Obtaining large numbers of predatory mites, namely Amblyseius swirskii Athias-Henriot (Acari: Phytoseiidae), from cotton fields rich in it, and accumulating them on a few leaves

Mohamed Mahrous Youssef Elshazly (elshazly@alexu.edu.eg)
Faculty of Agriculture, Saba Basha; Alexandria University

Research Article

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Abstract

Preliminary studies on the population density of Amblyseius swirskii Athias-Henriot (Acari: Phytoseiidae) existing in a cotton field revealed that spraying Chlorpyrifos-methyl 48% E. C. and lambda-cyhalothrin 5% E. C. didn't affect the mite population density significantly. As regards the pesticide-untreated area, the maximum population density was recorded in September; meanwhile, the minimum one was recorded in June. The mites were at the highest population density on the leaves of the middle section of the plants; meanwhile, they were at the lowest population density on the leaves of the top section. The mite population found on the lower surfaces of the leaves was significantly more than the mite population found on the upper ones. The majority of the mite individuals existed at the pleats and the bases of the lower surfaces of the leaves. After spraying a defoliant by a week, the population density of the mites existing on the fresh leaves found beneath the defoliant-treated plants were 13.5 mites per leaf, which is considered to be comparatively high. Hence, large numbers of that mite could be obtained from that leaf category. By collecting 15 fresh leaves from the ground located under the plants treated with the pesticides and the defoliant, and stacking them in a jar, in certain conditions, for three days, most individuals congregated on the upper ones. The average population density of the mites found on the upper three leaves was 39.89 mites per leaf. Hence, large numbers of that mite could be accumulated on a few leaves.

Introduction

The predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) is a generalist predator with a high prey consumption capacity (Alipour et al. 2016; Fathipour et al. 2017; Riahi et al. 2017). It has become one of the most successful biocontrol agents in protected agriculture after its introduction into the market in 2005 (Calvo et al. 2015; Buitenhuis et al. 2010). *Amblyseius swirskii* has the potential to control several pest species including whiteflies, *Bemisia tabaci* (Gennadius) (Calvo et al. 2015); western flower thrips, *Frankliniella occidentalis* (Pergande) (Brodsgaard and Stengaard 1992; Messelink et al. 2005); chilli thrips, *Scirtothrips dorsalis* Hood (Young and Zhang 1998; Arthurs et al. 2009; Doğramaci et al. 2011); broad mites *Polyphagotarsonemus* spp. (Stansly and Castillo 2009); and spider mites, *Tetranychus urticae* Koch (Calvo et al. 2015). The study of Vafaie et al. (2021) supported that releasing *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) and *A. swirskii* can reduce insecticide applications by 25–78%. Results of Buitenhuis et al. (2010) demonstrated that *A. swirskii* did not disperse far from the release site, and presence of prey did not influence its dispersal.

Many investigators need large numbers of predatory mites, namely *Amblyseius swirskii*, to practice different tasks such as bioassay experiments. Hereupon, the first aim of the present study is to determine the periods within the growing season, the parts of the cotton leaf, and the parts of the plant that indicate availability of that mite. Moreover, the effects of spraying two pesticides and a defoliant on the population density of the mite were revealed.

Many studies tackled the methods of dispersing the predacious mites, which were previously reared, throughout a field. The commercially produced *A. swirskii* can be released by different methods. Slow-release sachets are small breeding units that are hung on the plant and produce predators over several weeks. Predatory mites in a bran or vermiculite carrier are generally placed as piles on the leaves or the substrate. Alternatively, this loose product can be broadcast, e.g., by air blast (Opit et al. 2005). Pezzi et al. (2015) verified the release quality of *A. swirskii* through evaluating the distribution patterns and the effects on the viability and the fecundity.

The present study tackles the opposite trend, *i.e.*, how to gather the predacious mites existing in a field. Hereupon, the second aim of the present study is to find a method by which the tremendous stock of *A. swirskii* mites existing on the leaves at the end of the growing season could be obtained to benefit from it instead of leaving it to be lost. The present study also dealt with the dispersal of that mite on stacked leaves, in the laboratory, aiming to exploit that phenomenon for gathering the mites found on some leaves on a few ones.

Materials And Methods

A cotton field in Abohommos, Behera, Egypt was utilized during the two successive growing seasons of 2020 and 2021 to conduct the present study; the conventional agricultural process was applied.

