

THE EFFECT OF ARTIFICIAL FEEDING THE DEVELOPMENT AND GROWTH OF THE THIRTEEN- SPOTED LADYBIRD *HIPPODAMIA VARIEGATE* THE SEVEN-SPOTED LADYBIRD *COCCINELLA SEPTEMPUNCTATA*.L

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Abstract. *The laboratory experiments were conducted by the biological control laboratory of the Graduate Studies Laboratory at Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University on 10/8/2020 to show the possibility of breeding and propagating two types of predatory ladybirds, namely the thirteen-spotted ladybird *Hippodamia variegata* and the seven-spotted ladybird *Coccinella septempunctata*.L on a group of different artificial foods (alfalfa leaf powder, chicken egg yolk powder, fish meal, sedge pollen powder, locust powder) in addition to live whitefly nymphs and frozen whitefly nymphs. The results showed:*

*Adults of the thirteen-spotted ladybird *H. variegata* and the seven-spotted ladybird *C. septempunctata*.L succeeded in surviving for twenty-three days when fed with different artificial foods and with significant differences compared with the control treatment.*

*The larvae of the adult thirteen-spotted ladybird *H. variegata* and the seven-spotted ladybird *C. septempunctata*.L succeeded in survival, reaching the highest period of 8.34 days for the second larval instar compared to the control treatment, which amounted to 2.72 days, with significant differences*

*None of the larval stages of the thirteen-spotted ladybird *H. variegata* and the seven-spotted ladybird *C. septempunctata*.L from moulting to the later instar when fed on the above-mentioned artificial foods. While the larvae that fed on live whitefly nymphs and frozen whitefly nymphs were able to complete their development and growth normally.*

*The thirteen-spotted ladybird *H. variegata* and the seven-spotted ladybird *C. septempunctata*.L could not lay eggs when fed on artificial foods. While the total number of eggs for the thirteen-spotted ladybird *H. variegata* during the study period was (315.40, 211.20 egg) when reared on live and frozen whitefly nymphs, respectively, while the total number of eggs for the seven-spotted ladybird *C. septempunctata*.L was (307.80, 245.2 egg) when reared on live whitefly nymphs and frozen whitefly nymphs, respectively.*

Keywords: *Hippodamia. Variegata , Coccinella. Septempunctata.L*

1. INTRODUCTION

Predatory Coccinellids (ladybirds) belong to the Coccinellidae family of the Coleoptera order, which includes a large number of species, up to 220 species, most of which are animal-feeding insects, except for

the suborder Epliachinae, which are phytotrophic, where they feed on some plant parts such as leaves, fruits and on the nectar of flowers, or honeydew. Iraq More than 60 species of ladybirds [14], [2]. They are called by different names according to the different countries and areas in which they are located, but the common name for them in some Arab countries is the Abi Eid beetles [15]. ladybirds have known importance since ancient times in the fields of natural and biological control. It was found that they have a wide range of families, especially aphids, whiteflies, scale insects, mealybugs, as well as some small stages of dream animals and some insects [11], [7], [4]. The seven-spotted ladybird is *C. septempunctata*.L and the eleven-spotted ladybird *C. undecipunctata*.L is one of the most common species of ladybirds in Iraq [16], [13]. The insect is controlled by many chemical pesticides in the world and Iraq, but the irrational use of chemical pesticides and the appearance of the trait of resistance to the insect and the residual effect of the environment made researchers search for other alternative methods that are safer for the environment and humans, and one of these methods is the use of vital numbers as a complementary method with other methods of resistance [8]. Several studies have been conducted on this predator that dealt with the prey that feeds on it, its life, and its predation efficiency [1]. Ease of breeding is considered one of the most important points to be provided in the biological enemy. The food of predation ladybirds is directly related to reproduction. There are certain foods that support growth and reproduction (basic foods) and others are dedicated only to their survival (alternative foods) [12]. The expansion of research and scientific studies in the use of natural enemies in Integrated Protection Management programs (IPM) contributes to finding safe, effective, and controlled methods for their breeding and reproduction and creating the appropriate environment for them. Natural foods in different parts of the world, The attempts were different in terms of success and failure. [11], [5]. In light of this, the research aims at the effect of nutritional media on the growth and development of larvae and adults of the seven-spotted ladybird *C. Septempunctata*. L and the thirteen-spotted ladybird *H. variegata*.

