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Functional response and predation efficiency of the flower bug *Orius albidipennis* (Hemiptera: Anthocoridae) to different densities of the cotton mealybug *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) under laboratory conditions

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Abstract:

The cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Sternorrhyncha: Coccoidea: Pseudococcidae) is considered a very dangerous pest of various vegetable crops in Egypt. Also, the predator *Orius albidipennis* Reuter (Hemiptera: Anthocoridae) is one of the most effective control methods of many insect pests infested vegetables crops in Egypt. This Study aimed to investigate the predation efficiency and functional response of *O. albidipennis* to different densities of cotton mealybug *P. solenopsis* (10, 15, 20, 25, 30 and 35) eggs, 1st and 2nd nymphal instars during 24 hrs. period under laboratory conditions at 28 ±2 °C, 60 ± 5 % RH. and L18:D6 photoperiod. The results showed that *O. albidipennis* preferred eggs compared with 1st and 2nd nymphal instars of the cotton mealybug. *O. albidipennis* good fitted to holling type II functional responses for each stage of this predator. The increasing in prey densities lead to increase in the search efficiency (a), decreasing in handling time (T_h) and increasing the predation efficiency of different stages of *O. albidipennis*. The handling time was shorter when fed on 2nd nymphal instar than the eggs and 1st nymphal instar (0. 026, 0.0128 and 0.00926 hrs.) for 4th, 5th and females of *O. albidipennis*, respectively. As well as, the rate of successful search (a) was higher when fed on 2nd nymphal instar than other stages (0.4841, 0.8318 and 1.1664 hrs.) for 4th, 5th nymphal instars and females of *O. albidipennis*, respectively. The maximum predation rate of females of predator was higher than the other stages (80.04 d^{-1}) for eggs when compared with other stages of the cotton mealybug. As well as, the females of this predator was consumed more eggs, 1st and 2nd nymphal instars of the cotton mealybug than 4th and 5th nymphal instars of the predator. The mean numbers of consumed different stages of *P. solenopsis* were increased by increasing prey densities. So, we can use the females, 4th and 5th nymphal instars of *O. albidipennis* in controlling eggs and small nymphal stages of the cotton mealybug in greenhouse or field on vegetables crops.

Introduction

The cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Sternorrhyncha: Coccoidea: Pseudococcidae) has become invasive worldwide mealybug species. This species is a polyphagous insect, it was reported to infest more than 200 plant species from almost 24 countries located in tropical and subtropical regions around the word. In Egypt, it was recorded for the first time by Abd-Rabou *et al.* (2010) and it was recorded on about 29 host plants belonging to 16 families (Abdel-Razzik *et al*., 2015 and Nabil *et al*., 2015).

P. solenopsis recorded about 8-12 generations a year. This mealybug species infests almost all plant parts with a destructive effect especially in heavy infestation as it sucks a large amount of plant sap which lead to plant welt, leaves turn to yellow then dry and fall. Also, it secrets a large amount of honey dew which consider a suitable media to the growth of the black sooty mold fungi on leaves which blocks photosynthesis and respiration operation (Wu and Zhang, 2009). In 2005-2009 in India and Pakistan, *P. solenopsis* outbreak caused loss in cotton yield ranged between 30-60% (Jeyakumar *et al*., 2009). Different natural enemies were recorded on *P. solenopsis* about 12 predators and 16 parasitoids (Attia and Awadallah, 2016 and Nabil and Hegab, 2019). That's why it was extremely important to control this pest to minimize its massive destructive effect on different plants.

Orius albidipennis Reuter (Hemiptera:Anthocoridae) is a very effective predator of many insect pests in Egypt (Sobhy *et al*., 2010 and Al-Kherb, 2013). As well as, it has a numerous host preys such as thrips (Tan *et al*. 2013), spidermites (Zhou *et al*., 2006), mealybugs (Elbahrawy *et al*., 2020) and the eggs of moth (Zhou and Lei, 2002, Butler and O'Neil, 2007 and Zhou *et al.*, 2006). Mass Rearing of this predator at laboratory and releasing its nymphal stage and adults in the field and greenhouse will support the controlling of this insect pests and suppressing it's population in the field, which leads to, reduce of using pesticides (Shapiro and Ferkovich, 2006).

