

Chemical Taxis of Dust Mites
~ Considering How to Get Rid of Dust Mites by Using Similar Substances
to Sex Pheromones or Fragrance ~

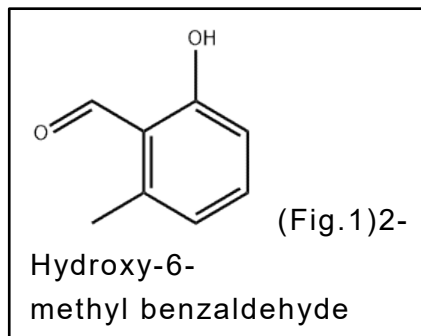
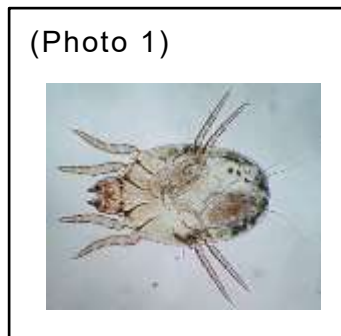
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Abstract

About 50% of Japanese people suffer from dust mite allergies. The purpose of this study is to find ways of decreasing the number of dust mites in homes. We can prevent dust mites from breeding by separating male dust mites and female dust mites. We investigated which material attracts only male dust mites in the same way dust mite attract each other using sexual pheromones. However, it was difficult to distinguish male dust mites from female dust mites, so we looked for materials which attract both of them. Fruit extract is used in a mite catching sheet on the market, so we examined the chemical taxis of dust mites to fruit candies. Our data suggested that dust mites are attracted by pineapple, peach, strawberry, and grape candies. Green apple and orange candies do not attract them.

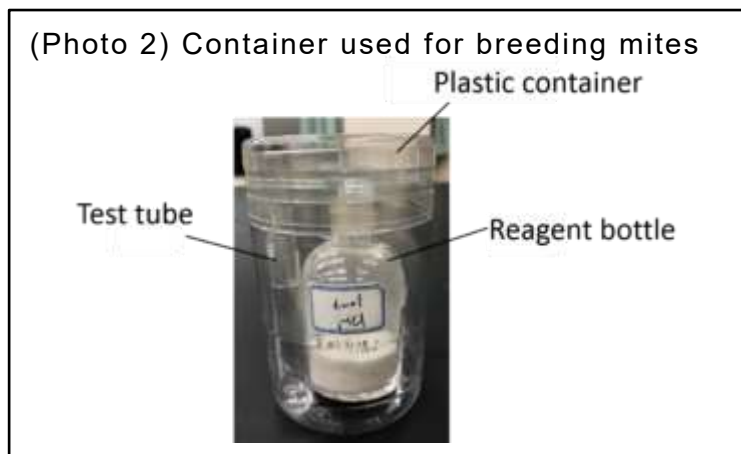
1. Research background and purpose

In recent years, allergic reactions such as urticaria and dyspnea have been caused by acaridaes. They breed in wheat flour and okonomiyaki flour stored at room temperature. Also, infectious diseases caused by mites living in grass and mountains have become social problems. Based on this, we decided to consider how to remove mites. In this study, we used *Dermatophagoides farinae* (Photo 1) provided by The Acarological Society of Japan. *Dermatophagoides farinae* is a type of dust mite that inhabits furniture such as beds and carpets. The corpses and excrement become allergens and have a bad effect on human. Previous studies have shown that *Dermatophagoides farinae* has a male-attracting (chemical taxis) pheromone called 2-Hydroxy-6-methylbenzaldehyde (Fig. 1). A sex pheromone is a type of pheromone which attracts the opposite sex. Dust mites can be attracted and removed using 2-Hydroxy-6-methylbenzaldehyde. However, 2-hydroxy-6-methylbenzaldehyde is expensive, so a cheap alternative, which has a similar structure to sex pheromones, needs to be found. We conducted research with the aim of finding substances that attract dust mites.



2. How to raise *Dermatophagoides farinae*

- (1) Put okonomiyaki <Hakubaku Co., Ltd.> as feed in a 120 mL reagent bottle, put a small amount of the breeding medium on it, and cover it. At this time, air is allowed to pass.
- (2) In order to maintain the optimum humidity (60-80%) for mites, prepare a 10 mL test tube containing saturated saline solution.
- (3) Put the reagent bottle of (1) and the test tube of (2) in an 8 cm x 14 cm plastic container. Keep them in an incubator (800-20000 Lux) <Nippon Ika Kikai Seisakusho> for a 12h : 12h, light to dark period, at 25°C.