2. MATERIALS AND METHODS

2.1 Breeding predation ladybirds:

2.1.1 seven-spot ladybird(*C. Septempunctata* L) :

The 20 pairs of adult male and female predator *C. Septempunctata*.L were brought from one of the fields of Kutha District, Babylon province on 10/8/2020. They were diagnosed by Prof. Razak Shaalan Akal and Prof. Dr. Hana Hani Al-Saffar (Research Center and the Natural History Museum / the University of Baghdad), It was placed inside a glass cage measuring (75 x 75 x 75 cm). The back and sides were covered with transparent glass, and the front was covered with muslin and the cloth was provided with a hole (50 cm) long with a zipper that could be controlled by opening and closing the box for the purpose of bringing in and taking out insects and food and to prevent the escape of adults. As for the upper and lower sides, they were covered with a piece of plywood, and each box was provided with a sufficient quantity of infected plants in the whitefly nymphs that were brought from the infected fields. Where parts of the affected plants are taken and their lower end is immersed in a container containing water added to it (5 g) of sugar to maintain the greenness of the plant to save moisture, small Petri dishes with a cotton wet with water were placed inside the cage, and the cage was placed in the laboratory at a temperature of 28 ± 2 °C and a period of 16 hours of illumination / 8 hours of darkness. The box was also provided with a layer of multi-cell sandwich made of two layers of corrugated cardboard on one side measuring (15 x 10 cm) and fixed to each other by pins, So that each corrugated side of one of the two pieces meets the corrugated side of the other, forming tubes and long cells for the purpose of preparing the appropriate

place for laying eggs inside them by the female maidens, The sandwiches that contain eggs are raised and replaced daily and transferred to other cages away from the mothers to prevent self-predation of eggs (cannibalism) for the purpose of perpetuating the colony [11] , [10]

2.1.2 *The thirteen- spotted ladybird H. variegata:*

Pairs of male and female *H. variegata* were brought from the fields indicated above, and they followed the same breeding steps mentioned in (2-1-1).

2.2 *Preparation of industrial foods:*

2.2.1 *Mixture of sedge pollen:*

The method of [11],[4] was followed by mixing 0.7 g of agar with 25 ml of cold distilled water and then leaving it for 20 minutes, then putting the solution on fire until it reaches the boiling point, then taking it off the fire and mixing with it 5 g of natural honey. and 8 g of cane sugar, then leave the mixture to cool to a temperature of 50°C. After that, mix the mixture with 5 g of sedge pollen, 0.3 g of yeast, 25 ml of cow's milk, 0.75 g of ascorbic acid and 10 mg of royal jelly, so that the mixture became of medium consistency, kept in the freezer at a temperature of (-20 °C) until use

2.2.2: *Locust Powder Mixture:*

Numbers of locusts were collected from the fields of farms in the Kutha region, and then dried in an electric thermal oven at a temperature of 45 °C, and then milled by an electric grinder, and the method mentioned in (2-2-1), except for replacing pollen with 5 g locusts

2.2.3: *Fish meal mixture:*

Quantities of small fish were collected, the internal entrails and their outer scales were cleaned and then dried in an electric thermal oven at a temperature of 45 °C, then they were ground by an electric grinder, and followed the same steps mentioned in (2-2-1), except for replacing sedge pollen with 5 g fish

2.2.4: *Chicken egg yolk powder mixture:*

Quantities of chicken eggs were collected and then dried by an electric thermal oven at a temperature of 45°C and only the yolk was taken from them, then dried by an electric grinder, and I followed the same steps mentioned in (2-2-1), except for the replacement of papyrus pollen grains with 5 g of chicken egg yolk.

