

# Population Dynamic of *Phenacoccus solenopsis* Tinsley on Cotton Plants and Its Susceptibility to Some Insecticides in Relation to the Exposure Method

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## ABSTRACT

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) was first recorded during 2014 as an invasive cotton pest in Egypt. Thus, field experiments were conducted during the seasons 2015 and 2016 to study the population dynamic of *P. solenopsis* on cotton in Kafr El-Sheikh governorate, Egypt. Also, chlorpyrifos, methomyl, deltamethrin, lufenuron, acetamiprid, thiamethoxam and imidacloprid were tested for their toxicity to the third instar nymphs of *P. solenopsis* under laboratory conditions using leaf-dip and insect-spray methods. In both seasons, the infestation of *P. solenopsis* started during the early June when the cotton plants aged about two months. The highest population densities of 142.9±15.6 and 191.5±18.1 insects per twig were recorded on September 6<sup>th</sup> 2015 and September 11<sup>th</sup> 2016, respectively. The infestation was positively correlated with the maximum ( $r = 0.77$ ) and minimum ( $r = 0.71$ ) temperature and the relative humidity ( $r = 0.49$ ). Based on the multiple regression analysis and the coefficient of determination values ( $R^2$ ), the maximum and minimum temperature and the relative humidity were responsible for the changes in the insect population by 54.2 -- 63.7%. Thiamethoxam was the most effective insecticide ( $LC_{50} = 23.6 \text{ mg AI L}^{-1}$ ) against the third instar nymphs of *P. solenopsis* using the leaf-dip method, while lufenuron was the least toxic one with  $LC_{50}$  value of 556.1 mg AI L<sup>-1</sup>. When the insect-spray method was adopted, the toxicity of deltamethrin and lufenuron increased, whereas the toxicity of the others decreased comparing to that obtained in the leaf-dip method.

**Key words:** *Phenacoccus solenopsis*, cotton, population dynamic, weather factors, insecticides, exposure method.

## INTRODUCTION

The cotton mealybug, *Phenacoccus solenopsis* Tinsley is a highly polyphagous sap sucking insect, native to USA (Fuchs *et al.*, 1991), and later spread over a wide range of tropical and subtropical countries. Cotton is considered of the most preferred hosts of *P. solenopsis* (Abbas *et al.*, 2010). This solenopsis mealybug not only causes direct damages to cotton plants by sucking the cell sap, but also excretes large quantities of honey dew which encourages the black sooty mold growth and hinders the photosynthesis process (Arif *et al.*, 2012). Besides this, ants feed on the sugary honey dew, playing a very serious role in the spread of mealybug infestation and deter biological

control agents (Cudjoe *et al.*, 1993). The infested cotton plants become stunted, weak and fruit a few small bolls resulting in severe economic yield losses (Dhawan *et al.*, 2007; El-Zahi *et al.*, 2016). In Egypt, *P. solenopsis* was first recorded in 2014 as a new invasive pest attacking the cotton plantations (El-Zahi *et al.*, 2016), and it is predicted to become one of the most dangerous cotton pests within the next few years, where the agro-ecosystem is closely adequate to its development and spread. This insect has a high reproductive potential in wide ranges of temperature (Prasad *et al.*, 2012) and relative humidity (Hameed *et al.*, 2012), and a waxy secretion on the body surface that protect it from insecticides and natural mortality factors. The cotton growers mostly rely on insecticides to control the insects infesting their crops because the insecticides act rapidly on the infestation, less cost and easily applied (Rashid *et al.*, 2011). *Phenacoccus solenopsis* infestations on different hosts could be effectively controlled using biological control agents, plant extracts, mineral oils and synthetic insecticides (Aheer *et al.*, 2009; Suresh *et al.*, 2010; Kumar *et al.*, 2012; El-Zahi *et al.*, 2016). The information about the biological activities of *P. solenopsis* in the cotton fields under the Egyptian condition is not available till now, and no insecticides are recommended to its control as well. Thus, the present investigations were planned to study the population dynamic, seasonal abundance and peak activity of *P. solenopsis* on cotton, and to determine its susceptibility to some synthetic insecticides using leaf-dip and insect-spray methods under laboratory conditions.

