A Study on Nutrients for Culturing the Allergen-Producing Mites, *Dermatophagoides Pteronyssinus*

Mont Panichjeerasin[1] and Nat Malainual[2*]

[1]Graduate Program in Immunology, Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[2]Siriraj Dust Mite Center for Services & Research, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[*]Corresponding author, e-mail: nat.mal@mahidol.ac.th

Abstract

Dermatophagoides pteronyssinus is a major indoor allergen-producing mite in Thailand, which causes allergic symptoms including allergic rhinitis, allergic conjunctivitis, atopic asthma and atopic dermatitis. Allergenic extract made from this mite species is widely used for diagnosis and immunotherapy of allergic patients worldwide. Cultivation of the mites is simple but to obtain high production at low cost is considered. Nutrition from food sources is the major concern for allergen production in mites. In this study, we tested several food sources and formulations to obtain the high production yield. Mites were cultured in various foods for six weeks. Then they were harvested, and counted. We found that the mixture of 10% yeast with carp food gave the highest yield of mites compared to other formulations (p <0.0001, one-way ANOVA). This food formulation would be used for the mass production of this mite species.

Keywords**:** *Dust mite, mite culture, Dermatophagoides pteronyssinus, food*

บทคัดย่อ

ไรฝุ่นบ้าน *Dermatophagoides pteronyssinus* เป็นต้นเหตุสำคัญของโรคภูมิแพ้อาทิโรคจมูกอักเสบ เยื่อบุตาอักเสบ หอบหืดจากภูมิแพ้ รวมถึงโรคผิวหนังอักเสบ สารสกัดจากไรฝุ่นชนิดนี้ใช้เป็นชุดทดสอบและวัคซีนภูมิแพ้ต่อไรฝุ่นโดยทั่วไป การเพาะเลี้ยงไรฝุ่นนั้นทำได้โดยง่ายแต่การเลี้ยงให้ได้ผลผลิตตัวไรในปริมาณมากนั้นมีเรื่องของต้นทุนเข้ามาพิจารณาร่วมด้วย สารอาหารในอาหารที่ใช้เลี้ยงไรฝุ่นเป็นสิ่งที่ส่งผลต่อการผลิตสารก่อภูมิแพ้ในตัวไร ในการศึกษานี้ผู้วิจัยได้ทดสอบอาหารชนิดต่างๆ รวมถึงอัตราส่วนผสมที่ใช้เลี้ยงไรฝุ่นเพื่อให้ได้ผลผลิตไรฝุ่นที่มากที่สุด ผู้วิจัยได้เลี้ยงไรฝุ่นในอาหารชนิดต่างๆ เป็นเวลาแปดสัปดาห์ มีการเก็บผลผลิตเป็นรายสัปดาห์เพื่อทำการนับจำนวนไร ผู้วิจัยได้พบว่า อาหารที่ใช้เลี้ยงไรฝุ่นซึ่งเป็นอาหารปลาผงและมียีสต์ผงร้อยละสิบ ให้ผลผลิตไรฝุ่นมากที่สุดเมื่อเทียบกับอาหารชนิดอื่น (p <0.0001, one-way ANOVA) ซึ่งอาหารสูตรนี้จะนำมาใช้เลี้ยงไรฝุ่นในระดับอุตสาหกรรมต่อไป

คำสำคัญ: *ไรฝุ่น การเพาะเลี้ยงไรฝุ่น Dermatophagoides pteronyssinus*

1. Introduction

House dust mites, *Dermatophagoides pteronyssinus*, infest home worldwide, belong to the family Pyroglyphidae. House dust mites mainly infest bed, carpet or upholstered furniture. A typical double bed can be a home of 200 million mites which thrive with unlimited food and humidity (Colloff, 2009) The human skin scale and humidity from human body allow mites to thrive indefinitely.

An allergy to house dust mites mostly occurs through inhalation of house dust mite faecal pellet which contain mite allergens (Arlian & Platts-Mills, 2001). The clinical features of allergic diseases are allergic rhinitis, allergic conjunctivitis, allergic asthma and atopic dermatitis, which reflect the portal of allergens entry (Delves, 2011).

