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Population Densities of the Predaceous Mite *Amblyseius swirskii* on Cotton Plants, and A New Technique to Gather the Mite Individuals

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ABSTRACT: The population densities of the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) were observed in a pesticide-free cotton field in Egypt. The highest population density of was noted in September, whereas the lowest was noted in June. The plant's central section indicated the maximum population density, whereas the plant's top section indicated the lowest population density. The population density of mites found on the undersides of the leaves was significantly higher than that of mites found on the upper sides of the leaves. The bases and pleats of the undersides of the leaves harboured the mite majority. Neither Lambda cyhalothrin (5%) E.C. nor Chlorpyrifos methyl (48%) E.C. had a significant effect on the mite numbers. A defoliant was sprayed in the pesticide-free area. A week later, the recently fallen leaves had the greatest mite population density. A few recently fallen leaves were collected from an area treated with both defoliant and the two pesticides. When they were stacked in a glass jar covered with opaque fabric apart from the opening, in a continuously lit laboratory, the majority of the mites congregated on the topmost leaves.

Keywords: *Amblyseius swirskii*; cotton; population; pesticides; defoliated; gather

INTRODUCTION

Different insect species can be managed by the predaceous mite *A. swirskii* Athias-Henriot (**Alipour et al., 2016; Riahi et al., 2017**). Various pests like thrips, whiteflies, and mites can be controlled by it **(Messelink *et al*., 2005; Arthurs *et al*., 2009; Stansly and Castillo, 2009; Dogra Maci *et al*., 2011; Calvo *et al*., 2015)**. Since its commercial production, it has been amongst the most effective biological control agents **(Buitenhuis *et al*., 2010)**.

To carry out various investigations, a great number of *A. swirskii* mites are required. Accordingly, the primary goal of the current study was to determine the months of the growing period, the plant sections, and the places on the cotton leaf that serve as indicators of mite abundance. Additionally, two insecticides were sprayed, and their impact on the mite numbers was measured. The impact of spraying a defoliant on the mite numbers was also studied.

The methods of discharging predaceous mites across fields have been studied in the past. Different tools, like small packets, can be used to spread mites that are created for commercial purposes. They are tiny units that hang from the plant and unleash predators for a period of time. Predatory mites kept in roughage are usually applied to the foliage. As an alternative, an air blast can disseminate the mites (**Opit et al., 2005**).

The current study focuses on the contradictory direction, specifically how to collect predatory mites that are present on cotton plants. Accordingly, the second goal of the current study is to discover a way to get the enormous store of *A. swirskii* that is on the leaves before the end of the growing period in order to use it rather than let it perish.

The restricted dissemination of *A. swirskii* from the point of discharge was revealed by **Buitenhuis et al. (2010)**. The current study focused on how *A. swirskii* disperses on piled cotton leaves. This phenomenon might be used to gather predatory mites. The pattern of the mite's dispersal should be the guiding principle to designate the best location of the plant for releasing that mite.

MATERIALS AND METHODS

The current study was carried out in a cotton field in Behera governorate, Egypt over two seasons (2020 and 2021).

Apart from the application of pesticides, standard agricultural procedures were followed. In Egypt, cotton is planted in March, April, and May **(El-Debaby *et al*., 1995; Attia *et al*., 2021)**. Consequently, cotton plants remain in some fields till the middle of November. The present study was conducted in a belated cotton field. Individuals of *A. swirskii* were counted, considering the taxonomic identification.

1. Estimating the population densities of the mites found in the different months from mid-June to mid-November

Replicates were selected at three different field locations. Monthly, ten leaves were picked at random from each location, and the number of *A. swirskii* individuals present on each leaf was noted. Each location's monthly mite population density was estimated. That was done over the course of two seasons, from mid-June to mid-November (2020 and 2021).

2. Estimating the population densities of the mites found in the different sections of the plant

The plants' average height in September was around 180 cm. Three plants represented the replicates. The bottom, the center, and the top of each designated plant were supposed to be separate sections. The section had a height of roughly 60 cm. From every section, ten leaves were picked at random. Each leaf was picked apart to count the mites found on it. The mite population density was estimated for each of the three sections.