## MATERIALS AND METHODS

### Insecticides

Commercial formulations of lufenuron (Match 5% EC, Syngenta Agrosiences, Switzerland), chlorpyrifos (Dursban H 48% EC, Dow Agrosiences, UK), methomyl (Neomyl 90% SP, Rotam Agrochemical Co. Ltd., Hong Kong), deltamethrin (Decis 2.5% EC, Bayer Crop Science, Germany), acetamiprid (Newcet 20% SP, Jinan Leader Chemical Co. Ltd., China), thiamethoxam (Actara 25% WG, Syngenta Agrosiences, Switzerland) and imidacloprid (Ecomida 30.5% SC, Bharat

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Insecticides Ltd., India) were tested for their toxicity to *P. solenopsis* under laboratory conditions.

### Insect colony establishment

To establish a culture of *P. solenopsis*, infested cotton twigs were collected from unsprayed fields and brought to the laboratory. Adult females were separated and inoculated on cotton plants of two months age, potted under laboratory conditions of  $30\pm 2^\circ\text{C}$ ,  $65\pm 5$  RH and 13:11(L: D) photoperiod. Two days later, the females settled on plant leaves and stems and started egg laying. The newly moulted third instar nymphs were used in the laboratory experiments.

### Field experiments

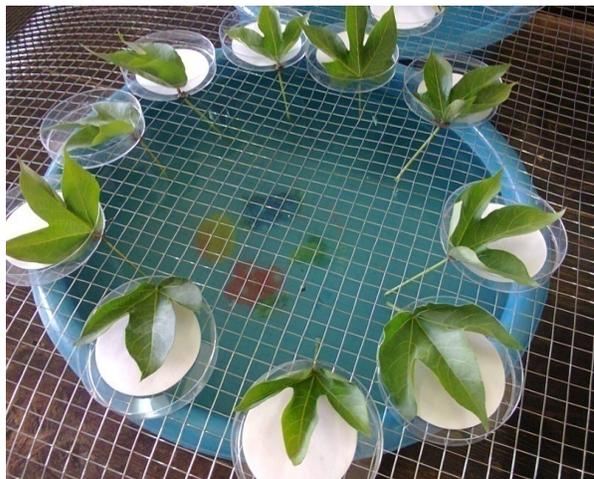
The survey for *P. solenopsis* infestations on cotton plants (*Gossypium barbadense* var. Giza 92) was conducted at the farm of Sakha Agricultural Research Station, Kafr El-Sheikh governorate, Egypt during the cotton growing seasons 2015 and 2016 in an area of approximately 2000 m<sup>2</sup>. This area did not receive any insecticidal treatments during the two seasons. The survey started one month after plant emergence and continued till harvesting at fortnightly observations. In each observation, 100 apical twigs of the same age were randomly chosen from this area (20 twigs from each corner plus 20 from the center) to count the adult females and third instar nymphs of the mealybug. The mean population per twig was calculated and correlated with the weather factors: maximum temperature, minimum temperature and relative humidity which were obtained from the Meteorological Station of Sakha, Egypt.

### Laboratory experiments

#### 1. Leaf-dip method

A serial of concentrations was prepared from each insecticide using tap water for dilutions. Fresh cotton leaves with petioles were washed thoroughly with tap water and shade dried. Ten leaves were dipped in each concentration for 20 seconds and in the tap water only for the control, and then shade dried. Circular openings were created in nine cm diameter plastic Petri dishes. The treated leaves were placed individually inside the dishes, while their petioles passed through the openings and immersed in 1% sucrose solution to keep the leaves alive (Fig. 1). A filter paper was put underneath each leaf to absorb any water vapor. Twenty newly moulted third instar nymphs of *P. solenopsis* were transferred to each Petri dish using a fine camel hair brush, representing one replication. Ten replications were made for each concentration and the control. The dishes were covered with their lids to prevent the insect escape, and maintained under constant conditions of  $30\pm 2^\circ\text{C}$ ,  $65\pm 5$  RH and 13:11(L: D) photoperiod. A binocular

