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INFLUENCE OF FOOD TYPE AND COLD STORAGE DURATION ON THE LONGEVITY AND EMERGENCE PERCENTAGE OF *Aenasius arizonensis* (GIRAULT) ASSOCIATED WITH *Phenacoccus solenopsis* TINSLEY

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ABSTRACT: *Aenasius arizonensis* (Girault) is considered as associated parasitoid with the cotton mealybug, *Phenacoccus solenopsis* Tinsley which infesting many vegetable and field crops. This investigation was conducted under laboratory conditions of 25±1˚C with 70±5% RH and a photoperiod of 12:12 (L:D) hrs. to study the influence of different food sources on the longevity of the parasitoid, *A. arizonensis*. Also, to evaluate the effects of cold storage temperatures and storage durations on the emergency percentages of the parasitoid and the longevities of adults. The obtained results indicated that bees honey, molasses and bees honey + royal jelly were the best food sources for mass rearing of the parasitoid. The adult emergence percentages of *A. arizonensis* and the longevities of adults were significantly decreasing with the periods of storage compared with control treatment for females and males. The 50% Emergence Percentage (EP_{50}) was assessed after 9.14 days of storage female pupae at 5˚C and after 12.72 days of storage at 10˚C. The EP_{50} for male pupae was assessed after 9.20 days of storage at 5˚C and after 14.56 days of storage at 10˚C. These results can be beneficial when choosing the best food source in mass production for the parasitoid and providing an opportunity to release cold stored parasitoids synchronously in fields during outbreak of pests. These data may help us in developing an integrated pest control program against the aforementioned mealybug.

Key words: *Aenasius arizonensis*, food sources, cold storage temperatures, longevity.

INTRODUCTION

Mealybugs are usually considered as one of the most important pests which infesting many horticultural and field crops in many countries. The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Coccoidea: Pseudococcidae) attacks numerous crops, weeds, ornamental and medicinal plants. It infests the different parts of plants, feeding on phloem sap and producing sugary honeydew. Large populations of mealybugs cause general weakening and death of susceptible plants. Indirectly, it may also damage plants by transmition diseases (**Miller *et al*., 2005; Meyer *et al*., 2008; Nakaune *et al*., 2008; Mahfoudhi *et al*., 2009; Garcia Morales *et al*., 2016; Nabil and Hegab 2019**). In Egypt, the first record of *P. solenopsis* infestation was on weed plants by **Abd-Rabou *et al.* (2010). Ibrahim *et al.* (2015)** recorded *P. solenopsis* for the first time on tomato plants at Qalyoubia Governorate. **Nabil *et al.* (2015)** registered *P. solenopsis* for the first time on four economical crops at Sharkia Governorate. The outbreaks often occur when mealybugs get introduced to new locations in the absence of their natural enemies. So, the biological agent should be considered an effective factor for decreasing the populations of insect pest **(Sagarra *et al*., 2001; Bokonon-Ganta *et al*., 2002; Muniappan *et al*., 2006; Roltsch *et al*., 2006).** Accordingly, the objective of the present work is to study the influence of different food sources on the longevity of the parasitoid, *Aenasius arizonensis* (Girault) to choose the best food source in mass production for the parasitoid. Also, to evaluate

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the effects of cold storage temperatures and storage durations on the emergency percentages and the adult longevities of the parasitoid because the cold storage provides an opportunity to accumulate sufficient number of parasitoid for field release at proper weather conditions.

MATERIALS AND METHODS

This experiment was carried out under laboratory conditions of 25±1ºC, 70±5% R.H. and 12 hours of photoperiod at Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt.

Collection of Insects

The populations of the cotton mealybug, *P. solenopsis* and its associated parasitoid, *A. arizonensis* were collected from okra plants, *Abelmoschus esculentus* L. (Malvaceae) at Hihya district, Sharkia Governorate, Egypt.

Effect of food sources on the longevity of *A. arizonensis*

Mummies of *P. solenopsis* with *A. arizonensis* were placed in glass lamp container (15cm long and 10cm diameter) covered with muslin cloth under laboratory conditions and observed daily for the emergence of adults. Newly emerged individual females and males parasitoid were transferred to a separate glass container covered with muslin cloth under the same conditions. Eight food sources including bees honey, royal jelly, bees honey + royal jelly 1:1, glucose 1M, fructose 1M, sucrose1M, molasses, distilled water and a control with no food served. Five replicates each treatment had ten females or males individuals which were newly emerged. Food sources were placed on the inside of the glass lamp wall as droplets. The food provision was repeated till the death of individuals and thus the longevity was determined.

