TEST REPORT

“Precise Climate Controller for Eliminating House Dust Mites”

Performed by

Siriraj Dust Mite Center for Services and Research

Department of Parasitology, Faculty of Medicine Siriraj Hospital

Mahidol University

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Introduction

Allergy to house dust mites (HDM) is an important cause of asthma and rhinitis in Thailand. The percentage of sensitization to house dust mites was reported to be more than 40% of the population in Bangkok. The climate of Thailand is tropical, high both in temperature and relative humidity. Certainly, the warm and humid climate favors growth of dust mites. To thrive, dust mites need very warm temperatures (23°C - 35°C) and high humidity levels 70% - 80% RH. Most mite allergens are formed by adult mites during their active phase. The survival of active adult mites is limited to 4 to 11 days at relative humidity below 50% RH at 25°C.[1] However, if maintaining daily indoor relative humidity is below 50% RH and the relative humidity rises above 50% for more than 2 hours daily, it cannot prevent the dust mite's population growth. This is the answer why a portable dehumidifier in home use cannot control the dust mite population.

Exposure to indoor allergen is recognized as the most important risk to trigger the development of asthma and allergies in children living in urban area. Among the indoor allergens, house dust mites have been accounted as the major sources of indoor allergens and considered to be the most important allergen. The association between indoor allergens and humidity has been interested. It has been established that the levels of dust mite allergen correlated with humidity levels in the ambient and indoor microenvironment. The optimal relative humidity for mites ranged from 75% - 85%, so they cannot survive where the relative humidity is lower than 60%.[2] The survey of HDM allergen in Florida revealed that level of dust mite allergen in the room having relative humidity between 69% and 80% was over 1,000 ng/gm whereas in the relative humidity between 51% and 64%, the level of mite allergen was lower around 100 ng/gm.[3] Moreover, dampness in school building also showed a relationship with the intensity of dust mite allergen. Visible signs of dampness on the floor had 25% higher Der p 1 ng/m^2 level. Upper floors had lower dust mite levels compared with ground floors according to the condition of lower moisture.

Therefore, we aim to study that in the condition with relative humidity of 55% controlling by invented humidity inverter can kill mites and decrease mite population and hence mite allergen.

Materials and Methods

Precise Climate Controller

The precise climate controller is designed by Thai scientist and invented in Bangkok, Thailand. Its size is about 0.3 x 1.0 x 1.2 m (W x L x H) similar to an air condition. The device is consisted of a unit of humidifier and a unit of dehumidifier installed within the same case as shown in Figure 1. The device is installed and running incorporated with air-conditioning under the automatic control by microprocessor. Therefore, the temperature and humidity in testing room was controlled to be as 25°C and 55% RH.

Figure 1. A precise climate controller with the dimension of 0.3 x 1.0 x 1.2 m (W x L x H) which is similar to a 10,000 BTU air condition.

