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Effect of Essential and Factitious Foods and their Mixing on Developmental Performance and Reproductive Fitness of the Ladybeetle, *Hippodamia variegata*

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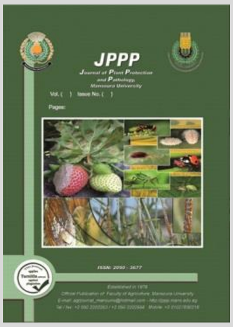

ABSTRACT

Food quality has a direct influence on growth, survival and reproduction of predatory ladybirds. Among the foods of ladybirds, the majority (essential foods) supports both development and reproduction and the rest (factitious foods) is meant for survival. Effect of four food types on development and reproduction fitness of the ladybird *Hippodamia variegata* has been examined at 25.0±1.0 °C and 65±5% RH. The first larval group was provisioned daily with the essential cowpea aphid prey, *Aphis craccivora*, the second with the factitious eggs of Mediterranean flour moth, *Ephestia kuehniella*, the third with the factitious pollen, and the fourth one with a mixed diet of the first (essential) and second (factitious) food types. Unfortunately, the third group larvae failed to complete their development on pollen. The total developmental time (egg-adult) was shortest with heavier males and female body weights when larvae fed with a mixed diet of essential and factitious food. Pre-adult survival rates for *Hippodamia* progenies significantly differed among the treatments with no mortality for those larvae provided with a mixed diet of essential and factitious food. Food type significantly affected the 10-cultch egg fecundity for *Hippodamia* females with the highest fecundity estimated for females that their progenies fed with a mixed diet of essential and factitious prey, whereas the 10-cultch egg fertility did not vary among treatments. The combination of *Ephestia* eggs plus cowpea aphid holds promise as an alternative food for the mass production of *H. variegata* since both enable faster development and greater egg production.

Keywords: *Aphis craccivora*, Fecundity, Fertility, Survival

INTRODUCTION

Ladybeetles consider the most effective predators of aphids in worldwide and maximizing their impact on their prey is necessary for the successful augmentative biocontrol in comparison with the classical biocontrol approach of using alien species (Hodek et al., 2012). In evolutionary biology, one of the most important challenges is identifying and quantifying traits that significantly impact performance of an organism. Thus, great attention, both empirically and theoretically, to the traits that influence lifetime reproductive success has been paid (Godfray, 1994; Roff, 2000). Hypothetically, organism endeavors to evolve life-history traits through optimizing its fitness, such as accelerating development, rapid sexual maturity, attaining large adult body size, prolonging lifetime longevity, and produce many offspring. However, these advantages are unreachable in nature because of many limitations including lack of adequate food, metabolic restrictions, and environmental heterogeneity (Roff, 2000).

The variegated ladybeetle, *Hippodamia* (Adonia) *variegata* (Goeze) (Coleoptera: Coccinellidae) has been docummented as the most important predator of aphids that infesting many crops in several countries (e.g., Fan and Zhao 1988; Kontodimas and Stathas, 2005). It was recorded in aphid-rich crops including alfalfa, clover, rye, sweet corn, and vetch, and in weedy field borders (Ellis et al., 1999). The voracious feeding of aphids by *Hippodamia* spp. keep them as appropriate effective biocontrol agents (Pedigo, 2004; Kontadimas and Stathas, 2005). Its origin is Palearctic, with a cosmopolitan distribution (Franzmann, 2002), and is found in Asia (Hameed et al., 1977), Africa (Badawy, 1969; Saharaoui and Gourreau, 1998), and Europe (Pekin, 1996; Burgio et al., 2006).

Development and reproduction of predators are affected by several factors such as, prey availability (Korpimäki, 1992; Brommer et al., 1998), prey type (Mendes et al., 2002; Omkar and James, 2004; Berkvens et al., 2008; Mirhosseini et al., 2015; de Castro-Guedes et al., 2016), weather (Kostrzewa and Kostrzewa, 1990; Wang et a., 2013), the presence of competing species (Hakkarainen and Korpimäki, 1996; Michaud et al., 2016; Bayoumy et al., 2018) and habitat quality (Newton, 1989; Sim et al., 2001). Bueno (2000) proposed that among the ecological information, nutrition constitutes a powerfully important factor in studies that interesting with maximizing the impact of predators in biological control of insect pests.