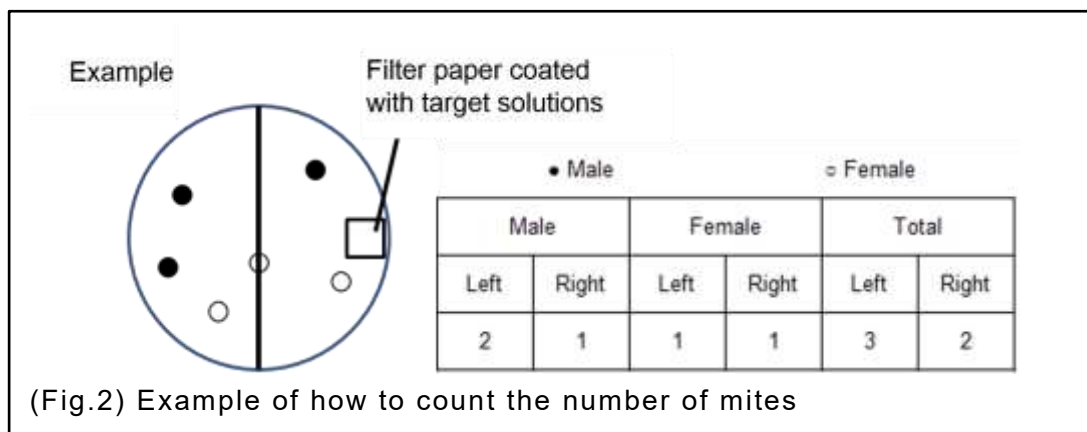


3. Experiment 1 (Search for attractants Search I)

3-1. Method

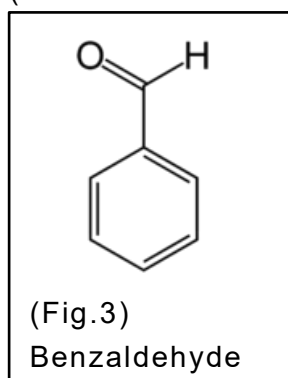
- (1) Put 0.01 g *Dermatophagoides farinae* together with the breeding medium in a $\phi 60$ mm petri dish (number of mites: 300 to 400), spread it, and then turn the petri dish upside down to drop the breeding medium. The *Dermatophagoides farinae* that remained without falling from the petri dish were used for experiments.
- (2) Add 5 μ L of the solution to a 9 mm \times 9 mm filter paper using a micropipette and place it on a corner of the petri dish containing *Dermatophagoides farinae*.
- (3) Store the petri dish for 24 hours in an incubator kept at a suitable temperature and humidity for mites.
- (4) After 24 hours, observe the petri dish with a microscope and attach a blue

sticker if the tick found is a male and a red sticker if the tick found is a female to the top lid of the petri dish so that the taxis can be visually discriminated. (5) Divide the petri dish in half and count the number of ticks, defining those on the side with the filter paper as having shown chemotaxis.



[Substance to be examined]

- Benzaldehyde (Fig. 3) (Diluted with ethanol in *5 steps)
 - Oil-based pen <Zebra Co., Ltd.> (black, red, blue)
 - Fruit extract (Diluted attractant for commercially available mite catching sheet)
 - Ethanol
 - Vanilla essence (Diluted in *5 steps with undiluted solution / ethanol)
- (*: Dilution ratio is 0.02%, 0.05%, 0.10%, 0.15%, 0.20%)