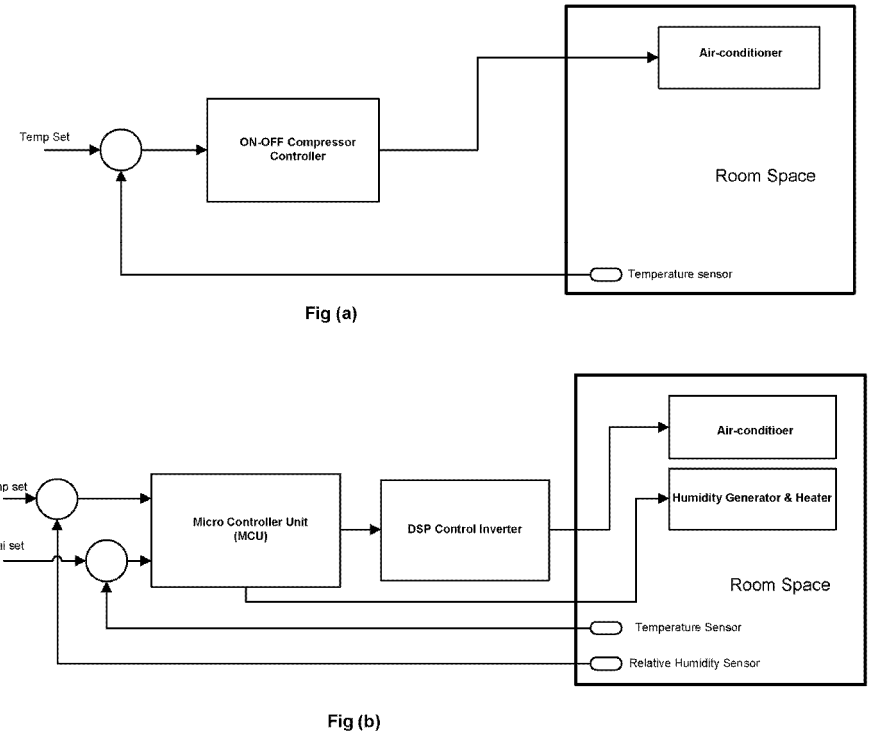

Figure 2. Diagram comparing the conventional air conditioning system (a) and the precise climate controller system (b).

Testing Room

The testing was performed in a building located in Pak-kret District, Nonthaburi. The testing room is about 4.0 x 3.5 x 3.0 metres (equals 42 m^3). There was no activity other than sample collections was conducted in this room but the heat was periodically generated to simulate human activity. Temperature and humidity of both inside and outside the testing room were recorded hourly along the whole testing period (6 weeks).