Effect of cold storage on some biological parameters of *A. arizonensis*

Cold storage experiments were carried out on the pupal stages of *A. arizonensis*. To obtain parasitoid pupae, mummies of *P. solenopsis* with *A. arizonensis* were collected and separated in test tube as female and male mummies. Ten female and male mummies were placed in test tubes closed with cotton wool and storage under constant laboratory conditions of 5 and 10±1˚C, 70±5% RH and a photoperiod of 12:12 (L:D) hrs. There were 4 replicates of each treatment. The control was kept under standard conditions of 25±1˚C, 70±5% RH and a photoperiod of 12:12 (L:D) hrs. For all the treatments, the effect of storage periods after one, two, three and four weeks of storage and the effects of storage temperatures on performance of the parasitoid was evaluated by measuring the percentage of emergence and the longevity for females and males. The percentage of emergence was measured by checking the number of adults that emerged after the period of storage on the total number of mummies. The longevity of the adult parasitoid was evaluated as newly adult emerged separated individually in test tubes, closed with cotton wool, storage under the laboratory conditions and provided daily with droplets of bees honey until the individuals died to calculate the longevity.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using a software package, **CoStat Statistical Software (2005),** a product of Cohort Software, Monterey, California. Means were compared by calculated least significant differences at P≤0.05 level of probability. Moreover, the Simple correlation (r) between the periods of storage and the emergence percentages of adult was detected. To calculate the 50% Emergence Percentage (EP_{50}), the partial regression formula was used according to **Hendi (1969)**, the dependence variance (Y) represented the percentage of emergence and the independent variance (X) was the periods of storage, the slop (b) of the straight regression line was calculated to obtain the corrected values of the percentage of parasitism by using the formula: $Y = a \pm bX$.

RESULTS AND DISCUSSION

Effect of Food Sources on the Longevity of *Aenasius arizonensis* (Girault)

Data presented in Table 1 showed the effect of eight food sources on adult longevity of *A. arizonensis*. In all cases adult females of *A. arizonensis* lived longer than males. In adult female, the longevity arranged descendingly as

Table 1. Effect of different food sources on the adult longevity on the actoparasitoid *Aenasius arizonensis* (Girault)

Different food sources	Adults	
	Female	Male
No food (control)	$4.0^{e} \pm 0.3$	$2.9^{f} \pm 0.1$
Distilled water	$6.3^{de} \pm 0.3$	$4.7^{f} \pm 0.2$
Royal jelly	$4.4^{e} \pm 0.2$	$3.0^{f} \pm 0.1$
Bees honey + Royal jelly 1:1	$19.9^{c} \pm 1.0$	$14.5^{c} \pm 0.9$
Molasses	$28.1^{b} \pm 3.0$	$23.1^{b} \pm 1.7$
Bees honey	$53.1^{a} \pm 3.0$	$41.5^{a} \pm 2.1$
Glucose 1 M	$10.5^{d} \pm 1.1$	$7.6^{de} \pm 0.4$
Fructose 1 M	$6.4^{de} \pm 0.4$	$5.1^{ef} \pm 0.3$
Sucrose 1 M	$10.4^{d} \pm 1.1$	$8.4^{d} \pm 0.7$
$LSD_{0.05}$	4.28	2.75
P	<0.0001**	<0.0001**

Means in row with the same letter are not significantly different at 0.05 levels.

bees honey, molasses, bees honey + royal jelly, glucose, sucrose, fructose, distilled water and royal jelly with values of 53.1, 28.1, 19.9, 10.5, 10.4, 6.4, 6.3 and 4.4 days, respectively. While, in the adult male , the longevity arranged in descending order as bees honey, molasses, bees honey + royal jelly, sucrose, glucose, , fructose, distilled water and royal jelly with values of 41.5, 23.1, 14.5, 8.4, 7.6, 5.1, 4.7 and 3.0 days, consecutively.

Statistical methods showed that there were significant differences between the female and male longevities when the individuals feed on bees honey, molasses and bees honey + royal jelly with all treatments. On the other hand, there were no significant differences between the female longevities when the individuals were fed on glucose or sucrose and those that were fed on fructose or distilled water.