Food for ladybirds is directly converted to the reproduction. Among the accepted foods for ladybirds, the majority supports both development and reproduction (essential foods) and the rest is meant only for their survival (alternative foods) (Hodek et al., 2012). Essential prey supports development and reproduction, whereas factitious food confers the energy for adults to survive when essential prey is absent or/and scarce (Mills, 1981; Evans et al., 1999). Thus, most ladybird predators use both kind of food “mixed

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diet" to survive, develop and reproduce (Hauge et al., 1998; Evans et al., 1999; Nielsen et al., 2002; Omkar, 2006). Some reports show that some ladybird predators are able to reproduce on non-aphid items, such as *Ephestia* eggs which may explain the significance of these resources **(**Hodek et al., 1978**;** Lundgren and Wiedenmann, 2004; Lundgren, 2009**).**

Quality of food has a direct influence on the growth, development and reproduction of predatory ladybirds (Dixon, 2000). The suitability of a prey species can be assessed by estimating its impact on biological characteristics of the predator (Kalushkov and Hodek, 2001). Reproduction needs high energy elements that are provided by food, which is a key regulatory factor in reproductive success (Houck, 1991). Thus, to understand the biology, ecology and behaviour of an insect species and to establish pest management strategies, it is important to understand how insect interacts with its food sources. Further, to initiate a successful biocontrol program, it is crucial to depict growth, survival, stage structure, fecundity and predation rate of predators (Farhadi et al., 2011). Due to the need to reduce costs, use of factitious prey/foods as an alternative to natural prey may be a fundamental step in the commercial production of ladybird predators (Cohen, 2003; De Clercq, 2005; Sun et al., 2017). This information would help to select the most effect food source that may facilitate the production of *H. variegata* for future biological control of aphids in Egypt and elsewhere.

MATERIALS AND METHODS

The broad bean (*Vicia fabae* L.) seeds were soaked in warm water for 48-72 hours before germination. Once they germinated, they were planted in plastic pots containing sandy-loam perlite and watered daily until plants were 4 cm in length, and kept indoors under natural light. Once they reached this length, the cowpea aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae) was moved onto the plants. The cowpea aphid colony was maintained in natural light supplemented with metal halide lamps to supply aphids with 24 h of light per day throughout period of rearing. Constantly, new plants were entered the rearing process.

Colony of variegated ladybeetle, *H. variegata* was initiated from ca. 50 beetles per species that directly collected, from green bean and their surrounding weeds that grow at the experimental farm of Faulty of Agriculture, Mansoura University, Egypt, by shacking them from the plants into perforated plastic vials. In the laboratory, adult ladybeetles were provisioned with on the frozen eggs of Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Beneficial lab, Cairo University), and water on a cotton wick. Both *Ephestia* eggs and water were renewed every 48 h. In each experiment, one generation for each species was reached under laboratory condition before the experiment took place, i.e. the experiments started from the F2 progeny.

The ladybird beetle species was kept in incubator set to a temperature of 25.0 ± 1.0 °C and a relative humidity of 65 ± 5% RH. Ladybird larvae and oviposited adults were isolated in Petri-dishes sized 5.5 and 9.0 cm diameters, both with 2.0 cm ht, for development and reproduction, respectively.

The parental beetles of *H. variegata* that reared from the field-collected beetles were isolated in 9.0 cm plastic Petri dishes to lay eggs. Twenty females were isolated and one day of eggs was collected at the same time from all females to ensure homogeneity of development. Eggs were kept at 25.0 ± 1.0 °C and 65 ± 5% RH. After hatching (3 days for all eggs), the neonate larvae were divided into four groups. In each group, 50 neonate larvae were reared until adult emergence. Larvae of each group were isolated in 5.5 cm Petri-dishes. Dishes were kept at 25.0 ± 1.0 °C and 65 ± 5% RH. Larvae of the first group were provisioned daily with the essential cowpea aphid prey, *A. craccivora*, the second group with the factitious frozen eggs of *Ephestia*, the third group with the factitious pollen item, and the fourth group with a mixed diet of the cowpea aphid and *Ephestia* eggs. Water was provided on pieces of sponge for all larvae. Larvae were checked daily and all developmental transformations were recorded until adult emergence. Data of individual beetles that failed to complete their development were excluded from analysis. Upon emergence, beetles were sexed and weighted on an analytical balance to obtain their fresh body mass. The third group larvae were failed to complete their development on pollen and water, and thus excluded from data analysis.