This species of dust mite has six stages in life cycle: egg, prelarva, protonymph, tritonymph and adult. Life cycle of mite begin at egg which is white, oval and elongate, and then it develops into prelarva within eggshell. Larva then emerges and develops into protonymph which is immobile. Tritonymph will develop following protonymph in which male mite guard female tritonymph for mating. Adult will emerge from tritonymph which sexual maturity will be attained (Colloff, 2009).

Besides the allergen avoidance, antihistamine and immunosuppressive agents practice as obvious choice for treatment of allergy. An allergy to house dust mites can also be treated by desensitisation to house dust mite allergen called specific allergen immunotherapy. The procedure requires small amount of dust mite extract to be injected or ingested by a patient. The small amount of allergen which enter the patient in tolerogenic zone will induce antigen presenting cells to become tolerogenic which also turn the conventional T cells to become regulatory T cells by means of immunosuppressive cytokines. The regulatory T cells will suppress the activity of pathogenic T and B cells and finally the partial or complete control of allergic pathogenesis (Eifan, Calderon, & Durham, 2013).

Diagnosis of house dust mite allergy by skin prick test or treatment by allergen immunotherapy requires house dust mite extract. The variation of allergen contents in the extract is mainly due to mite culture conditions especially culture medium (Casset et al., 2012; Frati et al., 2012).

Mites can be grown in various sources of foods, however the food can influence the population growth and endotoxin accumulation within the mite culture (Avula-Poola, Morgan, & Arlian, 2012). Temperature is also an important factor that influences the mite growth (Yella, Morgan, & Arlian, 2011). The previous study demonstrated that food medium that contains powdered carp food and powdered yeast in equal quantity gives highest yield of mite culture (Ree, Lee, Kim, Jeon, & Hong, 1997).

2. Objective

To study on the mite culture medium which gives the high yield of mites.

3. Materials and methods

3.1 Study Design

This study was divided into Phase I and Phase II.

Phase I was carried out to determine which food source is suitable for mite culture.

Phase II was to determine the appropriate food formula for mite rearing.

Experiments were performed for weeks for both phases.

3.2 Dust Mites

A house dust mite, *Dermatophagoides pteronyssinus*, stock culture was obtained from Siriraj Dust Mite Centre for Service and Research, Department of Parasitology, Faculty of Medicine Siriraj Hospital. Mite population was maintained in powdered mouse food at 75 ± 5% relative humidity and 25 ± 3 degree Celsius. The mites were used in the experiment once the population reached the peak (about 4 weeks).

Mite population was randomly divided into six groups in phase I and five groups in phase II. Four replicates per individual group were prepared in phase I and five replicates per individual group were prepared in phase II.

3.3 Culture Medium

Phase I:

Several kinds of food sources including ground mouse food, soybean, wheat germ, rice bran and powdered yeast were examined. The food sources were allocated as shown in Table 1.

Culture medium was transferred into culture flasks and pre-moisted 24 hrs prior to mite seeding. The mite seeding was done by fetching stock mite culture into a pre-moisted culture medium at the ratio of 1:10 (v/v). All culture containers were placed in the culture chamber maintained at 75 ± 5 %RH until harvesting.

Table 1. Group allocation of food sources examined in Phase I

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
ground mouse food	Soybean (powdered)	Soybean (powdered) + wheat germ (1 : 1 v/v)	wheat germ	rice bran	ground mouse food + powdered yeast (10 : 1 v/v)

Phase II:

As of Phase I result, the mixture of ground mouse food and powdered yeast (10:1 v/v) gave the highest yield of mite population. Therefore, it was selected to be used as the control formula in further experiment in Phase II. Also, in order to obtain the better yield of mite growth, carp food (in powdered form) which contains rich proteins was compared to the control formula. The experiment was designed as in Table 2.