Numerical responses and daily prey consumption of *O. albidipennis* to different pests is very imperative studies on controlling these pest and decreasing population densities on different crops (Lia and Yano, 2010; Ganjisaffar and Thomas, 2015 and Wang *et al*., 2018). This study will provide us the primary data about using *O. albidipennis* in biological control programs for controlling *P. solenopsis* on vegetable crops. Therefore, the present work aimed to evaluate the feeding response and predation consumption of *O. albidipennis* Reuter in different densities and stages of cotton mealybug *P. solenopsis* under laboratory conditions. As well as, the laboratory experiment was conducted to evaluate the efficacy of the predator *O. albidipennis* against *P. solenopsis* as a biological control agent which may play an important role in IPM program.

Materials and methods

1. Mass rearing of the predator *Orius albidipennis:*

O. albidipennis adults were obtained from unsprayed sunflower *(Helianthus annuus* L.) fields in Qalyoub region, Qalyubiya Governorate, Egypt. *O. albidipennis* had been reared under laboratory conditions at 28 ±2 °C, 60 ± 5 % RH. and L18:D6 photoperiod. The predators were reared using the methods described by (Isenhor and Yeargan, 1981a). Adults and nymphs of *O. albidipennis* were kept in plastic jars of (10 cm ×20 cm) covered with muslin and held in place by means of rubber bands. Each jar was provided with both small pieces of cardboard to reduce cannibalism behavior and put 2 gm of frozen *E. kuehniella* eggs as nutrition source for the enclosed predators (Bonte and De Clercq, 2008). A piece of bean pod (3cm) (*Phaseolus vulgaris*) was placed in each jar as an ovipostional substrate and piece of cotton soaked in sugar water (1:1)

(Isenhor and Yeargan, 1981 b). Eggs are inserted into the tissue of bean pods. Newly deposited eggs inside bean pods were kept in new plastic jars. Jars were examined daily until hatching. Soon after hatching; newly-hatched nymphs were carefully transferred to new plastic jars and provided with frozen eggs of *E. khunellia.*

2. Mass rearing of the prey cotton mealybug *Phenacoccus solenopsis*:

Cotton mealybug *P. solenopsis* was collected from eggplant, *Solanum melongena* L. (Solanaceae) orchards in Benha district, Qalyubiya Governorate, Egypt and placed in plastic bags. Then, the mealybugs were reared on sprouted potatoes. Potatoes tubers were placed in cartoon box (80 cm length x 45 cm width x 45 cm height). Potato tubers, *Solanum tuberosum* L. (Solanaceae) were washed thoroughly in water and put it on plastic dishes 40 cm and kept it in a refrigerator at 7°C for 10 days and then returning them to room temperature again. After three weeks, potatoes sprout of 6-8 cm height. Then the insects were transferred carefully with the aid of a fine camel hair brush to the potatoes sprouts. The mealybug females settled on potatoes sprouts started to lay eggs. The crawlers emerged out and started feeding and developed to adults. The eggs, 1st and 2nd nymphal instars were used as a prey.

