J. Plant Prot. and Path., Mansoura Univ., Vol.10 (1): 65 – 68, 2019

Comparative Studies between an Artificial Diet and *Ephestia kuhniella* Zeller Eggs on the Biological Aspects of *Coccinella undecimpunctata* L. at Three Different Temperatures

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ABSTRACT

The objective of the present investigation was to evaluate rearing of *Coccinella undecimpunctata* L. on an artificial diet and *Ephestia kuhniella* eggs. Significant differences were observed between the artificial diet and *E. kuhniella* eggs on the incubation period and the total immature stages duration at the three tested temperatures. The life cycle of *C. undecimpunctata* was the shortest when reared on the artificial diet and *E. kuhniella* eggs at 30 ± 1ºc and 65 ± 5% R.H. and lasted 27.5 ±0.15 and 22.7 ± 0.20 days, respectively. The total larval survival rate was the highest for *C. undecimpunctata* when reared on *E. kuhniella* and recorded 86.67 %, 96.67% and 90% at the three different temperatures. The longest female and male longevity were observed when reared at 20 ± 1 ºC on two diets. The high fecundity value was found at 25± 1 ºC for two diets followed by 30 ± 1 ºC. While, it was lowest at 20 ± 1 ºC for *C. undecimpunctata*.

Keywords: *Coccinella undecimpunctata* L. , artificial diet and *Ephestia kuhniella*.

INTRODUCTION

Biological control is one of the beneficial actions in managing pests and their damage. It is defined as the reduction of pest population by natural enemies. This biological control agents, which called "natural enemies", is especially important for reducing the numbers of pest insects and mites (Dreistadt and Statewide 2014).

The eleven-spotted ladybird, *Coccinella undecimpunctata* L. (Coleoptera : coccinellidae) is an important polyphagous predator and use in the biological control for a large wide of pests such as aphids , eggs and new hatching of lepidopterian insects and it can be found in different parts of the world with different environmental conditions (Hodek and Honêk 2012). *C. undecimpunctata* L. is one of the most intensively studied predators because of its importance as biocontrol agent, wide geographical distribution and the diversity of their feeding habits. Because of the sharp reduction on the number of natural preys on some times of the year that's required for the predator mass rearing *C. undecimpunctata.* So, it was important to study some factitious preys or artificial diets. (El-Heneidy *et al.,* 2008 and Song and Swinton 2009).

There are two approaches for developing artificial diets for living organisms. The complicated methods are to prepare a mixture of chemically defined substances (holidic), and the easier technique by mixing natural substances (meridic diets) (Okada, 1970 ; Chen and Qin 1982 ; Hattingh and Samways, 1993 ; Abd El-Salam, 1995 ; Ghanim and El-Adl 1996 and 1997 ; Ghanim *et al.* 2000 ; Mohamed, 2001 ; El- Serafi, *et al.* 2002 and Bahy El-Din, 2014).

Temperature is one of the most important environmental factors has effects on the biological aspects of the predators such the developmental duration, ovi-positional periods and reproductive ability. The knowledge of environmental factors that can affect the increase in the insect populations is crucial for predicting potential changes in population dynamics and developing sustainable an environment-friendly pest control strategies. (Ghanim and El-Adl, 1987; El-Serafi, 2002 ; Mohamed, 2013 ; Bahy El-Din, 2014 ; Bayoumy, *et al.* 2015 ; El-Batran, 2015 and Said, 2018). The objective of this study to compare effects of artificial diet and *E. kuhniella* on the biological aspects of *C. undecimpunctata* at three different temperatures.

MATERIALS AND METHODS

The present investigation was carried out at the laboratory of the department of Entomology, Faculty of Agriculture, Mansoura University. *Coccinella undecimpunctata* adults were collected from guava and peach trees on the Faculty of Agriculture farm, Mansoura University and reared in the laboratory at 25 ± 1ºC and 60 ± 5% relative humidity (RH).

