Comparison of Siriraj Chamber and Other Apparatus for Restraining House Dust Mites

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Abstract

The aim of this study was to introduce a newly developed device for restraining house dust mites, called "the Siriraj chamber" and compare this new device with five different chamber types in terms of their ability to restrain mites, recovery techniques and other functions. Six different chambers were used in this study; model 1: a well designed by Kalpaklioglu; model 2: a 24-well tissue culture plate; model 3: a 15 cm diameter petri dish sealed with grease; model 4: a 15 cm diameter petri dish covered with filter paper; model 5: a mite cage; and model 6: a Siriraj chamber. Twenty adult mites (*Dermatophagoides pteronyssinus*) were placed in the test set up. The mites were observed and counted every day for 1 week. There was a significant difference between the chambers in their ability to restrain mites (P-value < 0.001). Models 1 and 3 had the lowest restraining ability as a result of an insufficient lock. With model 2, all the mites died due to a lack of ventilation. Model 4 could restrain the mites, but the large area made it unsuitable for observing them. Model 4 was unable to restrain mites throughout the experiment. Model 5 had the drawback of a long preparation time. The Siriraj chamber was superior to the other chambers. It was simple to use and the study area could be adjusted to the number of mites used. It can be used not only to study the biological aspects of dust mites in laboratory conditions, such as the assessment of anti-mite agents, and the protective ability of impermeable covers, but also for other studies such as scanning electron microscopy and establishing a pure local mite culture from house dust. The Siriraj chamber is recommended as a standard chamber for house dust mite studies.

Keywords: mite apparatus, acaricidal test, anti-mite agents, mite cage, mite trap

Introduction

The discovery of an effective anti-mite agent is important in controlling house dust mites, since a reduction in the mite population results in reduced allergen production. Dust mites can produce up to 200 times their own weight of feces in their life time [1]. They are on average only 300 µm in length, are whitish in color and fast moving [2]. These characteristics make it difficult to observe dust mites with the naked eye resulting in the chance of mites being missed during a study. Several studies have been carried out to evaluate the acaricidal properties of chemical and natural anti-house dust mite agents under either semi-

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natural conditions [3-5], or in field studies. Most *in-vitro* studies have determined the contact effect of study agents on live mites, usually the Pyroglyphid mites, ie, *Dermatophagoides pteronyssinus* and *D. farinae* due to their close association with allergic diseases [6-7]. Techniques and devices have usually been specially developed in individual laboratories. For example, Mitchell (1985) used the Robinson chamber to study the effect of pirimiphos methyl as an acaricide in the reduction of house dust mite allergen levels [3]. Kalpaklioglu *et al* tested chemical compounds using specially-designed wells with black filter paper and a glass slide [4]. Raynaud *et al* tested acaricidal compounds on a hard surface using a 24-well tissue culture plate as a test chamber [5]. These studies reflect variations in how experts carried out testing in their own laboratories. Different types of chamber have also made it difficult to compare results among studies. Our own experience indicated that badly designed chamber would not be able to restrain the mites throughout the experiment and make it difficult to recover the mites accurately. Therefore, an ideal chamber should have three basic properties; 1) to be able to restrain mites in the chamber throughout the experiment and ensure that none die as a result of physical effects, 2) to be able to observe mite activities easily, and 3) the recovery method should make it easy to distinguish between live and dead mites, and to determine the time between administration of the agent and mite death, which indicates the effectiveness of the agent studied. Therefore, the aim of this study was to introduce a newly developed device called "the Siriraj chamber" and compare this new device with five different chamber types in terms of their ability to restrain mites, recovery techniques and other functions.

Materials and methods

Apparatus (Fig 1)

Model 1. Wells specially designed by Kalpaklioglu were used [4]. These wells had a volume of approximately 1 cm^3 with one side covered by black filter paper and the other side with a glass slide. An ordinary hair clip was used to prevent mites from escaping.

Model 2. A 24-well tissue culture plate (Costar No. 3524) was used. After putting mites in the well, grease was applied to the upper rim of the well and it was covered with a cover slide [5].

Model 3. A 15 cm diameter petri dish was used. After putting mites in the dish, the dish was sealed inside with grease and covered with its own lid.

Model 4. A 15 cm diameter petri dish was used. After putting mites in the dish, the dish was sealed on top with latex glue and covered with filter paper.

Model 5. A mite cage developed by Boczek in Warsaw, Poland was used. It was made of 2.5x6.0x0.2 cm plastic with a conical hole 0.5 cm diameter in the middle. To use the apparatus, the bottom of the hole is first covered with filter paper sealed with melted candle wax. Mites are then put into the cage through the top hole, which is finally covered with another glass slide and sealed again using melted candle wax to prevent mite escape.

Model 6. The Siriraj chamber, a 5x5x3 cm acrylic box with a 4.5x4.5x0.3 cm plastic sheet inserted at the top with a 1-cm diameter hole in the middle. To use the apparatus, the hole was first covered with a 2x2 cm piece of filter paper, followed by an acrylic ring. The acrylic ring can vary in size to accommodate different amounts of study agent. Mites are placed in the middle of the acrylic ring, covered with the chamber lid, which is then locked to prevent mite escape.