3. Estimating the population densities of the mites found on the two leaf sides and on different leaf parts

In September, ten leaves from the central part of the plants were selected as ten replicates. Each leaf was examined immediately after picking. Certain cotton leaves had five lobes and four pleats. Others had three lobes and two pleats (**Figs. 1 and 2**). The pleat typically has a convex upper side and a concave underside (**Fig. 3**).

The underside of the cotton leaf contained either five or three main, noticeable veins. They converge at the base of the leaf. The mites were counted, taking into account the ones that follow:

3. 1. Locations appointed on the upper side

The number of mites on the upper side of each leaf was recorded, paying particular attention to the base, the pleats, and the remainder of the upper side. The total number of mites on the upper side was then determined.

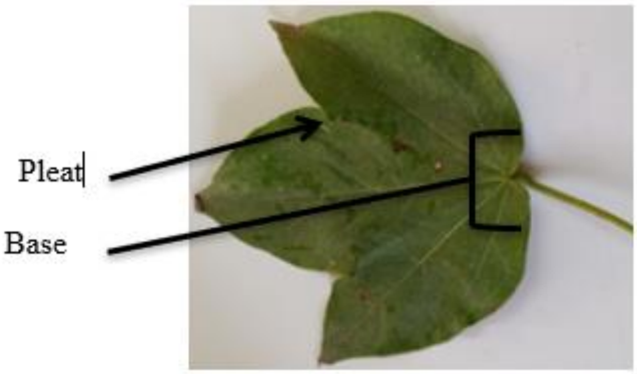
3. 2. Locations appointed on the underside

The number of mites on the underside of each leaf was recorded, paying particular attention to the base, the pleats, the edges of all the major veins, and the remainder of the underside. The total number of mites on the underside was then determined.

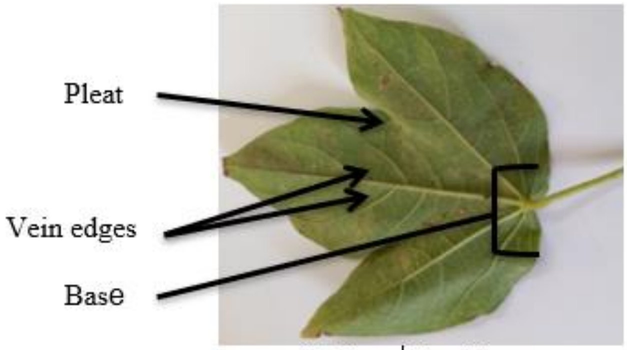
(a) The upper side

(b) The underside

Fig. 1 Leaf with five lobes



(a) The upper side



(b) The underside

Fig. 2 Leaf with five lobes

Fig. 3 Portion of the upper side, comprising a pleat in the center

4. Inspecting the leaves after spraying the pesticides

In a determined region, Lambda cyhalothrin (5%) E. C. and Chlorpyrifos methyl (48%) E. C. were alternately sprayed. Following the manufacturer's guidelines, each insecticide was applied three times. The interval was 20 days. The initial application (chlorpyrifos methyl) was made on the first of July. The final application (lambda cyhalothrin) took place on August 20th. Accordingly, a total of six sprays were performed. The interval was 10 days. Three sites were chosen to represent three replicates. Furthermore, three pesticide-free sites were chosen to represent the other three replicates. The pesticide application programme ended on August 20th. Five days later, *A. swirskii* mites found on each of the ten leaves that were randomly selected from each site were noted. Each site's mite population density was estimated.

5. Inspecting the leaves after spraying a cotton defoliant

Following the manufacturer's guidelines, a cotton defoliant, namely dropp® 50 w. p., was sprayed on the first of October. That was applied on a pesticide-free plot, leaving an area untreated with the defoliant. After a week, there were four-leaf categories:

1. The old leaves underneath the defoliated plants
2. The recently fallen leaves underneath the defoliated plants
3. The leaves remaining on the plants treated with the defoliant
4. The leaves of the plants that haven't been treated with the defoliant

Each category was represented with three plants as replicates. From each replicate, ten leaves were picked and inspected to evaluate the population density of the considered mite.

