A New Method to Prevent the Individuals of the Predatory Mite, *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae), from Exiting the Area Allocated to them in the Laboratory

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ABSTRACT: Forty-eight methods were tried out to evaluate the efficiency of each regarding preventing the individuals *of Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) from exiting the area allocated to them in the laboratory.
The most efficient method was based on surrounding that area with a 5-millimeter-high plastic wall and laying a thin cotton thread on the outer bevel of the wall top, besides saturating the thread continuously with phosphoric acid 3%. There was a groove around the wall; meanwhile, there was a narrow space between the wall and the groove. The groove was filled with phosphoric acid 3%. One of the thread tips was immersed in the diluted acid located in the groove to ensure continuous saturation. Over 24 hrs., no mite individual could pass across the wall, but 0.33% of the individuals stuck to the thread. In contrast, by saturating the thread with water, 4.33% of the mite individuals could pass across the wall, and 2% of the mite individuals stuck to the thread.
The least efficient method was based on surrounding the area allocated to the mites with a groove and filling it with citric acid 25%. Over 24 hrs., no mite individual could pass across the groove, but 7% of the mite individuals drowned in the surrounding acid. In contrast, when filling the groove with water, no mite individual could pass across the groove, but 4% of the individuals drowned in the surrounding water.

Keywords: *Amblyseius swirskii*, pass, barrier, thread, phosphoric acid, stick

INTRODUCTION

The generalist predator mite, *A. swirskii*, has a high prey consumption capacity (**Fathipour *et al.*, 2017**). Several studies were conducted in the laboratory to develop its usage. **Vázquez-Benito *et al.* (2022)** studied the combined application of it and fungal pathogens for biological control of mite pests under laboratory conditions.

Crucial measures have to be taken to avoid the mite escaping while rearing it. Closed containers may be necessary, especially while dealing with flying insects. To observe the mites placed on bean leaflets with abundant prey, **Nguyen and Amano (2009)** placed each leaflet on a strip of filter paper; each filter strip was inserted into a small transparent plastic vial. The top of the vial was covered with a polyvinyl sheet and fastened shut with a rubber band to prevent the mites from escaping. **Messelink *et al.* (2011)** tested the effect of the predatory mite *A. swirskii* on aphid populations. For this, leaf discs were embedded in water agar, each in a separate plastic box. The mites and the insects were placed on the leaf discs. **Wu *et al.* (2018)** used *Tetranychus urticae* Koch mites to feed predatory mites on excised kidney bean leaves placed on black plastic mulch in a covered plastic container. A hole was cut in the lid and covered with fine mesh to provide ventilation. **Park and Lee (2020)** reared mite individuals in Petri dishes with a nylon-mesh-covered hole in the lid.

For better detection, open containers such as Petri dishes may be preferable to closed ones, but appropriate measures must be taken to prevent the mites from escaping. **Baier and Moll (2002)** conducted a bioassay using bean leaf discs that were surrounded by a glue barrier to prevent mite escape. **Bostanian *et al.* (2009)** modified a method based on leaf discs that fit tightly into the bottom halves of Petri dishes. The top half of each Petri dish had a window. For phytophagous mites, the window was covered with a Pecap polyester screen. That method reduced losses due to escapees and allowed observations to be made. **Nguyen *et al.* (2015)** reared phytoseiid mites on green plastic arenas placed on a wet sponge in a plastic tray containing water. The edges of the arenas were covered with tissue paper immersed in the water to provide moisture and deter the mites from escaping. However, some mites were able to escape. **Kakkar *et al.* (2016)** created an experimental arena consisting of a Petri dish lined on the bottom with a thin layer of moist cotton wool. A cucumber leaf disc was placed in the center of the Petri dish onto which a mite individual and larvae of thrips were released. **Gyuris *et al.* (2017)** placed mites in plastic cups on bean leaf discs on wet cotton to prevent them from leaving. **Fathipour *et al.* (2019)** covered the margins of Petri dishes with paraffin layers to prevent mites from escaping. **Schausberger *et al.* (2020)** reared *A. swirskii* on artificial arenas. Each arena consisted of an acrylic resting on a water-saturated foam cube in a plastic box half-filled with tap water. The edges of the tile were surrounded with moist tissue paper to prevent the mites from escaping.