3. Predation consumption by 4th, 5th nymphal instars and females of *Orius albidipennis*:

Newly individual emerged nymphs of *O. albidipennis.* (0-12 hrs.) were collected from the stock colony which reared in laboratory and were put in separate plastic petri dishes (7 cm diameter) and provided with different densities of prey (10,15, 20, 25, 30 and 35) eggs, 1st and 2nd nymphal instars of *P. solenopsis*. The number of consumed prey by 4th and 5th nymphal instars of *O. albidipennis* were checked by using a stereomicroscope (20×) after 24 hrs. Couples of newly emerged adults (male and female) were placed separately in plastic petri dish (7cm diameter) without preys to stimulate mating occurrence. Later after12 hrs., males were removed. Then, these dishes were supplied with different densities of prey (10, 15, 20, 25, 30 and 35 individuals of eggs, 1st and 2nd nymphal instars of *P. solenopsis*). The tests were conducted for a 24 hrs. period. The numbers of consumed prey by adults of *O. albidipennis* were checked by using a stereomicroscope (20×). There were 6 replicates per each cotton mealybug's density. A predator was tested only once. A control experiment was conducted of similar setups but without the predator.

4. *Orius albidipennis* functional response to *Phenacoccus solenopsis* different densities at 28 ±2 °C, 60 ± 5 % RH. and L18:D6 photoperiod:

The predation ability of the developmental stages (4th, 5th nymphal instars and females) of *O. albidipennis* feeding on eggs, 1st and 2nd nymphal instars of *P. solenopsis* at various densities were compared by analysis of variance (ANOVA) using SPSS program for Windows Version 16.0. Also, analysis of variances were used to compare differences in the mean numbers of different densities of *P. solenopsis* different stages consumed by *O. albidipennis*. The mean values of different treatments were compared using Duncan's multiple range test at probability P<0.05. The functional response data were fitted to type-II responses (Holling, 1959). Parameters of a type-II model were estimated by the Holling disc equation:

$$N_a = \frac{aTN_0}{1 + aT_h N_0}$$

Where(N_a)is the number of eggs consumed,) N0$_)$ is the initial density of eggs,) a) is the predator attack rate or instantaneous searching rate, T is the time of exposure of predator to prey (T = 24 h) and T_h is the handling time.

Results and discussion

1. Predation capacity for 4thand 5th of nymphal instars and females of *Orius albidipennis* on different densities of eggs, 1st and 2nd nymphal instars of *Phenacoccus solenopsis* during 24 hrs. under laboratory conditions:

Predation capacity for 4th and 5th of and nymphal instars and females of *O. albidipennis* on different densities of eggs, 1st and 2nd nymphal instars of *P. solenopsis* at 28ºC during 24 hrs. Results in Table (1) showed that there was a positive relation between the densities of mealybug and the mean number of consumed different stages of mealybug (Eggs, 1st and 2nd nymphal instars). Where there were increase in predator consumption with the increase of the prey numbers. Also, the consumption of the *O. albidipennis* different stages to *P. solenopsis* can be arranged ascendingly as follows; 4th then 5th nymphal instar followed by females. Where females consumed higher numbers of the prey than the other nymphal instars (Figure 1).

In addition, it was clear that the consumption of the predator to the eggs of *P. solenoids* was higher than the consumption of 1st nymphalid followed by the 2nd nymphal instar. That's may be because the eggs are steady and easier to locate on the plant. Besides females of the predator prefer the egg stage of the prey where females of the predator need large portion of protein (found in eggs) for the eggs development. Statistical analysis showed that, almost there were no significant differences between mean numbers of consumed *P. solenopsis* different stages (eggs, 1st and 2nd nymphal instars) in case of the prey densities 10 and 15 individuals in all treatments, the same was recorded between the prey densities 20 and 25 individuals and between 30 and 35 individuals. I.e. the 4th nymphal instar of *O. albidipennis* consumed about 2.57±0.173 eggs when the prey density was 10 eggs with no significant difference with the prey density 15 eggs with 4.14 ±0.295 eggs.