Preliminary experiments

Preliminary experiments were done to determine the effect of two treatments on the mite population density. Moreover, different inspections were done to determine the periods within the growing season and the places on the plant that indicate availability of the mite.

1. Spraying two pesticides

In a specific area, chlorpyrifos-methyl 48% E. C. and lambda-cyhalothrin 5% E. C. were sprayed alternately. Each pesticide was sprayed three times, at the recommended doses, with about 20-day intervals. The first spray, with chlorpyrifos-methyl 48% E. C., was on July 1st whereas, the last spray, with lambda-cyhalothrin 5% E. C., was on August 20th. Accordingly, six sprays were applied with 10-day intervals. Three pesticide-treated plots were designated as replicates. Moreover, three pesticide-untreated plots were designated as other replicates. After completing the pesticide application program by five days, 10 leaves were collected randomly from each plot; *A. swirskii* mites existing on each collected leaf were counted and recorded. The mite population density of each plot was calculated.

2. Inspecting the leaves monthly during the growing season

In the pesticide-untreated area, three plots were designated as three replicates. Ten leaves were collected randomly from each plot every month; *A. swirskii* mites existing on each collected leaf were counted and recorded. The mite population density of each plot was calculated monthly. That ancillary investigation was done during the period from June 15 th to November 15 th in the two successive seasons of 2020 and 2021.

3. Inspecting the plant sections

In the pesticide-untreated area, when the plants expanded to a height of about 180 cm., *i.e.*, in September, three plants were designated as three replicates, each of which was considered to be a three-section plant, *i.e.*, the top, the middle, and the base; each section was about 60 cm high. Ten leaves were collected randomly from each section. *Amblyseius swirskii* mites existing on each collected leaf were counted. The mite population density of each section was calculated.

4. Inspecting the leaf parts

In the pesticide-untreated area, 10 leaves existing in the middle part of some plants were designated in September as 10 replicates, each of which was picked separately and inspected individually. As shown in **Figs. 1**, **2** and **3**, each examined leaf had either three lobes with two pleats or five lobes with four pleats. At each pleat, the upper surface of the leaf is usually convex whereas, the lower surface is usually concave. On the lower surface, there were either three or five main prominent veins, which originate at the leaf base. Mites were counted immediately after picking each leaf, considering the following:

4. 1. Different locations on the upper surface

Mites found on the upper surface of each designated leave were counted considering three location categories, *i.e.*, the base, where the veins converge; all the pleats; and the rest of the upper surface. Moreover, the total number of the mites found on the upper surface of each leaf was recorded.

4. 2. Different locations on the lower surface

Mites found on the lower surface of each designated leave were counted considering four location categories, *i.e.*, the base, where the veins converge; all the pleats; the sides of all the main veins; the rest of the lower surface. Moreover, the total number of the mites found on the lower surface of each leaf was recorded.

5. Spraying a cotton defoliant

In the pesticide-untreated area, the cotton defoliant dropp® 50 w. p. was sprayed at the recommended dose on October1st with leaving a plot without defoliant application. Three defoliant-treated plants were designated as replicates. Moreover, three defoliant-untreated plants were designated as other replicates. A week later, an inspection was conducted to estimate the population densities of *A. swirskii* mites existing on the four leaf categories, *i.e.*, the leaves collected from the defoliant-untreated plants, the leaves collected from the defoliant-treated plants, the fresh green leaves found beneath the defoliant-treated plants, and the dry pale leaves found beneath the defoliant-treated plants. Ten leaves were collected randomly from each replicate. *Amblyseius swirskii* mites existing on each collected leaf were counted and. The mite population density of each of the four leaf categories, *i.e.*, the leaf sources, was calculated.

Obtaining large numbers of the mites and accumulating them on a few leaves

In the pesticide-treated area, a plot was designated for that task; three steps were conducted to gather the mites.

1- Spraying the cotton defoliant dropp® 50 w. p. at the recommended dose on October 1st.

2- Collecting the fresh green leaves which are found on the ground under the defoliant-treated plants. That step was on October 8th; collected leaves were transferred immediately to the laboratory.

3- Amassing the mites on a few leaves.