Culture medium was transferred into culture flasks and pre-moisted 24 hrs prior to mite seeding. The mite seeding was done by fetching stock mite culture into a pre-moisted culture medium at the ratio of 1:10 (v/v). All culture containers were placed in the culture chamber maintained at 75 ± 5 %RH until harvesting.

Table 2. Food formulation examined in Phase II

Group 1 (Control)	Group 2	Group 3	Group 4	Group 5
ground mouse food	ground mouse food + powdered yeast (10 : 1 v/v)	ground mouse food + powdered yeast (5 : 1 v/v)	carp food + powdered yeast (10 : 1 v/v)	carp food + powdered yeast (5 : 1 v/v)

Mite harvesting and mite count

During the 8th week, 0.05 gram of mite culture in each container was transferred into a test tube and soaked with 70% ethanol for overnight. On the following day, ethanol was aspirated, and the saturated sodium chloride solution was filled and left for 10 minutes. Floating dust mites were poured off over a filter paper, rinsed and collected for slide mounting. Then, number of mites were counted under microscope. Mite counts were recorded as numerical data for each week for data analysis.

Data Analysis

Data from the experiments were analysed with PASW statistics software version 18. Descriptive statistics was employed to characterise the data and determine normality. One-way analysis of variance (ANOVA) was used to compare the mean between experimental groups. Tukey HSD was used for multiple comparison.

4. Results

Phase 1

From the first week, mite population were stationary. The ground mouse food group and ground mouse food+ yeast group had similar population growth and significantly grew better than other group except wheat germ group as shown. This was due to the fact that mite population began to thrive in ground mouse food group and ground mouse food +yeast and not to begin starvation. At week 2, mite population grew more than week 1. Ground mouse food was slightly higher than ground mouse food +yeast and wheat germ group. Mite population at other groups began starving and died off. The mite population growth continued at week 3. Ground mouse food group and ground mouse food +yeast had equivalent population and exhibited faster growth rate than other groups. At week 4, mite population growth of ground mouse

food group and ground mouse food +yeast were significantly higher than other groups (p-value <0.001). The population growth peaked at week 6 in which mite population in ground mouse food +yeast were significantly highest among other groups (p-value <0.0001).

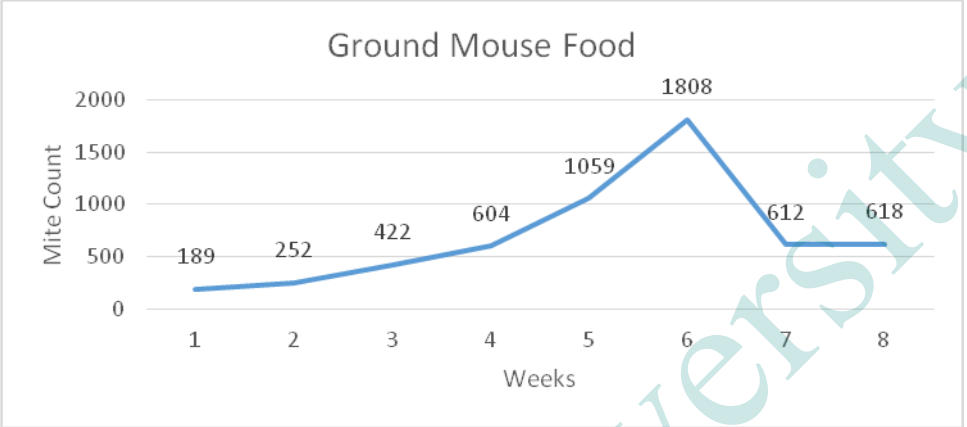

Figure 1 Growth of mite population in ground mouse food group

Mite population in ground mouse food was used as control. They grew on food on influence of optimum humidity and temperature. The population peaked at week 6 which was significantly higher than other groups (p value<0.0001) except ground mouse food+ yeast 10% which was the highest (p value <0.0001).