A cost-effective method for mass-rearing of phytoseiid predatory mites is an essential prerequisite **(van Lenteren, 2003)**.

While rearing predaceous mites in laboratories, many individuals get lost due to escaping or sticking to the surrounding barrier. On the other hand, mite dispersion in the laboratory is considered undesirable. The present study has presented a solution to that problem. By applying the new method, it would be more prosperous to rear the predatory mite, *A. swirskii*, for study or for commercial purposes. Most studies depend on using water as an escape inhibitor. The present study dealt with different kinds of liquids to determine the most effective one as an escape inhibitor.

MATERIALS AND METHODS

Source of mites

Individuals of the predatory mite *A. swirskii* were obtained from cotton leaves. They were transferred gently from the leaves by using a thin brush with a little sodden hair. By using a water-saturated brush, mite pickup is easy.

Preventing the mites from escaping

A hermetic barrier has to be set up to prevent the mites from escaping. For this, four main methods were carried out, three of which involved placing a liquid in a groove located along the margin of a 13-cm-diameter round plastic dish; the middle part of the dish was allocated to the mites. Meanwhile, the fourth method involved placing a liquid in a Petri dish into which a table-shaped unit was put; the table board was allocated to the mites.

1. Placing a liquid in a groove located along the dish margin

Round plastic dishes (13 cm in diameter) were utilized to carry out three of the main methods. Each dish had a groove along its margin. The grooves were allocated to the liquids.

The three main methods were distinguished as follows:

1. 1. Without laying a thread around the area allocated to **the mites**

As shown in **Fig. 1**, no thread was laid on the dish surface.

1. 2. Besides laying a thread around the area allocated to the mites

As shown in **Fig. 2**, a 0.2-mm-diameter cotton thread was laid around the area allocated to the mites. One of the thread tips was immersed in the liquid located in the groove to ensure continuous saturation. There was a narrow space between the thread and the surrounding groove.

1. 3. Besides erecting a wall around the area allocated to the mites and laying a thread on the wall top

As shown in **Fig. 3**, a 5-mm-high wall was set up around the area allocated to the mites. The wall worked as a fence. The wall was erected by using a hot melt glue stick and an electric gun. There was a narrow space between the wall and the surrounding groove. A 0.2-mm-diameter cotton thread was laid on the outer bevel of the wall top. One of the thread tips was immersed in the liquid located in the groove to ensure continuous saturation. A cross section of the thread located on the wall top is illustrated in **Fig. 4**.

2. Placing a liquid in a Petri dish into which a table-shaped unit is put

As shown in **Fig. 5**, a table-shaped unit was made. The table board was represented by a foam cuboid. It was 3 cm long, 1 cm wide, and 1 cm high. The table legs were represented with wooden parts of matches. Each leg was 3 cm tall. A small part of each leg was inserted into the foam for fastening. The unit was placed in a Petri dish in which a liquid was put. The liquid was 1 cm high.

Liquids used, and the total number of ancillary methods tried out

As regards each of the four above mentioned methods, four main liquids were utilized with the aim of estimating their comparative efficiency as escape inhibitors. Two of the liquids were used at different concentrations. Hence, a total of 12 liquids were utilized for each of the four main methods. The 12 liquids were water; camphor oil; phosphoric acid at the concentrations of 1, 2, 3, 4, and 5%; and citric acid at the concentrations of 5, 10, 15, 20, and 25%. Thus, a total of 48 ancillary methods were tried out.