2-2-5: *Alfalfa Leaves Powder Mixture:*

The leaves of the Alfalfa plant were collected from the Alfalfa fields in the Kutha region, and then dried by an electric thermal oven at a temperature of 45°C. Then it was milled by an electric grinder, and it followed the same steps mentioned in (2-2-1), except for the replacement of sedge pollen with 5 g of Alfalfa leaf powder.

2.3: *Effect of Feeding on the Development of ladybirds Larvae:*

2.3.1: The seven- spotted ladybird *C.Septempunctata L.* :

2.3.1.1: First larval age:

Dishes with a diameter of 6 cm and a height of 3 cm were taken and the dishes were divided into eight groups (treatments), each group included five dishes (replicators), and each group was provided daily with the following:

- 1- The first group compared without food.
- 2- The second group 10 whitefly nymphs.
- 3- The third group 1g mixture of sedge pollen.
- 4- Fourth group 1 g of locust powder mixture.
- 5- Sixth group 1g fish meal mixture
- 6- Seventh group 1 g of chicken egg yolk powder mixture.
- 7- The eighth group: 1 g of a mixture of the leaves of the Alfalfa plant. Provide each dish with one larva of one day-old *C. septempunctata L.* seven- spotted larvae and that the larvae remain immobile after hatching, immobile for up to 12 hours, and then leave the eggshells that came out of them and begin to move and search for food, and the larvae are transferred by a small paintbrush.

2.3.1.2: The second larval age:

The second instar larvae of the seven- spotted ladybird *C. septempunctata L.* were taken from the larvae of the first instar and fed on whitefly nymphs until they molt and transform into the second larval instar and the same steps of the experiment were mentioned in the first larval instar, except for replacing the first larval instar with the second larval instar .

2.3.1.3: The third larval age:

The third instar larvae of the seven- spotted ladybird *C.Septempunctata L.* were taken. From the larvae of the second larval instar referred to above, the same steps of the experiment mentioned in the first larval instar were performed, except that the first larval instar was replaced by the third larval instar.

2.3.1.4: Fourth larval age:

Larvae of the fourth larval instar of *C.Septempunctata L.* were taken from the larvae of the third larval instar referred to above, and the same steps of the experiment mentioned in the first larval phase were performed, except that the first larval instar was replaced by the fourth larval instar . The period of development of the larval role was calculated from the beginning of hatching of the eggs to the entry of the larvae into the pupae role, as well as observing the emergence of adults or their failure to exit from the pupae role.

2.3.2: The thirteen- spotted ladybird *H. variegata*:

The same steps of the experiment mentioned in (2-3-1) were conducted except that the seven- spotted ladybird *C. septempunctata*.1 larvae were replaced by the thirteen- spotted ladybird *H.variegata* larvae.

2.4: The effect of artificial feeding on the life of adult predation ladybirds:

2.4.1: The seven- spotted ladybird *C.Septempunctata* .L :

The 40 plastic dishes with a diameter of 12 cm and a height of 6 cm were brought. The dishes were divided into eight groups (treatments), each comprising five dishes (replicators), and each dish was provided with a piece of multicell sandwich, as much as half a base for each dish for the purpose of laying eggs inside it by the females. Then, one pair of ladybirds (male and female) was placed in each dish, and at the age of (7 days), each group was provided daily with the following:

- 1- The first group compared without food.
- 2- The second group 2 g of whitefly nymphs.
- 3- The third group 2 g mixture of sedge pollen.
- 4- Fourth group 2 g of locust powder mixture.
- 5- The sixth group: 2 g of fish meal mixture.
- 6- The seventh group: 2 g of chicken egg yolk powder mixture.
- 7- The eighth group: 2 g mixture of Alfalfa leaves powder.

The opening of each dish was covered with a piece of muslin cloth fixed by a rubber band with the same steps of the experiment mentioned in (3-3-1), the number of eggs laid by each female per day and the percentage of hatching until the end of the experiment was calculated, in addition to recording the age of the adults.