6. Gathering the mites on some leaves

The mites were gathered from the pesticide-treated region by following three successive steps:

1. The defoliant, namely, Dropp®, was applied at the on the first of October.
2. Some of the recently fallen leaves were gathered on October 8th and transferred to the laboratory right away.
3.The mites were accumulated on some leaves in the laboratory as follows:

The petioles of the leaves as well as the tips of the broad leaves were removed. In a glass jar, fifteen leaves were stacked on top of one another, as seen in **Fig. 4**. Nine replicates of the leaf-filled jar were made. The margins of the leaves made no direct contact with the inside surface of the jar wall. The jar rim had been previously treated with vaseline mixed with camphor oil. The exterior surfaces of the jar sides were covered with an opaque fabric.

The jars were left open in a continuously lit laboratory.

Three replicates were considered on the next day to count the mites that were present on each of the leaves. The same inspection was performed on three replicates every day. Consequently, the nine jars were considered within three successive days. Five groups, each of which comprises three leaves, were thought to make up the 15 leaves of the jar. Following that, the numbers from one, which stands for the highest group, to five, which stands for the lowest group, were used to signify the five groups. Each group's mite population density was estimated. The laboratory experiment to assemble the mites was done at a temperature of 30 ± 5° C and a relative humidity of 70 ± 5%.

Fig. 4 A jar's top view before the sides are covered

7. Statistical analysis

An ANOVA was used for examining the data. The computer application COSTAT software was used to determine the least significant differences (L.S.D.) at the 0.05 level.

RESULTS

1. Population densities of the mites in the different months of the season

According to **Table 1** and **Fig. 5,** the highest population density occurred in September, while the lowest occurred in June.

Table 1. The mites' average numbers, per leaf, in the different months

Months	Season of 2020		Season of 2021	
	Mites' average numbers	**Ranks**	**Mites' average numbers**	**Ranks**
June	0.833 d	6	1.000 d	6
July	1.700 c	5	1.867 cd	5
August	5.433 ab	2	5.967 a	2
September	5.833 a	1	6.133 a	1
October	5.300 b	3	4.867 b	3
November	1.833 c	4	2.267 c	4
L.S.D., 0.05	0.4725414421		0.9061425	

Three replicates were used for the samples.

The order of the ranks is downward.

Averages in the same column that are followed by the same letter do not differ significantly at $P < 0.05$ level.

