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Biological and Life Table Studies of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) Reared on the Factitious Prey, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae).

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Abstract: The developmental times of immature stages, survival percent, longevity, fecundity, and life table parameters of *Harmonia axyridis* were studied under laboratory conditions by feeding on fresh and frozen grain moth eggs (GME), *Sitotroga cerealella*. The effect of crowding adults on fecundity of females was also determined. The total developmental time from egg hatching to adult eclosion ranged from 18.89 ± 0.32 to 22.5 ± 0.21 days on fresh and frozen GME, respectively. Survival percent from egg hatching to adult emergence was differed significantly when the predator fed on the two diets of GME. Also, pupal and adult weights were affected by feeding on fresh and on frozen GME. There were no significant differences in longevity of females, while there were significant variations in fecundity of females and longevity of males. The calculated values of T, DT, R_o, r_m, and e^{rm} were high by feeding on fresh GME. Morever, rates of survivorship (*Lx*), and maximum oviposition per female per day (*Mx*) were high when the predator reared on fresh than on frozen GME. Crowding conditions of predator adults affected the fecundity of females due to egg cannibalism by both males and females of the predator.

Key words: Predators, Coccinellidae, *Harmonia axyridis, Sitotroga cerealella,* biological characteristic, life table parameters, mass rearing, egg cannibalism.

Introduction

The multicolored Asian ladybeetle, *Harmonia axyridis* Pallas is an important non-specific predator of many insect pests (McClure 1987; Chapin and Brou, 1991; Abdel-Salam 1995; Ellington *et al.*, 1995, Brown and Miller, 1998). It could make a good candidate for mass rearing and release in pest hot spot infestations in open fields and greenhouses, because it has a good search activity and a high consumption rate (Tedders and Schaefer, 1994 and Ferran *et al.*, 1997). It originated in Japan, Korea, Formosa, China, and other parts of Asia (Chapin, 1965). Due to the efficieny of *H. axyridis*, the predator had been imported to many countries (i.e. France, USA, Greece, Egypt, and Syria) (Gordon 1985; Ongagna *et al.* 1993; Abdel-Salam 1995; Ghanim and El-Adl, 1996; Abdel-Salam *et al.*, 1997; "a & b"; ICARDA 1997; Katsoyannos *et al.*, 1997; El-Arnaouty *et al.*, 1998). It has not been yet recorded in the Egyptian fauna (Tawfik, 1997).

In order to use this predator in pest management programs, it is necessary to develop a mass production method for this predator. Factitious preys or hosts are insects (immature or adult stages offered either life or dead by freezing or exposure to U.V. radiation) which are not normally attacked by an entomophagous insects, but they are suitable for their development. They are often easier and inexpensive to rear than the natural preys (Waage *et al.* 1985; Schanderl *et al.*, 1988; Greco and Stilinovic, 1998). The angoumois grain moth eggs, *Sitotroga cerealella* Olivier were used as factitious hosts for mass production of Trichogrammatidae (Hassan, 1981; Abbas *et al.*, 1989); and several insect predators (e.g. *Chrysoperla rufilabris* Burmeister, *Orius majusculus* Reuter and *Leis dimidiata* Fabr.) (Legaspi *et al.*, 1994, Semyanov 1996, Hejzlar and Kabieck, 1998).

Life table parameters are essential to know the general biology of an insect. From these parameters, the intrinsic rate of natural increase (r_m) is regarded as the best available single description of the population growth of a species under given conditions (Southwood, 1978). It can be determined by its developmental time and reproduction rate. It has been used to compare a species under different environmental conditions and as an index of population rate response to selected preys.

However, scanty information is available concerning the suitability of fresh and frozen angoumois grain moth eggs, *S. cerealella* for mass rearing of *H. axyridis*, the effect of these diets on the biology, life table, and the influence of crowding adults on fecundity of females. Therefore, the objective of the current study was to investigate the suitability of fresh and frozen angoumois grain moth eggs, *S. cerealella* as factitious preys for rearing *H. axyridis*, the effect of these diets on the biological characters of this predator, the influence of the above diets on life table parameters, and the effect of crowding adults on the fecundity of the predator female.