However, there were significant differences between the pairs of prey densities (15 and 20) individuals and (20 and 25) individuals and between (30-35) individuals .For example, the consumption of *O. albidipennis* 4th nymphal instar to mean number of *P. solenopsis* eggs in case of the densities 10 and 15 eggs has significant differences with both eggs densities 20 and 25 eggs as well both eggs densities 30 and 35 eggs with F value = 6.071. It was clear from the review of literature collected that the trials conducted to estimate the efficiency of *O. albidipennis* against *P. solenopsis* was very rare but there were other trials evaluated different preys which we can be taken as a pilot review. Kaewpadit (2018) stated that the 4th and 5th nymphal of *Orius maxidentex* Ghauri (Hemiptera: Anthocoridae) could feed on the averaged 7.17±1.71 and 9.28±2.27 nymphs of whiteflies, respectively. The individual adult could feed on the averaged 85.73±32.61 nymphs of whiteflies under laboratory conditions. Sánchez *et al*. (2018) in Spain, they mentioned that *O. laevigatus* preyed on the eggs and larvae of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) as well as the 4th and 5th nymphal instars had a greater consumption of eggs than younger ones. The intrinsic rate of natural increase did not differ between *O. laevigatus* fed with *S. exigua* eggs and those offered eggs of the substitute host *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae). Thus, *O. laevigatus* is a good candidate for the biological control of *S. exigua*, a cosmopolitan pest of many crops. They mentioned that *O. laevigatus* preyed on the eggs and larvae of *S. exigua* as well as the 4th and 5th nymphall instar had a greater consumption of eggs than younger ones. The intrinsic rate of natural increase did not differ between *O. laevigatus* fed with *S. exigua* eggs and those offered eggs of the substitute host *E. kuehniella* . Thus, *O. laevigatus* is a good candidate for the biological control of *S. exigua*, a cosmopolitan pest of many crops.

Table (1): Predation capacity for 4th, 5th of nymphal instars and females of *Orius albidipennis* on different densities of eggs, 1st and 2nd nymphal instars of *Phenacoccus solenopsis* at 28ºC during 24hrs.

Predator stages	**Mean No. of *Phenacoccus solenopsis* consumed**			
	Densities of prey	**Mean No. of consumed *Phenacoccus solenopsis* eggs±SE**	**Mean No. of consumed *Phenacoccus solenopsis* 1st nymphal instar ±SE**	**Mean No. of consumed *Phenacoccus solenopsis* 2nd nymphal instar ±SE**
4th Nymphal instar	**10**	2.57±0.173a	1.14±0.163a	0.28±0.129a
	15	4.14±0.295a	2.0±0.310a	1.05±0.173a
	20	6.42±0.369b	3.14±0.463a	2.07±0.275b
	25	8.71±0.258b	4.85±0.129b	2.47±0.264b
	30	10.09±0.632c	5.42±0.238b	3.85±0.163c
	35	12.85±0.547c	8.42±0.258c	6.42±0.369c
F value		6.071	3.71	1.093
5th Nymphal instar	**10**	6.14±0.12a	4.66±٠.13a	3.9±0.15a
	15	9.57±0.23a	7.28±0.17a	5.71±0.13a
	20	12.85±0.37b	10.14±0.23b	8.85±0.16a
	25	17.14±0.33b	11.71±0.47b	10.29±0.17b
	30	20.14±0.51c	15.36±0.54c	12.57±0.32b
	35	24.28±1.28c	20.71±0.57c	17.0±0.40c
F value		11.615	24.848	10.292
Females	**10**	11.57±0.31a	8.714±0.21a	5.85±0.21a
	15	16.71±0.588a	11.0±0.42a	7.85±0.47a
	20	20.57±0.435b	13.57± 0.61b	10.57±0.25b
	25	2٢.04±0.282b	17.42±0.25b	12.28±0.55b
	30	26.85±0.310c	19.71±0.34c	14.57±0.40c
	35	30.0±0.489c	23.71±0.60c	16.85±0.40c
F value		12.240	11.708	9.46

In the same column, figures followed by the same letters are not significantly differed by Duncan's Multiple (1955) Range Test, P= 0.05.