Generally, it is clearly shown that the food sources may differ in their attractively to different species of parasitoids where, longevity of parasitoids influenced by feeding on various foods. So this may play an important role in biocontrol programs, but the carbohydrate sources as sugar, honey, royal jelly and honeydew are not always readily available in the agro-ecosystems.

These results are in agreement with those obtained by **Chong and Oetting (2006)** who observed that the male and female of *Anagyrus* sp. Nov. Nr. Sinope lived 3-13 times longer when provided with a carbohydrate source in the form of honey solution than those provided with only distilled water or starved. Distilled water apparently did not provide any nutritional value to *Anagyrus* sp. Nov. Nr. Sinope because the longevity of the hydrated parasitoids was identical to that of the starved parasitoids. **Poorani *et al.* (2009)** in India, mentioned that the adults of *Aenasius bambawalei* Hayat survived for 2-4 days on water but the mean longevity was considerably increased on 50% honey (70.95+2.30 days in females and 49.00+2.35 days in males). Unmated females produced 100% male progeny. **Nalini (2015)** in India, studied the effect of food sources on the longevity of *A. bambawalei*. He stated that *A. bambawalei* survived 11-14 times when the individuals fed with 100% honey longer than those maintained under starved (control). Adult females of *A. bambawalei* lived longer than males.

Effects of Cold Storage on the Percentage of Emergence and Longevity of *Aenasius arizonensis* (Girault)

Data presented in Tables 2 and 3 indicated that the adult emergence percentages of *A. arizonensis* and the longevities of adults were significantly decreasing with the periods of storage compared with control treatment which showed 87.50% emergence and 46.52 days longevity for females and 82.50%emergence and 38.75 days longevity for males.

At 5˚C storage, the adult emergence percentages were 47.50, 25.00, 15.00 and 10.00% for females and 45.00, 35.00, 20.00 and 7.50 % for males after 1, 2, 3 and 4 weeks of storage, respectively. The adult female longevities were 32.33, 28.81, 22.63 and 17.25 days. While, the adult male longevities were 29.91, 27.02, 21.38 and 12.00 days after one, two, three and four weeks of storage, successively.

At 10˚C storage, the adult female emergence percentages were 55.00, 42.50, 32.50 and 17.50% and the emergence percentages of males were 57.50, 47.50, 40.00 and 27.50 % for males after one, two, three and four weeks of storage, consecutively. The adult female longevities were 42.93, 38.60, 34.21 and 27.57 days, while the male longevities were 32.14, 27.64, 23.19 and 17.21 days after one, two, three, four weeks of storage, respectively.

The correlation coefficient between the periods of storage and the emergence percentages of adult female and male were negative and highly significant (r1= -0.935**, r1=-0.964**), (r2=-0.970**, r2=-0.972**) under 5˚C and 10 ˚C storage, successively.

The statistical analysis showed that there were significant differences between of the adult female and male longevities and the periods of storage where the LSD values were 1.95 and 5.55, respectively at 5˚C storage, while LSD values were 2.31 and 1.18 at 10˚C storage, successively.

At 5˚C storage, data presented in Figs. 1 and 2 according to regression coefficient and straight line between the emergence percentages of female and male of *A. arizonensis* and the periods of storage with the helping of the formula Y= 74.50 – 2.68X and Y= 73.00 - 2.50X, respectively and the 50% Emergence Percentage (EP_{50}) were assessed after 9.14 and 9.20 days of storage .

At 10˚C storage (Figs. 3 and 4), the regression coefficient and straight line between the emergence percentages of female and male and the periods of storage with the helping of the formula Y=76.50 – 1.82X and Y= 79.50 – 2.32X, conclusively and the 50% Emergence Percentage (EP_{50}) were assessed after 14.56 and 12.72 days of storage.

Generally, the results provided that the storage period to one week at 10 ˚C was the most suitable and favorable for ideal cold storage of *A. arizonensis* pupae. So, the cold storage project of pupae parasitoid can be used to improve mass rearing and commercial production of its.