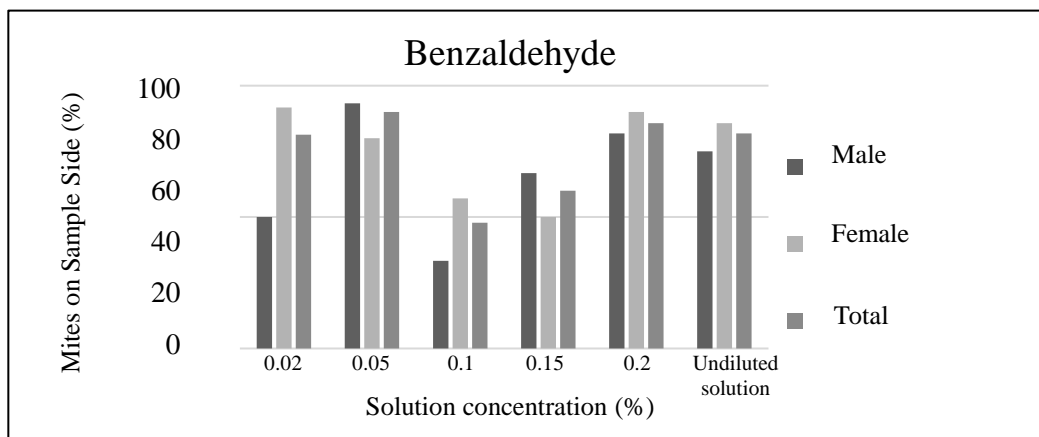
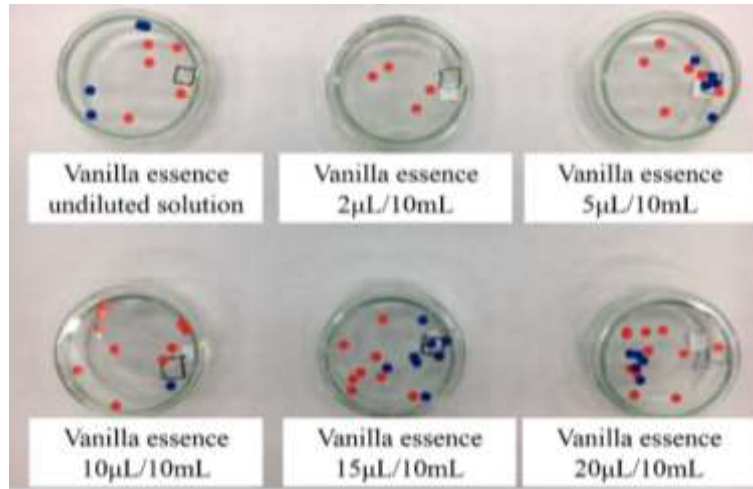


Benzaldehyde, which has a structure similar to that of 2-hydroxy-6-methylbenzaldehyde, which is the sex pheromone of *Dermatophagoides farinae*, is used in this studies (Reference (2)). It is a component of almond and apricot kernel scents and emits a sweet scent. Similarly, vanilla essence also emits a sweet odor.

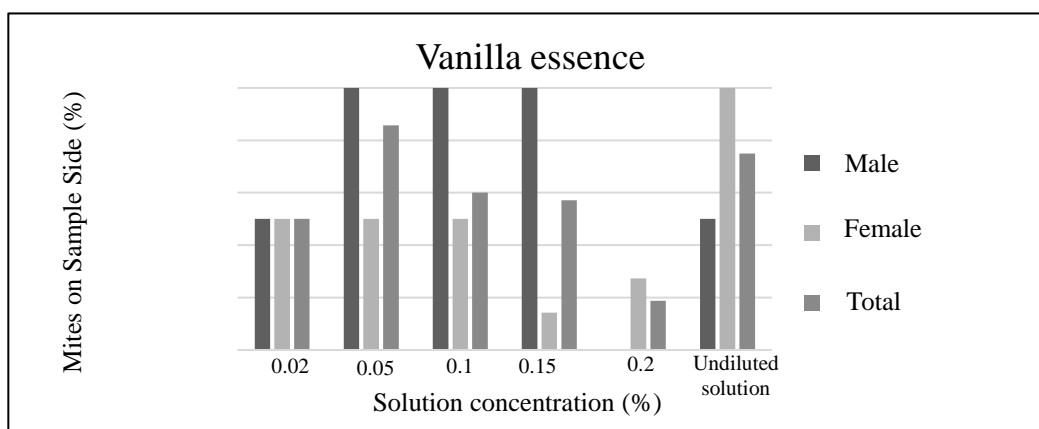
3-2. Experimental results and discussion

One experiment was performed for each substance. As a result, benzaldehyde attracted both sexes regardless of the degree of dilution. In addition, vanilla essences attracted mites at some concentration.