Materials and Methods

Rearing immature stages of *H. axyridis*: Since it was brought in October, 1994 from New Mexico State University, Las Cruces, NM, USA for further experimental studies, *H. axyridis* adults were reared on the cotton aphids, *Aphis gossypii* Glover and released in the open fields at the Experimental Research Station, Mansoura University (Abdel-Salam, 1995). The eggs laid by females were removed daily, and monitored until hatching. To avoid cannibalism, the hatched larvae of this predator were reared individually in petri dishes (9 cm in diameter). A piece of filter paper was placed on the bottom of each dish to provide a walking surface for the larvae. Twenty five *H. axyridis* larvae were reared on fresh or on frozen (that had been killed in freezer to prevent egg hatching) grain moth eggs (GME), *Sitotroga ceralella* Olivier which reared according to the method described by Hassan (1981) and Abbas *et al.*, (1989). Each reared larva was considered to be a replicate. The developmental time of immature stages, survival from eggs to adult eclosion, and sex ratio were recorded. Pupal and adult stages body weight were also weighed.

Rearing H. axyridis adults: After eclosion, 20 males and 20 females of *H. axyridis* were also fed on the diets of GME until development was completed. The duration of the pre-oviposition, oviposition, post-oviposition periods, the fecundity of female and the longevity of males was recorded. The effect of the two diets on life table parameters was calculated using a BASIC computer program (Abou-Setta *et al.*, 1986) for females reared on both diets. This computer program is based on Birch's method (1948) for the calculation of an animal's life table. Effect of factitious preys on population growth of the predator was assessed by constructing a life table, using rates of age-specific (Lx), and fecundity (Mx) for each age interval (x). To compare the biotic potential of the predator fed on fresh or frozen GME, the following population growth parameters were determined: the mean generation time (T), the net reproductive increase (R_o), the intrinsic rate of increase (r_m), and the finite rate of increase (e^{rm}). The doubling time (DT) was calculated according to Mackauer's method (Mackauer, 1983). The life tables were prepared from data recorded daily on developmental time (egg to first egg laid), sex ratio, the number of deposited eggs, the fraction of eggs reaching maturity, and the survival of females. Interval of one day was chosen as the age classes for constructing the life table.

Effect of crowding on the fecundity of females: Adults of the predator were divided into four treatments, each had four replicates. Ten males and 10 females in the first treatment, 20 males and 20 females in the second treatment, 30 males and 30 females in the third treatment, and 40 males and 40 females in the fourth treatment were tested. Plastic containers (24X16X11 cm) were used as a rearing unit for each replicate. Paper stripes were criss-crossed in layers to minimize egg cannibalism by both males and females, and to serve as oviposition sites. Each container was covered with muslin and tightened with rubber band. A surplus fresh grain moth eggs were distributed in the containers for feeding. Twenty five pairs of *H. axyridis* were reared in petri dishes as checks. Number of eggs was counted daily for a 30 day observation period, since the highest oviposition rate of *H. axyridis* was generally reached within this period when there was a high food supply (Abdel-Salam *et al.*, 1997 b).
All of the experiments were run in an incubator set for a constant temperature of 27.0±1.0°C,75.0±5.0% RH and a light regime of 16 h L : 8 h D.

Data analysis: Data for developmental time of immature stages, survival, weight of pupae and adults, longevity and fecundity of females, and longevity of males reared on fresh or on frozen GME were subjected for one way analysis of variance (ANOVA), and the means were separated using Duncan's Multiple Range Test (Costat Software, 1990).

Results

Developmental times of immature stages: The ANOVA indicated that there were no statistically significant variation in the incubation period of *H. axyridis* eggs when females reared on fresh and frozen GME (Table 1). Also, data in the same table showed that the average developmental time of the four larval instars was 2.34±0.18, 2.00±0.10, 2.95±0.11 and 3.90±0.08 days, respectively, when larvae fed on fresh GME. While on frozen GME, the developmental time was 3.04±0.12, 2.47±0.09, 3.09±0.10, and 4.80±0.19 days, in succession, with no significant difference. The developmental time of larval and pupal stages was 11.19±0.14 and 4.9±0.10 days, successively on fresh GME. However, on frozen GME, the developmental time of both stages was 13.4±0.17 and 6.0±0.21 days, with no significant differences. Based on statistical analysis, the duration from egg hatching to adult eclosion was significantly differed between fresh and frozen GME. Survival percent from egg to adult of *H. axyridis* varied from 84% on fresh to 80% on frozen GME (Table 1). The average pupal and adult body weight were 27.72±1.22 and 26.79±1.12 mg when larvae fed on fresh GME. Whereas on frozen GME, the body weights were 24.53±1.39 and 23.14±1.65 mg, respectively, with significant variation among the two diets (Table 2).

