppius (Tan *et al.* 2013) and *O. insidiosus* Say (Ferkovich and Shapiro 2004a, b).

However, there has been no effort to develop an effective artificial diet for mass rearing of *O. albidipennis*. Therefore, the objective of this study was to develop an artificial diet and compare it with a factitious diet, which was obtained from the bodies of an arthropod including *Ephestia* eggs.

Materials and Methods

Insect collection

Orius albidipennis adults were collected from unsprayed sunflower fields in the Mollasani region, Khuzestan province, southwest Iran (31°36'01.5"N 48°52'58.1"E). Female bugs were isolated in a plexiglas cylinder (18 cm high, 7.5 cm diameter) covered with a fine gauze lid on the top and margin for ventilation. The bugs were reared on eggs of *E. kuehniella* + date palmat 25°C (±1°C), 60% (±5%) RH (relative humidity), and 16 : 8 h (light : dark) in an incubator. Bean pod, *Phaseolus vulgaris* L., was used as oviposition substrate. At least one male selected from the offspring was then identified by using keys of Woodroffe (1973).

Artificial diet

The compositions of the artificial diets for rearing *O. albidipennis* are shown in Table 1. The compositions were selected from various formulations. If fed other formulations the predatory bugs did not feed on them or they did not develop to the adult stage or reproduce.

Table 1. Composition of artificial diet for rearing *Orius albidipennis*

Ingredient	Weight
Distillated water	30 g
Lamb liver	50 g
Fresh hen yolk	50 g
Whey protein (80%)	20 g
Honey	25 g
Royal jelly	2 g
Ascorbic acid	1 g
Vitamin E	400 mg
Vitamin A	300 µg
Thiamin (vitamin B1)	1 mg
Riboflavin (vitamin B2)	0.4 mg
Niacin	8 mg
Vitamin B6	0.4 mg
Folic acid	55 µg
Vitamin B12	2 µg
Sesame oil	8 g
Bean pod	20 g
Homogenized extract of *Ephestia kuehniella* egg	0.4 g
Date palm pollen	10 g
Gentamicin (as preservative)	3 mg

Preparation and combination of the ingredients were done in the Food Science Laboratory of Ramin Agriculture and Natural Resources, University of Khuzestan. The ingredients were weighed with a precise digital scale (AND model HR200). The ingredients were mixed and homogenized with an electric blender (Model track X, Black and Decker, USA) for 20 min (500 rounds per minute). Two milliliters of the diet were taken in a microcentrifuge tube. The tube opening was sealed with Parafilm® M. The packed diet was stored in a freezer at −18°C when it was not being used.

Life table parameters

One female and one male, 2 days old, were placed in the plexiglas cylinder with a bean pod for 24 h, and then the bugs were removed. One egg was maintained on the pod and other eggs were removed by using a fine needle. The pod was placed in a rearing cylinder with a tube of artificial diet at 25°C (±1°C), 60% (±5%) RH, and 16 : 8 h (light : dark) in an incubator. In the control, 0.005 g of *E. kuehniella* egg + date palm pollen were used as a factitious diet. The insect was checked and its life stages were recorded every day. After adult emergence, a fresh bean pod was placed in the cylinder every day. Each day, the number of eggs was recorded, then the pod was transferred to a new container and replaced with another pod for oviposition. Observations continued until bug death. Each treatment had 40 replications.