These results were closely agree with those of **Ayvaz *et al.* (2008)** determined the effects of cold storage, rearing temperature, parasitoid age, and irradiation on the performance of the egg parasitoid, *Trichogramma evanescens* Westwood. Pupae of *T. evanescens* can be stored at 4 ˚C for up to 3 weeks without much loss of performance. The longevity and walking speed of adults emerging from chilled pupae significantly decreased after longer storage periods. **Liu *et al.* (2014**) in China, studied the effect of temperatures and cold storage on performance of *Tetrastichus brontispae*, a parasitoid of *Brontispa longissimi*. They findings that a period of 10 days at 10˚C may be more suitable and acceptable for ideal cold storage of parasitized pupae of *T. brontispae*. **Spinola-Filho *et al.* (2014)** in USA, evaluated that the suitability of *Anagasta kuehniella* eggs stored at 5 ˚C for different time periods as a host for *Trichogrammatoidea annulata*. The percentage of parasitized eggs decreased when the storage period increased. Among the tested parasitoids, *T. acacioi* parasitized eggs stored for longer periods and showed the highest percentage both of parasitism and adult emergence. **Tunca *et al.* (2014)** in Turkey, studied the effects of cold storage on the biology of the larval parasitoid *Venturia canescens* (Gravenhorst) and recorded that the decreasing of temperature and increasing of storage time resulted in increasing

Table 2. Correlation coefficient on *Aenasius arizonensis* (Girault) females and males emergence percentage and longevity under 5˚C at four storage periods

Storage period	Female				Male			
	Replicates	No. of emergence	% of emergence	Longevity	Replicates	No. of emergence	% of emergence	Longevity
1 week	**1**	5	50.00	33.00±0.71	**1**	5	50.00	31.00±1.41
	2	5	50.00	32.20±0.86	**2**	4	40.00	29.25±1.11
	3	5	50.00	34.60±2.71	**3**	5	50.00	29.40±0.60
	4	4	40.00	29.50±0.65	**4**	4	40.00	30.00±0.41
	Mean ±SE		**47.50**	**32.33^{b}±1.07**	**Mean ±SE**		**45.00**	**29.91^{b}±0.40**
2 weeks	**1**	4	40.00	29.75±0.25	**1**	4	40.00	27.50±0.65
	2	2	20.00	29.50±0.51	**2**	3	30.00	27.00±0.59
	3	2	20.00	27.50±0.51	**3**	4	40.00	26.25±1.03
	4	2	20.00	28.50±0.51	**4**	3	30.00	27.33±0.90
	Mean ±SE		**25.00**	**28.81^{c}±0.51**	**Mean ±SE**		**35.00**	**27.02^{b}±0.28**
3 weeks	**1**	1	10.00	24.00±0.00	**1**	2	20.00	23.00±1.01
	2	2	20.00	22.00±1.01	**2**	3	30.00	22.00±0.59
	3	2	20.00	22.50±0.51	**3**	1	10.00	20.00±0.00
	4	1	10.00	22.00±0.00	**4**	2	20.00	20.50±0.51
	Mean ±SE		**15.00**	**22.63^{d}±0.47**	**Mean ±SE**		**20.00**	**21.38^{c}±0.69**
4 weeks	**1**	1	10.00	17.00±0.00	**1**	1	10.00	17.00±0.00
	2	1	10.00	19.00±0.00	**2**	1	10.00	16.00±0.00
	3	1	10.00	17.00±0.00	**3**	1	10.00	15.00±0.00
	4	1	10.00	16.00±0.00	**4**	0	00.00	00.00±0.00
	Mean ±SE		**10.00**	**17.25^{e}±0.63**	**Mean ±SE**		**7.50**	**12.00^{d}±4.02**
Control	**1**	10	100.00	46.00±0.44	**1**	9	90.00	38.33±0.44
	2	8	80.00	47.50±0.44	**2**	9	90.00	38.22±0.22
	3	9	90.00	46.56±0.46	**3**	8	80.00	38.88±0.35
	4	8	80.00	46.00±0.44	**4**	7	70.00	39.57±0.37
	Mean ±SE		**87.50**	**46.52^{a}±0.26**	**Mean ±SE**		**82.50**	**38.75^{a}±0.31**
P		**< 0.0001****			**P**	**< 0.0001****		
LSD		**1.95**			**LSD**	**5.55**		
r_1		**-0.935***			**r_1**	**-0.964****		

Means in column with the same letter are not significantly different at 0.05 levels.