Testing

Only male mites were selected, since female mites may produce eggs during the study, which makes it difficult to maintain the same number of mites during the study. Twenty adult male mites were put in each type of apparatus, which was then kept at the optimum conditions for mite growth, of 25 ^oC and 75% relative humidity. The number of mites was recorded at 24, 48, 72 hours and 1 week. Each experiment was repeated 10 times.

Statistical analysis

The restraining ability of each of the six different apparatus, for each day, was presented using mean and standard deviation. Kruskal-Wallis

one-way analysis of variance (ANOVA) was used to compare the restraining ability each day. All statistical hypothesis tests were performed with a 2-sided significance level of 0.05 using SPSS/PC Version 10.0.

Results

Table 1 shows the different ability of each apparatus to restrain mites. Restraining ability ranked from lowest to highest was model 1 (Kalpaklioglu), model 3 (petri dish with grease), model 2 (tissue culture plate), model 4 (petri dish with filter paper), model 5 (mite cage), and model 6 (Siriraj chamber). It was found that for model 1, most of the mites were missing within 24 hours. The use of an ordinary hair clip seemed to provide an insufficient lock. Using the petri dish with grease model, about 50% of the mites remained on day 1, which decreased linearly over time to 10% on day 7. Sealing with grease did not prevent mite escape. The other chambers ie, the 24-well tissue culture plate, petri-dish with filter paper, mite cage and Siriraj chamber had 100% restraining ability throughout the 7-day period. However, in the 24-well tissue culture plate, all the mites died due to a lack of ventilation.

With regard to recovery technique, the purpose was to differentiate and recover the correct number of dead and live mites following treatment. The number of mites observed/recorded over time is also shown in Table 1. The petri-dish with filter paper was found to be too large relative to the number of study mites. Mites were able to wander around the plate which made them very difficult to locate and count. The mite cage had the drawback of a long preparation time and the need to check that it was sealed properly.

Discussion

To evaluate the effectiveness of anti-mite agents or acaroids, assessment needs first to be carried out under laboratory conditions. We found that the apparatus independently developed by each investigator varied in type, size and recovery technique. The main purpose of the recovery technique was to clarify whether treated mites were dead or alive. Two common mite recovery methods were: 1) forcing mites to move either upward or downward using heat [8], and 2) investigating any sign of movement by prodding. Some models were found to cause mite death due to physical effect. For example, mites got stuck in grease or were killed by an inappropriate recovery technique. Therefore, we carefully designed a standardized apparatus called "the Siriraj chamber". It not only had the required properties mentioned above but other uses, as well. First, it can be used for the presentation of unique dust mite pictures via electron microscopy and light microscopy, as shown in Fig 2. Second, it was very useful for studying of the biological aspects of dust mites under laboratory conditions. Third, it was very convenient to produce a pure local mite culture from house dust. This was done by first rearing either a pair of mating mites or a pregnant

Table 1 Ability of each test chamber to restrain mites.

Model	Number of mites : mean ± SD (min, max)					% dead
	Day 0	Day 1	Day 2	Day 3	Day 7	(Day 7)
1. Kalpaklioglu's well	20	1 ± 1.1 (0, 3)	0 ± 0 (0, 0)	0 ± 0 (0, 0)	0 ± 0 (0, 0)	-
2. Tissue culture plate	20	20 ± 0 (20, 20)	20 ± 0 (20, 20)	20 ± 0 (20, 20)	20 ± 0 (20, 20)	100
3. Petri dish with grease	20	11.8 ± 5.0 (5, 20)	8.4 ± 3.6 (2, 15)	5.1 ± 2.8 (0, 9)	1.80 ± 1.4 (0, 4)	10
4. Petri dish with filter paper	20	20 ± 0 (20, 20)	20 ± 0 (20, 20)	20 ± 0 (20, 20)	20 ± 0 (20, 20)	0
5. Mite cage	20	20 ± 0 (20, 20)	20 ± 0 (20, 20)	20 ± 0 (20, 20)	20 ± 0 (20, 20)	0
6. Siriraj chamber	20	20 ± 0 (20, 20)	20 ± 0 (20, 20)	20 ± 0 (20, 20)	20 ± 0 (20, 20)	0
P-value*	-	< 0.001	< 0.001	< 0.001	< 0.001	