microscope was used to distinguish dead insects from live ones. Number of dead insects and the percentages of mortality were recorded after 72 h of the treatment. In the case of the chitin synthesis inhibitor (lufenuron) the data were recorded after 6 days of the treatment. Where, the third instar nymphs of *P. solenopsis* moult to the next stage after  $5.20 \pm 0.45$  days (Prishanthini and Vinobaba, 2013).



**Fig.1. Preparing of Petri dishes: Seen are the petioles of the cotton leaves passed through the openings and were immersed in 1% sucrose solution to keep the leaves alive for about a month**

#### 2. Insect- spray method

Adult females of *P. solenopsis* were transferred to cotton plants of two months age. When the offspring emerged to the 3<sup>rd</sup> instar nymphs, the cotton plants were closely sprayed with 15 ml insecticide solution per plant. Each concentration was applied to five plants using a hand sprayer. The concentrations of the test insecticides and the Petri dishes were prepared as previously described. Untreated cotton leaves were placed in all the Petri dishes. After complete dryness of the sprayed plants, 20 newly moulted third instar nymphs of *P. solenopsis* were transferred to each Petri dish with aid of a fine camel hair brush, representing one replication. For the control, the nymphs were transferred from plants sprayed with water only. Ten replications were made for each concentration and the control. The dishes were covered with their lids to prevent the insect escape, and maintained under constant conditions of  $30\pm 2^\circ\text{C}$ ,  $65\pm 5$  RH and 13:11(L: D) photoperiod. The data were recorded as previously mentioned.

#### Statistical analysis

The mortality data were corrected using the formula of Abbott (1925), and subjected to probit analysis based on Finney (1971). Failure of 95% confidence limits to overlap at a particular lethal concentration indicated a

significant difference. SPSS 16.0 Software for Windows was used to determine the correlation and multiple regression coefficients between the weather factors and the mean population of *P. solenopsis*.

## RERSULTS AND DISCUSSION

### Population dynamic of *P. solenopsis*

During the season 2015, the infestation of *P. solenopsis* on cotton plants started at low density ( $2.4 \pm 0.6$  insects per twig) on June 14<sup>th</sup> (Table 1). The population increased gradually and its highest peak ( $142.9 \pm 15.6$  insects per twig) was observed on September 6<sup>th</sup>. During 2016, the first incidence of *P. solenopsis* was recorded on June 5<sup>th</sup> ( $0.6 \pm 0.2$  insects per twig). The highest population density ( $191.5 \pm 18.1$  insects per twig) was noticed on September 11<sup>th</sup>. The population averages were  $54.8 \pm 9.7$  and  $70.5 \pm 13.6$  insects per twig in 2015 and 2016, respectively. It was observed that the petioles and among sepals of the flower buds and the terminal bud were the first infested parts of the cotton plant with *P. solenopsis*, which later spread over the entire plant. Also, in both seasons the infestation started when the cotton plants aged about two months, with the first formation of the flower buds. The present results are in parallel with Sahito *et al.* (2011) who observed the highest infestation of *P. solenopsis* on cotton during September and October, while Shahid *et al.* (2012) recorded the peak of the mealybug population in August and September, when the temperature ranged from 28-30 °C. The highest *P. solenopsis* population ( $222.3 \pm 22.8$  insects per cotton twig) was observed during the second half of September (Shah *et al.*, 2015).