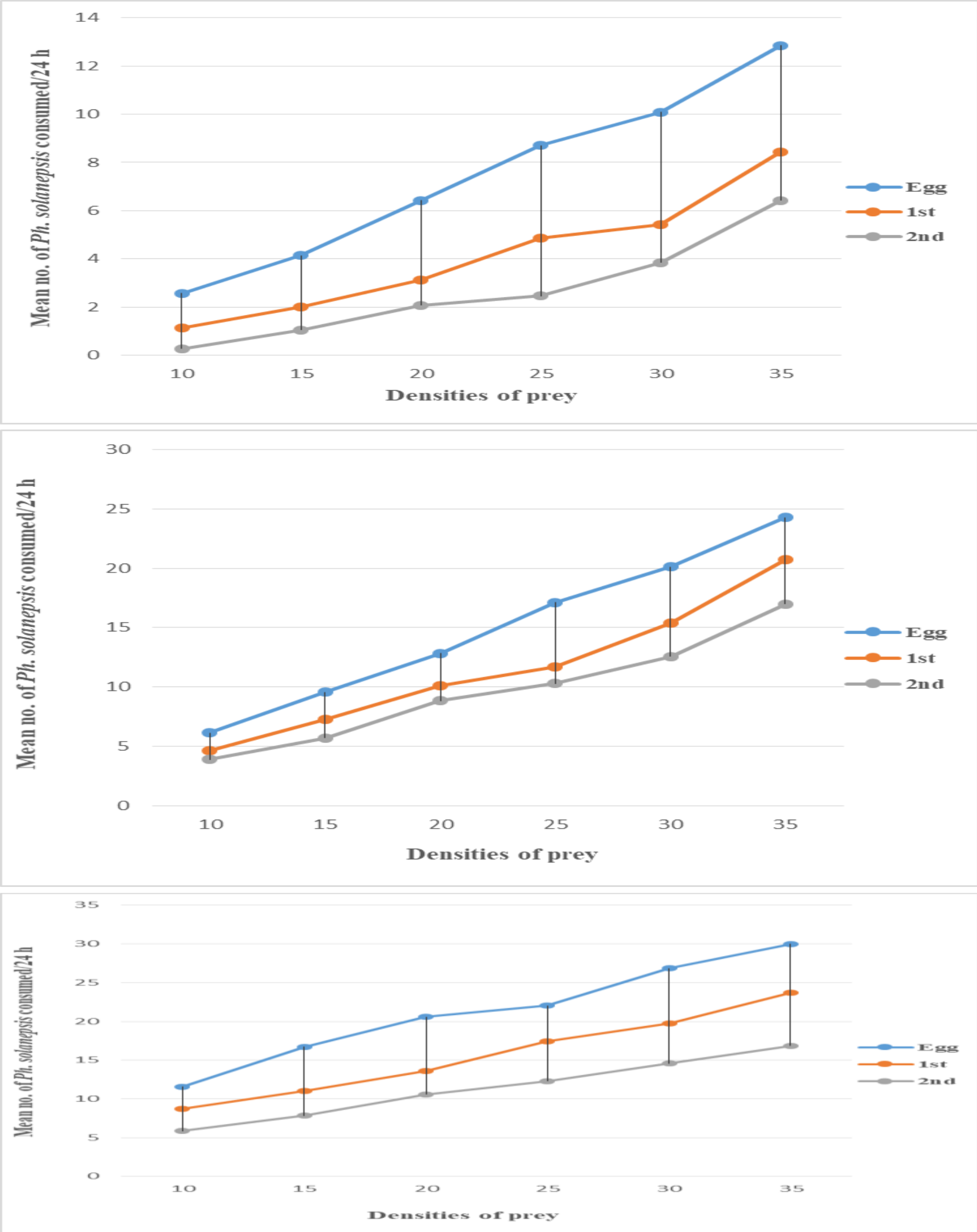

Figure (1 A, B, C): Mean No. of *Phenacoccus solenopsis* consumed by *Orius albidipennis* under laboratory conditions during 24 hrs.