In the laboratory, the tips of the large leaves, and the petioles of all the leaves were cut out. Fifteen straight leaves were stacked on top of each other in a glass jar, with replicating the jars nine times. There was no contact between the edges of the leaves and the inner side of the jar wall. The rim of each jar was previously coated with a mixture of vaseline and camphor oil, as shown in **Fig 4**.

Black cloth was used to cover the outer sides of the jars. The jars were kept open in a laboratory, which was lit up constantly. A day later, the leaves of three replicates were inspected to count the mite individuals found on each leaf. Every day, other three replicates were subjected to the same inspection. Thereupon, the nine jars were inspected within three days. The 15 leaves in each jar were considered to consist of five groups on top of each other, each of which consists of three leaves. Thereupon, the five groups were symbolized with the numbers from one, which represents the upper group, to five, which represents the bottom group. *Amblyseius swirskii* individuals were counted considering the taxonomic identification. The mite population density of each group was calculated. The experiment of amassing the mites in the laboratory was conducted at a temperature of 30° C ± 5, and a relative humidity of 70% ± 5.

In all the experiments, the leaves were inspected using a stereo microscope. Each counted mite was picked and removed immediately to avoid counting it again.

Statistical analysis

The data were analyzed using ANOVA and the "F" Test. The least significant differences (L. S. D.) at the 0.05 ≤ level were determined according to the computer program COSTAT software.

Results

The effect of spraying the two pesticides

As shown in Table 1, the average number of the mites found on the pesticide-treated leaves and the average number of the mites found the pesticide-untreated leaves were 4.57 and 5.6 mites per leaf, respectively. Thereupon, chlorpyrifos-methyl 48% E. C. and lambda-cyhalothrin 5% E. C didn't affect the population density of *A. swirskii* significantly.

Table 1 Average numbers of the mites, per leaf, in the plots treated with chlorpyrifos-methyl 48% E. C. and lambda-cyhalothrin 5% E. C., and in the pesticide-untreated plots

Plots	Average number of the mites, per leaf
Pesticide-treated	4.57 a
Pesticide-untreated	5.6 a
L.S.D., 0.05	1.54862709026

Samples were based on three replicates.

Averages followed by the same letter are not significantly different at $P < 0.05$ level.

The period within the growing season that indicates availability of the mite

As shown in **Table 2**, the mite maximum population density, was recorded in September whereas, the minimum one was recorded in June.

Table 2 Average numbers of the mites, per leaf, in the months of the two seasons

Months	First season		Second season	
	Average number of the mites, per leaf	Rank	Average number of the mites, per leaf	Rank
June	0.83 d	6	1 d	6
July	1.7 c	5	1.87 cd	5
August	5.43 ab	2	5.97 a	2
September	5.83 a	1	6.13 a	1
October	5.3 b	3	4.87 b	3
November	1.83 c	4	2.27 c	4
L.S.D., 0.05	0.47254144205		0.90614246	

Samples were based on three replicates.

Ranks are arranged in descending order.

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

The plant section that indicates availability of the mite

As shown in **Table 3**, the mites were at the highest population density, 9.97 mites per leaf, on the leaves of the middle section of the plants whereas, they were at the lowest population density, 1.63 mites per leaf, on the leaves of the top section.

Table 3 Average numbers of the mites, per leaf, in the three sections

Plant section	Average number of the mites, per leaf	Rank
The top	1.63 c	3
The middle	9.97 a	1
The base	6.97 b	2
L.S.D., 0.05	1.31010746916	

Samples were based on three replicates.

Ranks are arranged in descending order.

Averages followed by the same letter are not significantly different at $P < 0.05$ level.

The leaf part and the leaf surface that indicate availability of the mite

As shown in **Table 4**, by inspecting 10 leaves as replicates, the average number of the mites found on the pleats of the lower surfaces was five mites per leaf. In other words, the total number of the mites found at all the pleats of the lower surfaces of ten leaves was 50 mites. In contrast, it is more than the average numbers of the mites found on the other locations on the leaves. Moreover, the average number of the mites found on the bases of the lower surfaces was three mites per leaf, which is considered to be comparatively high. Thereupon, the majority of the mite individuals existed at each of the pleats and the bases of the lower surfaces of the leaves. Furthermore, the average number of the mites found on the lower surfaces was 9.2 mites. In other words, the total number of the mites found at all the locations of the lower surfaces of ten leaves was 92 mites. In contrast, the average number of the mites found on the upper surfaces was 2 mites. Thereupon, the mite population found on the lower leaf surfaces was significantly more than the mite population found on the upper ones.