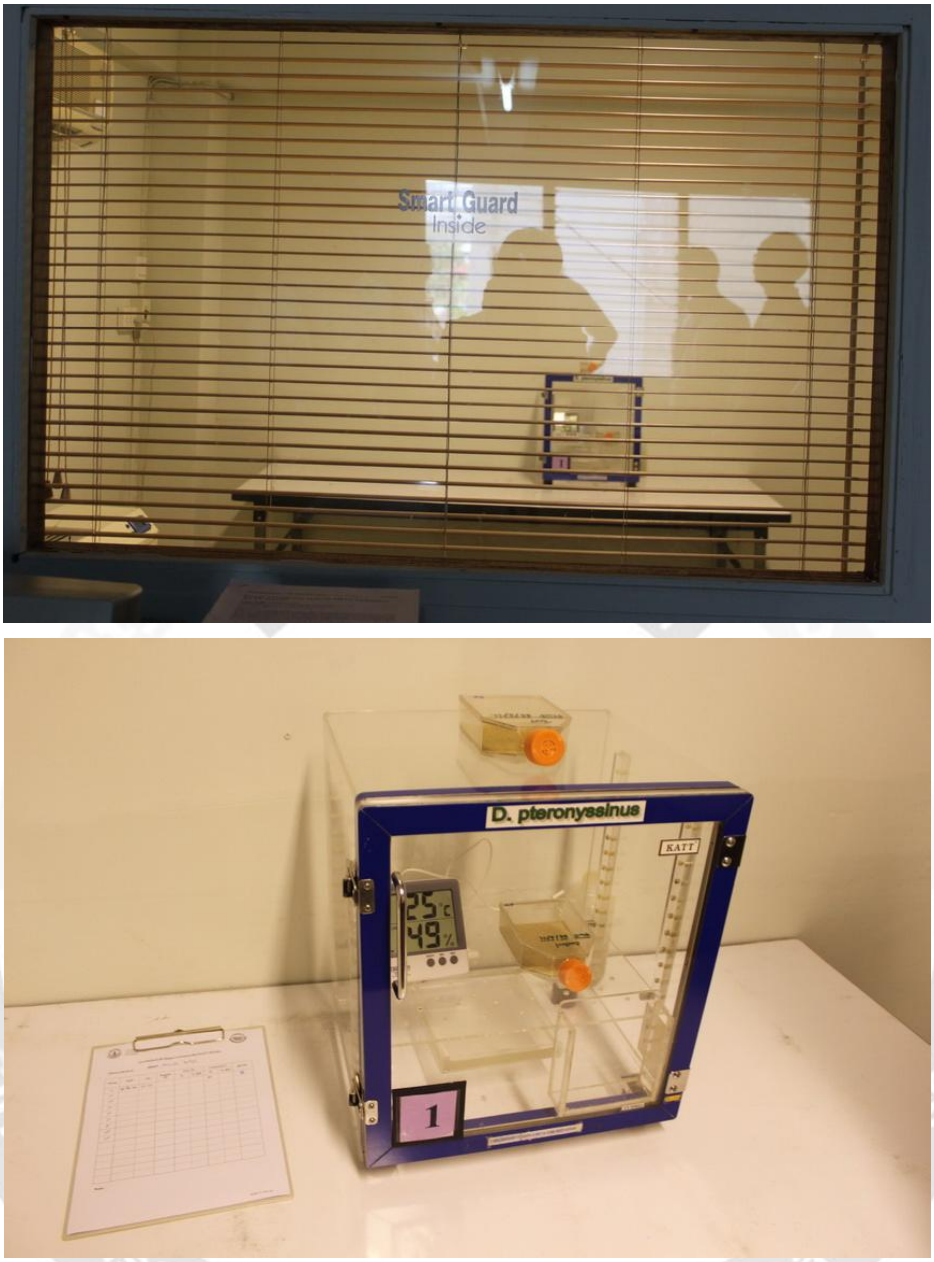

Figure 3. The testing room located in Pak-Kred District, Nonthaburi province. The room size is 4.0 x 3.5 x 3.0 m. Two bottles of dust mite cultures were placed in the room; one was inside the 75% RH cabinet and the other was outside with the setting 50% RH climate controller.

House Dust Mites

The tested house dust mites *Dermatophagoides pteronyssinus* (Dpt) was obtained from the laboratory culture maintained at the Faculty of Medicine Siriraj Hospital. The mites were cultured in a 250-cm^2 flat tissue culture bottle.

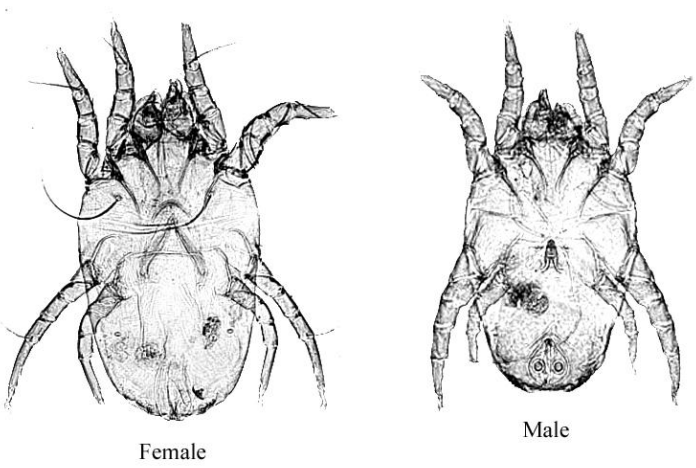

Figure 4. Morphology of *Dermatophagoides pteronyssinus* mites under microscopic magnification. Their natural sizes are 100-300 μm.

Testing Method

Once installed in the testing room, the device was operated and the climatic information was investigated until the 25°C and 55% RH had been met and the device operation was continued for 30 minutes further prior to starting the experiment. Two sets of mite culture flasks were divided into the Treat (T) and the Untreat (C) groups. The untreated flask was placed in the closed acrylic chamber (30 x 30 x 45 cm), whereas the treated flask was placed on top of the chamber without cover. The humidity in the chamber was controlled at 75% RH with saturated NaCl solution.

At the day 0 of experiment, 0.2 gm of the mite cultures was sampling and the same amounts of culture were collected again on the following weeks (1st week to 6^{th} week). The samples were then divided into 2 x 0.1 gm, one was for further examination of mite number and the other was for mite allergen content.

Mite Count

Mite numbers were examined by counting the live mite bodies. In order to obtain the mites, floatation technique developed by Hart & Fain[4] was applied. Basically, mites in the cultures were soaked overnight in 80% ethanol before floating over the saturated NaCl solution. Mites recovered from this technique with plump shape and pale yellow were identified as live mites whereas mites with shrinkage and brown were identified as dead mites. All immature stages of mites including eggs, larvae and nymph can also be recovered.

Mite Allergen Measurement

Levels of Der p 1, the major allergen of *Dermatophagoides pteronyssinus* mites was examined with the commercial sandwiched ELISA reagents (Indoor Biotechnology, U.K.). 0.1 gm of mite culture was extracted in 1 ml of phosphate saline buffer (PBS) at 4°C overnight. The supernatant was then used in the assay performed under manufacturer protocol. Briefly, the wells of 96-well microtiter plate were overnight coated with anti-Der p 1 monoclonal antibody, then the samples containing Der p 1 were added and incubated. After several rinse, the detecting biotinylated antibody was added and incubated. Then, a 1:1000 dilution of streptavidin-peroxidase was added. At the final step, the ABTS substrate was added prior to measure the color development by microtiterplate reader at 405 nm. The concentrations of Der p 1 in sample cultures were interpolated against standard curve and the data were converted into ng per gram by the extraction dilution (x20).