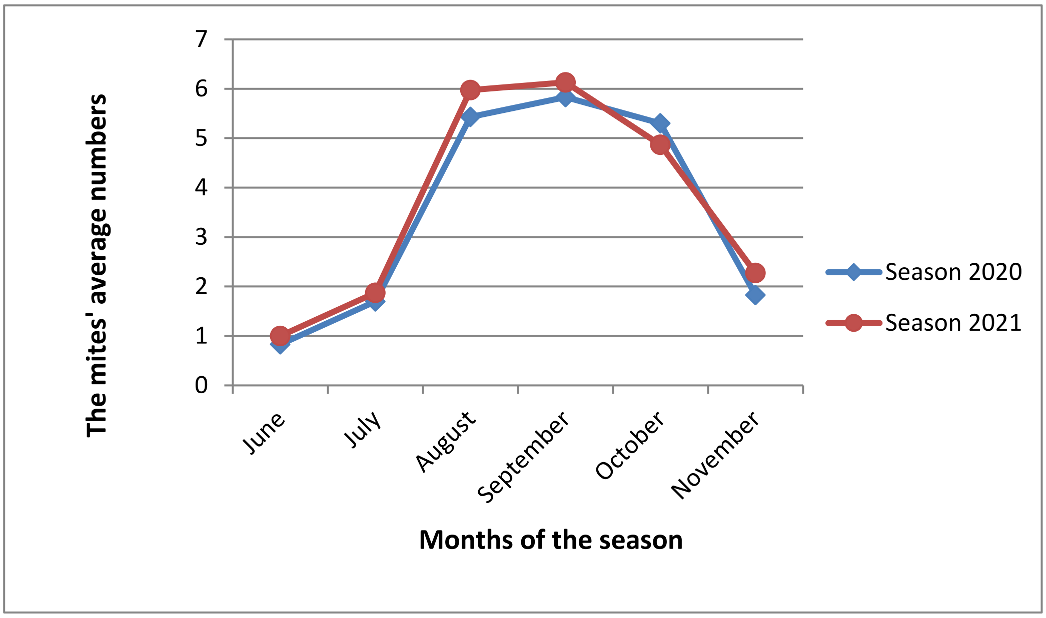


Fig. 5. Average numbers of the mites, per leaf, during the months of the season

2. Population densities of the mites in the different sections of the plant

According to **Table 2**, the leaves located in the central section of the plants had the highest mite population density (an average of 9.967 mites per leaf), while the leaves located in the top section of the plants involved the lowest mite population density (an average of 1.633 mites per leaf).

Table 2. The mites' average numbers, per leaf, in the different sections

sections	Mites' average numbers	Ranks
Bottom	6.967 b	2
Center	9.967 a	1
Top	1.633 c	3
L.S.D., 0.05	1.3101074692	

Three replicates were used for the samples.
The order of the ranks is downward.
Averages in the same column that are followed by the same letter do not differ significantly at $P < 0.05$ level.

3. Population densities of the mites on the two leaf sides and on different locations of each side

Considering the undersides of ten leaves, the average number of mites detected in the pleats amounted to five, as indicated in **Table 3**. Simply said, there were 50 mites on the undersides of all the pleats of ten leaves. Hence, most mites were observed in that location. The average number of mites detected on the undersides of the ten leaves amounted to 9.2. Simply said, there were 92 mites on all the locations of the undersides of ten leaves. Comparatively, the average number of mites detected on the upper sides of the ten leaves amounted to just two. Thus, the mite population was substantially greater on the undersides of the leaves than it was on the upper sides.

Table 3. The mites' average numbers, per leaf, in different locations

Sides	Locations	Parameters of the mites detected in the seven locations		Parameters of the mites detected on the two sides	
		Mites' average numbers	Rank	Mites' average numbers	Rank
Upper side	Base	1.80 c	3	2.40 b	2
	Pleats	0.30 d	5		
	Remainder of the side	0.30 d	5		
Underside	Base	3.00 b	2	9.20 a	1
	Pleats	5.00 a	1		
	edges of the major veins	0.60 d	4		
	Remainder of the side	0.60 d	4		
L.S.D., 0.05		1.1066308825		2.1471016676	

Ten replicates were used for the samples.
The order of the ranks is downward.
Averages in the same column that are followed by the same letter do not differ significantly at $P < 0.05$ level.

4. Population densities of the mites after spraying the pesticides

Leaves that had been exposed to pesticides had an average of 4.567 mites per leaf, while leaves that had not been exposed to pesticides had an average of 5.60 mites per leaf. Accordingly, the two pesticides had no significant impact on the mite population density (Table 4).

Table 4. The mites' average numbers, per leaf, on the pesticide-untreated leaves and the pesticide-treated leaves

Pesticide treatment	Mites' average numbers
Untreated	5. 600 a
Treated	4. 567 a
L.S.D., 0.05	1.5486270903

Three replicates were used for the samples.
Averages in the same column that are followed by the same letter do not differ significantly at $P < 0.05$ level.

5. Population densities of the mites after spraying a cotton defoliant

In spite of applying the defoliant, a few leaves lingered on the stems' lower portions for a few days.