Data analysis

The life table and life history data of *O. albidipennis* fed an artificial diet and control bugs were analyzed according to the theory of age-stage, two-sex life table (Chi and Liu 1985; Chi 1988). To facilitate data analysis, life table analysis, and the bootstrap method, a user-friendly computer program, TWOSEX-MS Chart for the Windows operating system, was made available at http://140.120.197.173/Ecology/prod02.htm (Chi 2017). The age-stage specific survival rate (s_{xj}; where x – age and j – stage), the age-stage specific fecundity (f_{xj}), the age-specific survival rate (l_x), the age-specific fecundity (m_x), and the population parameters [r – the intrinsic rate of increase; λ – the finite rate of increase ($\lambda = e^r$); R_0 – the net reproductive rate; T – the mean generation time] were calculated accordingly. The bisection method can be found in most text books of numerical analysis (Burden and Faires 2005). The mean generation time is defined as the time length that a population needs to increase to R_0-fold of its size as the stable age-stage distribution and the stable increase rate are reached. In other words, this means $e^{rT} = R_0$ or $\lambda^T = R_0$. The mean generation time is calculated as $T = (\ln R_0)/r$. The gross reproductive rate (GRR) is calculated as $GRR = \Sigma m_x$. Also, the adult pre-oviposition period (APOP – the period between the emergence of an adult female and her first oviposition), total pre-oviposition period (TPOP – the time interval from birth to the beginning of oviposition) were also calculated using the experimental data. Finally, the standard errors and variances of the population parameters were estimated via the bootstrap technique (Efron and Tibshirani 1993), which is contained in the TWOSEX-MS Chart program. Sigma plot 12.5 was used to create graphs.

Results

Life table parameters of *O. albidipennis* reared on an artificial diet and controls are shown in Table 2. The results indicated that APOP and TPOP with artificial diet treatment were significantly more than the control. However, GRR, λ, r_m and R_0 with artificial diet treatment were significantly lower than the control. The values of GRR, λ, r_m and R_0 with control treatment were 70.1, 3.29, 35.9 and 91.7% higher than artificial diet treatment. Also, the parameter T in artificial treatment was significantly (9.3%) more than the control.

Curves of age-specific survival rate (l_x), age-specific fecundity of total population (m_x), age-specific maternity ($l_x m_x$), age-stage specific survival rate (s_{xj}) and age-stage life expectancy (e_{xj}) of *O. elbidipennis* reared on artificial diet and the control are presented in Figures 1–3.

Curves of l_x, m_x and $l_x m_x$ with artificial diet treatment are totally similar to the control. Moreover, curve trends with artificial diet treatment and the control were similar. There was a difference between e_{xj} curves in artificial diet and the control. The age-stage life expectancy was extended to 46 days with artificial diet treatment in comparison with 52 day in the control.

Table 2. Life history and life table parameters ± SE of *Orius albidipennis* reared on *Ephestia kuehniella* egg + date palm pollen (control) and an artificial diet

Parameter [unit]	Artificial diet	Control
Male longevity (day)	38.35 ± 0 .437 a	38.06 ± 0.799 a
Female longevity (day)	40.58 ± 0.641 a	39.95 ± 0.888 a
APOP	2.642 ± 0.28 b	1.437 ± 0.155 a
TPOP	24.357 ± 0.338 b	20.75 ± 0.429 a
GRR	18.097 ± 3 b	30.792 ± 6.056 a
λ	1.093 ± 0.009 b	1.129 ± 0.0091 a
r_m	0.089 ± 0.0082 b	0.121 ± 0.0081 a
R_0	13.857 ± 3.162 b	26.558 ± 5.299 a
T	29.488 ± 0.36 a	1.969 b

APOP – the period between the emergence of an adult female and her first oviposition, TPOP – the time interval from birth to the beginning of oviposition, GRR – the gross reproductive rate, λ – the finite rate of increase, r_m – intrisic growth rate, R_0 – the net reproductive rate, T – the mean generation time; the same letters in each row indicate non significant difference (T-test)

Discussion

Our data indicated that *O. albidipennis* could complete its life stages and reproduce when reared on the recommended artificial diet. However, its fecundity and survival rate when fed the artificial diet were lower than the control.