The population of *P. solenopsis* was significantly and positively correlated (Table 2) with the maximum ( $r = 0.77$  and  $0.69$ ) and minimum ( $r = 0.71$  and  $0.67$ ) temperatures in 2015 and 2016, respectively. The correlation between the relative humidity and the population was positive and insignificant in 2015 and 2016 with  $r$  values of  $0.33$  and  $0.49$ , respectively. The multiple regression analysis revealed that the increase in the maximum temperature by 1°C increased the *P. solenopsis* population by 14.87 and 12.38 insects per twig in 2015 and 2016, respectively (Table 2). Similarly, 1°C increase in the minimum temperature caused an increase in the insect population by 4.02 and 3.76 insects per twig in 2015 and 2016, respectively. An increase in the relative humidity by 1% resulted in increasing of 3.22 and 3.94 insects per twig in 2015 and 2016, respectively. The development duration of *P. solenopsis* decreased as the temperature increased from 18 to 32°C (Prasad *et al.*, 2012). Hanchinal *et al.* (2010) recorded a positive and significant correlation between the mealybug population and the maximum temperature with  $r$  value of 0.78. Also, the infestation of *P.*

*solenopsis* on cotton was positively correlated with the maximum and minimum temperature (Babu and Meghwal, 2014), and showed a positive correlation with the relative humidity with  $r$  value range of 0.23- 0.76 (Hameed *et al.*, 2014).

According to the coefficient of determination values ( $R^2$ ) of this study, the maximum and minimum temperatures and the relative humidity were responsible for the change in the population density of *P. solenopsis* by 63.7 and 54.2% during 2015 and 2016, respectively. The obtained results of correlation and regression analysis clearly showed that weather factors play important role in the development of *P. solenopsis* population.

### Susceptibility of *P. solenopsis* to some insecticides under laboratory conditions

The results presented in Table (3) indicated that thiamethoxam proved to be the most effective insecticide ( $LC_{50} = 23.6$  mg AI L<sup>-1</sup>) against the third instar nymphs of *P. solenopsis* using the leaf-dip method. Methomyl, acetamiprid and imidacloprid showed insignificant differences among them, recording  $LC_{50}$  values of 34.3, 39.0 and 43.5 mg AI L<sup>-1</sup>, respectively. Based on the 95% confidence limits overlap, the  $LC_{50}$  values of chlorpyrifos (73.4 mg AI L<sup>-1</sup>) and deltamethrin (94.8 mg AI L<sup>-1</sup>) were not significantly different from each other. The chitin synthesis inhibitor (lufenuron) was the least toxic with  $LC_{50}$  value of 556.1 mg AI L<sup>-1</sup>. The susceptibility of the third instar nymphs of *P. solenopsis* to the tested insecticides varied when the insect-spray method was adopted (Table 4). The  $LC_{50}$  values of acetamiprid, thiamethoxam, imidacloprid, methomyl and chlorpyrifos increased to be 56.5, 59.3, 66.4, 112.8 and 295.9 mg AI L<sup>-1</sup>, respectively. On the contrary, the  $LC_{50}$  values of deltamethrin (39.6 mg AI L<sup>-1</sup>) and lufenuron (397.9 mg AI L<sup>-1</sup>) declined comparing to that obtained in the leaf-dip method.

These results indicated that the systemic insecticides: thiamethoxam, imidacloprid, acetamiprid and methomyl may have the ability to penetrate the cotton leaf tissue in the leaf-dip method resulting in their high toxic action compared to the non-systemic tested insecticides. The increased toxicity of deltamethrin and lufenuron in the insect-spray method comparing to that in leaf-dip method, may be attributed to their high molecular weights (505.2 and 511.2, respectively) and high solubility in lipids (Tomlin, 2003), which allow them to pass through the waxy secretions of the insect. Thiamethoxam, imidacloprid, methomyl and chlorpyrifos have been reported as effective insecticides against the cotton mealybug (Aheer *et al.*, 2009; Suresh *et al.*, 2010; El-Zahi *et al.*, 2016).