Table 4 Average numbers of the mites, per leaf, found on the seven locations and on the two leaf surfaces

Leaf surface	Location on the surface	Parameters of the mites found in each location		Parameters of the mites found on each surface	
		Average number of the mites found at the location	Rank	Average number of the mites found on the surface	Rank
Upper	Base	1.8 c	3	2.4 b	2
	Pleats	0.3 d	5		
	Rest of the leaf surface	0.3 d	5		
Lower	Base	3 b	2	9.2 a	1
	Pleats	5 a	1		
	Sides of the main veins	0.6 d	4		
	Rest of the leaf surface	0.6 d	4		
L.S.D., 0.05		1.10663088245		2.14710166763	

Samples were based on ten replicates.

Ranks are arranged in descending order.

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

The effect of spraying a cotton defoliant

Despite spraying the defoliant, some leaves existing on the lower parts of the stems remained without falling for several days.

As shown in **Table 5**, after spraying the defoliant by a week, the average number of the mites, per leaf, found on each of the four leaf categories, *i.e.*, the leaves collected from the defoliant-untreated plants, the leaves collected from the defoliant-treated plants, the fresh green leaves found beneath the defoliant-treated plants, and the dry pale leaves found beneath the defoliant-treated plants were 5.43, 7.37, 13.5 and 1.43 mites per leaf, respectively with a ratio of 2.1: 5.15: 9.44: 1, respectively. Thereupon, the population density of the mites found on the fresh green leaves located beneath the defoliant-treated plants were the highest. In other words, the majority of the mites existed on those leaves.

Table 5 Average numbers of the mites, per leaf, found on the four leaf categories

Category of the inspected leaves	Average number of the mites, per leaf	Rank	Ratio
Leaves collected from the defoliant-untreated plants	5.43 c	3	2.1
Leaves collected from the defoliant-treated plants	7.37 b	2	5.15
Fresh green leaves found beneath the defoliant-treated plants	13.5 a	1	9.44
Dry pale leaves found beneath the defoliant-treated plants	1.43 d	4	1
L.S.D., 0.05	1.86154198314		

Samples were based on three replicates.

Ranks are arranged in descending order.

Averages followed by the same letter are not significantly different at $P < 0.05$ level.

Obtaining large numbers of the mites and gathering them on a few leaves

Large numbers of the mites congregated on the upper leaves. As shown in **Table 6**, the average number of the mites found on the upper three leaves, group one, after stacking the leaves in the jars by three days, was 39.89 mites per leaf. As far as the passed days are concerned, the average number of the mites, per leaf descended daily. As regards the preys, no living individual was detected in the final inspection.

Table 6 Average numbers of the mites, per leaf, in each of the five groups and on each of the three days

Number of the passed days	Group	Parameters of the mites found in each group		Parameters of the mites found on each day	
		Average number of the mites, per leaf	Rank	Average number of the mites, per leaf	Rank
One	1	16.89 d	4	12.35 a	1
	2	20.22 c	3		
	3	14.11 e	6		
	4	8.22 f	8		
	5	2.34 g	10		
Two	1	36.78 b	2	11.78 b	2
	2	14.22 e	5		
	3	7 f	9		
	4	0.33 g	13		
	5	0.56 g	12		
Three	1	39.89 a	1	10.78 c	3
	2	12.56 e	7		
	3	0.89 g	11		
	4	0.22 g	15		
	5	0.33 g	14		
L.S.D., 0.05		2.4133981027		0.2027642134	

The experiment is based on three replicates

Ranks are arranged in descending order.

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

Discussion

The effect of spraying certain pesticides

The used pesticides may have little lethal effect on the studied mites due to the introduced resistance resulted from high application rates according to Pimentel (2005). The numbers of the remaining preys, after the pesticide application, may be sufficient to keep the mite population at a semistable density. However, the use of insecticides may not be the best solution owing to its adverse effects on natural enemies and environment (Herron et al. 2007, Jensen 2000). In this regard, it is important to choose selective pesticides that are non-toxic to natural enemies, and at the same time are detrimental to the pests (Duso et al. 2020). Also, these pesticides are mainly insecticides and may have less effect on mites.

The period within the growing season that indicates availability of the mite.