The present results are in agreement with Arjis and De Clercq (2004) who showed that fecundity and

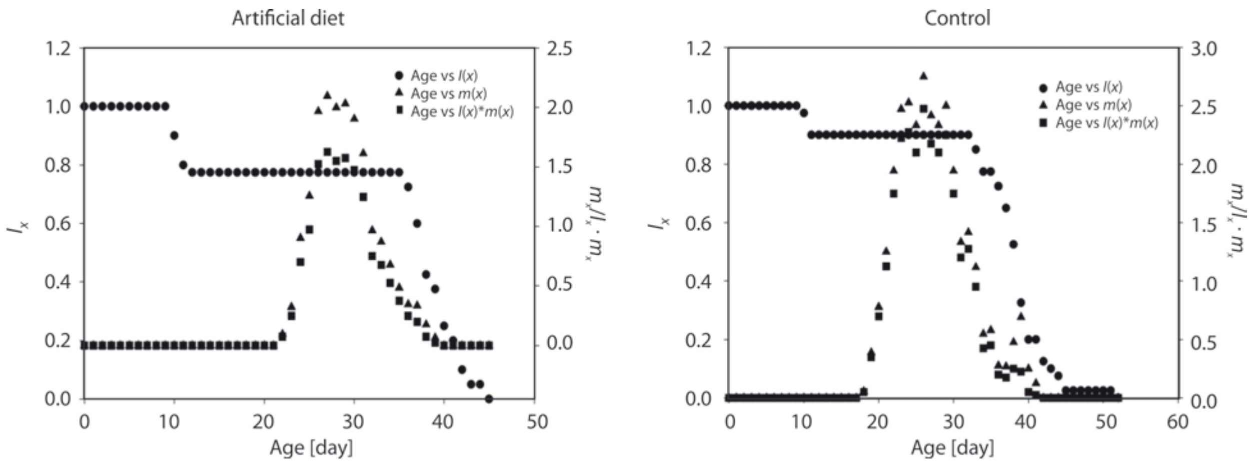

Fig. 1. Curves of age-specific survival rate (l_x), age-specific fecundity of total population (m_x), age-specific maternity ($l_x m_x$) of *Orius albidipennis* reared on an artificial diet and the control

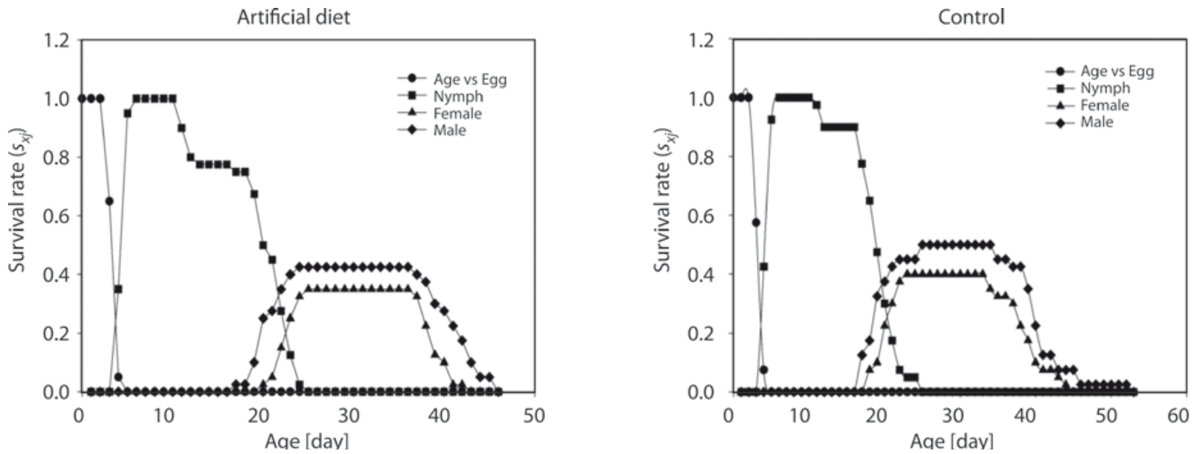

Fig. 2. Curves of age-stage specific survival rate (s_{xj}) of *Orius albidipennis* reared on an artificial diet and the control

