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Fitness evaluation of *Oenopia conglobata contaminata* (Menetries) (Col.: Coccinellidae) fed on different diets

Ocena reprodukcijske uspešnosti pri *Oenopia conglobata contaminata* (Menetries) (Col.: Coccinellidae) po različnih prehranskih dietah

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Abstract: Due to the importance of coccinellid predators as biological control agents of insect pests, the effects of different factitious and artificial diets on reproductive parameters of *Oenopia conglobata contaminata* (Menetries) were studied under laboratory conditions (26±1ºC, 60±5% relative air humidity and a photoperiod of 16h light : 8 h dark). In this study, various combinations of *Ephestia kuehniella* (Zeller) eggs, bee pollen, cysts of *Artemia urmiana* Günther and lyophilized artificial diet were used. All diets affected fitness parameters of the ladybeetle. The adult weight (12.00±1.30 mg), fecundity (725±29.31 eggs), oviposition rate (15.72±0.85 eggs), fertility (4.86±0.34 larvae) and number of egg clusters (66.16±2.87) were higher on unlimited *E. kuehniella* eggs plus pollen (UEkP) than those on the other diets. The adults lived longer when fed on EkPLd (the *E. kuehniella* eggs plus pollen and a mixture of lyophilized diet) and UEkP diets (58.57±2.38 and 62.77±3.64 days, respectively) than when fed on the other offered diets. More eggs were hatched when adults reared on EkPLd and EkLd (the *E. kuehniella* eggs plus a mixture of lyophilized diet) diets (36.44±1.93 and 40.01±2.58 %, respectively). The parameter values were increased when the *E. kuehniella* eggs were used in a mix with bee pollen and artificial lyophilized diet and resulted in producing the heavier adults with longer longevity and more fertile eggs. Certainly, the diet will be more cost-effective than natural prey for mass production of the predatory coccinellid, *O. conglobata contaminata*.

Keywords: artificial diet, biological control, biological parameters, factitious diet, predator

Izvleček: Polonice predstavljajo pomemben naraven način za kontroliranje in zatiranje škodljivcev. V tej raziskavi so preučevali vpliv prehrane na razmnoževanje povrste polonice *Oenopia conglobata contaminata* (Menetries) v laboratorijskih razmerah (26±1 ºC, 60±5 % zračne vlage in fotoperiodo 16 h svetlobe : 8 h teme). Prirast polonic so spremljali po uporabi različnih diet; kombinacije jajc močne vešče *Ephestia kuehniella* (Zeller), cvetni prah (osmukanec), čisti solinski rakec *Artemia urmiana* in liofilizirana umetna hrana iz mletega govejega mesa, jeter kokoši, jajčnega rumenjaka in raztopine saharoze. Najboljša prehrana je bila tista, ki je vključevala neomejeno

količino jajc močne vešče in cvetni prah. V primeru tovrstne prehrane so imeli odrasli osebki večjo telesno maso (12.00±1.30 mg), celotno število jajc (725±29.31), hitrost odlaganja jajc (15.72±0.85 jajc/dan), in število izleženih larv/dan (4.86±0.34). Odrasli, ki so bili hranjeni z dieto jajca vešče in mešanica liofilizirane hrane (EkPLd) in neomejeno količino jajca vešče in cvetnega prahu (UEkP) so živeli dlje (58.57±2.38 in 62.77±3.64 dni v primeru EkPLd in UEkP) kot ostale skupine. V primeru diet EkPLd ter jajc vešče in liofilizirane hrane (EkLd) se je izleglo za 36.44±1.93 in 40.01±2.58 % več jajc. Vsi testirani parametri so bili povečani v primeru, ko so bile živali hranjene z jajci vešče, cvetnim prahom in umetno liofilizirano prehrano. V tem primeru so bili odrasli osebki težji, z daljšo dobo preživetja in večjim številom oplojenih jajc. Uporaba umetnih diet je ekonomsko bolj ugodna kot uporaba naravne hrane.

Ključne besede: umetna hrana, biološki parametri, energetsko-bogata prehrana, plenilec

Introduction

The pistachio psylla, *Agonoscena pistaciae* (Burckhardt and Lauterer) is the most important pest of pistachio trees in Iran and its neighboring countries (Mehrnejad 2001). The nymphs and adults feed on pistachio plants and increase the economic damages to pistachio fruits (Mehrnejad 2003). Since implementation of integrated pest management programs based on using natural enemies is crucial, predatory coccinellids can play an important role as the most beneficial insects in agricultural ecosystems to suppress population of plant pests such as aphids, mites, psyllids, mealybugs and scales (Obrycki and Kring 1998, Omkar and Pervenz 2005) along with reducing chemical pesticide applications (Hoffman and Frodsham 1993).