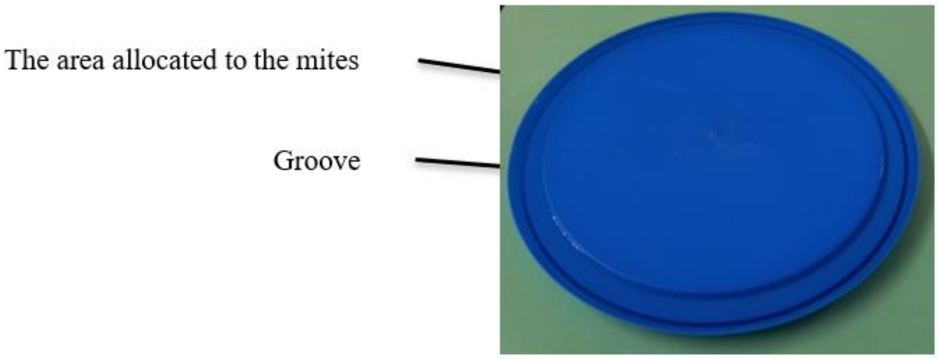

Fig. 1 A plastic dish without a thread around the area allocated to **the mites**

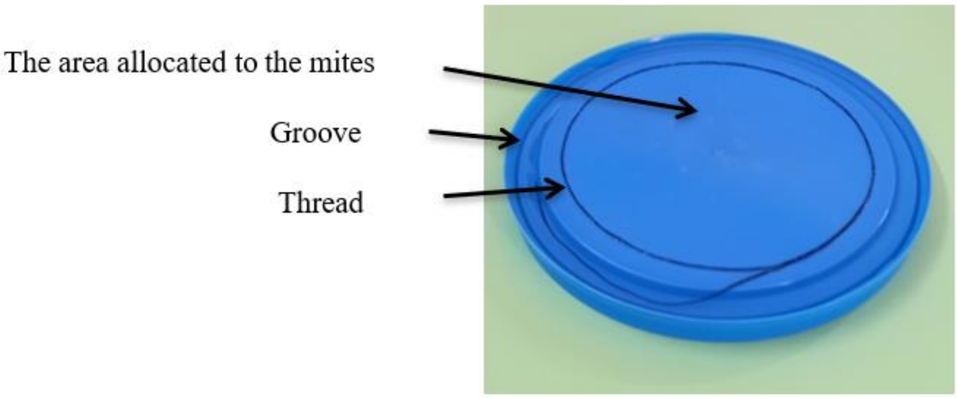

Fig. 2 A plastic dish with a thread around the area allocated to the mites

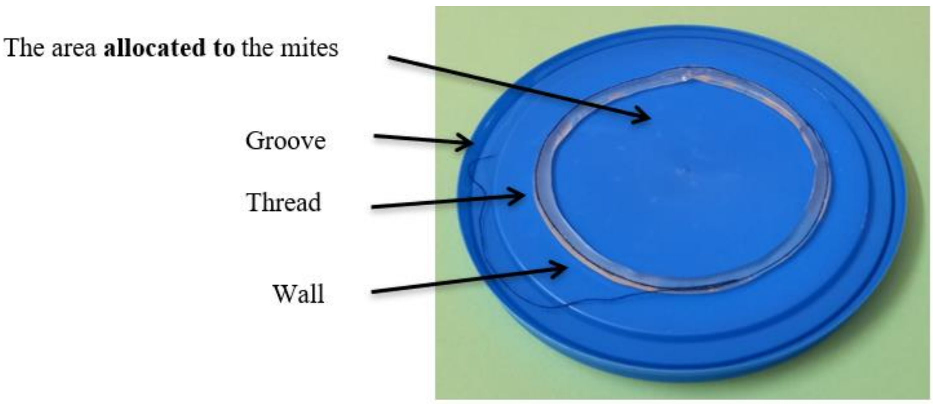

Fig. 3 A plastic dish with a thread on the top of the wall erected around the area allocated to the mites