Table 3. Correlation coefficient on *Aenasius arizonensis* (Girault) females and males emergence percentage and longevity under 10 ˚C at four storage periods

Storage period	Female				Male			
	Replicates	No. of emergence	% of emergence	Longevity	Replicates	No. of emergence	% of emergence	Longevity
1 week	**1**	6	60.00	45.50±0.77	**1**	6	60.00	34.00±0.58
	2	6	60.00	44.83±1.14	**2**	5	50.00	31.40±0.50
	3	5	50.00	41.00±0.71	**3**	6	60.00	32.67±0.42
	4	5	50.00	40.40±0.81	**4**	6	60.00	30.50±0.22
	Mean ±SE		**55.00**	**42.93[a]±1.30**	**Mean ±SE**		**57.50**	**32.14[b]±0.76**
2 weeks	**1**	4	40.00	37.50±0.65	**1**	4	40.00	27.75±0.48
	2	4	40.00	39.00±0.71	**2**	5	50.00	27.20±0.58
	3	4	40.00	38.50±0.87	**3**	5	50.00	28.20±0.58
	4	5	50.00	39.40±0.51	**4**	5	50.00	27.40±0.51
	Mean ±SE		**42.50**	**38.60[b]±0.41**	**Mean ±SE**		**47.50**	**27.64[c]±0.22**
3 weeks	**1**	3	30.00	34.67±0.34	**1**	4	40.00	23.25±0.48
	2	3	30.00	34.67±0.34	**2**	4	40.00	23.25±0.25
	3	3	30.00	32.00±0.59	**3**	4	40.00	23.25±0.48
	4	4	40.00	35.50±0.29	**4**	3	30.00	23.00±0.59
	Mean ±SE		**32.50**	**34.21[c]±0.76**	**Mean ±SE**		**40.00**	**23.19[d]±0.06**
4 weeks	**1**	2	20.00	28.50±0.51	**1**	3	30.00	17.33±0.34
	2	2	20.00	27.00±1.01	**2**	3	30.00	16.67±0.34
	3	2	20.00	26.50±0.51	**3**	2	20.00	17.50±0.51
	4	1	10.00	29.00±0.00	**4**	3	30.00	17.33±0.34
	Mean ±SE		**17.50**	**27.57[d]±0.60**	**Mean ±SE**		**27.50**	**17.21[e]±0.18**
Control	**1**	10	100.00	46.00±0.44	**1**	9	90.00	38.33±0.44
	2	8	80.00	47.50±0.44	**2**	9	90.00	38.22±0.22
	3	9	90.00	46.56±0.46	**3**	8	80.00	38.88±0.35
	4	8	80.00	46.00±0.44	**4**	7	70.00	39.57±0.37
	Mean ±SE		**87.50**	**46.52[a]±0.26**	**Mean ±SE**		**82.50**	**38.75[a]±0.31**
P	< 0.0001**				**P**	< 0.0001**		
LSD	1.95				**LSD**	1.18		
r_2	-0.970**				r_2	- 0.972**		

Means in column with the same letter are not significantly different at 0.05 levels.