Oenopia conglobata contaminata (Menetries) is a polyphagous and native species of Palearctic regions (Delplanque 1998). The species lives on trees higher than two meters (Hodek 1973). The predatory coccinellid can be introduced in pistachio orchards as biological control agent as well as other species such as *Coccinella undecimpunctata* L., *C. septempunctata* L., *Adalia bipunctata* L., *Hippodamia variegata* (Goeze) and *Exochomus nigripennis* (Erichson) (Jalali 2001, Mehrnejad 2002).

The essential food for *O. conglobata contaminata* is *A. pistaciae* (Mehrnejad and Jalali 2004), however using artificial diets is the most important research aim for mass rearing of this predatory coccinellid. To this end, researchers attempted to find alternative diets instead of natural prey. Several studies investigated the effects of artificial and factitious diets on biological properties of predatory coccinellids such as *A. bipunctata* (Kariluoto et al. 1976) and *H. convergens* (Guérin-Méneville) (Hussein and Hagen 1991).

Most researchers demonstrated that the mediterranean flour moth, *Ephestia kuehniella* (Zeller) eggs had favorable effects on fitness of *H. convergens*, *Harmonia axyridis* (Pallas), *Coleomegilla maculata* (De Geer), *A. bipunctata*, (Kato et al. 1999, Specty et al. 2003, Michaud and Jyoti 2008, Jalali et al. 2009) and *O. conglobata contaminata* (Mirhosseini et al. 2015).

Nevertheless, mass rearing of *O. conglobata contaminata* is not economical on *E. kuehniella* eggs, hence, other alternative diets should be examined to select high quality diets with low cost rather than *E. kuehniella* eggs. For instance, nutritional value of various pollens was examined for rearing *C. maculata* by Michaud and Grant (2005). Also, Farag and Moniem (2011) could rear *C. undecimpunctata* on some bee products as diet substitutes.

Various researchers managed to rear other predators on meat products such as *H. axyridis* on whole chicken egg and chicken liver (Dong et al. 2001), *Geocoris punctipes* (Say) on beef liver and ground beef (Cohen 2000), *Dicyphus tamaninii* (Wagner) (Iriarte and Castane 2001, Castane et al. 2002, Zapata et al. 2005) and *Podisus maculiventris* (Say) on beef liver, fatty ground beef and egg yolk (Mahdian et al. 2006).

Also, the brine shrimp, *Artemia franciscana* (Kellogg) has been used as an alternative food for rearing coccinellid (Lundgren and Weber 2010, Pilorget et al. 2010, Li et al. 2011), heteropteran (Arijs and De clercq 2001) and mite predators (Nguyen et al. 2014). The current paper aims were to investigate the value of several factitious and artificial diets to rear the predatory coccinellid, *O. conglobata contaminata* and to compare influence of the diets on its developmental and reproductive parameters such as preoviposition period, longevity, oviposition rate, egg fertility and fecundity.

Materials and sethods

Insect culture

The predator, *O. conglobata contaminata*, was collected from pistachio trees infested by *A. pistaciae* in a pistachio garden at Shahid Bahonar University of Kerman, Kerman, Iran. The predator colony was fed on pistachio psylla, *A. pistaciae,* obtained daily from a pistachio garden for two successive generations at 26 ± 1ºC, 60 ± 5% relative humidity and a photoperiod of 16 h light : 8 h dark.

Diet preparations

In the present experiment, six diets were tested including the *E. kuehniella* eggs (Ek), the *E. kuehniella* eggs plus pollen (EkP) (1:1)*,* the *E. kuehniella* eggs plus pollen and *Artemia urmiana* Günther cysts (EkPAf) (1:1:1), the *E. kuehniella* eggs plus a mixture of lyophilized diet (EkLd) (1:1), the *E. kuehniella* eggs plus pollen and a mixture of lyophilized diet (EkPLd) (1:1:1) and unlimited *E. kuehniella* eggs (*ad libitum*) plus pollen (UEkP).

The eggs of *E. kuehniella* were prepared from the laboratory of Entomology, Plant Protection Department, Shahid Bahonar University of Kerman and were maintained in freezer at -18 ºC for two months. The lyophilized diet was prepared according to De Clercq et al., (1998). The diet was a mixture of ground beef (100 g), hen liver (100 g), egg yolk (10 g) and 12 ml of sucrose solution (5%) that was frozen at -18 ºC until it was used. The decapsulated cysts of *A. urmiana* were supplied by Urmia Lake Research Institute, Urmia, Iran.