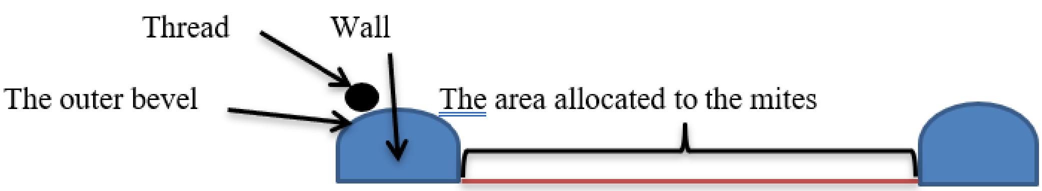

Fig. 4 An illustration of a cross section showing the location of the thread on the wall

The cuboid allocated to the mites

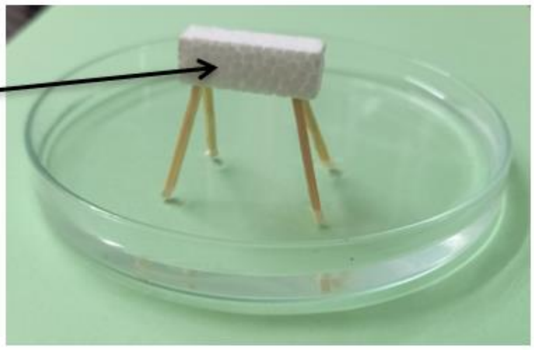

Fig. 5 A Petri dish, in which a table-shaped unit is put

Replicates

Three replicates were used for each of the 48 ancillary methods.

Placing the mites and detecting them

After taking the measurers of escape prohibition, 100 mite individuals were used for each replicate. As regards the methods involving placing a liquid in a groove, mites were placed in the middle of the dish. As regards the methods involving placing a liquid in a Petri dish, mites were placed on the cuboid representing the table board.

All experiments were conducted at a temperature of 30 ± 5° C, a relative humidity of 70 ± 5% and a 16:8 hrs. (L: D) photoperiod.

Inspections were done in 24 hours. The mite individuals that managed to pass across the thread were counted; the average percentages of them were calculated. The mite individuals that drowned in the liquids, or stuck to the thread were counted; the average percentages of them were calculated.

Statistical analysis

Data were analyzed using ANOVA and ″F″ Test, with 3 replicates for each treatment. The least significant differences (L.S.D.) at the 0.05 ≤ level were determined according to the computer program CoStat software and Duncan's Multiple Range.

RESULTS

Average percentages of the mites which managed to pass across the barrier

No mite individual could pass across the liquids located in the grooves or in Petri dishes. As regarding using a liquid-saturated thread to prevent mite escaping, as shown in **Table (1)**, no mite individual could pass across the thread saturated with any of the following liquids: camphor oil; phosphoric acid at the concentrations of 3, 4 and 5%; or citric acid at the concentrations of 15, 20 and 25%. Some individuals were able to pass across the thread saturated with the other liquids, but the liquid existing in the surrounding groove prevented the mites from escaping and being lost. The highest average percentage of the mites which managed to pass across the thread was 4.33%; it was attained by applying the wall-based method, using water as an escape inhibitor.

Table 1 Average percentages of mite individuals which managed to pass across the liquid-saturated thread

Liquids		Parameters of the mites which managed to pass across the liquid-saturated thread					
		With laying a thread around the area allocated to the mites			With erecting a wall around the area allocated to the mites, and laying a thread on the wall top		
		Average percentage		Rank	Average percentage		Rank
water		4	ab	2	4.33	a	1
Camphor oil		0	d	11	0	d	19
Phosphoric acid	1%	3.67	ab	3	3.33	b	4
	2%	0.67	cd	10	1	c	7
	3%	0	d	20	0	d	14
	4%	0	d	24	0	d	23
	5%	0	d	22	0	d	21
Citric acid	5%	1	c	6	0.67	cd	9
	10%	0.67	cd	8	1	c	5
	15%	0	d	12	0	d	18
	20%	0	d	15	0	d	16
	25%	0	d	17	0	d	13
L.S.D., 0.05		0.6702					

The experiment was based on three replicates.
Averages followed by the same letter are not significantly different at $P < 0.05$ level.
Ranks are arranged in descending order.