In the three feeding treatments, all adults received from each feeding regime were kept together in plastic containers (20 cm diam. x 30 cm ht) at the same physical conditions as above. Adult beetles were only provisioned with aphids for two days to induce spermatogenesis or oogenesis, then provided only with *Ephestia* eggs until the time of reproduction. Once mating took place, each female from the same feeding regime was marked, isolated in 9.0 cm Petri dish and provided daily with the same type of food that received during its pre-adult development. Water considered as well. These females were kept at the same physical conditions, as above. 10-egg clutches were collected from each female. From each feeding type, ten mated females were used. Eggs were recorded daily and then incubated at 25.0 ± 1.0 °C and 65 ± 5% RH until hatching. The total number of eggs (female fecundity) and the total number of viable eggs (female fertility) were counted for each female. The number of days required to obtain these ten-clutches of eggs were also determined for each female in each feeding type.

Data (developmental times, adult fresh weights, and female fecundity and fertility) were first checked for normality (Shapiro–Wilk test). After passing the assumption test, data were statistically analyzed using one-way ANOVA. Once significance appeared, means were compared by the Bonferroni Multiple Comparison test ($\alpha < 0.05$). The proportions of progeny surviving to adulthood were analyzed using chi-squared (χ goodness-of-fit tests). All tests were conducted using SigmaPlot 12 (2011; Systat Software, San Jose, CA, U.S.A.).

RESULTS AND DISCUSSION

Results

Development, body weight, and pre-adult survival

Over the three food types, one-way ANOVA revealed that there were significant differences in periods of larval stage ($F_{2,164} = 60.29$, $p < 0.001$), total development ($F_{2,136} = 20.15$, $p < 0.001$), male fresh body weight ($F_{2,62} = 40.32$, $p < 0.001$), female fresh body weight ($F_{2,74} = 75.54$, $p < 0.001$), and an individual ladybird body weight ($F_{2,136} = 36.79$, $p < 0.001$), however food types did not affect the pupal period ($F_{2,136} = 0.95$, $p = 0.39$). In general, the development time (egg-adult) was shortest when ladybird larvae fed on diet consisted of both cowpea aphid and frozen moth eggs of *E. kuehniella*. In addition, heavier males and female body weights were obtained from larvae reared on such mixed food type (Table 1).

Survival rates for progeny of *H. variegata* females significantly differed among the three food types ($\chi^2 = 2.56$, df = 2, $p < 0.05$) with the highest pre-adult mortality was estimated for larvae provided with frozen moth eggs, while there was no mortality for those provided with a mixed diet of cowpea aphid and frozen moth eggs.

Maternal reproduction

Food type significantly affected the 10-cultch egg fecundities for *H. variegata* females ($F_{2,29} = 42.13$, $p < 0.001$) with the highest number of eggs laid by females that their progenies fed with a mixed diet of cowpea aphid and frozen eggs of *E. kuehniella,* while the 10-cultch egg viabilities (i.e. fertility) for *H. variegata* females did not significantly vary ($F_{2,29} = 2.91$, $p = 0.07$) under the three different food types (Fig. 1). Statistical analysis revealed that there were significant differences between females of *H. variegata* of the three treatments in the number of days required to obtain 10-clutches of eggs ($F_{2,29} = 22.99$, $p < 0.001$) with the shortest number of days for mothers fed during their larval stage on mixed diet of cowpea aphid and frozen eggs of *E. kuehniella* (Fig. 2).

Table 1. Mean (± SE) life history parameters and adult weights for progeny of *Hippodamia variegata* reared on four food types (cowpea aphid, *Aphis craccivora*; frozen moth eggs of *Ephestia kuehniella*; *A. craccivora* + frozen moth eggs of *Ephestia kuehniella*; and pollen). All larvae completed successfully their development on food types except pollen. Neonate larvae were kept with their food at 25.0 ± 1.0 C and 65 ± 5.0 % RH till adult emergence.