Effect of diets on fitness of O. conglobata contaminata

The egg clusters of *O. conglobata contaminata* were collected from the main coccinellid colonies reared on each diets described above. The hatched larvae were transferred separately in Petri dishes (9 cm diameter) under laboratory conditions (26 ± 1ºC, 60 ± 5% RH and 16 L : 8 D photoperiod). Each larva was provided every other day with 12 mg of one diet until pupation, except with UEkP diet which was *ad libitum*. Newly emerged adults were used after 24 h starvation and weighed by using Mettler microbalance (accuracy readability 0.1 mg) (Bonte et al. 2010). The emerged adults were then transferred into a container (40 × 10 × 20 cm) for two successive days. The each mated couple was transferred into one Petri dish lined with filter paper and fed similar to their larvae. All couples were daily checked and pre-oviposition and oviposition periods, oviposition rate (the number of laid eggs per day), fecundity (the total egg production), longevity, number of egg clusters, hatching rate, percentage of hatched eggs and egg fertility were calculated.

Data analysis

One-way analysis of variance (ANOVA) was performed for all data and the averages were compared by the Tukey' test at 5% probability. The percentage data were arcsine square-root transferred. All data were statistically analyzed using SAS Software (SAS 1989).

Results

The weight of heaviest (12.00 ± 1.30 mg) and lightest adults (7.20 ± 0.24 mg) had significant difference when they were reared on UEkP and EkPAf diets, respectively (F=3.19; df=473;

Table 1: Effect of different diets on adult weight, preoviposition period, longevity and fecundity of *Oenopia conglobata contaminata*. Mean values ± SE are shown (n – numerus). Different letters in the same column indicate statistically significant differences (Tukey's test, P< 0.05).

Tabela 1: Vpliv različnih diet na maso, čas odlaganja jajc, dolgoživost in število odloženih jajc odraslih osebkov *Oenopia conglobata contaminate.* Prikazane so povprečne vrednosti ± SN (n – numerus). Različne črke v istem stolpcu kažejo statistično neznačilne razlike (Tukey test, P< 0.05).

Diet	Adult weight (mg)	Preoviposition period (days)	Longevity (days)	Fecundity (eggs/♀)
Ek	10.40 ± 0.19 [b] n=99	9.60 ± 0.37 [b] n=15	40.00 ± 5.07 [b] n=14	278.77 ± 11.70 [b] n=14
EkLd	10.20 ± 0.18 [b] n=100	9.25 ± 0.35 [b] n=16	38.14 ± 2.46 [b] n=14	115.83 ± 7.04 [c] n=14
EkP	11.40 ± 2.4 [b] n=64	11.93 ± 1.01 [a] n=15	44.57 ± 4.93 [b] n=14	74.18 ± 4.58 [cd] n=14
EkPAf	7.20 ± 0.24 [c] n=41	13.45 ± 1.03 [a] n=11	34.27 ± 3.98 [b] n=11	28.20 ± 1.57 [d] n=11
EkPLd	10.30 ± 0.18 [b] n=91	9.53 ± 0.43 [b] n=15	58.57 ± 2.38 [a] n=14	252.25 ± 12.51 [b] n=14
UEkP	12.00 ± 1.30 [a] n=79	8.42 ± 0.35 [b] n=19	62.77 ± 3.64 [a] n=18	725.06 ± 29.31 [a] n=18

Abbreviations / Okrajšave: EK - *Ephestia kuehniella* eggs; EkLd - *Ephestia kuehniella* eggs plus a mixture of lyophilized diet; EkP - *Ephestia kuehniella* eggs plus pollen (1:1); EkPAf - *Ephestia kuehniella* eggs plus pollen and *Artemia urmiana* cysts; EkPLd - *Ephestia kuehniella* eggs plus pollen and a mixture of lyophilized diet (1:1:1); UEkP - unlimited *Ephestia kuehniella* eggs (*ad libitum*) plus pollen.