According to **Table 5**, The average numbers of mites per leaf, considering the four-leaf categories, *i.e.*, the old leaves underneath the defoliated plants, the recently fallen leaves underneath the defoliated plants, the leaves remaining on the plants treated with the defoliant, and the leaves of the plants that haven't been treated with the defoliant, were 1.433, 13.500, 7.367, and 5.433 mites per leaf, respectively, with a ratio of 1: 9.42: 5.14: 3.79. Hence, the recently fallen leaves underneath the defoliated plants had the highest population density of mites. Simply said, those greenish and freshly fallen leaves were home to the greatest number of mites.

Table 5. The mites' average numbers, per leaf, on the four-leaf categories

Leaf category	Mites' average numbers	Ranks	Ratio
Old leaves underneath the defoliated plants	1.433 d	4	1
Recently fallen leaves underneath the defoliated plants	13.500 a	1	9.42
Leaves remaining on the plants treated with the defoliant	7.367 b	2	5.14
Leaves of the plants that haven't been treated with the defoliant	5.433 c	3	3.79
L.S.D., 0.05	1.8615419831		

Three replicates were used for the samples.
The order of the ranks is downward.
Averages in the same column that are followed by the same letter do not differ significantly at the $P < 0.05$ level.

6. Gathering the mites on some leaves

The uppermost leaves in the jars acquired the mite most densely.

Table 6 demonstrates the mite population densities after three days of stacking the leaves in the jars. An average of 39.89 mites were detected on the uppermost three leaves of group 1. In contrast, the mite population density on the defoliant-untreated plants in October 2021 was just 4.867 mites per leaf.

As regards the elapsed days, the total mite numbers in the jars have declined day after day.

As far as the prey is concerned, no living prey was observed on the jar leaves at the end of the experiment.

Table 6. The mite's average numbers, per leaf, in each group and on each day

Number of the elapsed days	Groups	Parameters of the mites detected in the group		Parameters of the mites detected daily	
		The mite's average number	Ranks	The mite's average number	Ranks
One	1	16.890 d	4	12.353 a	1
	2	20.223 c	3		
	3	14.110 e	6		
	4	8.220 f	8		
	5	2.337 g	10		
Two	1	36.777 b	2	11.777 b	2
	2	14.220 e	5		
	3	7.000 f	9		
	4	0.33 g	13		
	5	0.56 g	12		
Three	1	39.887 a	1	10.776 c	3
	2	12.557 e	7		
	3	0.887 g	11		
	4	0.220 g	15		
	5	0.333 g	14		
L.S.D., 0.05		2.413398103		0.202764213	

Three replicates were used for the samples.
The order of the ranks is downward.
Averages in the same column that are followed by the same letter do not differ significantly at $P < 0.05$ level.

DISCUSSION

1. Population densities of the mites in the different months of the season

That study was carried out to find out the months within the season in which the mites are most abundant. The mite numbers were low in June because the population was in the instituting stage. The drop in temperature or lack of prey may be to blame for the low mite counts in November. Data obtained from the meteorological station showed a temperature decrease in November. As mentioned by **Park and Lee** (**2020**), *A. eharai*, which feeds on *Tetranychus urticae* (Koch), develops differently depending on the temperature. The results of the current study match those of **Yu *et al*. (2018)**. At 27 degrees C, *Thrips hawaiiensis* had the highest rate of reproduction.

2. Population densities of the mites in the different sections of the plant

The goal of that investigation was to identify the section of the plant in which the mites are most prevalent. The appropriateness of the light may be responsible for the mite abundance in the central section.

3. Population densities of the mites on the two leaf sides and on different parts of each side

The purpose of that study was to discover the leaf side and the location on the leaf that harbours the mites most densely. The cavities were where the mite individuals prefer to hide out. Mites can find a comfortable home in the concave areas of the leaf surface that are at the pleats.

The leaf bases also do this. Domatia primarily serve as safe havens for some mites against predators, as demonstrated by **Gustavo et al. (2005)**, who stated that domatia increase the number of beneficial mites on plants.