To get the maximum obtainable mite individuals, a preliminary experiment was done to determine the period of the season, when the mites exist in the largest numbers. Few mite numbers in June and July may be attributed to the fact that the population is in the establishing phase. Few mite numbers in November may be attributed to the temperature decrease or prey scarcity. Park and Lee (2020) studied the temperature-dependent development of *A. eharai* preying on *Tetranychus urticae* (Koch) (Acari: Tetranychidae). The intrinsic rate of increase was the highest (0.2619) at 27.4 °C and the lowest (0.0792) at 18.0 °C. Environmental temperature has a significant impact on insect behavior. Both the highest intrinsic rate of increase, at 0.200, and net reproduction rate, at 44.97, for *Thrips hawaiiensis* were observed at 27 degrees C whereas, the lowest values of 0.114 and 25.56, respectively were observed at 18 degrees C (Yu et al. 2018).

The plant section that indicates availability of the mite

That preliminary experiment was done to determine the part of the plant, where the mites exist in the largest numbers. The mite abundance in the middle part may be attributed to the suitability of light and relative humidity.

The leaf part and the leaf surface that indicate availability of the mite

This preliminary experiment was done to determine the surface of the leaf and the part of the leaf, where the mites exist in the largest numbers. Apparently, the mite individuals prefer the cavities of these sites as a shelter. It is obvious that the concave parts of leaf surface which are at the pleats, represent a good shelter for the mites. And so do the leaf bases. Gustavo et al. (2005) proved that domatia function primarily as refuges for beneficial mites against predators, and presence of domatia therefore results in more beneficial mites on leaves.

The effect of spraying a cotton defoliant

It is obvious that large numbers of the mites which exist on the previously falling leaves make for the green recently falling leaves in search of a better substrate and prey abundance. likewise, more studies have to be done to investigate the effect of spraying a defoliant on other plants at the end of the growing season. Thence, accumulating predatory mites could be obtained to benefit from.

Obtaining large numbers of the mites and gathering them on a few leaves

The experiment dedicated for that purpose was conducted in the pesticide-treated area, which matches most cotton fields. Hence, the described new method could be applied in most cotton fields. The rim of each jar was coated with a mixture of vaseline and camphor oil to prevent the mites from escaping. Before stacking the leaves in the jars, the angle-shaped tips of the lobes of the large leaves, and the petioles of all the leaves were cut out to avoid the contact between the leaves and the inner side of the jar wall.These measures were taken to prevent the mites from moving from the leaves, except the lower one, to the jar wall. The jars were kept open so that moisture resulted from the leaf transpiration couldn't accumulate inside the jars; transpiration moisture may cause undesirable mold. The sides of the jars were covered with black cloth to make them dark aiming to urge the mites upwards.

By following that technique, it is possible to condense large numbers of *A. swirskii* mites on a few leaves for different purposes. It is worth mentioning that the mite population density in the defoliant-untreated plants in October 2021was just 4.87 mites per leaf. In contrast, by following that technique in October 2021, the mite population density was 39.89 mites per leaf. As regards the preys, no living individual was detected in the final inspection; they may have been consumed or may have died of hunger.

It's obvious that that mites can move from one leaf to another. That matches Casey and Parrella (2005) who said that when the plant canopy closes or inter-plant bridges are present, more *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) move from plant to plant. That also matches Lopez et al. (2017) who evaluated the dispersal of *A. swirskii*, using the ornamental pepper as a banker plant. Within 24 h., *A. swirskii* dispersed four plants away from the banker plants. The upward track of the mites in the jars matches that shown in the results of Buitenhuis *et al*. (2010), which demonstrated that only a quarter of the *A. swirskii* eventually attempted to disperse by going down to the ground. With regard to their study and the present study, it's advisable to release *A. swirskii* individuals which are brought numerously in commercial bottles, at the lower parts of the target plant so that the mites can spread upright to the whole plant.

Increasing the leave contact via stacking, encouraged the mites to disperse upwards. That matches the results of Buitenhuis *et al*. (2010) which revealed that the presence of inter-plant contact greatly improved movement of *A. swirskii* between plants. Likewise, Zemek and Nachman (1999) said that in greenhouses, the primary means of dispersal for predatory mites is by ambulatory movement because wind speed in the greenhouse is often too low for effective aerial dispersal. They also said that the main limitation to mechanical release is that the beneficial organisms may be damaged by the machine parts during their handling and distribution due to possible contact with mechanical elements and abrasion against carrier materials. Also, other technical and operational conditions restrict mechanical intervention (Pezzi et al. 2015). Hence, the upward mite movement revealed in the present study recommends releasing the mites at the plant base instead of using dispersing machines.