2.4.2: The thirteen- spotted ladybird *H. variegata*:

The same steps were performed in the experiment mentioned in (3-4-1), except for the replacement of the seven- spotted ladybird *C.Septempunctata*. L The thirteen- spotted ladybird *H. variegata*

2.5 STATISTICAL ANALYSIS:

The experiments were designed according to the Complete Randomized Design (C.R.D) and the results were analyzed using the Anova Table and the means were compared using the choice of the least significant difference (L.S.D) under the level of significance (0.05) to test the significance of the results [6]

3. RESULTS AND DISCUSSION

3.1 The effect of artificial foods on the development of predation ladybirds:

3.1.1 Effect of breeding the predation ladybird *C.septempunctata* .L on different artificial foods on the development of larvae:

Table (1) indicated that the larvae of the seven- spotted ladybird *C.septempunctata* L., which were fed artificially, died after several days from the beginning of the feeding and did not molt to the next stage, while the larvae fed on natural foods (live whitefly nymphs) completed Frozen whitefly nymphs) developed and moulted into the later stage. The results showed that the highest average lifespan of first-stage larvae fed on live whitefly nymphs was 5.64 days and frozen whitefly nymphs reached 5.96 days, while the average lifespan of first-stage larvae fed on a mixture of Alfalfa leaf powder, chicken egg yolk powder and powder Fish, sedge pollen powder mixture and locust powder mixture (2.08, 2.06, 1.94, 1.88, 1.98) days, respectively, compared with the control treatment, which amounted to 1.12 days, and there were non-significant differences. As for the second instar larvae, it was noted that there were significant differences between their survival average in all treatments, as it reached 8.34, 7.54, 4.92, 3.66, 5.02, and

2.72 days at Feeding on the mixture of Alfalfa leaf powder, chicken egg yolk powder mixture, fish meal, sedge pollen powder mixture and locust powder mixture, As for the larvae that were fed on live whitefly nymphs and frozen whitefly nymphs, they moulted to the later stage. As for the effect of feeding on the average of the third larval instar, it was shown in Table (1) that the average of the third larval stage was 3.78, 3.98 days when feeding on live whitefly nymphs and frozen whitefly nymphs, respectively, without significant differences between them, while there are clearly significant differences Between the averages of industrial foods, which amounted to (7.04, 6.82, 6.08, 5.46, and 6.12) days, respectively, and the control treatment, which amounted to 0.57 days. As for the effect of these food media in the fourth larval instar period, Table (1) showed the average length of the fourth larval instar amounted to 4.18 and 4.92 days, which were fed on live whitefly nymphs and frozen whitefly nymphs, respectively, with significant differences. As for the duration of larvae survival, The life that was left without food or fed on artificial foods amounted to 7.58 and 3.14 days, with significant differences between them. The results also showed that the fourth instar larvae fed on powdered Alfalfa leaves, chicken egg yolk powder, fish meal, sedge pollen powder, and locust powder entered the pupa stage, but could not exit to the adult stage.

Table (1) Effect of breeding on different industrial foods on the development of the seven- spotted ladybird larvae *C. Septempunctata*. L

food type	The average period of larval age (day)			
	First instar	Second instar	Third instar	Fourth instar
Alfalfa leaves powder	*2.08	*8.34	*7.04	*7.58
chicken egg yolk powder	*2.06	*7.54	*6.82	*6.36
Fishmeal	1.94*	*4.92	*6.08	*4.64
sedge pollen powder	*1.88	*3.66	*5.46	*4.18
locust powder	*1.98	*5.02	*6.12	*5.96
Live whitefly nymphs	5.64	3.84	3.78	4.18
frozen whitefly nymphs	5.96	4.00	3.98	4.92
without food (control)	*1.12	*2.72	*3.98	*3.14
L.S.D	0.80	0.78	0.57	0.67

The larvae died before they moulted.