Frozen eggs of *Ephestia khuniella* (Pyralidae: Lepidoptera) were brought from the Natural Enemies lab, Faculty of Agriculture, Cairo University.

Thirty freshly hatched larvae (< 24h – old) as replicates were collected and placed individually in a Petri-dish (10 cm in diameter) and placed in controlled incubators at 20, 25, 30ºC with 60 ± 5% RH and every day fed with artificial diet and *E. kuhniella* eggs for each temperature. The duration of each larval instar and the number of surviving larvae were recorded and then when larvae were in the pupae stage the duration of pupae stage were recorded. The gender of adults was recorded for each diet on each temperature. After three days of emerging the females and males were collected for copulation. Each pair was placed in a Petri-dish and in the next day every pair were separated in two petri dish to record the longevity of males and the ovipositional periods and fecundity of females.

2. Statistical analysis:-

Data for developmental duration of the immature stages, survival percentage, longevity and fecundity of *C. undecimpunctata* were analyzed by one-way ANOVA, and the means were separated using Student- Newman-Keuls Test by using Costat Software, (2004).

Table1. Artificial diet ingredients used to rear predator, *Coccinella undecimpunctata* in the laboratory.

Ingredients	Amounts (g)
1- Sucrose	56
2- Bee Honey	10
3- Royal jelly	5
4- Pollen grains	9
5- Water	4
6- Yeast	2.5
7- Dry milk	8
8- Dry aphid	3.5
9- Streptomycin	2.0

RESULTS AND DISCUSSION

1- Duration of immature stages:-

Data illustrated in Table (2) showed significant differences on the incubation period for the artificial diet ($f_{2, 87}$ = 98.67 and p= 0.0000***) and for *Ephestia kuhniella* eggs ($f_{2, 87}$ = 139.90 and p= 0.0000***) between the tested temperature for each diet. On the other hand, there were not significant differences between the two diets with the same temperature at 20ºC ($f_{1, 58}$ = 0.23 and p= 0.6339 ns, respectively), at 25ºC ($f_{1,58}$ = 0 and p= 1 ns) and at 30ºC ($f_{2, 87}$ = 2.43 and p = 0.1246). Temperature shortened the incubation period similar effect of temperature. Recorded by Ramesh Babu and Azam, (1987) and Abdel-Salam *et al.*(2018).

The longest incubation period for the eleven spotted ladybird *C. undecimpunctata* were at 20 ±1°C for the artificial diet and *E.kuhniella* eggs and represented by 6.5 ± 0.10, 6.6 ± 0.09 days, respectively. While, the shortest incubation period were at 30 ±1°C and 65 ± 5 % RH and presented by when reared on the artificial diet and *E.kuhniella* eggs 4.6 ± 0.09 and 4.4 ± 0.09 days respectively. The reduction of the incubation period may be due to the elevated of the embryonic development rate at the higher temperature. These results in consistent with those of Srivastava and Omkar (2003).

Data in Table (2) showed that there were significant differences between the three temperature degrees studied for each diet in the total larval stages when *E. kuhniella* eggs ($f_{2,81}$= 310.95 and p = 0.0000***) and the artificial diet ($f_{2, 68}$ = 337.61 and p=0.0000***), pupal stage when *E. kuhniella* eggs ($f_{2, 79}$=135.39 and p = 0.0000***) and the artificial diet ($f_{2, 68}$= 238.61 and p = 0.0000***). And total immature stages when *E. kuhniella* eggs ($f_{2,79}$= 575.95 and p = 0.0000***) and for the artificial diet ($f_{2, 63}$= 591.51 and p= 0.0000***). But there were significant differences with the artificial diet and *E. kuhniella* eggs for the total larval stage at 20 ±1°C, ($f_{2, 81}$ = 310.95 and p = 0.0000***), at 25 ±1°C ($f_{2, 68}$ = 337.51 and p = 0.0000***) and at 30±1°C ($f_{2, 68}$ = 337.51 and p = 0.0000***). Significant differences were showed in the pupal stage between artificial diets and *E. kuhniella* eggs at 30±1 °C ($f_{2, 72}$ = 7.46 and p = 0.0011**) and at 25 ±1°C ($f_{2, 78}$ = 2.57 and p = 0.0830 ns), while, it was not significant at 20 ±1°C ($f_{2 , 73}$ = 2.88 and p = 0.0625 ns) for the three different diets. Because of the increasing of temperature increased the metabolism of larvae voracity this leads to increasing the supply of the nutrients and resulting rapid development according to Ponsonby and Copland (1996) and Srivastava and Omkar (2003).