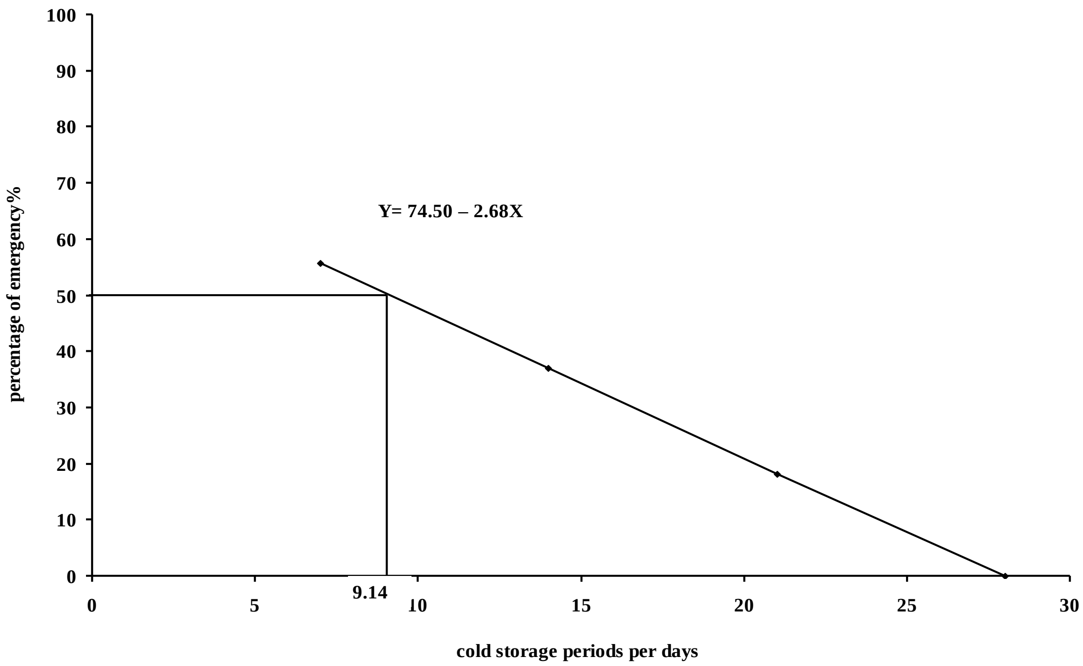

Fig.1. The calculated 50% Emergence Percentage (EP_{50}) of the adult female pupae of *Aenasius arizonensis* (Girault) storage under 5˚C storage

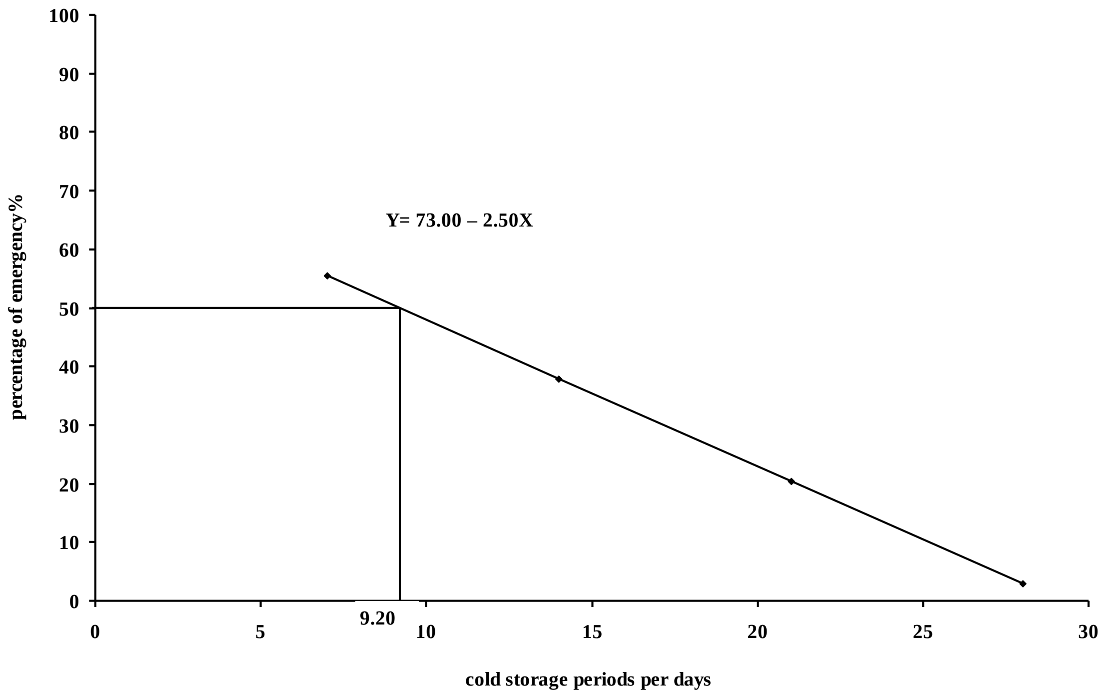

Fig. 2. The calculated 50% Emergence Percentage (EP_{50}) of the adult male pupae of *Aenasius arizonensis* (Girault) storage under 5˚C storage