1.2.1 Effect of breeding thirteen- spotted predation ladybird *H. variegata* on different artificial foods on larval development: Table (2) indicated that all larvae of the thirteen- spotted ladybird *H. variegata* that were fed artificial foods failed to reach the later stages, although they survived for a longer period than those left without food. The average length of the first larval instar was 3.64, 4.14 days over respectively when feeding on live whitefly nymphs and frozen whitefly nymphs and without significant differences, while the larval survival time was between 1.76 and 1.42 days when fed on artificial foods with significant differences between them compared with the control treatment 1.04 days. As for the second larval instar, it reached 3.54 and 3.68 days when fed on live whitefly nymphs and frozen whitefly nymphs, respectively, without significant differences. As for the larvae in the other treatments, they survived for a period ranging between 2.54 and 3.02 days, with significant differences compared to the control treatment. 1.36 days. As for the third larval instar , it reached 3.22 and 3.68 days when feeding on live whitefly nymphs and frozen whitefly nymphs, respectively, and there were no significant differences, while the larvae raised on

artificial foods remained live for a period ranging between 6.54 and 3.38 days compared with the control treatment that It reached 2.62 days and there were significant differences. As for the fourth larval instar, the average instar reached 4.18, 4.32 days when feeding on live whitefly nymphs and frozen whitefly nymphs, respectively, and there were no significant differences, where the larvae fed on artificial foods remained for a period ranging between 7.78 and 6.62 days, transformed All of them reached the role of the Virgin, but she did not succeed in reaching the role of the adult. The failure of the larvae of the predatory ladybirds under study that were raised on artificial foods to molt and move to the later stages despite their survival for quite a while. for predation ladybirds, While the development or molting of the fourth larval stage of the ladybird may be the sufficiency of the fourth larval stage from the food obtained from the different larval stages that preceded it, where the results agreed with what was found [4] when rearing larvae on baby food powder, while it did not agree with The results of [3] when rearing larvae on sheep liver mixture The reason for the difference in the results may be due to the environmental conditions under which the experiment was conducted. However, the rearing of the larvae succeeded when fed on live whitefly nymphs and frozen whitefly nymphs, where large quantities of whitefly nymphs can be collected from the field during the breeding season, sufficient to support the rearing of large numbers These results are consistent with what was reached by [4] , [16] , [11].

Table (2) Effect of breeding on different artificial foods on the development of thirteen- spotted ladybird larvae *H. variegata*

food type	The average period of larval age (day)			
	First instar	Second instar	Third instar	Fourth instar
Alfalfa leaves powder	*1.76	* 3.02	*6.54	*7.78
chicken egg yolk powder	*1.76	*2.86	*5.14	*7.64
Fishmeal	*1.56	*2.28	*3.38	*6.62
sedge pollen powder	*1.42	*2.84	*5.02	*6.78
locust powder	*1.56	*2.56	*6.12	*7.44
Live whitefly nymphs	3.64	3.54	3.22	4.18
frozen whitefly nymphs	4.14	3.86	3.68	4.32
without food (control)	*1.04	*1.36	*2.62	*3.22
L.S.D	0.20	0.64	1.13	0.78

The larvae died before they moulted.

3.1.3 Effect of feeding on artificial foods on longevity and fertility of adults *C. Septempunctata.L*: Table (3) indicated that the average age of the seven- spotted ladybird *C. septempunctata.L* adults that were fed artificial foods was significantly and significantly higher than the survival rate of the adults that were left without food, where most of the adults fed on artificial foods remained alive for a period ranging from between 17.36 and 22.98 days when fed on a mixture of AlfAlfa leaf powder and fish meal mixture, respectively, While the average survival time of adults that were left without food was 5.14 days, which is significantly different from the other treatments compared with the control treatment. It also indicated that

there were significant differences between the lifespan rates of adult predation ladybirds fed on artificial foods with those fed on live whitefly nymphs and whitefly frozen, As for laying eggs, the adults who were fed artificial foods were not able to lay eggs despite their survival for a long time. The average number of eggs laid by each female of the predation ladybirds that fed on live whitefly nymphs and frozen whitefly nymphs reached 307.80, 245.20 eggs on respectively and with significant differences between them and with other treatments .