Longevity and fecundity of adult stage: Average longevity and fecundity of *H. axyridis* adults fed on the two diets of GME is given in Table 3. Pre-oviposition period was shorter when females reared on fresh GME (8.1±0.31 days), while this period lasted 9.5±0.52 days on frozen GME. There were significant differences between oviposition period when reared on the two diets of GME. Whereas, no significant variation has been noted in both of post-oviposition period and the total longevity of females by feeding on the two diets (Table 3). Concerning the fecundity of females, the average number of eggs per female fed on the two diets of GME varied from 715.3±33.62 (fresh) to 606.6±21.87 (frozen GME), respectively, with significant difference.
In addition, the results in Table 3 showed that the average longevity of males was significantly longer by feeding on fresh than on frozen GME.

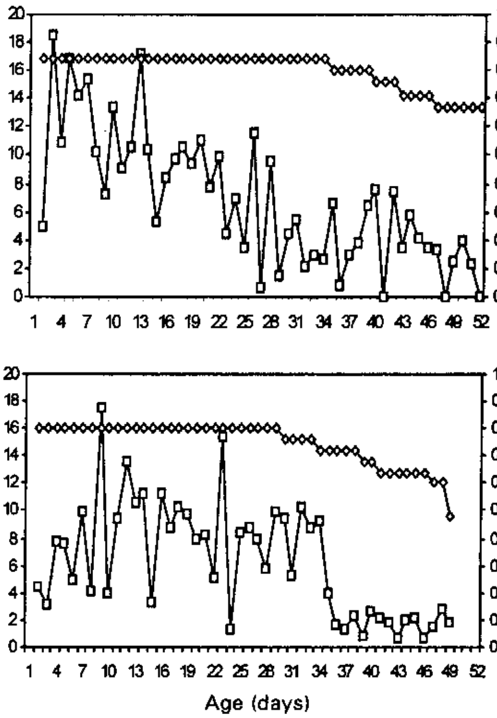

Fig. 1: Age-specific fecundity (Mx) and survivorship (Lx) of *H. axyridis* reared on fresh and frozen grain moth eggs under laboratory conditions (A: fresh, B: frozen).

Life table parameters: Data presented in Table (4) illustrate the life table parameters of *H. axyridis* females. The mean generation time (T) was shorter when the females reared on fresh GME (37.8 days), while on frozen GME, (T) was 45.04 days. The population of this predator could be doubled every 4.53 and 5.72 days when females fed on the two diets. The value of net reproductive rate (R_o) was higher by feeding on fresh than on frozen GME. The higher values of the intrinsic rate of increase (r_m), and the finite rate of increase (e^{rm}) were achieved when the females fed on fresh GME, whereas the lower values were obtained on frozen GME (Table 4). From data illustrated in Fig. 1, it could be noted that the survivorship (Lx) for female age intervals was high (0.84) on fresh GME. Which means that most of eggs had developed to maturity and death happened gradually after an extended oviposition period. While on frozen GME, the value of (Lx) was relatively low (0.80). Maximum oviposition rate per female per day (Mx) was 18.44 on 2nd day, and 17.44 on 8th day when females fed on fresh and frozen GME, respectively.

Effect of crowding on fecundity of females: Average number of eggs per *H. axyridis* female reared under crowding conditions and check during 30 day of observation period is presented in Table (5). It was interesting that there were significant differences between treatments which reared under crowding conditions

Table 1: Duration of the developmental stages of *H. axyridis* reared on fresh and frozen grain moth eggs under laboratory conditions.