Average percentages of the liquid-adhered mites

As shown in **Table (2)**, some mite individuals adhered to the liquids. In other words, some individuals drowned in the liquids; others stuck to the liquid-saturated thread. Whatever liquid was used, the wall-based method indicated the lowest average percentage of the liquid-adhered mites. As far as the 48 methods are concerned, the lowest average percentage of the liquid-adhered mites, 0.33%, was attained by applying the wall-based method, besides saturating the thread with phosphoric acid 3%. On the contrary, the highest average percentage of the liquid-adhered mites, 7%, was attained with applying the method involving surrounding the area allocated to the mites with a groove and filling it with citric acid 25%.

Table 2 Average percentages of the liquid-adhered mites

liquids		Main methods: Placing a liquid in the groove located along the dish margin — Without laying a thread around the area allocated to the mites — Parameters of the individuals which drowned in the liquid: Average percentage		Rank	With laying a thread around the area allocated to the mites — Parameters of the individuals which stuck to the thread: Average percentage		Rank	With erecting a wall around the area allocated to the mites, and laying a thread on the wall top — Parameters of the individuals which stuck to the thread: Average percentage		Rank	Placing a liquid in a Petri dish in which a table-shaped unit is put — Parameters of the individuals which drowned in the liquid: Average percentage		Rank
Water		4	efg	11	2.67	hijk	26	2	jklm	34	3.33	ghi	16
Camphor oil		2.33	ijkl	29	1.67	klmn	37	1	mno	42	2	jklm	33
Phosphoric acid	1%	3.67	fgh	13	2.67	hijk	25	1.67	klmn	38	3.67	fgh	12
	2%	3.33	ghi	17	2.67	hijk	27	1.33	lmno	40	3	ghij	19
	3%	1	mno	43	0.67	no	47	0.33	o	48	0.67	no	46
	4%	1.67	klmn	35	1.33	lmno	41	0. 67	no	45	1.33	lmno	39
	5%	2.67	hijk	24	2.33	ijkl	28	2	jklm	32	2.33	ijkl	30
Citric acid	5%	2.67	hijk	23	2	jklm	31	0.67	no	44	3	ghij	18
	10%	3.67	fgh	14	2.67	hijk	21	1.67	klmn	36	2.67	hijk	22
	15%	4.67	def	6	4	efg	9	3	ghij	20	4	efg	8
	20%	6.33	ab	2	5	cde	5	4	efg	10	4.67	def	7
	25%	7	a	1	6	abc	3	3.33	ghi	15	5.33	bcd	4
L.S.D., 0.05		1.1618											

The experiment was based on three replicates.
Averages followed by the same letter are not significantly different at P < 0.05 level.
Ranks are arranged in descending order.

DISCUSSION

As for the efficacy of the evaluated methods regarding preventing the mite individuals from escaping, they were all effective, but the average percentage of the liquid-adhered mites that resulted from each method determined the method efficiency. The lowest average percentage of the liquid-adhered mites, 0.33%, was attained with applying the method based on surrounding the area allocated to the mites with a short wall and laying a thin cotton thread on the outer bevel of the wall top, besides saturating the thread continuously with phosphoric acid 3%. Thereupon, it was considered to be a new efficient method in this regard. That comparatively low average percentage may be attributed to the following:

1- Surrounding the area allocated to the mites with a liquid-saturated thread instead of a liquid-filled groove. The thread may have enabled the mites to extricate themselves, *i.e.*, avoid drowning after touching the liquid. In this respect, a thin thread was preferred to a broad one to decrease the spread of the liquid around the thread. Hence, the number of mites that are prone to sticking to the thread could be reduced.