Table (3) The effect of breeding on different artificial feeding on adult survival and the average number of eggs for the seven- spotted ladybird *C.Septmpunctata L*

food type	Average adult survival time (day)	Average number of eggs during the experiment period
Alfalfa leaves powder	22.98	0.00
chicken egg yolk powder	21.58	0.00
Fishmeal	17.36	0.00
sedge pollen powder	17.64	0.00
locust powder	19.66	0.00
Live whitefly nymphs	26.34	307.80
frozen whitefly nymphs	24.34	245.20
without food (control)	5.14	0.00
L.S.D	3.26	27.43

3.1.4 *Effect of feeding on artificial foods on the length of life and fertility of the adults of the thirteen spotted predation ladybird H.variegata*: Table (4) showed that the survival rates of the thirteen- spotted ladybird *H.variegata* also increased when fed on artificial foods, significantly and clearly, then the survival rate of adults that were left without food, where most of the adults fed on artificial foods remained alive for a period ranging between 19.12, 13.54 days when fed on a mixture of AlfAlfa leaf powder and fish meal mixture, respectively, and the average survival time of adults left without food was 6.04 days. The results also showed significant differences between the life expectancy of adults fed artificial foods with those fed live whitefly nymphs and frozen whitefly nymphs, which amounted to 27.00 and 23.96 days, respectively. As for laying eggs, the adults failed to lay eggs when fed on artificial foods despite their survival for a long time, while the average number of eggs per female reached 315.40, 211.20 eggs when fed on live whitefly nymphs and frozen whitefly nymphs, respectively, with a significant difference between them. The reason for the failure of the thirteen- spotted ladybird *H. variegata* and the seven- spotted ladybird *C.Septmpunctata.L* when fed on different artificial foods in this study may be due to a lack of one or more of the basic requirements of nutrients necessary for egg maturation, such as amino acids and vitamins necessary in the adult diet for the process of maturation of eggs to take place naturally, and these vitamins are riboflanin, thymine, pyridoxine, pantothenic acid and folic acid, [17]. The reason may be due to the failure of breeding predation ladybirds on the aforementioned industrial foods to the heterogeneity of the proportions of their components, as [7] explained that insects may fail to develop and lay eggs when raised on industrial foods in the case of the heterogeneity of the proportions of the components of those food media, and the reason is due to the nature of the behavior of these insects in laying eggs. These results agreed with what was found by [11] when three types of baby food were used in raising the predator *C.Septmpunctata.L*. However, his attempt to obtain successful food could support the insects to complete their development or to lay eggs that failed. Nevertheless, he succeeded in extending the lifespan of the insects. Adults for more than two months on artificial foods. The results also agreed with [9]. when the female predator *Stethorus punctillum* was fed a diversified food alternative to her natural feeding, as she did not succeed in laying eggs despite her survival on artificial food. When obtaining natural and mature

eggs when rearing predation ladybugs on live whitefly nymphs and frozen whitefly nymphs, we conclude from this the possibility of using this method in breeding predatory ladybirds because it is an easy and cheap method and the possibility of conducting it in the laboratory, In addition to the abundance of the secondary host of bush plants for breeding whitefly insects, which can be a fertile environment for the reproduction of whitefly and its natural enemies in general and predation ladybirds in particular, and large quantities of whitefly nymphs can be collected and kept frozen in plastic containers inside frozen at a temperature (-20 C) for a long period of time.

Table (4) The effect of breeding on different artificial feeding on adult survival and the average number of eggs for the thirteen- spotted female ladybird *H. variegata*

food type	Average adult survival time (day)	Average number of eggs during the experiment period
Alfalfa leaves powder	19.12	0.00
chicken egg yolk powder	16.76	0.00
Fishmeal	13.54	0.00
sedge pollen powder	17.40	0.00
locust powder	18.60	0.00
Live whitefly nymphs	27.00	315.40
frozen whitefly nymphs	23.96	211.20
without food (control)	6.04	0.00
L.S.D	3.02	27.48

4. CONCLUSIONS

The inability of the larvae of the thirteen- spotted ladybird *H. variegata*, and the ladybird with the capacity of *C. Septempunctata*.L Growth and molting egg laying mortality when feeding on the food media under study. The larvae succeeded when feeding on frozen whitefly nymphs, where large quantities of field can be collected during the breeding season to raise large numbers of predation ladybirds

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