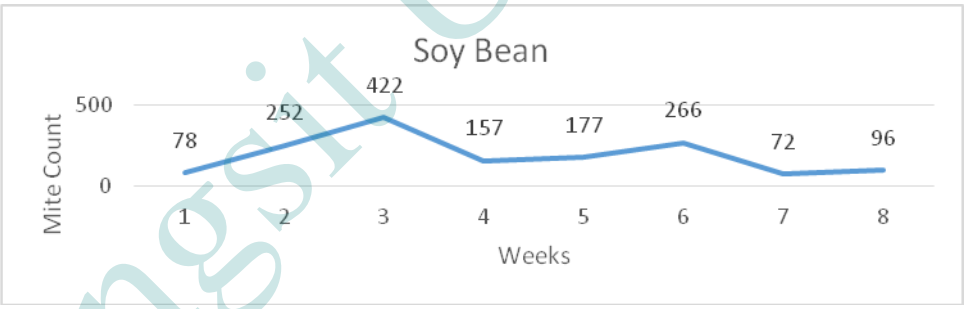

Figure 2 Growth of mite population in soy bean group

Mite population in powdered soy bean grew in small quantity relative to other groups. Soy bean was not suitable for mites to thrive.

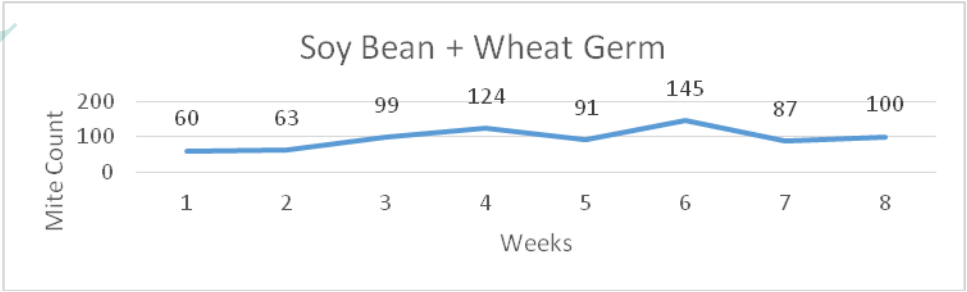

Figure 3 Growth of mite population in soy bean + wheat germ group

Soy bean + wheat germ allowed small population of mites size to thrive. This food medium was not suitable for mite.

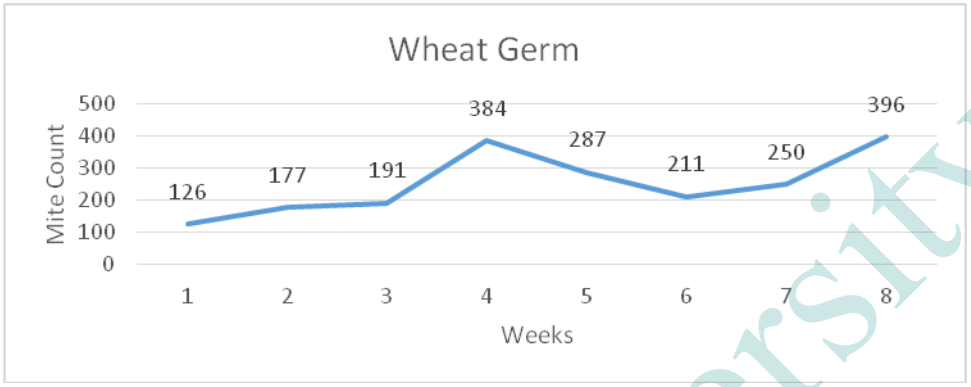

Figure 4 Growth of mite population in wheat germ group

Mites in wheat germ grew well as equally as mite reared with soy bean did, but it supported modest population growth, and it was not considered as good food for mite.