Data Analysis

The means and standard deviations of mite numbers and Der p 1 levels in both treated and untreated culture were calculated and analyzed for statistical significance.

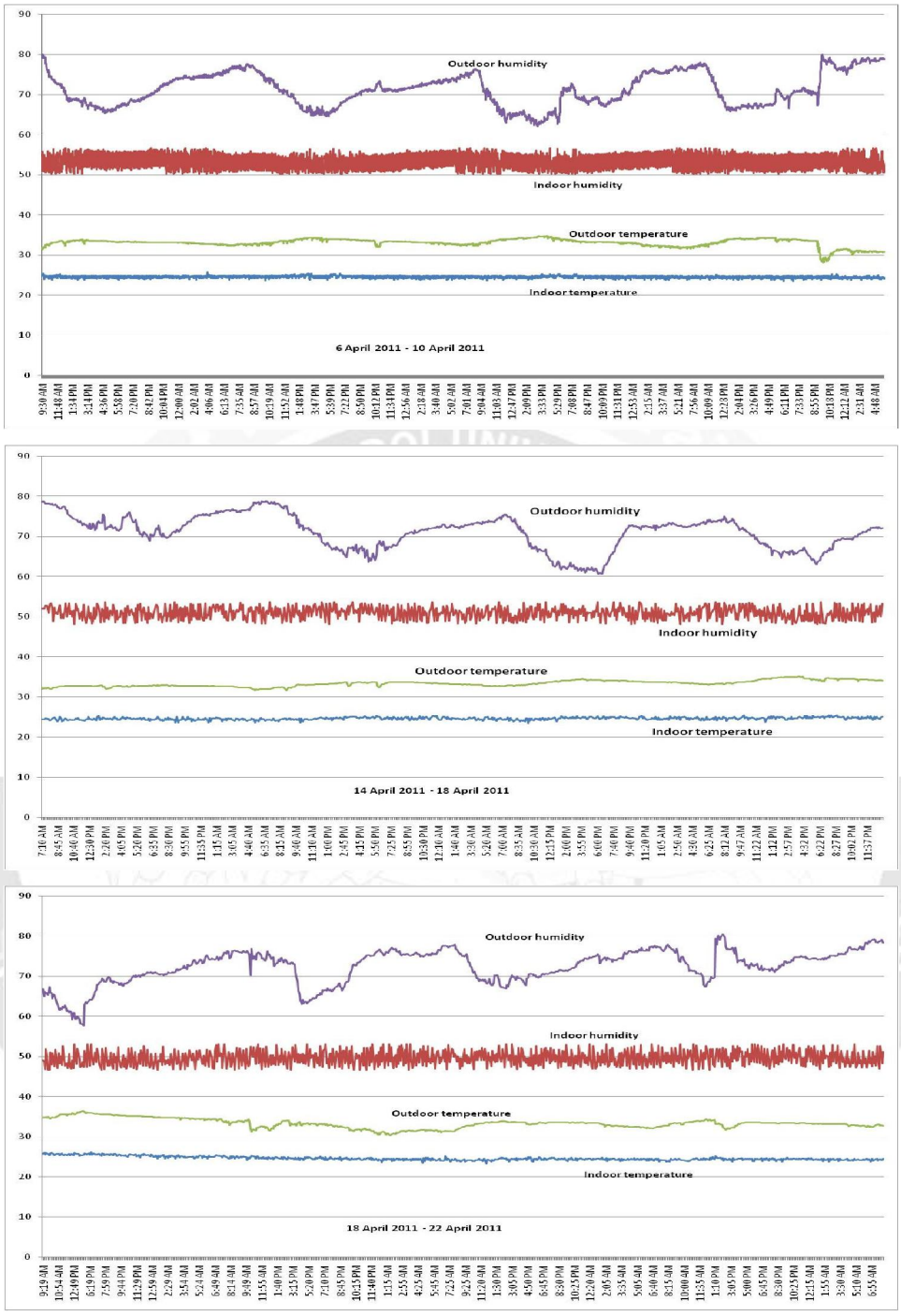

Figure 5. Climate data log during the testing period**.** The top two lines represent the outdoor and indoor humidity whereas the bottom tow lines represent outdoor and indoor temperatures respectively.