Diet	Incubation period (Mean ± SE)	Larval instars duration (Mean ± SE) [a]					Pupal stage (Mean ± SE)	Total duration (from egg to adult) (Mean ± SE)	Surv-ival %
		1 st	2 nd	3 rd	4 th	Total			
Fresh GME	2.8±0.10a	2.34 a ±0.18	2.00 a ±0.10	2.95a ±0.11	3.90 a ±0.08	11.19a ±0.14	4.9 a± 0.10	18.89 b± 0.32	84 a
Frozen GME	3.1±0.11a	3.04a ±0.12	2.47a ±0.09	3.09a ±0.10	4.80a ±0.19	13.4a ±0.17	6.0a±0.21	22.5 a±0.21	80 b

[a]Means followed by the same letter in a column are not significantly different at the 1% level of probability (Duncan's Multiple Range Test).

Table 2: Weight of pupal and adult stages of *H. axyridis* when larvae reared on fresh and frozen grain moth eggs under laboratory conditions.

Diet	Pupal stage (Mean[a] ± SE)	Adult Stage
Fresh GME	27.72a±1.22	26.79a±1.12
Frozen GME	24.53b±1.39	23.14b±1.65

[a]Means followed by the same letter in a column are not significantly different at the 1% level of probability (Duncan's Multiple Range Test).

Table 3: Longevity, and fecundity of *H. axyridis* adults reared on fresh and frozen grain moth eggs under laboratory conditions.

Diet	Sex	No.	Longevity (Mean ± SE) [a]				No. of eggs/ Female (Mean ± SE)
			Pre- oviposition	Oviposition	Post oviposition	Total longevity	
Fresh	M	20	-	-	-	47.5 *A*± 1.31	-
GME	F	20	8.1 a± 0.31	49.0a±2.78	5.1 a± 0.27	62.2 a± 2.70	715.3 a± 33.62
Frozen	M	20	-	-	-	43.9 *B*± 2.05	-
GME	F	20	9.5 a± 0.52	45.3b±2.95	6.8 a± 0.38	61.6 a± 3.09	606.6 b± 21.87

[a]Means followed by the same small or italic capital letter in a column are not significantly different at the 1% level of probability (Duncan's Multiple Range Test).

Table 4: Life table parameters of *H. axyridis* fed on fresh and frozen grain moth eggs under laboratory conditions.

Diet	Initial No. of Females	Mean generation time (T) (in days)	Doubling time (DT)(in days)	Net reproductive rate (R_0)	Intrinsic rate of increase (r_m)	Finite rate of increase (e^{rm})
Fresh GME	20	37.87	4.53	289.11	0.153	1.166
Frozen GME	20	45.04	5.72	234.96	0.121	1.128

Table 5: Average number of eggs per *H. axyridis* female reared under crowded condition and check during 30 day of observation period under laboratory conditions.

Treatment	No. of females/container	Average[a] no. of eggs/female (± SE)
First	10	264.8 b ± 43.03
Second	20	176.32 c± 53.99
Third	30	73.64 d± 56.96
Fourth	40	46.54 e± 43.57
Check	1	391.96 a ± 39.38

[a]Means followed by the same letter in a column are not significantly different at the 1% level of probability (Duncan's Multiple Range test).

Table 6: Values of life table parameters of *H. axyridis* and other coccinellid species reared on different preys.

Coccinelid species	Preys	*T*	*DT*	R_0	r_m	e^{rm}	Reference
Stethorus picipes Casey	*Oligonychus punicae*	21.00	-	-	0.122	-	Tanigoshi and McMurtry (1977)
Rodolia iceryae Janson	*Icerya pattersoni* Newstead	61.17	10.83	50.16	0.064	1.067	Kairo and Murphy (1995)
Coccinella septempunctata L.	*Acyrthosiphon pisum* Harris	37.20	-	559.6	0.170	1.200	Phoofolo and Obrycki (1995)
Nephaspis oculatus (Blatchley)	*Bemisia argentifolii* Bellow and Perring	51.27	8.89	54.27	0.078	1.080	Liu *et al.* (1997)
Coleomegilla maculata DeGeer	*A. pisum*	41.10	-	54.00	0.100	1.100	Phoofolo and Obrycki (1997)
	Ostrinia nubilalis (Hubner) eggs	36.38	-	37.89	0.100	1.110	
Harmonia axyridis	Fresh GME	37.87	4.53	289.11	0.153	1.166	This study
	Frozen GME	45.04	5.72	234.96	0.121	1.128	