P<0.05) (Tab. 1). The latter diet produced the adults that were 40% lighter than the UEkP diet. The pre-oviposition period of the adult predatory coccinellid significantly varied from 8.42 ± 0.35 to 13.45 ± 1.03 days in all treatments (F=5.08; df=93; P<0.05). When the predator consumed EkPAf and EkP, the pre-oviposition periods were significantly higher than those of the other treatments. The adults reared on the UEkP and EkPLd significantly (F=12.85; df=79; P<0.05) had longer longevity than those were offered the other diets. The diet differentially affected the fecundity of the ladybeetle (F=18.73; df=72; P<0.05) and when *O. conglobata contaminata* was fed on the UEkP (725.06 ± 29.31 eggs), the highest value was significantly assessed.

The number of eggs laid by female per day (oviposition rate) on the UEkP diet was significantly higher than those of the other diets (F=13.90; df=87; P<0.05) (Tab. 2). More larvae were produced when the predator was offered the UEkP (4.86 ± 0.34 larvae per female per day) which was significantly higher than the other treatments (F=5.11; df=70; P<0.05). The hatchability percent of the eggs produced by *O. conglobata contaminata* over its lifetime was about 40% when the predator fed on the EkLd and was twice higher than that of the Ek diet (F=5.76; df=84; P<0.05). The number of egg clusters laid by the predatory female was differentially influenced by the diets (F=28.53; df=65; P<0.05). The UEkP diet consuming by the coccinellid was resulted in being produced the highest number of egg clusters.

Table 2: Effect of different diets on oviposition rate, egg fertility, hatched eggs and number of egg clusters of *Oenopia conglobata contaminata*. Mean values ± SE are shown. Different letters in the same column indicate statistically significant differences (Tukey's test, P< 0.05).

Tabela 2: Vpliv različnih diet na stopnjo odlaganja jajčec, oploditev jajčec, delež izleženih jajc in število jajčnih skupkov pri *Oenopia conglobata contaminate.* Prikazane so povprečne vrednosti ± SN. Različne črke v istem stolpcu kažejo statistično neznačilne razlike (Tukey test, P< 0.05).

Diet	No. of examined females	Oviposition rate (eggs/♀/day)	Egg fertility (larvae/♀/day)	Hatched eggs (%)	Egg clusters
Ek	14	8.13 ± 0.59 ^{b}	2.09 ± 0.14 ^{bc}	20.63 ± 1.29 ^{c}	26.75 ± 1.76 ^{b}
EkLd	14	5.12 ± 0.43 ^{cd}	1.97 ± 0.13 ^{bc}	40.01 ± 2.58 ^{a}	11.18 ± 0.71 ^{d}
EkP	14	5.28 ± 0.31 ^{cd}	1.50 ± 0.11^{cd}	26.55 ± 1.83 ^{b}	8.81 ± 0.55 ^{d}
EkPAf	11	3.69 ± 0.23 ^{d}	1.13 ± 0.04 ^{d}	25.20 ± 1.26 ^{bc}	2.87 ± 0.30 ^{e}
EkPLd	14	6.10 ± 0.37 ^{c}	2.39 ± 0.17 ^{b}	36.44 ± 1.93 ^{a}	19.83 ± 1.35 ^{c}
UEkP	18	15.72 ± 0.85 ^{a}	4.86 ± 0.34 ^{a}	27.05 ± 1.04 ^{b}	66.16 ± 2.87 ^{a}

Abbreviations / Okrajšave: EK - *Ephestia kuehniella* eggs; EkLd - *Ephestia kuehniella* eggs plus a mixture of lyophilized diet; EkP: *Ephestia kuehniella* eggs plus pollen (1:1); EkPAf - *Ephestia kuehniella* eggs plus pollen and *Artemia urmiana* cysts; EkPLd - *Ephestia kuehniella* eggs plus pollen and a mixture of lyophilized diet (1:1:1); UEkP - unlimited *Ephestia kuehniella* eggs (*ad libitum*) plus pollen.

Discussion

The present results demonstrated that the quality and quantity of different diets affected all the reproduction performance of *O. conglobata contaminata*. Almost, all parameters were significantly improved when the predator was given the UEkP diet than those of the other diets. It implies that the factitious prey with hexapod origin plus plant materials effectively enhances the reproduction of coccinellid predators. Compared to the EkP diet, the UEkP with higher quality, made the predatory coccinellid to impressively show better biological control performance. The eggs of *E. kuehniella* have been known as protein-rich sources for insect predators (Michaud and Qureshi 2005) and th diet has been extensively used as a main factitious prey for rearing predators (Hamasaki and Matsui 2006, Vandekerkhove et al. 2006, Specty et al. 2003) especially, *Orius laevigatus* (Fieber) (Bonte and De Clercq 2008) and *A. bipunctata* (De Clercq et al. 2005b).