On the other hand there were significant differences between the artificial diet and *E. kuhniella* eggs on the total immature stages duration at the three tested temperatures when *E. kuhniella* eggs ($f_{2,81}$= 310.95 and p = 0.0000***) and for the artificial diet ($f_{2, 68}$= 337.51 and p= 0.0000***). And there was significant variation at the three different temperatures at 20 ±1°C ($f_{1, 50}$ = 220.92 and p = 0.0000***), at 25 ±1°C ($f_{1,51}$ = 1094.62 and p = 0.0000***) and at 30±1°C ($f_{1,48}$ = 316.74 and p = 0.0000***). The total larval duration, the pupal stage and total immature stages a t 30 ±1°C and 65 ± 5 % RH were the shortest when this predator reared on *E. kuhniella* eggs and represented by 13.5 ± 0.17, 4.7 ± 0.09 and 22.7 ± 0.20 days, respectively. Meanwhile, there were the longest when reared on the artificial diet and recorded 22.9 ± 0.16, 7.4 ± 0.11 and 36.7± 0.19 days, respectively.

Bahy El-Din (2014) recorded that the total larval period of *C. undecimpunctata* lasted 15.97; 18.11; 12.85; 14.02 and 11.98 days on four artificial diets and *A. gossypii* under constant temperature of 27 ± 2ºC. He added that averages of mean number of deposited eggs by *C. undecimpunctata* were 204.15; 124.69; 518.35; 262.76 and 761.75 eggs/ female when the predator fed on the four artificial diets tested and *A. gossypii*, respectively. Said (2018) noticed that the developmental times of C. indecimpunctata preyed aphids reared on three eggplant varieties were decreased with increasing the temperature, whereas the developmental rates at 30±1°C. These results in consistent with those of Ghanim and El-Adl (1987) ; El- Serafi, *et al.* (2002); Mohamed, (2013); Bayoumy *et al.* (2015) and Saleh *et al.* (2017).

Table 2. Effect of artificial diet and natural preys on the immature stages of *Coccinella undecimpunctata* at 20°C, 25°C and 30°C and 65 ± 5% RH.

Type of diet	Temp. (°C)	Duration periods			
		Incubation period	Total larval stages	Pupal stage	Total immature stage
Artificial diet	20	6.5 ± 0.10 a^A	22.9 ± 0.16 a^A	7.4 ± 0.11 a^A	36.7 ±0.19 a^A
	25	5.7 ± 0.09 b^A	21.5 ± 0.12 b^A	6.0 ± 0.08 b^A	33.2 ± 0.19 b^A
	30	4.6 ± 0.09 c^A	17.7 ± 0.12 c^A	5.0 ± 0.33 c^A	27.5 ± 0.15 c^A
Ephestia kuhniella eggs	20	6.6 ± 0.09 a^A	19.2 ± 0.18 a^B	7.1 ± 0.13 a^A	33.0 ± 0.25 a^B
	25	5.7 ± 0.09 b^A	15.9 ± 0.12 b^B	5.7 ± 0.09 b^B	27.2 ± 0.18 b^B
	30	4.4 ± 0.09 c^A	13.5 ± 0.17 c^B	4.7 ± 0.09 c^B	22.7 ± 0.20 c^B

Values followed by the same lowercase small letters in a column among temperatures within each diet and the same uppercase capital letters among diets in each temperature are not significantly different at the 5% probability level (ANOVA, Student- Newan-Keuls Test).