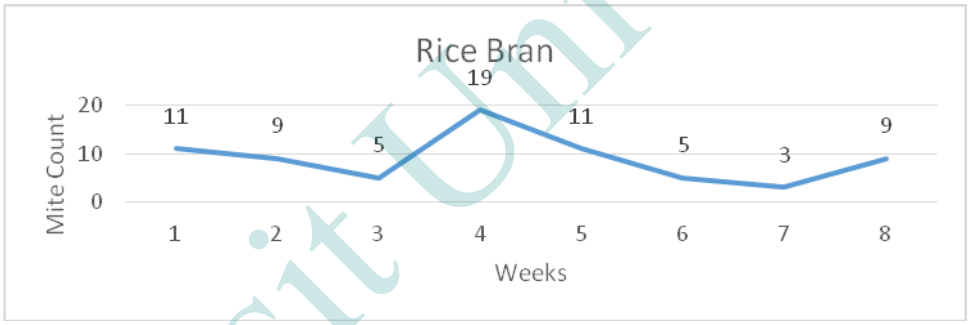

Figure 5 Growth of mite population in rice bran group

Rice bran could not support mite population to thrive. As seen from the first week, rice bran could not supply nutrient to mite population; thus, it did not make mite population thrive and reproduce.

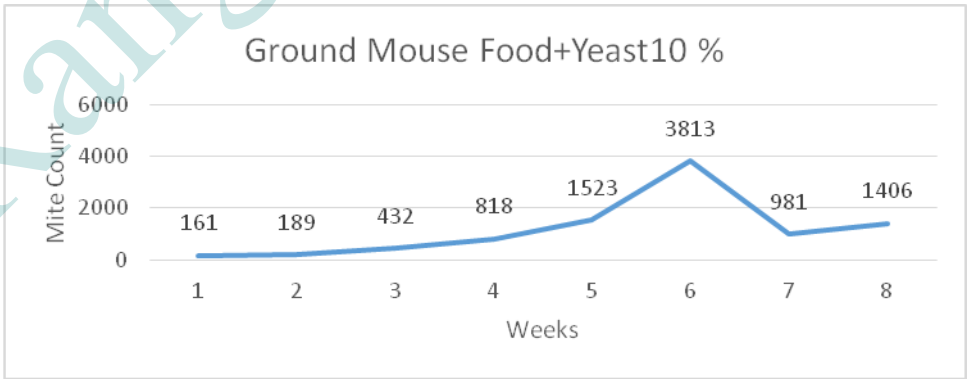

Figure 6 Growth of mite population in control+ yeast group phase 1

This group had excellent result. The mite population grew at highest rate among the others (p value <0.0001).

Phase 2

Mite population was seeded at equivalent quantity to all experimental unit of all group. The groups were divided into five groups as shown in materials and methods. All groups were reared at the same condition as phase 1. From the first week, mite population was in stationary and gradually increased in week 2. Mite population growth between groups became significantly different at week 3, and it continued to be extremely different (p-value <0.0001) until week 5. Then, population declined.

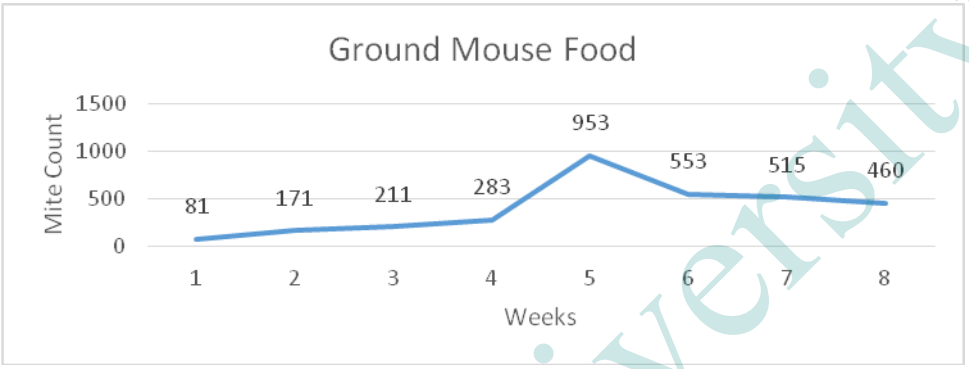

Figure 7 Growth of mite population in control group phase 2

This group served as control which was used to compare with other groups. The population growth was similar to ground mouse food in phase 1.