2. Functional response of 4th and 5th and females of *Orius albidipennis* to different densities of eggs, 1st and 2nd nymphal instars of *Phenacoccus solenopsis* during 24 hrs. under laboratory conditions:

Data in Table (2) obtained from logistic regression analysis revealed that the functional response of 4th, 5th nymphal instars and females of *O. albidipennis* were fitted to Type II (Holling, 1959), the functional response curves of different stages of *O. albidipennis* on *Ph. solenopsis* were shown in Figures (2-4) and all parameters of a type-II functional response were estimated by the Holling's disc equation. The handling times (T_h) of 4th nymphal instar of *O. albidipennis* when fed on eggs, 1st and 2nd nymphal stages of *P. solenopsis* were 0.03583, 0.02940 and 0. 02662 h, respectively, and the attack rates (a) were 0.3581, 0.4075 and 0.4841 h^{-1}, respectively (Figure 2). For 5^{th} nymphal instar when fed on eggs, 1^{st} and 2^{nd} nymphal instars of *P. solenopsis* the handling time were 0.0237, 0.0155 and 0.0128 h, respectively, and the attack rates were 0.7215, 0.8318 and 0.8704 h–1 (Figure 3). The handling time for females of *O. albidipennis* when fed on eggs, 1^{st} and 2^{nd} nymphal instars of *P. solenopsis* were 0.0125, 0.00987 and 0.00926 h, respectively and the attack rate were 0.8925, 1.0761 and 1.1664 h, respectively (Figure 4). The Predation efficiency for 4^{th} nymphal instar of this predator when fed on eggs, 1^{st} and 2^{nd} nymphal instars of *P. solenopsis* were 20.34, 12.10 and 9.74 individuals, respectively. Whereas, the predation efficiency for 5^{th} nymphal stages of this predator when fed on eggs, 1^{st} and 2^{nd} nymphal instars of *P. solenopsis* were 53.84, 43.32 and 36.48 individuals, respectively. Finally, The Predation efficiency for females of *O. albidipennis* when fed on eggs, 1^{st} and 2^{nd} nymphal instars of cotton mealybugs were 55.40, 44.0 and 40.1 individuals, respectively. The expected maximum consumption/ day for 4^{th} nymphal instar of *O. albidipennis*. As well as, the expected consumption of for females of *O. albidipennis* were 8٠,٠٤, 74.07 and 55.07 individuals respectively. In this paper, the number of consumed eggs or nymphs of *P. solenopsis* by different stages of *O. albidipennis* increased with increasing prey density. As well as, the functional responses of *O. albidipennis* to different densities of the eggs, 1^{st} and 2^{nd} nymphal instars of cotton mealybugs were type II. Also, we found that the increasing in prey density lead to increase in attack rate and decreasing in handling time. Results indicate that, *O. albidipennis* preferred feeding on eggs and 1^{st} nymphal instar than 2^{nd} nymphal instars. As well as, the females of *O. albidipennis* consumed more eggs, 1^{st} and 2^{nd} than 4^{th} and 5^{th} nymphal instars. In many researches, indicated that, *Orius* spp. was fitted in type II functional response (Holling, 1965 and Serkan *et al*., 2020). Whereas, Hassanzadeh *et al*. (2015) reported that, *O. albidipennis* showed type II functional response to different densities of *Tetranychus turkestani* Ugarov and Nikolski (Acari: Tetranychidae). As well as, Kasap and Atlihan (2011) indicated that the consumption rate of predators is related to prey size. *Stethorus* species feed on all prey life stages, but eggs usually are consumed in the largest numbers (Houck, 1991) because eggs are immobile and it easy to handle than other stages and therefore predators must consume more eggs to take their needs of nutrients. On the other hand, functional response is very important tool for evaluating the role of natural enemies in biological control program and the behavior of predators on controlling pests. To evaluate the effectiveness of a predator in relation to its prey, handling time is very important parameter to show how long a predator takes to capture and kill its prey (Atlhan *et al.,* 2010). The handling time was shortest when adult female *O. strigicollis* were offered *T. vaporariorum* nymphs. The handling time was higher when adult female *O. albidipennis* fed on nymphs of *B. tabaci* (Shahpouri *et al*., 2019). This study showed that, the handling time was shortened when females of *O. albidipennis* fed on 2^{nd} nymphal instars of cotton mealybugs. Increasing in prey densities due to increase in the predator attack rates and reducing handling time (Hassell *et al*., 1977).