2. Survival rate:-

Data presented in Table (3) indicated that, There were considerable variations survival percentage from egg to adult of *C. undecimpunctata* (at 20, 25 and 30°C) on *E.kuhniella* eggs and the artificial diet and the natural preys at 20°C (f= 0.28, df=2 and p= 0.7560), at 25°C (f= 4.77, df=2 and p= 0.0109*) and at 30°C (f= 4.91, df=2 and p= 0.0096). While there were no significant differences between different temperatures under study when the artificial diet (f= 1.13, df=2 and p= 0.3265ns) and for *E. kuhniella* eggs (f= 0.95, df=2 and p= 0.3913ns). In Table (3), it can be noticed that the maximum value of larval survival percentage was 96.7% at 25 °C on *E. kuhniella* eggs and 96.7% at 25 and 30 °C on *A. gossypii* and the minimum values were 73.3% at 30 °C on the artificial diet. And the maximum value total survival rate of the predator reared on the artificial diet was 83.33% at 20 °C, and (96.67% and 96.67) at 25 °C on *E. kuhniella* eggs. These results are in agreement with those of El- Serafi, (2002) who reported that all immature stages of coccinellid predators showed

Significantly better response in acquiring the high survival rate and faster development when fed on live aphids *sitobion avanae* compared with artificial diet. Results indicate that the highest mortality rate was on the first and second instar of the larval stage it may be because of their thinner and softer cuticle. While the other larval instars and the pupal stage have a thicker cuticle protecting it from unsuitable abiotic condition similar results reported by Ponsonby and Copland (1996), Srivastava and Omkar (2003) and Said, 2018.

Table 3. The survival rate for larval instars and adult emergence percentage of the eleven- spotted ladybird *C. undecimpunctata* under laboratory conditions (20, 25 and 30±1°C and 65±5% R.H) when fed on natural preys and artificial diet

Type of nutrition	Temp. (°C)	Total larval survival (%)	Emergency rate (%)	Total survival percentage
Artificial diet	20	83.3	92	83.33 a^{A}
	25	80	95.8	76.67a^{B}
	30	73.3	90.9	66.67a^{B}
Ephestia kuhniella eggs	20	90	96.3	86.67a^{A}
	25	96.7	100	96.67a^{A}
	30	93.3	96.4	90a^{A}

Values followed by the same lowercase small letters in a column among temperatures within each diet and the same uppercase capital letters among diets in each temperature are not significantly different at the 5% probability level (ANOVA, Student- Newan-Keuls Test).

3. Longevity

The obtained results arranged in Table (4) indicated that there were significant differences between the artificial diet and *E. kuhniella* eggs for the pre-oviposition period at 20 ±1°C ($f_{1,18}$ = 12.8 and p = 0.0022**), at 25 ±1°C ($f_{1.18}$ = 7.36 and p = 0.0142*) and at 30 ±1°C ($f_{1,18}$ = 5.9 and p= 0.0258*). And there were significant differences between the three temperature degrees were studied when *E. kuhniella* eggs ($f_{2, 27}$ = 22.40 and p = 0.0000***) and for the artificial diet ($f_{1.18}$ = 7.36 and p = 0.0142*).

The obtained results arranged in Table (4) indicated that the preovipsition period at 30° C ± 1 and 65 ± 5 % R.H. was the shortest when females of *C.undecimpunctata* reared on *E. kuhniella* eggs and presented by 5.4±0.16days. While, the longest pre-ovipsition period when the predator females fed on the artificial diet and lasted 9.4 ± 0.40 days.

Oviposition period showed significant differences at 30 ±1°C for the three diets while significant differences between *E. kuhniella* eggs and the artificial diet at (20 and 25 ±1°C) when *E. kuhniella* eggs ($f_{2, 27}$ = 4.92 and p = 0.0151*) and for the artificial diet ($f_{2, 27}$ = 7.53 and p = 0.0025**).