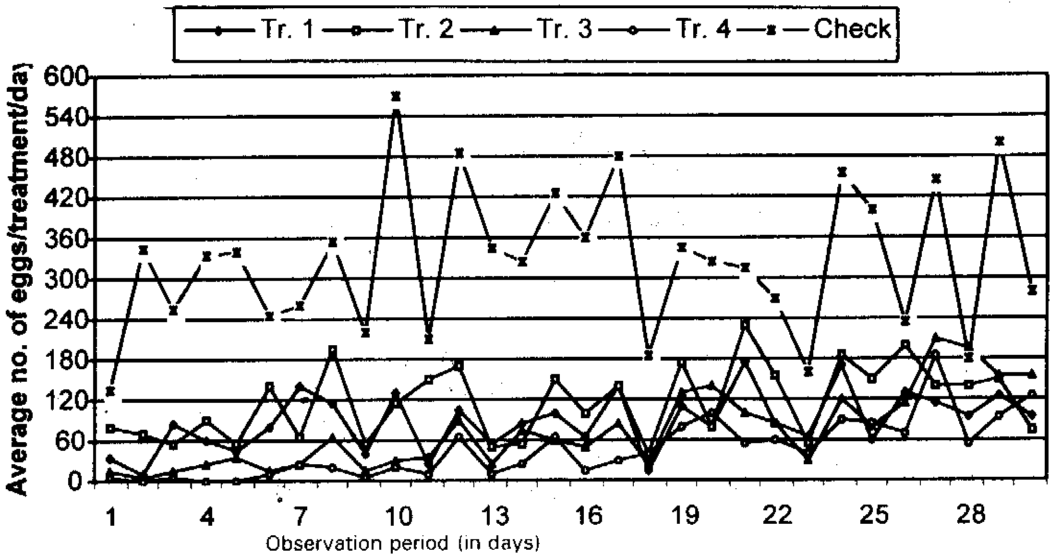

Fig. 2. Daily rate of oviposition per treatment during 30 day of observation period under crowding condition and check.

Average number of eggs per female was 264.8 ± 43.03 in the first, 76.32 ± 53.99 in the second, and 73.64 ± 56.96 in the third treatment. While, the lowest number was achieved when adults were reared under the conditions of the fourth treatment (40 males and 40 females/container). Whereas, adults which reared individually in petri dishes were laid the highest number of eggs during the observation period than those reared under crowding conditions (Table 5 and Fig. 2).

In addition, daily rate of oviposition per female was fluctuated during the 30 day of observation period under crowding conditions and the check (Fig. 2). This rate was influenced significantly by number of males and females in each treatment. The highest daily rate of oviposition was obtained when the adults were reared individually in petri dishes, while the lowest one was achieved in the fourth treatment.

Discussion

In mass rearing of entomophagous insects for inoculative or inundative release, it may be more economical to develop artificial diets or use factitious preys (Waage *et al.*, 1985). The artificial diets have been developed for both larvae and adults of many coccinellid predator species, but it is not yet suitable for use in mass production. Also, artificial diets that support normal rates of coccinellid egg production are not commercially available (Hagen 1987, Hatting and Samways 1993, Abdel-Salam *et al.* 1997 "a & b", Obrycki and Kring 1998). Focus is warranted on suitability of alternate or factitious preys (Obrycki and Kring 1998). *Ephestia kuehniella* and *Sitotroga cerealella* eggs are widely used for rearing coccinellid, chrysopid, and anthocorid species (Legaspi *et al.*, 1994; Ferran *et al.*, 1997; Hejzlar and Kabicek 1998).

The developmental time of immature stages of *H. axyridis* for a given time reported in the current study follow similar trend to those reported by Kim and Choi (1985) (19.92 days on *A. gossypii*), Niijima *et al.* (1986) (18.4 days on *Aphis rumicis* powder), McClure (1987) (18.6 days on *Acyrthosiphon pisum* Harris), Schanderl *et al.* (1988) (14.1-16.0 days on *Anagasta kuehniella* Zell eggs killed by U.V. radiation), Brun (1993) (18.0 days on *E. kuehniella*), He *et al.* (1994) (18.07 days on *A. gossypii*), Abdel-Salam *et al.* (1997 a) (13.59 and 17.55 days for developmental time of larval and pupal when the larvae fed on pink bollworm "PBW" eggs, *Pectinophora gossypiella* Saunders, and yellow pecan aphids" YPA", *Monelliopsis pecanis* Bissell), respectively, and Phoofolo and Obrycki (1998) (14.6 days from first instar larvae to adult eclosion on *A. pisum*).