2- Laying the thread on the outer bevel of the wall top instead of laying it directly on the dish surface. In this regard, positioning the thread on the outer bevel may have caused the excess liquid to accumulate on the thread's outer side, keeping it away from the inner side that faces the mites. Hence, the number of mites that are prone to sticking to the thread could be reduced.

3- The chemical and physical properties of phosphoric acid 3%, which may have made it a good escape inhibitor compared with the other utilized liquids. Likewise, Rambla *et al*. (2021) found that tomato trichomes are deadly hurdles limiting the establishment of *A. swirskii* Athias-Henriot. The trichomes and their exudates negatively impacted mite dispersal on the plant.

As regards the method based on placing the mites on a table-shaped unit which is put in a Petri dish, the purpose of creating that method was to reduce the liquid surface to which mites are exposed, aiming to reduce the percentage of the mites which drown in the surrounding liquid. However, the comparatively high average percentage of the liquid-attached mites may be attributed to the vertical orientation of the mites when touching the liquid. The vertical orientation may have led to extra drowning in the liquid.

The main methods mentioned above could be tried out in the future to reveal the efficacy of other kinds of liquids as mite-escape inhibitors. Moreover, they could be tried out to evaluate the efficiency of each in preventing other mite species from escaping.

CONCLUSION

To prevent the individuals of the predaceous mite, *A. swirskii*, from exiting the area allocated to them in the laboratory, it is advisable to surround that area with a short wall and lay a thin cotton thread on the outer bevel of it, besides saturating the thread with phosphoric acid 3%. In addition, it is advisable to set up a groove around the wall, leaving a narrow space between the wall and the groove and filling it with the same liquid. One of the thread tips has to be immersed in the liquid found in the groove to ensure continuous saturation. However, a few individuals can pass across the thread, but the liquid found in the groove would prevent them from escaping; hence, they could be returned to the area allocated to them.

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الملخص العربى

طريقة جديدة لمنع أفراد الأكاروس المفترس *Amblyseius swirskii* من الخروج من المنطقة المحددة لهم بالمعمل

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تم تجربة 48 طريقة لتقدير كفاءة كل منها في منع هروب أفراد الأكاروس المفترس *Amblyseius swirskii* من المنطقة المحددة لهم بالمعمل.

كانت الطريقة الأعلى كفاءة هي المبنية على أساس إحاطة هذه المنطقة بجدار بلاستك ارتفاعه خمسة مليمتر، ووضع خيط قطن رفيع على الحافة المائلة الخارجية المتواجدة على قمة الجدار، مع تشبيع الخيط باستمرار بحمض فوسفوريك 3% وذلك بغمر أحد طرفي الخيط فى حمض فوسفوريك 3% متواجد بخندق محيط بالجدار. حيث توجد مسافة قصيرة بين الجدار والخندق المحيط به. على مدى 24 ساعة لم يستطع أى أكاروس عبور الجدار، لكن 0,33% من الأكاروسات التصقوا بالخيط. أما فى حالة تشبيع الخيط بالماء، إستطاع 4,33% من الأكاروسات عبور الخيط، كما أن 2% منهم التصقوا به. مع إمكانية إستعادة الأكاروسات التى عبرت الخيط والتى تواجدت فى المسافة المحصورة بين الجدار والخندق.

كانت الطريقة الأقل كفاءة هي المبنية على أساس إحاطة المنطقة المخصصة للأكاروسات بخندق، وملئه بحمض خليك 25%. على مدى 24 ساعة لم يستطع أى أكاروس عبور الخندق لكن 7% من الأكاروسات غرقوا بالحمض الموجود به. أما فى حالة ملء الخندق بالماء، لم يستطع أى أكاروس عبور الخندق لكن 4% من الأكاروسات غرقوا بالماء الموجود به. تطبيق الطريقة الجديدة (الأعلى كفاءة) يجعل تربية الأكاروس المفترس *Amblyseius swirskii* بالمعمل سواء للدراسة أو لأغراض تجارية، أكثر نجاحاً.