Regarding to the oviposition period recorded longest period were when the predator females reared on *E. kuhniella* eggs at 25 ± 1°C and 65 ± 5% RH as compared to 20 and 30± 1°C and 65 ± 5% RH, and lasted 53.4 ± 0.37, 50.9 ± 1.49 and 46.6 ±2.12 days, respectively.

Data presented in Table (4) showed that when females reared on *E. kuhniella* eggs at 20 ± 1°C and 65 ± 5% RH gave the longest longevity and was 77.6 ± 1.42days, while the lowest longevity was 62.9 ± 1.19 at 30 ± 1°C and 65 ± 5% RH when the female reared on the artificial diet.

Data in the Table (4) showed that significant differences between the artificial diet and *E. kuhniella* eggs for the female longevity when *E. kuhniella* eggs (f 2, 27 = 22.40 and p= 0.0000***) and for the artificial diet (f 2, 27 = 10.00 and p = 0.0006***). And male longevity when *E. kuhniella* eggs (f 2, 27 = 4.19 and p= 0.0259*) and for the artificial diet (f 2, 27 = 7.16 and p = 0.0032**). The results presented in Table (4) showed that when males reared on *E. kuhniella* eggs at 20 ± 1°C and 65 ± 5% RH gave the highest longevity and was 52.2 ± 1.43 days, while the lowest longevity observed when the male reared on the artificial diet at 25 ± 1°C and 65 ± 5% RH and it was 42.9 ± 1.36 days.

Table 4. The adult longevity and fecundity of the ladybeetle *C. undecimpunctata* reared on artificial diets and natural preys three constant temperature degrees.

	Temp. (°C)	Female longevity				Fecundity	Fecundity /female/day	Male longevity
		Pre-oviposition period	Oviposition period	inter-oviposition	Total			
Artificial diet	20	9.4 ± 0.40 a^{A}	45.7 ± 0.69 a^{B}	16.4 ± 0.75 a^{A}	71.5 ± 1.13 a^{B}	209.6 ± 26.77 b^{B}	4.57 ± 0.58 b^{B}	46.2 ± 2.46 a^{B}
	25	8.2 ± 0.25 b^{A}	39.4 ± 2.2 b^{B}	18.2 ± 0.46 a^{A}	65.8 ± 1.75 b^{B}	296.3 ± 17.74 a^{B}	7.65 ± 0.52 a^{B}	42.9 ± 1.36 ab^{B}
	30	7.4 ± 0.34 b^{A}	36.5 ± 1.63 c^{B}	19.0 ± 0.70 a^{A}	62.9 ± 1.19 b^{B}	248.4 ± 25.30 ab^{B}	6.98 ± 0.81 a^{B}	39.8 ± 0.68 b^{B}
Ephestia kuhniella eggs	20	7.8 ± 0.20 a^{B}	50.9 ± 1.49 a^{A}	18.9 ± 0.77 a^{A}	77.6 ± 1.42 a^{A}	868.6 ± 22.75 b^{A}	17.27 ± 0.87 a^{A}	52.2 ± 1.43 a^{A}
	25	7.0 ± 0.37 b^{B}	53.4 ± 0.73 a^{A}	14.7 ± 0.84a ^{A}	75.1 ± 1.37 a^{A}	978.4 ± 20.0 a^{A}	18.36± 0.48 a^{A}	48.4 ± 1.54 ab^{A}
	30	5.4 ± 0.16 c^{B}	46.6 ± 2.12 b^{A}	16.2 ± 0.92 a^{A}	68.2 ± 1.63 b^{A}	898.4 ± 44.07 ab^{A}	19.71 ± 0.98 a^{A}	45.6 ± 1.86 b^{A}

Values followed by the same lowercase small letters in a column among temperatures within each variety and the same uppercase capital letters among varieties in each temperature are not significantly different at the 5% probability level (ANOVA, Student- Newan-Keuls Test).