Results

Mite Growth

Mite numbers at the beginning (Day 0) in the treated (55% RH) and untreated (75% RH) cultures were similar (5,160 and 6,600 mites/gm, respectively). After one week, all mites in the treated culture were died as seen by their brown shrinkage bodies (Figure 6), then the mite growth stopped (Figure 7). Meanwhile, the mite growth in untreated culture gradually increased every week. The minimum growth rate was in the week 2nd whereas the maximum growth rate was in the week 6th (Table 1). The average growth of mites was 31.9% per week. At the final week of testing (week 6th), the mite number was increased to 31,940 mites per gram which was about five times of the number at beginning. The mite density seemed to increase further but we did not continue the investigation.

Life stage composition

The life stage compositions of mites in the cultures are shown in Table 2 and Figure 8. Since all mites in treated culture were died after 1 week so the life stage compositions were changed in the untreated culture. As expected, number of eggs was increased every week but was slightly constant after the 2nd week. The ratio was ranged from 0.003 to 0.28 times to all life stages. Although the nymph was the majority life stage in the culture but its ratio was relatively constant since the beginning to 6 weeks of testing. Meanwhile, the ratio of adult mites was declined to about half of the ratio at the beginning.

Level of Der p 1 Allergen

Levels of Der p 1 allergen in the treated culture was 23.1 μg/gm on the beginning day and did not have much change along the period of testing (Figure 9). Although there was a slightly difference week-by-week but Der p 1 allergen levels in the untreated culture gradually increased from the beginning (30.8 μg/gm) to the 6th week (36.3 μg/gm). The allergen levels in the untreated group tended to be increased further according the increasing number of mites.

Table 1. Number of live *Dermatophagoides pteronyssinus* mites in the treated (55% RH) and untreated (75% RH) cultures during the test period (6 weeks). Percentage of mite increased is the percentage of difference between mite numbers on each individual week against mite number at the beginning.

Week	No.of live mites /gm		% of mite increased	
	Treat	Untreat	Treat	Untreat
0	5,160	6,600	-	-
1	0	8,420	0	27.6 %
2	0	14,880	0	125.4 %
3	0	16,120	0	144.2 %
4	0	18,640	0	182.4 %
5	0	22,160	0	235.8 %
6	0	31,940	0	383.9 %

Table 2. Population densities and stage compositions of *Dermatophagoides pteronyssinus* dust mites in the treated (55% RH) and untreated (75% RH) cultures during the testing period (6 weeks). Numbers in parentheses are the ratio of each life stage against total mite number. Abbreviation for stage composition: A = Adults, L = larvae, N = nymphs, E = eggs. ND = not done.

Weeks	Number of mites and stages							
	Treat (55% RH)				Untreat (75% RH)			
	A	L + N	E	Total	A	L + N	E	Total
0	2840 (0.55)	2320 (0.45)	0 (0.0)	5,160	3,640 (0.55)	2,940 (0.44)	20 (0.003)	6,600
1	2840 (0.55)	2320 (0.45)	0 (0.0)	5,160	4,792 (0.57)	3,594 (0.43)	34 (0.004)	8,420
2	2840 (0.55)	2320 (0.45)	0 (0.0)	5,160	5,700 (0.38)	6,140 (0.41)	3,040 (0.20)	14,880
3	2840 (0.55)	2320 (0.45)	0 (0.0)	5,160	4,800 (0.30)	7,460 (0.46)	3,860 (0.24)	16,120
4	2840 (0.55)	2320 (0.45)	0 (0.0)	5,160	5,879 (0.32)	8,227 (0.44)	4,534 (0.24)	18,640
5	2840 (0.55)	2320 (0.45)	0 (0.0)	5,160	6,672 (0.30)	9,816 (0.44)	5,672 (0.26)	22,160
6	2840 (0.55)	2320 (0.45)	0 (0.0)	5,160	8,410 (0.26)	14,460 (0.45)	9,070 (0.28)	31,940