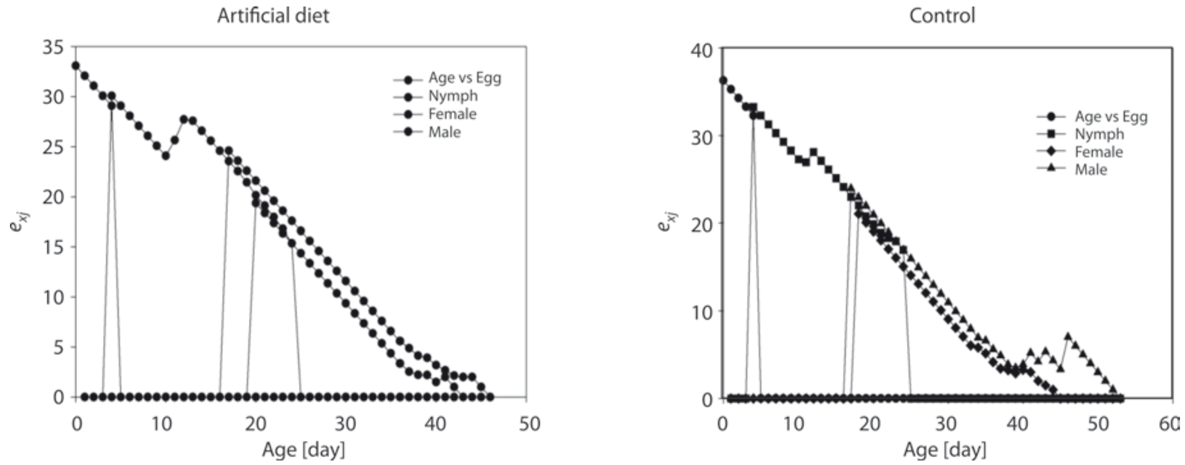

Fig. 3. Curves of age-stage life expectancy (e_{xj}) of *Orius albidipennis* reared on an artificial diet and the control

survival rate of *O. laevigatus* are reduced when fed a liver based artificial diet in comparison with factitious food, *E. kuehniella* egg. Similar results were obtained for *O. strigicollis* (Lee and Lee 2004) and *Podisus maculiventris* Say (Het., Pentatomidae) (Wittmeyer and Coudron 2001) fed an artificial diet.

The lower fecundity of the predatory bug fed an artificial diet than the control may be due to the specific lipid or protein content of *E. keuhniella* egg. Ferkovich and Shaprio (2004b) showed that higher fecundity of *O. insidiosus* when fed *Plodia interpunctata* Hübner eggs in comparison with an artificial diet was related to some specific soluble protein and lipid contents in the eggs. The *P. interpunctata* egg proteins significantly increased egg production and the mean number of oviposition days at concentrations of protein that were 83-, 557-, and 837-fold lower than the concentrations needed for beef liver, bovine serum albumin, and chicken egg albumin, respectively. Moreover, the lower reproduction and survival rate may be related to other factors including the artificial packet. It was demonstrated that the size of artificial diet packets and the size of moth eggs affect fecundity and the survival rate of *O. insidiosus* (Ferkovich *et al.* 2007). Tan *et al.* (2013) reported that microencapsulation of an artificial diet, provided the artificial diet in micro-capsule, and optimized its efficacy for *O. suaeri*.

Due to its lower production costs the artificial diet can be recommended for mass rearing of *O. albidipennis* despite the lower fecundity and survival rate. Riddick (2009) stated that one major benefit of an artificial diet for rearing natural enemies is its lower costs in comparison with factitious diet, *E. kuehniella* egg + date palm pollen.

In conclusion, the meridic artificial diet which was composed of many natural materials with some essential vitamins and microelements can be used for mass rearing of *O. albidipennis*. However, more studies are needed for enhancement of the diet efficacy.

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