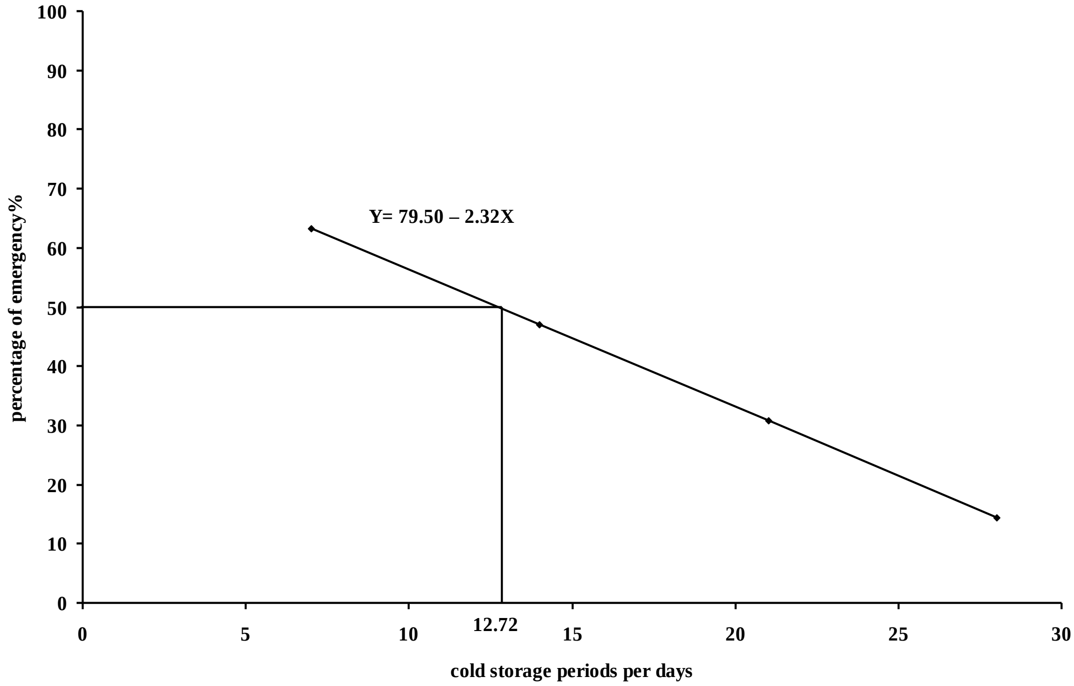

Fig. 3. The calculated 50% Emergence Percentage (EP_{50}) of the adult female pupae of *Aenasius arizonensis* (Girault) storage under 10˚C storage

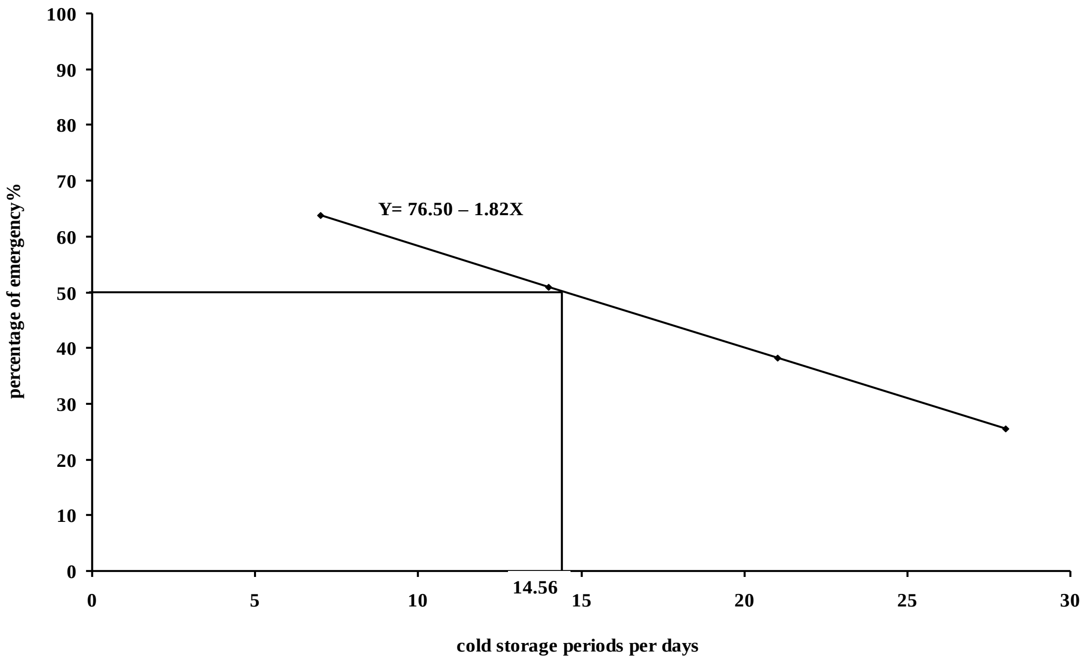

Fig. 4. The calculated 50% Emergence Percentage (EP_{50}) of the adult male pupae of *Aenasius arizonensis* (Girault) storage under 10˚C storage