Table 1. Mean population of *Phenacoccus solenopsis* per cotton twig at fortnightly observations during 2015 and 2016

		Date	17/5	31/5	14/6	28/6	12/7	26/7	9/8	23/8	6/9	20/9	4/10	18/10	1/11	Average
2015	Mean		0.0	0.0	2.4	10.7	25.3	49.8	90.1	103.6	142.9	130.5	70.6	53.4	32.9	54.8
	+SE		+0.0	+0.0	+0.6	+2.3	+5.4	+7.2	+11.0	+9.7	+15.6	+8.9	+10.4	+6.1	+4.5	+9.7
2016	Mean	Date	22/5	5/6	19/6	3/7	17/7	31/7	14/8	28/8	11/9	25/9	9/10	23/10	6/11	Average
	+SE		0.0	0.6	5.3	12.6	33.4	57.7	108.2	170.4	191.5	156.8	95.3	62.7	21.5	70.5
			+0.0	+0.2	+1.8	+3.8	+6.2	+5.9	+8.7	+13.6	+18.1	+14.2	+7.9	+8.3	+4.2	+13.6

Data are expressed as means  $\pm$  SE

**Table 2. Correlation (r) and multiple regression (b) coefficients between some weather factors and mean population of *Phenacoccus solenopsis* per cotton twig during 2015 and 2016**

Season	Maximum temperature (°C)		Minimum temperature (°C)		Relative humidity		% R <sup>2</sup>
	r	b	r	b	r	b	
2015	0.77**	14.87	0.71**	4.02	0.33 <sup>NS</sup>	3.22	63.7
2016	0.69**	12.38	0.67*	3.76	0.49 <sup>NS</sup>	3.94	54.2

\*\* Highly significant,  $P \leq 0.01$  — \* Significant,  $P \leq 0.05$  — <sup>NS</sup> Not significant.

**Table 3. Susceptibility of *Phenacoccus solenopsis* third instar nymphs to some insecticides using leaf-dip method under laboratory conditions**

Insecticide	LC <sub>50</sub> <sup>a</sup> 95% CL <sup>b</sup>	LC <sub>90</sub> <sup>a</sup> 95% CL	Slope value	$\chi^2$
Chlorpyrifos	73.4 59.0 – 98.5	309.0 157.5 – 432.6	1.46 ± 0.27	0.44
Methomyl	34.3 29.4 – 40.2	118.7 86.4 – 154.2	2.38 ± 0.35	2.71
Deltamethrin	94.8 81.3 – 113.7	227.8 194.3 – 309.8	3.15 ± 0.44	5.59
Imidacloprid	43.5 30.4 – 58.5	177.5 138.3 – 241.4	1.62 ± 0.36	2.65
Thiamethoxam	23.6 18.6 – 28.4	59.5 47.4 – 86.1	3.21 ± 0.42	0.32
Acetamiprid	39.0 28.9 – 52.7	130.3 89.9 – 190.2	1.72 ± 0.33	3.62
Lufenuron	556.1 401.8 – 734.1	2391.2 1673.8 – 3491.1	1.52 ± 0.36	2.96

<sup>a</sup> LC<sub>50</sub> and LC<sub>90</sub> are expressed in mg AI L<sup>-1</sup> — <sup>b</sup> 95% Confidence limits

**Table 4. Susceptibility of *Phenacoccus solenopsis* third instar nymphs to some insecticides using insect-spray method under laboratory conditions**

Insecticide	LC <sub>50</sub> <sup>a</sup> 95% CL <sup>b</sup>	LC <sub>90</sub> <sup>a</sup> 95% CL	Slope value	$\chi^2$
Chlorpyrifos	295.9 218.9 – 402.4	1334.5 1080.9 – 1848.4	1.34 ± 0.32	2.96
Methomyl	112.8 78.5 – 158.1	442.2 322.8 – 588.1	1.04 ± 0.33	0.31
Deltamethrin	39.6 22.3 – 62.7	130.7 105.8 – 185.6	1.69 ± 0.42	3.26
Imidacloprid	66.4 50.1 – 87.6	278.8 209.1 – 368.0	1.91 ± 0.39	0.25
Thiamethoxam	59.3 43.7 – 79.5	193.5 127.8 – 278.6	2.51 ± 0.57	2.66
Acetamiprid	56.5 43.8 – 69.5	183.9 132.7 – 305.3	2.50 ± 0.45	1.43
Lufenuron	397.9 297.0 – 557.1	1683.1 1279.2 – 2810.7	1.73 ± 0.35	2.67