Biological parameter	Food type		
	Aphid	Moth eggs	Aphid + moth eggs
Egg stage (d)[NT]	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
Larval stage (d)	11.46 ± 0.07 a	10.44 ± 0.27 b	9.21 ± 0.12 c
Pupal stage (d)	4.39 ± 0.07 a	4.56 ± 0.11 a	4.44 ± 0.08 a
Total development (d)	18.85 ± 0.07 a	18.00 ± 0.32 b	17.28 ± 0.11 c
Male fresh weigh (mg)	0.54 ± 0.01 b	0.42 ± 0.01 c	0.62 ± 0.02 a
Female fresh weight (mg)	0.70 ± 0.01 b	0.64 ± 0.02 c	0.89 ± 0.01 a
Total adult fresh weight (mg)	0.63 ± 0.01 a	0.53 ± 0.02 c	0.78 ± 0.02 a

Means bearing the same letters within a row are no significantly different (ANOVA followed by Bonferroni's Multiple Comparison test, α =0.05). NT = Not statistically tested.

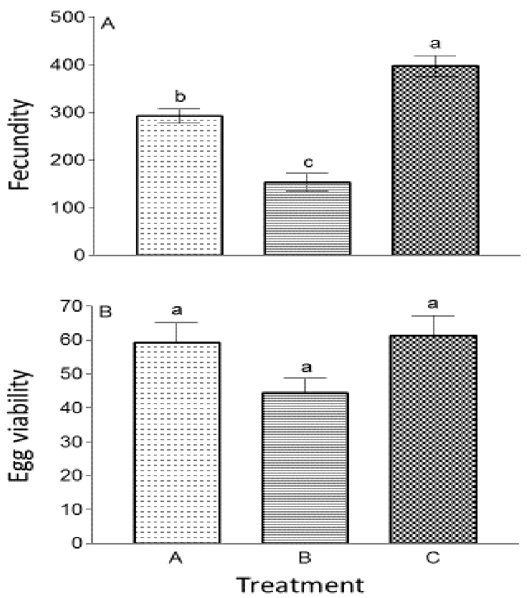

Figure 1. The 10-clutch egg fecundity and egg viability (±SE) for adult females of *Hippodamia variegata* that obtained from progenies reared under three different food types (A: cowpea aphid, B: frozen eggs of *Ephestia kuehniella*, and C: cowpea aphid + frozen eggs of *E. kuehniella*) at conditions of 25.0 ± 1.0 C and 65 ± 5.0 % RH. In case of treatment "B", females fed with aphids for two days prior to moth eggs to develop their eggs. Symbols bearing the same letters are not significantly different (ANOVA followed by Bonferroni's Multiple Comparison test, α =0.05).

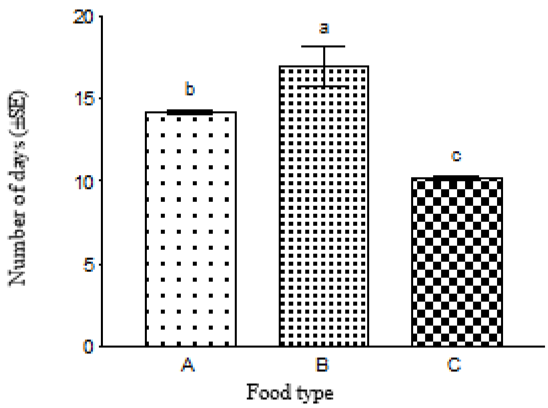

Figure 2. Number of days (±SE) required to obtain 10-clutches of eggs by adult female of *Hippodamia variegata* that obtained from progenies provided with one of three different food types (A: cowpea aphid, B: frozen eggs of *Ephestia kuehniella*, and C: cowpea aphid + frozen eggs of *E. kuehniella*) and provided with the same type of food during her oviposition, except females of treatment "B", that fed with aphids for two days prior to moth eggs to develop their eggs. Experiment was at conditions of 25.0 ± 1.0 C and 65 ± 5.0 % RH. Symbols bearing the same letters are not significantly different (ANOVA followed by Bonferroni's Multiple Comparison test, α =0.05).