The current results showed that the reproduction of *O. conglobata* was lower when female fed the *E. kuehniella* eggs supplement with bee pollen plus *A. urmiana* cysts than when the predator fed the *E. kuehniella* eggs supplement with pollen and lyophilized diet. The adult weight of predatory coccinellid strongly decreased when the cyst of *A. urmiana* was used as a portion of the diet. Also, the cyst of *A. urmiana* had the worst effects on the reproduction parameters of *O. conglobata contaminata*. However, previous studies stated that *A. franciscana* cysts were suitable diet for O. *laevigatus* (De Clercq et al. 2005a, Arijs and De Clercq 2001) and predatory mite, *Amblyseius swirskii* (Athias-Henriot) (Nguyen et al. 2014). Furthermore, Hongo and Obayashi (1997) found that despite having favorable effects of hydrated *A. franciscana* cysts diet on *H. axyridis* larvae, the predators did not become mature. Similar to our findings Riddick and Rojas (2014) demonstrated that *C. maculata* had lower fecundity and egg hatching rate when fed on cysts of *A. franciscana* than fed on the *E. kuehniella* eggs and it was related to the amount of soluble protein and lipid that were higher in the *E. kuehniella* eggs than in *A. franciscana* cysts. The nutrients of *A. franciscana*

only contains 3% water (De Clercq et al. 2005a) and are not physiologically digestable (Bonte et al. 2010). Therefore, it makes the nutrients difficult to be digested in the predator alimentary system and it should be examined for *A. urmiana*, as well.

The positive effects of the pollen were proved in the present research. The bee pollen, as a food source for insects, consists of necessary proteins, lipids and carbohydrates (Jervis et al. 1996) and Cocuzza et al. (1997) reported its positive effects on the number of eggs laid by the two predators, *O. laevigatus* and *O. albidipennis* (Reuter). Allen and Cohen (1985) reported that the number of eggs laid by the predator *G. punctipes* was directly dependent on the amount of lipid and cholesterol in its diets, therefore, it was concluded that the unlimited *E. kuehniella* eggs plus bee pollen could provide the essential components and had high quality for rearing *O. conglobata*.

Also, according to the present results, the *E. kuehniella* eggs plus pollen and lyophilized diet could be substituted as a suitable diet for the unlimited *E. kuehniella* eggs plus bee pollen diet.

The lyophilized artificial diet mixed with pollen and the *E. kuehniella* eggs was resulted in the higher fecundity, longevity and percentage of hatched eggs compared to the diet without the pollen. When predators were offered a food source containing various ingredients, they were able to access to a broad range of sufficient nutrients to complete their development rather than a food source containing a single ingredient (Bonte et al. 2010). However, the earlier studies indicated that the lyophilized artificial diet, *A. franciscana* cyst and the pollen alone or a mix of them as food were not effective for *A. bipunctata* compared to the *E. kuehniella* eggs or aphids as natural preys (De Clercq et al. 2005b, Bonte et al. 2010).

Considering the quantity and the quality of the diets examined in the current study, it seems that providing the predator with a mixed diets enriched with a broad range of nutrients not only causes the mass production of the predatory coccinellid, *O. conglobata contaminata* to be cost-effective but also increases the values of reproductive parameters (e.g. the percentage of hatched eggs). It has been proved that the diet quality can be effective on larval growth, survival period (Obrycki and Orr 1990, Isikber and Copland 2002, Kalushkov and Hodek 2004) reproduction and hatching rate (Blackman 1967, Michaud 2005) of predatory ladybirds.

Based on the current investigation the adult weight, longevity and fecundity of *O. conglobata contaminata* increase along with elevating its food quality. The adult weight is a criterion factor reflecting the amount of energy stored in insect body that affects the mating behavior (Omkar et al. 2006) and the number of eggs deposited (Silva et al. 2009). Previously, the direct relation between the weight and longevity of *O. conglobata contaminata* has been confirmed by Smith (1965).

Conclusions

In conclusion, *ad libitum* usage of the *E. kuehniella* eggs mixed with the pollen had significant influence on the reproductive parameters of *O. conglobata contaminata* and increased the percentage of hatched eggs. Therefore, a diet with variety of essential animal and plant nutrients is suggested for rearing of *O. conglobata contaminata* and was resulted in producing heavy ladybirds with longer longevity and high fertile eggs. Certainly, the diet will be more cost-effective than natural prey for mass production of the predatory coccinellid, *O. conglobata contaminata*.

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