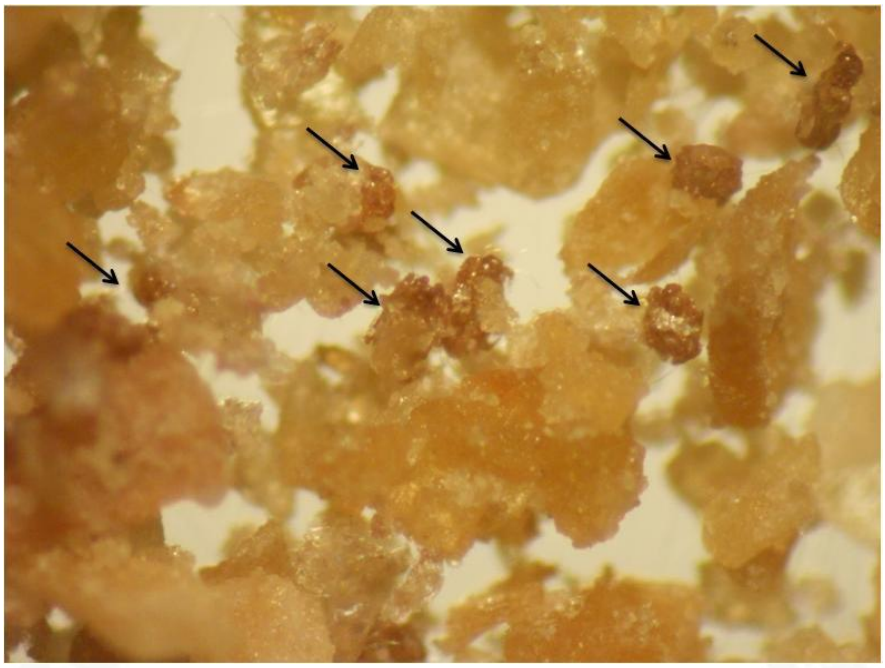

Figure 6. Microscopic image showing dust mites in the treated culture (55% RH). Arrows indicate the dead mites.

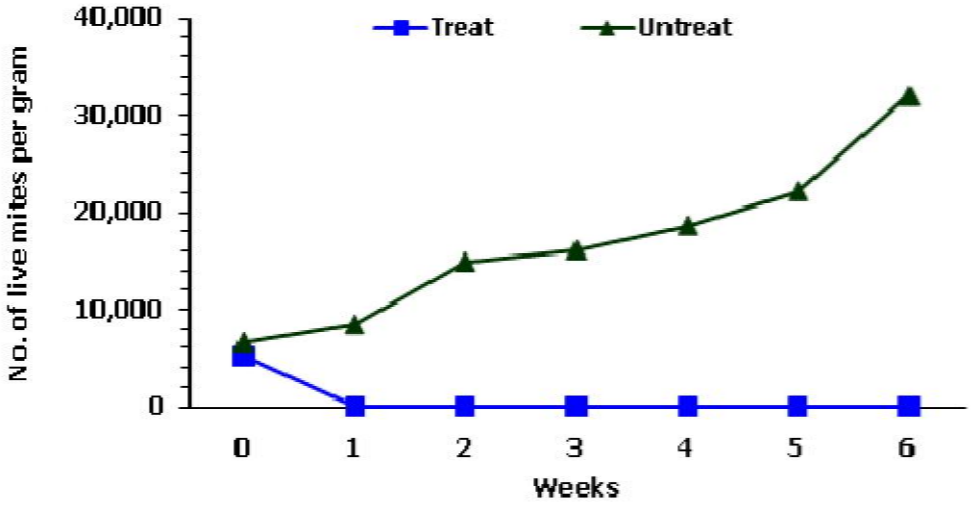

Figure 7. Mite population changes of *Dermatophagoides pteronyssinus* mites during the test period (6 weeks). The changes are represented by numbers of live mites in the treated (55% RH) and untreated (75% RH) cultures recovered from flotation method. All mites in the treated cultures were died within 1 week of testing.