in the parasitoid development time, but reducing emergence rate , the most suitable temperature for feeding and non-feeding condition was found to be at 10 ˚C for the adult. **Kidane *et al.* (2015)** in China, determined the effect of different durations of constant low temperature storage on the fitness traits of *Encarsia sophia* is an important bio-control agent of *Bemisia tabaci*. They indicated that there is a decrease in percentage emergence, longevity and ability to parasitize the longer and lower the temperature at which the pupae of *E. sophia* were stored. **Rathee and Ram (2018)** who studied the effects of cold storage temperature and storage duration of entomophagous insects on the biological parameters. They stated that low temperature storage is a valuable method for increasing the shelf life of entomophagous insects. Also, they mentioned that cold storage also helps to keep viable stock of natural enemies when not needed and to minimize laboratory operations by prolonging their survival and delaying exclusion.

These considered factors, food types and cold storage duration, have played a conspicuous role in detecting the biological parameters of *A. arizonensis* associated with *P. solenopsis* during the investigation. However, further investigation is needed to determine more information about parasitoid-host interaction, effect of different biological factors that may be implicated so as to release this parasitoid under protected cultivations which expanded greatly in Egypt.

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تأثير نوع الغذاء وفترة التخزين الباردة على فترة الحياة ونسبة خروج الطفيل *Aenasius arizonensis* (Girault) المرتبط بـبق القطن الدقيقى *Phenacoccus solenopsis* Tinsley

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يرتبط الطفيل *Aenasius arizonensis* (Girault) ببق القطن الدقيقي *Phenacoccus solenopsis* Tinsley والذي يصيب العديد من محاصيل الخضر والمحاصيل الحقلية. تم إجراء هذا البحث تحت الظروف المعملية عند 25 ± 1 درجة مئوية مع 70 ± 5% رطوبة نسبية وفترة ضوئية 12: ساعة لدراسة تأثير نوع الغذاء على طول فترة حياة الطفيل *A. arizonensis* وأيضًا تم تقييم تأثيرالتخزين البارد على درجتى حرارة 5 و 10 درجة مئوية وطول فترة التخزين على النسب المئوية لخروج الحشرات الكاملة للطفيل *A. arizonensis* وطول فترة الحياة. أشارت النتائج المتحصل عليها إلى أن عسل النحل، العسل الأسود، عسل النحل+ غذاء ملكات النحل كانت من أفضل المصادر الغذائية لإدراجها ضمن برنامج التربية الموسعة والانتاج التجارى للطفيل، وتناقصت نسب معدل خروج الأطوار الكاملة من الاناث والذكور للطفيل *A. arizonensis* وطول فترات الحياة بصفة معنوية مع طول فترات التخزين مقارنة بمعاملة الكنترول . تم تقييم النسبة المئوية لخروج 50% من الحشرات الكاملة(EP50) بعد 9.14 يوماً من تخزين عذارى إناث الطفيل عند درجات حرارة خمس درجات مئوية وبعد 12.72 يوماً من التخزين عند درجات حرارة عشر درجات مئوية ، بينما تم تقييم EP50 لعذارى ذكور الطفيل بعد 9.20 يوماً من التخزين على درجات حرارة خمس درجات مئوية. وبعد 14.56 يوماً من التخزين عند درجات حرارة عشر درجات مئوية. يمكن أن تكون هذه النتائج مفيدة بهدف إختيار أفضل مصادر للغذاء وادراجها في الإنتاج الموسع للطفيل وإتاحة الفرصة لإطلاق الطفيليات المخزنة بالتبريد فى الحقول بشكل متزامن اثناء انتشار الآفة. وقد تساعدنا هذه البيانات في تطوير برنامج متكامل لمكافحة تلك الآفة .

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