<sup>a</sup> LC<sub>50</sub> and LC<sub>90</sub> are expressed in mg AI L<sup>-1</sup> — <sup>b</sup> 95% Confidence limits

Under laboratory conditions, Kumar *et al.* (2012) found that chlorpyrifos at 8 ml L<sup>-1</sup> caused 61.3% mortality in the five days-old nymphs of *P. solenopsis*. The field rates of thiamethoxam and imidacloprid applied in the laboratory resulted in 95.2 and 81.6% mortality, respectively in the 2<sup>nd</sup> instar nymphs of *P. solenopsis*

(Rashid *et al.*, 2011). The results of the present study suggest that the complete coverage of treated plants in the case of systemic insecticides, and direct spray on the insects in the case of deltamethrin and lufenuron could be recommended to obtain a successful *P. solenopsis* control on cotton.

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## الملخص العربي

ديناميكية تعداد بق القطن الدقيقي *Phenacoccus solenopsis* Tinsley على نباتات القطن والعلاقة بين

حساسيته لبعض المبيدات وطريقة التعرض

الزاهي صابر الزاهي، عبد السلام إسماعيل فرج

درجات الحرارة العظمى ( $r = 0.77$ ) والصغرى ( $r = 0.71$ ) والرطوبة النسبية ( $r = 0.49$ ). طبقاً لنتائج تحليل الانحدار المتعدد وقيم معامل التقدير ( $R^2$ ) فإن درجات الحرارة العظمى والصغرى والرطوبة النسبية مجتمعة تتحكم في التغير في تعداد بق القطن الدقيقي بنسبة تتراوح بين ٥٤,٢ - ٦٣,٧%. كما أوضحت النتائج أنه عند استخدام طريقة غمر الورقة، كان مركب ثياميثوكسام هو الأكثر فاعلية ضد حوريات العمر الثالث للحشرة مسجلاً قيمة للتركيز القاتل لـ ٥٠% من التعداد تساوى ٢٣,٦ جزء في المليون، و كان مركب ليوفينورون هو الأقل سمية مسجلاً قيمة للتركيز القاتل لـ ٥٠% من التعداد تساوى ٥٥٦,١ جزء في المليون. من ناحية أخرى أوضحت النتائج أنه عند استخدام طريقة الرش المباشر على الحشرة زادت سمية مركبي دلتامثرين و ليوفينورون بينما إنخفضت سمية المركبات الباقية مقارنة بطريقة غمر الورقة.

لقد تم تسجيل بق القطن الدقيقي *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) لأول مرة كافة جديدة على القطن في مصر في عام ٢٠١٤م ومن ثم أجريت تجارب حقلية في محافظة كفر الشيخ — مصر خلال موسمي ٢٠١٥م ، ٢٠١٦م لدراسة ديناميكية تعداد هذه الآفة على القطن. كذلك تم تقييم فاعلية مركبات كلوربيريفوس، ميثوميل، دلتامثرين، ليوفينورون، أسيتامبريد، ثياميثوكسام، إميذاكلوبريد على طور الحورية الثالث لهذه الآفة تحت الظروف المعملية باستخدام طريقتي غمر الورقة، والرش المباشر على الحشرة. أظهرت النتائج أن الإصابة بهذه الحشرة بدأت مبكراً في شهر يونيه في كلا موسمي الدراسة عندما كان عمر نباتات القطن حوالي شهرين. سجلت أعلى كثافة تعداد للآفة  $142,9 \pm 15,6$  و  $191,5 \pm 18,1$  حشرة لكل فرع ثمري في ٦ سبتمبر ٢٠١٥ و في ١١ سبتمبر ٢٠١٦ على الترتيب. أوضحت النتائج أنه هناك علاقة ارتباط موجبة بين الإصابة وكل من