* Kruskal-Wallis 1-way ANOVA by rank

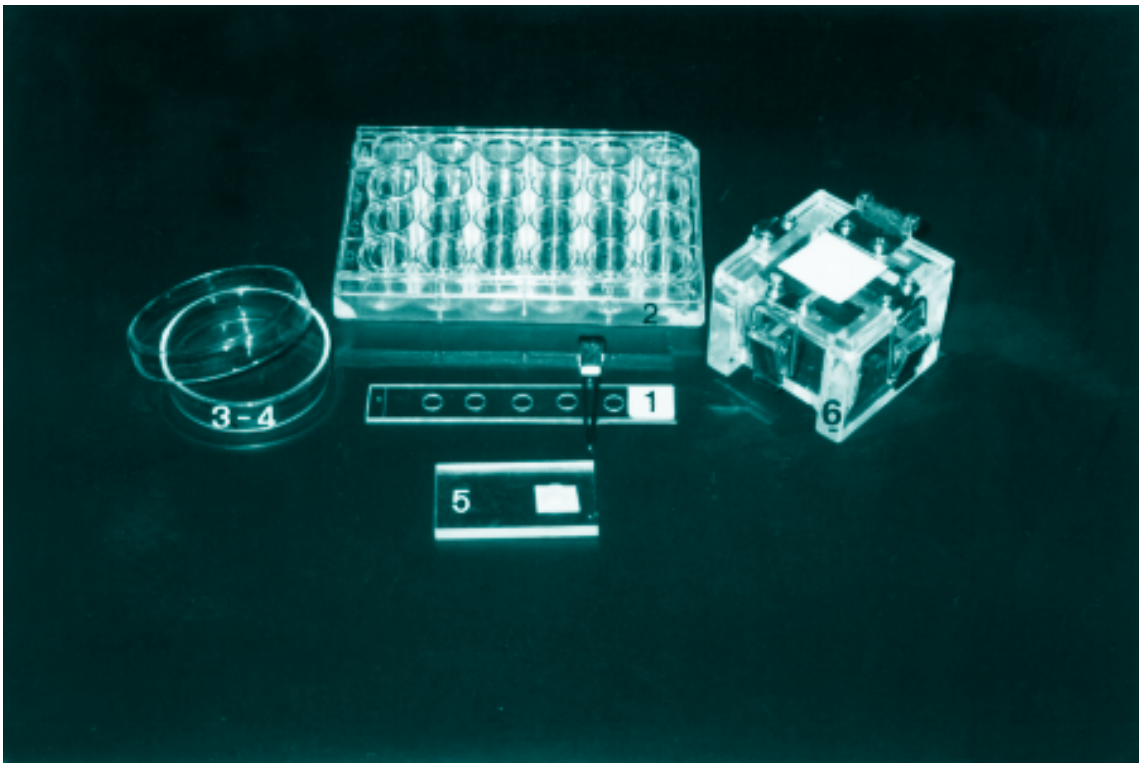

Fig 1 Kalpaklioglu's model (1), 24-well tissue culture plate (2), 15-cm petri dish (3-4), mite cage (5) and Siriraj chamber (6).

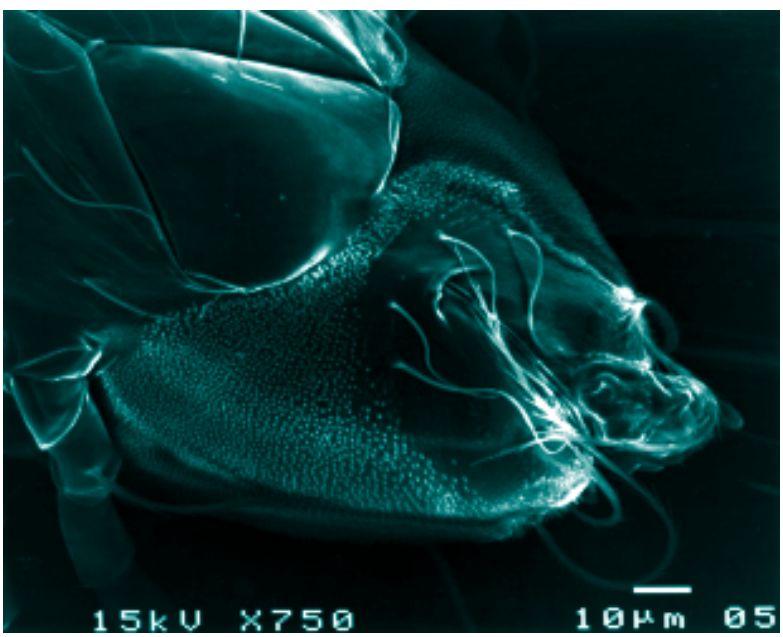

Fig 2 *Blomia tropicalis* with fecal pellet at the posterior (ventral view).

mite in the Siriraj chamber. The siblings from each chamber were then pooled over several generations (unpublished data). The use of acrylic material makes it easy to observe the number of mites, and their activity and morphology under a stereomicroscope. Furthermore, the size of the ring could be easily adjusted to the amount of study agent used. Also, the study area in the Siriraj chamber is so limited that all mites are thoroughly exposed to the study agent. Lastly, the chamber could also be used to study either the effectiveness of antimite-coated fabrics or the protective ability of impermeable covers [9-10]. In conclusion, the Siriraj chamber was superior to the other chambers. It was simple to use and the study area could be adjusted to the number of mites used. It can be used not only to study the biological aspects of dust mites in laboratory conditions, such as the assessment of anti-mite agents, and the protective ability of impermeable covers, but also for other studies such as scanning electron microscopy and establishing a pure local mite culture from house dust. The Siriraj chamber is recommended as a standard chamber for house dust mite studies.

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