Survival percent was found to closely match that of Kim and Choi (1985) (85.9% on *A. gossypii*), Niijima *et al.*, (1986) (80.0% on *Aphis rumicis* powder), Schanderl *et al.*, (1988) (88.3% days on *A. kuehniella* eggs), and Abdel-Salam *et al.* (1997 a) (88.0 and 90.0% on PBW and YPA, respectively. Similar results concerning pupal and adult weight were found by Niijima *et al.*, (1986), Schanderl *et al.* (1988), and Abdel-Salam *et al.*, (1997 a).

The results of longevity and fecundity are in complete agreement with those addressed by Kim and Choi (1985) who found that the adult longevity was 31.4 days on *A. gossypii*. The average of eggs per female on *A. pisum* was 718.7, pre-oviposition and oviposition periods were 7.3 and 45.2 days (McClure 1987). Schanderl *et al.*, (1988) reported that the fecundity was 522 eggs/female on *A. kuehniella* eggs. Brun (1993) mentioned that the females laid eggs after 8-10 days from emergence on *A. gossypii*. On *A. gossypii*, the pre-oviposition period was 13-30 days and females laid 751 eggs (He *et al.*, 1994). Abdel-Salam *et al.* (1997 b) found that the pre-oviposition, oviposition, and post-oviposition periods were 14.8, 49.0 and 37.0 days on PBW eggs. The longevity of males was 65.00, and the number of eggs/female was 257.0. On Russian wheat aphid, *Diuraphis noxia* and black bean aphid, *Aphis fabae*, the females laid a total of 1536 and 834 eggs/female (ICARDA, 1997).

There are no published data on the life table parameters of *H. axyridis* on preys tested in the current study or other preys for comparison. Whereas, there were some investigations on life table parameters of other coccinellid predator species. Comparison between life table parameters of *H. axyridis* and other coccinellids showed that the shortest mean generation time (T) was recorded with *Stethorus picipes* Casey, *Coleomegilla maculata* DeGeer and *H. axyridis* while the longest (T) was observed with *Rodolia iceryae* Janson (Table 6). *Harmonia axyridis* populations doubled in a shortest period than other coccinellid predators. The highest values of (R_o), (r_m), and (e^{rm}) were recorded with *H. axyridis* and *Coccinella septempunctata* L. than other coccinellid predators. The calculated values of (DT), and (r_m) for *H. axyridis* were higher than found in other species of coccinellid. These results were reflected the suitability of fresh and frozen GME for mass rearing of this predator. In addition, this predator had a highest capacity to multiply its population than other coccinelids.

Concerning the effect of crowding on the fecundity of *H. axyridis* females, it can be concluded that the higher fecundity were obtained when adults were reared individually in petri dishes followed by in the first treatment (10 males and 10 females). The due to cannibalism of laid eggs by adults (both males and females). Okada and Matsuka (1973) reported that predaceous coccinellids ordinarily are reared individually because of cannibalism. Crowding of predators with their preys causes competition and starvation, which in turn reduces survival and adult fitness. Relatively low levels of crowding may be undesirable due to cannibalism (Waage *et al.* 1985). Cannibalism was the only significant mortality factor during all developmental stages of *H. axyridis*, and the stage most heavily cannibalized was eggs by adults (McClure, 1987). Cannibalism by larvae and adults is a persistent problem in mass rearing of many coccinellid species (Agarwala and Dixon 1992). Adult females of *Chilocorus nigritus* Fabricuis have been observed on numerous occasions to oviposit and immediately turn to eat the eggs. Egg cannibalism by both males and females of *C. nigritus* showed that some individuals consumed up to two thirds of total laid (Ponsonby and Copland 1998).

The results of the current study assure that *H. axyridis* can survive, develop, and reproduce normally when reared on fresh or frozen GME. It can be concluded that the mass rearing of this coccinellid predator on the factitious prey tested in this study could offer a valid alternative than using normal preys (e.g. aphids, coccids and other soft bodied insects). The findings of the current study indicate that *H. axyridis* would be a suitable biological control candidate for mass rearing and release in the Egyptian fields.

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