4. Fecundity

Significant differences were found for the fecundity percentage between the artificial diets and the natural preys at (20 and 25 ± 1°C), while under 30± 1°C the results were not significant within each of artificial diet and *E. kuhniella* eggs as compared with 20 and 25 ± 1°C for *E. kuhniella* eggs ($f_{2, 27}$ = 3.38 and p = 0.0488*) and for the artificial diet ($f_{2, 27}$ = 3.39 and p= 0.0488*).

High fecundity values were observed at 25 ± 1°C and 65 ± 5% RH for the different diets as compared to 20 and 30± 1°C and 65 ± 5% RH in general. On the other hand, the highest fecundity values was recorded when the female fed on *E. kuhniella* at 25 ± 1°C and it was 978.4 ± 20.0 eggs, While the fecundity values was low when the female reared on the artificial diet under 20 ± 1°C and 65 ± 5% RH and it was 209.6 ± 26.77 eggs. The increased fecundity with increase in temperature in ladybeetles was also reported in *C. septempunctata* , *Adalia bipunctata* (Linnaeus) , *H . convergens* , *Coccinella undecimpunctata* Linnaeus (Frazer and Mcgregor 1992), *C . mntrouzieri* (Jalali *et a1.* 1999) and *Micrmpis discolor* (Fabricius) (Omkar and Pervez 2002).

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دراسات مقارنة بين بيئة صناعية وبيض فراشة دقيق البحر الأبيض المتوسط على الخصائص البيولوجية لأبو العيد إحدى عشر نقطة تحت ثلاث درجات حرارة مختلفة

هالة أحمد كامل الصيرفي ، عبدالبديع عبد الحميد غانم ، سمير صالح عوض الله و منى معتمد على شلبي
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أجريت هذه الدراسة بكلية الزراعة جامعة المنصورة وأجريت التجارب المعملية بمعمل قسم الحشرات الإقتصادية بهدف تقدير تأثير البيئة الصناعية و بيض فراشة دقيق البحر الأبيض المتوسط على فترة نمو الأطوار الغير كاملة و معدلات البقاء و الخصائص البيولوجية لحشرة أبوالعيد إحدى عشر نقطة *Coccinella undecimpunctata* تحت ثلاث درجات حرارة ثابتة و هي 20 ± 1 ، 25 ± 1 و 30± 1ºم و رطوبة نسبية 65 ± 5 % . أوضحت النتائج أن دورة حياة أبوالعيد إحدى عشرة نقطة كانت الأقصر عند تربيته على درجة حرارة 30± 1ºم على البيئة الصناعية و بيض فراشة دقيق البحر الأبيض المتوسط *Ephestia kuhniella* حيث إستمرت 27 ± 0.15 و 22.7 ± 0.20 يوم على التوالي و أظهرت النتائج أن معدل البقاء الكلي للطور اليرقي لأبوالعيد إحدى عشر نقطة سجلت أعلى القيم عند تربيته على بيض فراشة دقيق البحر الأبيض المتوسط *Ephestia kuhniella* و كانت % 86.67 ، %96.67 و %90 على درجات الحرارة 20 ± 1 ، 25 ± 1 و 30± 1ºم على التوالي و رطوبة نسبية 65 ± 5 %. سجلت أطول فترة حياة للإناث و الذكور عندما ربيت المفترسات على كلا من أنواع التغذية عند درجة حرارة 20 ± 1ºم بالمقارنة بدرجات الحرارة 25 ± 1 و 30± 1ºم . و في نفس الوقت سجل أعلى معدل للخصوبة عند درجة حرارة 25 ± 1ºم و ذلك لنوعى التغذية في حين كان أقل معدل للخصوبة لمفترس أبوالعيد إحدى عشر نقطة عند درجة حرارة 20 ± 1ºم .