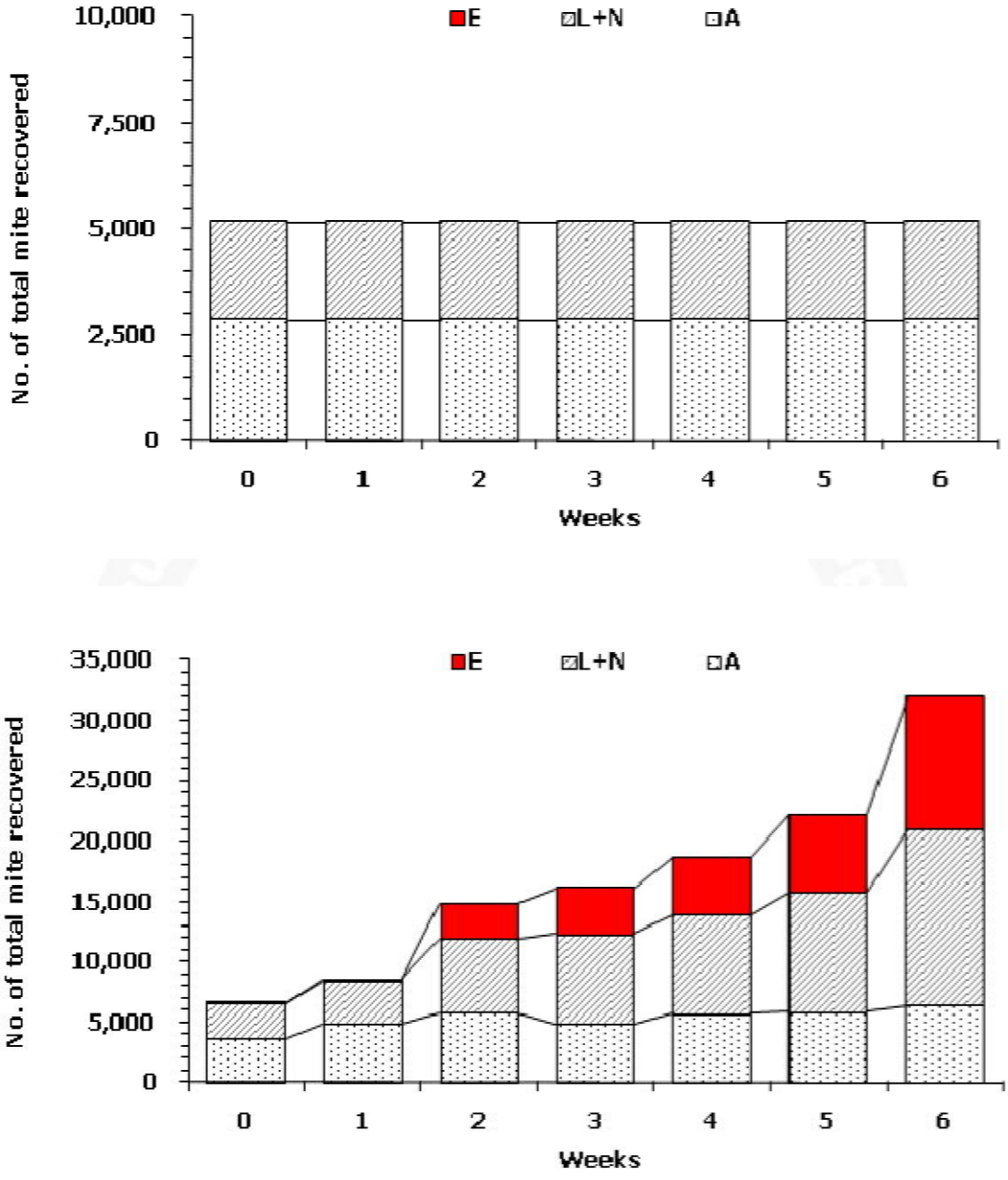

Figure 8. Growth stage compositions of *Dermatophagoides pteronyssinus* mites in the treated (55% RH) and untreated (75% RH) cultures during the test period (6 weeks). Numbers represents total amount of mites in the cultures recovered from flotation method. The bottom stack represents adult stage (A), whereas the middle stack is larval and nymph stages (L+N) and the top stack is egg (E).