4. Population densities of the mites after spraying two pesticides

After the pesticide application, there may have been enough prey left behind to maintain the mite population at a semi-stable level, so the two pesticides had no significant impact on the mite population density. The pesticides used in the current study may also have less impact on mites since they are mostly insecticides. Also, resistance to the insecticides may have been the reason for the insignificant effect. The resistance brought about by high treatment rates may make the utilized pesticides only marginally harmful to mites (**Pimentel**, **2005**). Due to their negative effects on the environment, insecticides, however, may not be the ideal answer for pest control (**Jensen, 2000; Herron et al., 2007**). In this respect, it's critical to select pesticides that are harmful to pests while being safe for the beneficial arthropods (**Duso et al., 2020**).

5. Population densities of the mites after spraying a cotton defoliant

Just three plants were considered for inspection, but ten leaves were collected from each replicate to get a sample large enough for the study.

Many mites migrated from the earlier-fallen leaves to the most recent ones. It's possible that they were looking for a better substrate and more prey.

6. Gathering the mites on some leaves

The experiment of gathering the mites on some leaves was initiated in a pesticide-treated area, similar to most cotton fields. Henceforth, the newly developed mite-gathering technique could be applied to most cotton fields.

To keep the mites from absconding, the jar rim was treated with vaseline mixed with camphor oil. To prevent contact between the leaves and the inner side of the jar wall, the petioles of all the leaves and the tips of the large leaves were removed before the leaves were stacked in the jars. With the exception of the lower leaf, these precautions were taken to stop the mites from wandering from the leaves to the jar wall. The jars were kept without lids to prevent the buildup of moisture produced by leaf transpiration inside the jars. Unwanted mould may grow in areas where there is moisture resulting from transpiration. To encourage the mites to rise higher, the sides of the jars were made dark by covering them with opaque cloth.

It is possible to concentrate a large number of *A. swirskii* mites on some leaves by using the strategy that was just presented. Afterwards, mite-enriched cotton leaves would be ready to be used in other cultivated fields for pest control.

Nowadays, there are easy and inexpensive ways to rear the predator, *A. swirskii*. However, the mites obtained from the field would be more valuable because they are expected to be resistant to pesticides. The present study proved that the field mite colony is rather resistant to two commonly used pesticides. Hence, those mites obtained from the field could be used in integrated pest control programs. According to a study by **Vafaie et al. (2021)**, the release of *Eretmocerus eremicus* and *A. swirskii* can cut the need for insecticides by 25–78%.

The method used in the current study could lead to the collection of many predatory mite species. If mites are available and the conditions are similar, the same technique can be effectively used in other places. The ability of mites to migrate from one leaf to the one above became clear. That is consistent with **Casey and Parrella's (2005)** assertion that *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) wanders among plants when the plant canopy is closed or plant bonds are present. Additionally, the results agree with the findings of **Lopez et al. (2017)**. They examined the spread of *A. swirskii*. They claimed that *A. swirskii* dispersed about four plants in less than 24 hours.

The rising trend of such mites in the glass jars is consistent with the findings of **Buitenhuis et al. (2010)**. They showed that just 25% of *A. swirskii* mites made an attempt to spread by moving downwards. In light of their research and the current study, it is suggested to discharge the individuals of *A. swirskii*, which are produced commercially, at the lower portions of the targeted plants so that mites can move upwards to the entire plant. The mites were encouraged to spread upwards by stacking several leaves due to the increased connection. That is in line with the findings of **Buitenhuis et al (2010)**. They discovered that the interaction between plants facilitated *A. swirskii* migration between them. Similar to this, **Zemek and Nachman (1999)** claimed that predatory mites in glasshouses are mostly dispersed via movements since the air speed in closed places is too low for efficient distribution.

Additionally, they claimed that the main obstacle to machine discharge was the risk of the arthropods being harmed by machine components during its use. Other operational factors constrain machine usage for mite discharge **(Pezzi et al., 2015)**. Hence, releasing such mites at the plant base would be preferable to using the machines.