Discussion

As it is known, pollen gives the generalist coccinellid predators the energy required to be alive with a low mortality when prey is rare, however in this study pollen failed to support larval development of the specialist predator, *H.*

variegata with more than 70% mortality. Pollen represents a factitious food which does not permit alone the oocytes to mature but may constitutes an earlier start for oviposition by *Adalia bipunctata* L. after aphids appear (De Clercq et al., 2005). Bee pollen reported as a less suitable food for the Asian ladybird, *Harmonia axyridis* (Pallas) than the frozen eggs of *E. kuehniella* (Berkvens et al., 2008). A single diet of pollen permitted 35–48% larvae to reach adulthood, prolonged development by 31–49% and reduced adult weights by 37–68%. When both larvae and adults provided exclusively with pollen, only 40% of females oviposited a small number of viable eggs. Hukusima and Itoh (1976) attempted to rear *H. axyridis, C. septempunctata brucki* and *Menochilus sexmaculatus* (F.) (Coleoptera: Coccinellidae) on pollen. They failed with 7 and 10% to reach adulthood on maize and rye pollen, respectively.

In the current study, the mixed diet of cowpea aphid and *Ephestia* eggs shorten the larval development and accordingly the total development (egg-adult) of *H. variegata*. By this mixed diet, higher immature survival rates than those on other sole diets (i.e. pollen, cowpea aphid or *Ephestia* eggs) have been obtained. Further, heavier males and females resulted from larvae developed on such mixed diet. Regards total development (Table 2) diet of *Ephestia* eggs was intermediate, but for fresh body weight of both males and females, cowpea aphid was intermediate in its quality. In the contrast, Phoofolo et al. (2007) did not observe any variation between single and mixed diets of *Schizaphis graminum* (Rondani) and *Rhopalosiphum padi* (L.) for larval development of *H. convergens*. This may be because they used a mixture of two usual prey compared to the current study in which I used usual and factitious prey. Combination of various dietary resources has been examined frequently in phytophagous insects with a positive impact in most cases (Unsicker et al., 2008). The coccinellids may balance between valuable nutrients from pollen and aphids or pollen and conidia of mildew etc. (Waldbauer and Friedman, 1991). Some researchers have aimed to examine the suitability of food mixtures for ladybirds. The synchronized presence of two prey species has been increased the predation efficiency of *H. axyridis* (Lucas et al., 2004). However, no benefits from mixing diets have been documented for four species of predatory ladybirds that reared on a mixed diet of eggs of *Leptinotarsa decemlineata* (Say) and *Myzus persicae* (Sulzer), since they had lower survival and development rates than those reared on aphid alone (Snyder and Clevenger, 2004). As well, *C. septempunctata* did not get any benefits from feeding on a mixed prey that composited of aphid species, *Sitobion avenae* (F.), *Metopolophium dirhodum* (Walker) and *R. padi* (Ozder and Saglam, 2003), likely because the low quality of nutrient content (Cohen and Brummett, 1997) or presence of allelochemicals (Hauge et al., 1998).

Developmental benefits and costs differed between coccinellid species. For example, frozen eggs of *Ephestia* were more valuable for development *H. axyridis* (Berkvens et al., 2008), for *C. maculata* (Michaud and Grant, 2005), for *A. bipunctata* (De Clercq et al., 2005) and for *C. septempunctata* (Sundby, 1966) than the pea aphid, *Acyrthosiphon pisum* (Harris) or pollen. *Ephestia kuehniella* eggs are, however, unsuitable for *P. quatuordecimpunctata* (Bazzocchi et al., 2004) and for *C. undecimnotata* (Iperti and Trepanier-Blais, 1972; Schanderl et al., 1988). Ueno (2003) found that the larval and pupal durations of *H. axyridis* were the shortest on *A. pisum*, longest on *A. craccivora*, and intermediate on artificial diet. Thus, *A. craccivora* seems to be an unsuitable food for *H. axyridis* (Hodek, 1996; Ueno, 2003), with the latter author found lower toxicity for cowpea aphid, *A. craccivora* than the former ones in his earlier studies. The poor suitability of *A. craccivora* may not because its nutritive value, as it has produced heavier males and females than those resulted from frozen, but lighter than those resulted from mixed diet (i.e., aphid + *Ephestia* eggs), but more likely because its difficulty in ingesting and digesting by *H. variegata* due to its hard cuticle. When larvae of *A. bipunctata* fed with a mixed diet of *A. kuehniella* eggs and pollen, heavier adults and higher oviposition rate were obtained than when larvae fed only with *A. pisum* (De Clercq et al., 2005). Thus, *H. variegata* used the mixed diet to gain developmental and reproductive benefits from feeding both prey to achieve the faster development through benefiting from more easily digestible *Ephestia* eggs and to attain the heavier body weights through benefiting from more nutritious cowpea aphid. The nutritional value of cowpea aphid can be proven and documented through the shorter time for obtaining 10-egg clutches by *H. variegata* females than those fed with *Ephestia* eggs. Accordingly, the heavier body weights, especially for females of the mixed diet, resulted in producing higher number of eggs (fecundity) than those from other two diets, but the number of viable eggs did not vary among diets. Further, mothers fed cowpea aphid laid intermediate number of eggs compared with the lowest number for those fed *Ephestia* eggs. The importance of animal protein from *E. kuehniella* for egg laying was documented in *H. convergens*, however the largest fecundity and shortest preoviposition period were obtained when the beetles permitted to fed afterwards on aphids (*S. graminum*). Accordingly, this food mixture resulted in higher fecundity than continuous feeding on *Ephestia* eggs or aphids (Michaud and Qureshi, 2006). The same was obtained in similar experiments on *P. japonica* that used *A. pisum* in combination with *Ephestia* eggs (Hamasaki and Matsui, 2006).