It can be concluded that *O. albidipennis* showed an efficiency as a predator against *P. solenopsis* under laboratory conditions therefore it encourage further application under field conditions as an effective and successful biological control agent where the adult females proved an influential predation against the mealybug *P. solenopsis* especially the egg stage therefore it can be included in the integrated pest management program (IPM). As well as, 4th, 5th nymphal stages and females of O. *albidipennis* showed type II of functional responses to different densities of cotton mealybugs *P. solenopsis* under laboratory conditions. Females of *O. albidipennis* was more effective in feeding on eggs and 1st nymphs than the larger nymphs. As well as, females of *O. albidipennis* consumed more *P. solenopsis* different stages than nymphs of predator. Increasing in density of preys causes increasing in attack rate and predation efficiency. This study in evaluating the response and studying the behavior of *O. albidipennis* in controlling *P. solenopsis* which cause various damage on vegetables crop. Biological control by using the *O. albidipennis* will be the most effective method to control *P. solenopsis* on greenhouse or on the field.

Table (2): Effect of different densities of eggs, 1st and 2nd nymphal instars of the cotton mealybugs, and *Phenacoccus solenopsis* on the parameters of functional response of 4th, 5th nymphal instars band females of the predator *Orius albidipennis* under laboratory conditions

Predator stages	*Phenacoccus solenopsis* stages	Coefficient of correlation (r)	The rate of successful search (a)	Handling time (T_h)	Predation efficiency	The expected maximum consumption/ day	R^2	χ^2	*P* -value
4th Nymphal instar	Egg	0.995	0.3581	0.03583	20.34	30.63	0.9909	0.564	0.01513
	1st	0.989	0.4075	0.0294	12.1	25.4	0.9988	0.721	0.02932
	2nd	0.995	0.4841	0. 02662	9.74	20.16	0.9995	1.012	<0.0001
5th Nymphal instar	Egg	0.972	0.7215	0.0237	53.84	74.62	0.9844	0.971	0.03066
	1st	0.996	0.8318	0.0155	43.32	64.51	0.9933	0.664	0.03641
	2nd	0.982	0.8704	0.0128	36.48	43.85	0.9645	0.845	0.05971
	Egg	0.918	0.8925	0.0125	55.4	80.04	0.9635	0.972	0.02342
Female	1st	0.999	1.0761	0.00987	44	74.07	0.999	1.194	<0.0001
	2nd	0.981	1.1664	0.00926	40.1	55.07	0.9639	1.068	<0.0001