The overall number of mites in the jars has decreased daily during the course of the elapsed days. Cannibalism, natural death, or moving to the jar wall are all possible explanations for that. According to **Rasmy et al. (2004)**, females of *A. swirskii* were shown to feed on their species' protonymphs. As for the prey, no living one was found during the final check. They might have been eaten or they might have perished from starvation. According to **Fathipour et al. (2017)**, *A. swirskii* is an effective predator**.**

CONCLUSION

Individuals of *A. swirskii* could be found in large numbers in the pleats of the underside of the leaves existing in the center of cotton plants, especially in September.

The population density of *A. swirskii* is not considerably impacted by neither chlorpyrifos methyl (48%) E. C. nor lambda cyhalothrin (5%) E. C.

When a defoliant is sprayed in a cotton field, that predatory mite is encouraged to congregate on the recently fallen leaves. To harvest a lot of *A. swirskii* mites from cotton fields that are plentiful in them, it is better to gather the recently fallen leaves that are found beneath the defoliated plants. Those leaves can be piled on top of one another to encourage the mites to condense onto the uppermost leaves. Cotton leaves that have been enhanced with mites are then ready to be utilized in other agricultural fields so that the mites can act as a biological control agent.

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الملخص العربى

الكثافات العددية للأكاروس المفترس *Amblyseius swirskii* على نباتات القطن، وتقنية جديدة لتجميع أفراده

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فى حقل قطن غير معامل بمبيدات حشرية، بمصر ، تواجد الأكاروس المفترس*Amblyseius swirskii* بأعلى كثافة عددية فى شهر سبتمبر بينما تواجد بأقل كثافة عددية فى شهر يونيو. وكان بأعلى كثافة عددية على الأوراق المتواجدة بالجزء الأوسط من النبات بينما تواجد بأقل كثافة عددية على الأوراق المتواجدة بالجزء القمى من النبات. وكانت الكثافة العددية للأكاروسات المتواجدة بالأسطح السفلية للأوراق أعلى من الكثافة العددية للأكاروسات المتواجدة بالأسطح العلوية للأوراق. تواجدت أغلبية الأكاروسات عند الثنايا والأجزاء القاعدية بالأسطح السفلية للأوراق. رش المبيدين Chlorpyrifos-methy 48% و lambda-cyhalothrin 5% لم يكن له تأثير معنوى على تعداد الأكاروس.

بعد اسبوع من رش مُسقط أوراق، كان متوسط الكثافة العددية للأكاروسات المتواجدة على أوراق النباتات كما يلي:

1- الأوراق الجافة المتواجدة أسفل النباتات المعامَلة بمسقط الأوراق: 1.43 أكاروس/ ورقة.

2- الأوراق الطازجة المتواجدة أسفل النباتات المعامَلة بمسقط الأوراق: 13.5 أكاروس/ ورقة.

3- الأوراق المتواجدة على النباتات المعامَلة بمسقط الأوراق: 7.37 أكاروس/ ورقة.

4- الأوراق المتواجدة على النباتات الغير معامَلة بمسقط الأوراق: 5.43 أكاروس/ ورقة.

عند تجميع 15 ورقة من الأوراق الخضراء الطازجة المتساقطة أسفل النباتات المعامَلة بكل من المبيدات المذكورة ومسقط الأوراق، ووضع هذه الأوراق مسطحة فوق بعضها البعض فى برطمان مغطى من الخارج بقماش أسود (ما عدا الجهة العلوية) فى معمل مضاء باستمرار لمدة 3 أيام، تجمعت معظم الأكاروسات على الأوراق العلوية، وبلغ متوسط الكثافة العددية للأكاروسات المتواجدة بالأوراق العلوية 39.89 أكاروس/ ورقة نبات.

وبذلك يمكن الاستفادة من الأكاروس المفترس *Amblyseius swirskii* المتواجد بحقول القطن بنهاية موسم النمو، من خلال تجميع الأوراق الطازجة المتساقطة على الأرض بعد رش مسقط الأوراق، ثم تكثيف أعداد الأكاروسات المتواجدة عليها (بطريقة رص هذه الأوراق فوق بعضها) بهدف استخدام الأوراق العلوية المحملة بكثافة بهذه الأكاروسات فى حقول أخرى، وذلك ببرامج المكافحة الحيوية.