The significance of factitious prey can be best evaluated in case of adding to a low amount of essential prey. Mass rearing of *H. variegata* could be improved by addition of moth eggs of *Ephestia*, as factitious prey, to the essential aphid prey. Both enable faster development and greater egg production, as *Ephestia* eggs suitable for development, and aphids more suitable for reproduction. Thus, the developmental rate of *H. variegata* larvae can be accelerated by feeding *Ephestia* eggs in addition to aphids, or separately even of case of aphid shortage.

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تأثير الأغذية الأساسية والبديلة والخلط بينهما على أداء النمو وقوة الإنتاج لمفترس الهيبوديميا فاريجاتا *Hippodemia variegate*

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لجودة الغذاء تأثير مباشر على النمو والبقاء والإنتاجية لمفترسات خنافس أبو العيد. من ضمن أغذية خنافس أبو العيد، فأن بعض الأغذية (الأغذية الأساسية) تدعم كل من النمو والإنتاج، فى حين أن البعض الاخر (الأغذية البديلة) تكون تستخدم فقط للبقاء علي قيد الحياة .تم فحص تأثير أربعة أنواع من الغذاء على قوة النمو والإنتاج لمفترس أبو العيد الهيبوديميا *Hippodemia variegata* على درجة حرارة 25 ± 1 م° ودرجة رطوبة نسبية 65 ± 5 % . تم تغذية المجموعة الأولى من اليرقات يوميا علي من البقوليات كغذاء أساسى، والمجموعة الثانية تم تغذيتها ببيض فراشة دقيق البحر الأبيض المتوسط *Ephestia kuehniella* كغذاء بديل، والمجموعة الثالثة تم تغذيتها علي حبوب اللقاح كغذاء بديل، والمجموعة الرابعة تم تغذيتها على خليط من من البقوليات (كغذاء أساسى) وبيض فراشة دقيق البحر الأبيض المتوسط (كغذاء بديل) . لسوء الحظ فأن يرقات المجموعة الثالثة فشلت في إكمال نموها على حبوب اللقاح . أظهرت النتائج أن الوقت الكلي للنمو من طور البيض إلى خروج الحشرة الكاملة كان أقصر مع أوزان أثقل لكل من ذكور وأناث المفترس عندما تم تغذية يرقاتها على خليط من الفريسة الأساسية والبديلة .كما أختلفت معدلات البقاء فى الأطوار ما قبل طور الحشرة الكاملة بصورة معنوية بين المعاملات الثلاث مع عدم حدوث موت فى حالة اليرقات التى تغذت على خليط من الفريسة الأساسية والبديلة . أظهرت النتائج أيضا أن نوع الغذاء أثر بصورة معنوية على الخصوبة الكلية لإناث مفترس الـ *Hippodemia* مع أعلى خصوبة كلية كانت فى حالة الإناث التى تم تغذية يرقاتها على خليط من الفريسة الأساسية والبديلة، فى حين أن الخصوبة الفعلية لإناث المفترس لم تختلف بصورة معنوية بين المعاملات الثلاث. يتضح أن المزج بين بيض الـ *Ephestia kuehniella* ومن البقوليات يبشر بالخير كغذاء بديل للأنتاج الكمى لمفترس الـ *Hippodemia variegate* حيث أدى إلى أسرع نمو وأعلي أنتاج للبيض في المفترس.