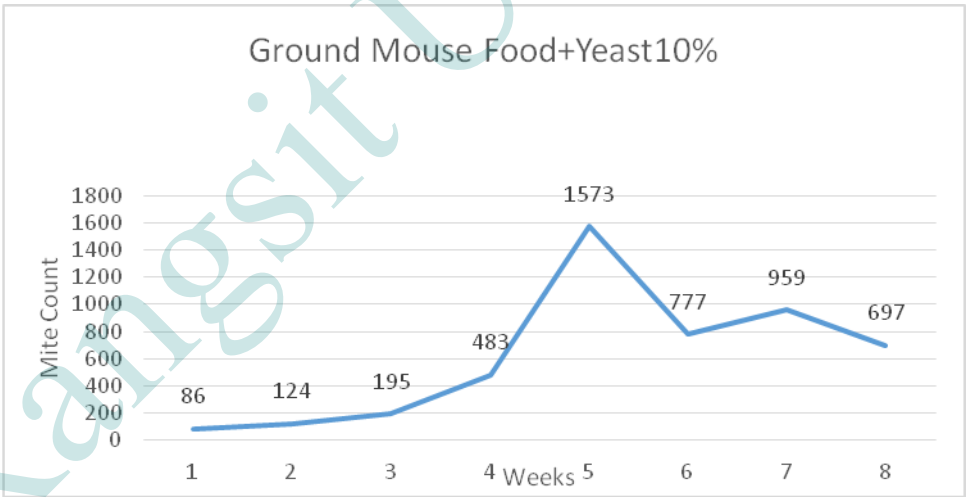

Figure 8 Growth of mite population in control+ yeast 10 percent group phase 2

The effect of yeast powder which gave higher production yield could be seen in this group. There was also accelerated population growth in this group.

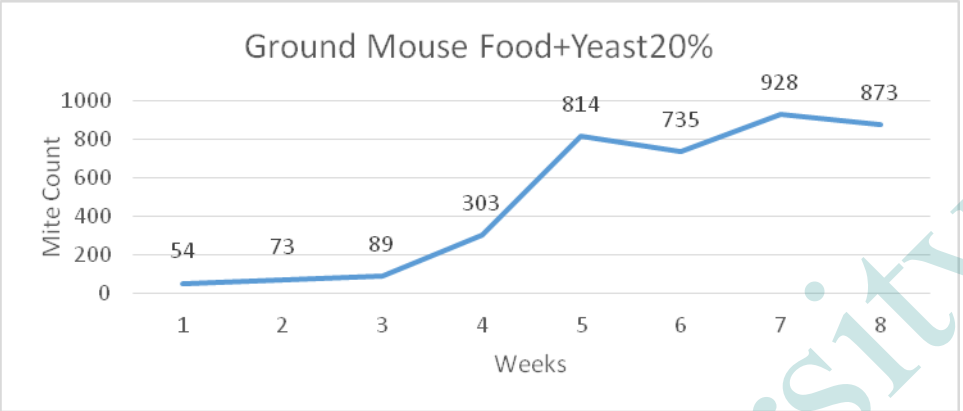

Figure 9 Growth of mite population in control+ yeast 20 percent group phase 2

Mite population in this group was slightly less than previous group.