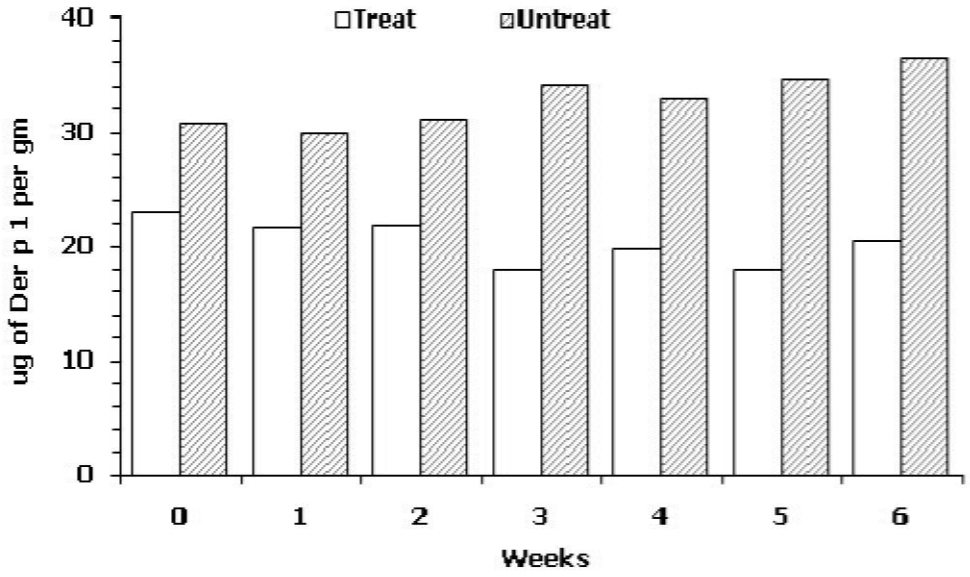

Figure 9. Levels of Der p 1 mite allergen in the mite culture of *Dermatophagoides pteronyssinus* during testing period (6 weeks). The treated culture was exposed to humidity at 55% RH whereas the untreated culture was placed in chamber under 75% RH. The room temperature was controlled at 25 °C.

Conclusion and Comment

It is almost impossible to regulate the relative humidity to eliminate the house dust mites in the dwelling in the tropical zone. With the state of art technology of a digital signal processor and a micro processor, the new proposed precise climate controller can be achieved. The results from the experiment and testing found that the house dust mites cannot survive beyond seven days after the operating of the precise climate controller system.

The dramatic decrease of HDM density in the climate-controlled testing room found in this study is not surprised. The most significant factor affecting the mite growth is humidity. A number of studies showed low mite growth under low humidity conditions. Pike *et al*.[5] investigated mite growths in the dry (60% - 38% RH) and damp (70% - 55% RH) conditions and found that all mites in the dry condition died within 18 days whereas those in the damp could survive and reproduced their offsprings.

The new proposed precise climate controller is applied to control the relative humidity all day long, even there is not occupying the room. There are two modes of the precise climate controller. First, a full control mode is used when both the temperature and the relative humidity are controlled. Second, a standby mode is used when only the relative humidity is controlled. This mode is applied during the room is not occupied. Therefore, the electrical energy is minimized in this mode. The precise climate controller differs from conventional air-conditioning system in which compressor works in on-off mode without humidity control. The precise climate controller comprises of a digital signal processor control inverter installed to control the speed of a compressor motor, an ultrasonic humidifier to increase water volume in the air, an electric heater to increase the temperature inside the room and a micro-processor control unit to control operation of each element in automation. The speed control of a compressor motor is employed to control the refrigerant flow in order to regulate the room temperature. Since, in the mathematical model of the relative humidity is a cross coupling function of the temperature. In other words, the relative humidity value depends on the temperature even at the same absolute humidity value. The new proposed precise climate controller applies decoupling control by separating the temperature variable and the relative humidity variable. The temperature value has to keep constant in order to separate the relative humidity variable from the

temperature variable. Practically, the room temperature is controlled within +/- 0.2°C of the set point by the variable-speed control of the compressor. Therefore, the relative humidity can control at any set point of the room temperature. During the standby mode, the relative humidity is continuously controlled less than the critical equilibrium humidity (CEH) at the ambient temperature.

Since the new proposed precise climate controller is utilized the conventional air-conditioner to operate as a cooling system and dehumidifier, in economic aspect the cost of the system is applicable to home uses. Moreover, not only the new proposed system can eliminate the house dust mites, but also it prevents the growth of fungi.

(Asst.Prof. Dr. Nat Malainual)
Examiner

(Assoc.Prof. Dr. Anchalee Tungtrongchitr)
Chairman of the Siriraj Dust Mite Center
For Services and Research

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