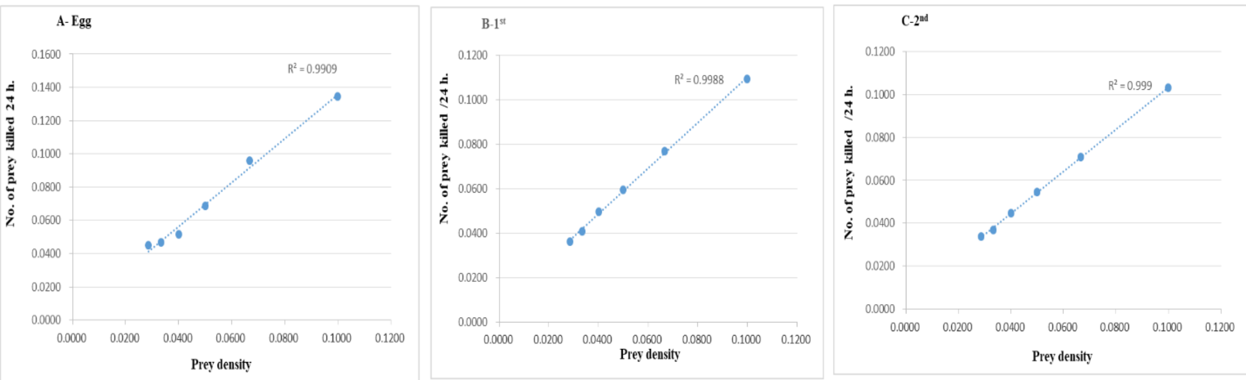

Figure (2 A, B , C): Functional response of 4th of *Orius albidipennis* to eggs, 1st and 2nd nymphal instars of *Phenacoccus solenopsis* under laboratory conditions.

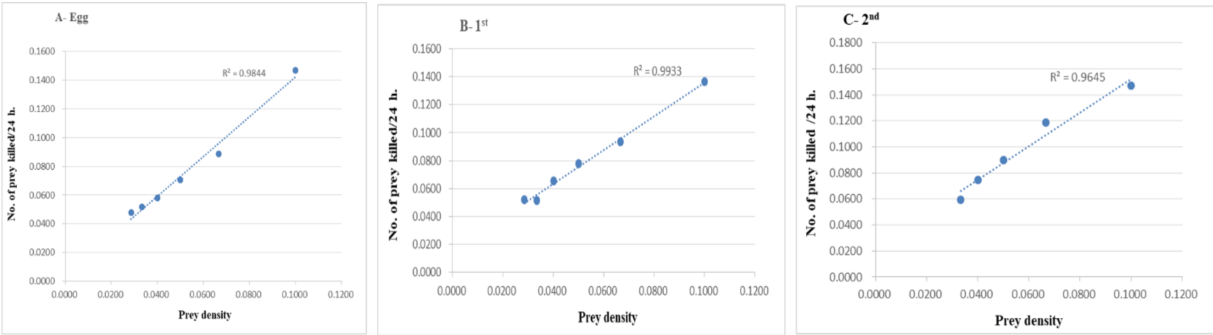

Figure (3 A, B , C): Functional response of 5th of *Orius albidipennis* to eggs, 1st and 2nd nymphal instars of *Phenacoccus solenopsis* under laboratory conditions.

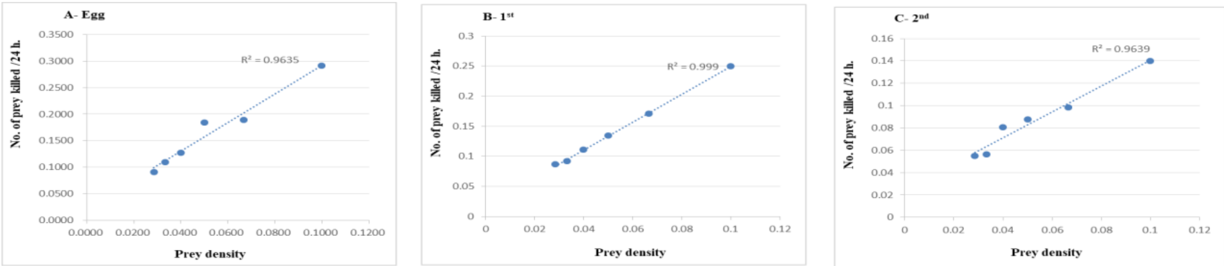

Figure (4 A, B , C): Functional response of female of *Orius albidipennis* to eggs, 1st and 2nd nymphal instars of *Phenacoccus solenopsis* under laboratory conditions.

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