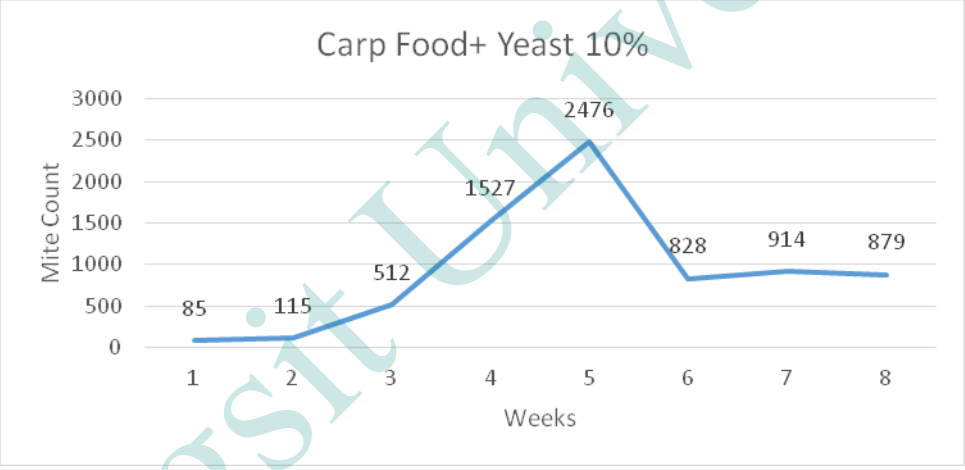

Figure 10 Growth of mite population in carp food+ yeast 10 percent group phase 2

Mite population grew well at this group and give the highest yield among other groups (p value <0.0001).

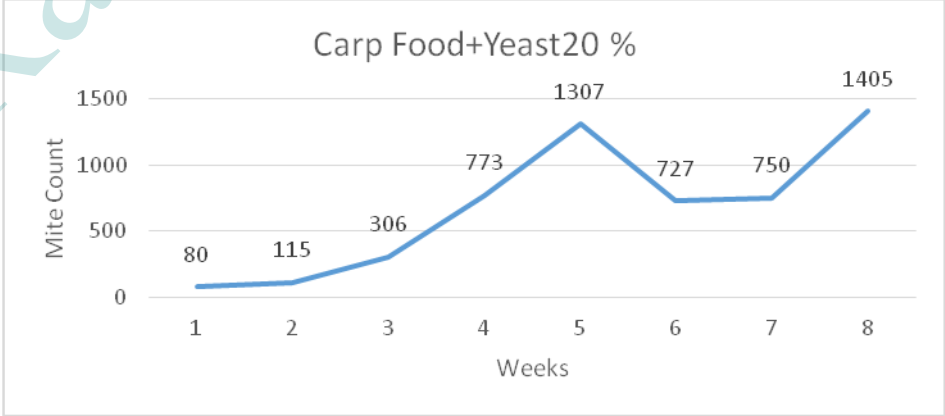

Figure 11 Growth of mite population in carp food+ yeast 20 percent group phase 2

This group grew less well than the group in figure 10. However this was more than the group with ground mouse food.

5. Discussion

Mite rearing requires optimum condition such as humidity and temperature as well as food availability. We evaluated the influence of food medium to the production yield of dust mite culture, in accordance with the previous study by Ree (Ree et al., 1997), which indicated that selection of food medium is an important factor of successful mite culture along with optimum temperature and humidity (Arlian, 1992; Crowther et al., 2006; Mahakittikun & Wongkamchai, 2009; Manuyakorn, 2015). Our result indicated some food sources which were suitable for mite culture but some food sources were not because mites could not thrive with this kind of food. Rice bran is not suitable because this food may be indigestible for mites, meanwhile powdered soy bean provided protein but other nutrient which was suitable for mite was still questionable. In case of food was suitable for mite to thrive and breed, the mite population became swell and gave production yield (Avula-Poola et al., 2012). The effect of supplement, referring to yeast, could be observed in group. only 10% by weight of yeastgave the highest yield due to the rich nutrient in yeast which supported mite population in thriving and breeding. Too much quantity of yeast was detrimental to yield probably due to the dilution effect of nutrient and too high humidity which promoted fungal growth that competed for available food with mites. The fine ground nature of carp food also influenced the population growth of mites by providing more available food compare for ground mouse food at the same weight (personal observation). Combination with rich nutrient and more available food allowed mite population to grow at maximum capability.

6. Conclusion

We can increase the mite production yield by optimum temperature, humidity and food medium selection. Temperature and humidity were kept at optimum to evaluate the effect of food medium alone; therefore, the food which gave the highest yield can be tested. We found that carp food with optimum yeast mixture gave the highest yield of mite and used lesser amount of yeast that previous study (Ree et al., 1997).

7. Acknowledgements

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8. References

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