The daily reduction in the total mite numbers found in the jars may be attributed to cannibalism, natural death or escaping to the jar wall. The laboratory study of Rasmy et al. (2004) revealed that *A. swirskii* females fed on protonymphs of their own species.

Finally, that method may result in gathering different species of predacious mites; it is contingent upon the diversity in the field. The same pattern can be repeated successfully elsewhere provided that the area is rich in *A. swirskii*, and distinguished with similar circumstances.

Conclusion

Chlorpyrifos-methyl 48% E. C. and lambda-cyhalothrin 5% E. C. don't affect the population density of *A. swirskii* significantly. In cotton fields which are rich in *A. swirskii* individuals, those mites are absolutely abundant at the pleats of the lower surfaces of the leaves located in the middle part of cotton plants in September. Spraying a defoliant in a cotton field urges the mites to accumulation on the green fresh leaves found on the ground underneath the plants. To obtain large numbers of *A. swirskii* mites from cotton fields which are rich in that predatory mite, it is advisable to collect the green leaves found underneath the defoliant-treated plants. To condense the mite individuals on a few leaves, the leaves can be stacked on top of each other to urge the mites to congregate on the upper leaves. After applying the above described gathering process, mite-enriched cotton leaves would be ready to be transferred to other cultivated fields to benefit from the mites as a biological control agent.

Declarations

Contributions

Elshazly M.M.Y. did all the duties, e.g., carried the experiments out, analyzed the data, wrote the manuscript, and discussed the results.

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Figures

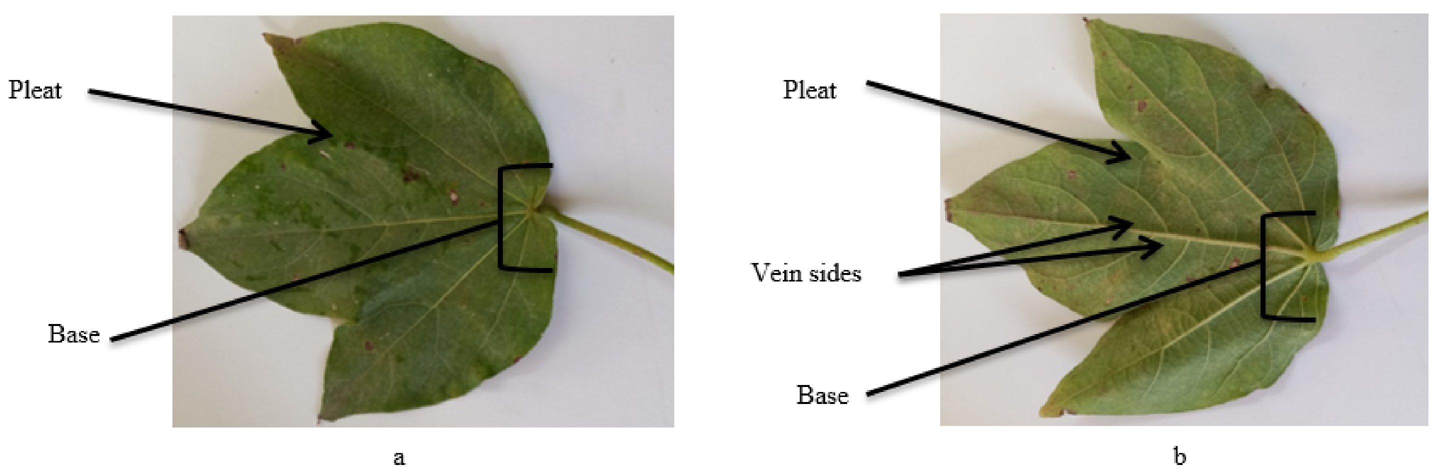

Figure 1

Three-lobed cotton leaf. Upper surface a lower surface b

a

b

Figure 2

Five-lobed cotton leaf. Upper surface a lower surface b

Figure 3

Part of the upper surface, with a pleat in the middle

Figure 4

Top view of a jar before covering the sides