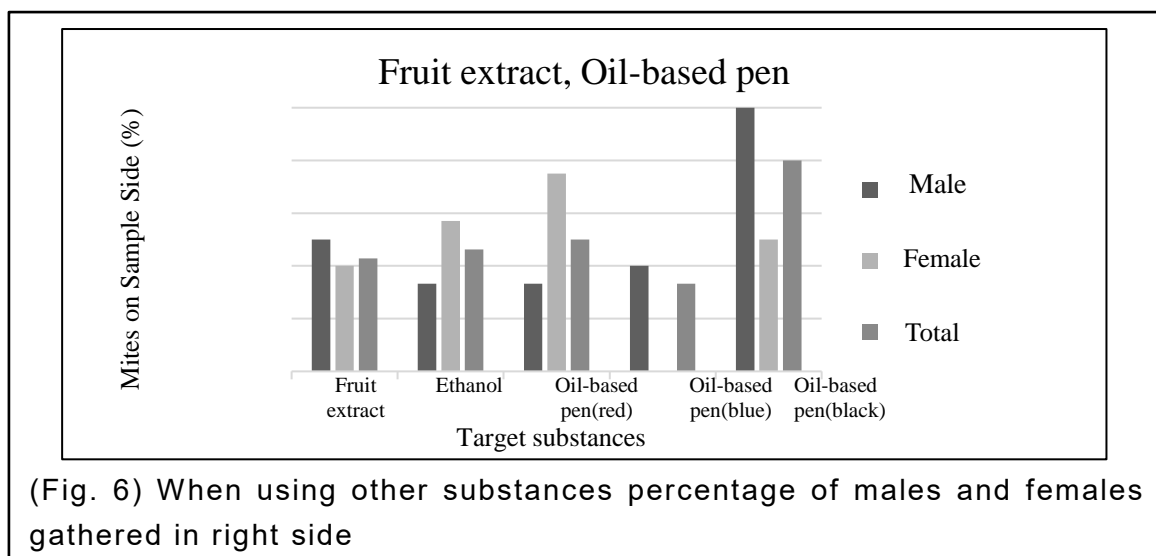
(Photo 3)



(Fig.4) When using benzaldehyde percentage of males and females gathered on the side



(Fig.5) When using vanilla essence percentage of males and females gathered



In FIGS. 4, 5 and 6, when the proportion of individuals gathered on the side with the filter paper exceeded 50%, it was judged to have positive chemical taxis.

From the results, it was not possible to confirm the difference between males and females, but positive taxis against benzaldehyde and vanilla essence were observed. It is considered that this is because benzaldehyde has the basic structure of the mite sex pheromone and vanillin also has a similar structure, so that it binds to the receptor of the sex pheromone. However, it is probable that the reason why the mite catching sheet could not attract mites was that the components of the mite catching sheet were diluted to allow it to soak into the filter paper. In this experiment, since the petri dish is divided in half and the number of mites is counted, there are many mites at positions that are difficult to distinguish. Therefore, as a method for measuring more accurate chemical taxis, we decided to carry out the experiment again by the following method.

Males and females are distinguished by the difference in the structure of the tail end. It is difficult to distinguish them. In addition, since the difference between males and females in taxis was not significant, we decided to examine only the number of mites in the next experiments.

4. Experiment 2 (Search for attractants II)

4-1. Method

The experiment was carried out by improving the method. We examined benzaldehyde, vanilla essence, and vanillin, which is the main component of the scent of vanilla, four times each.

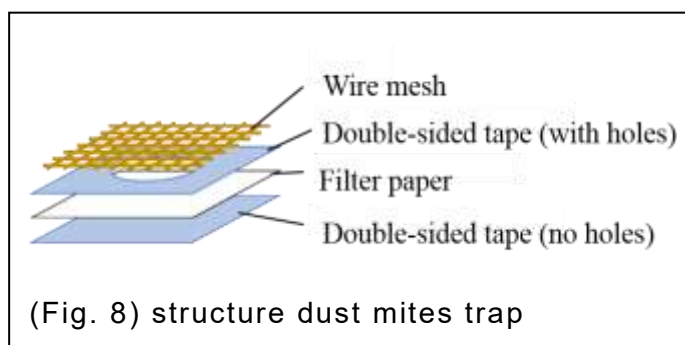
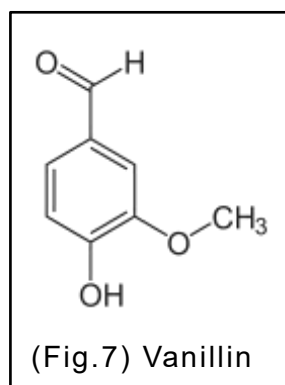
(1) Prepare one 8 mm square filter paper, two double-sided tapes, and one wire mesh, and make a hole with a diameter of 5 mm in one of the two double-

sided tapes.

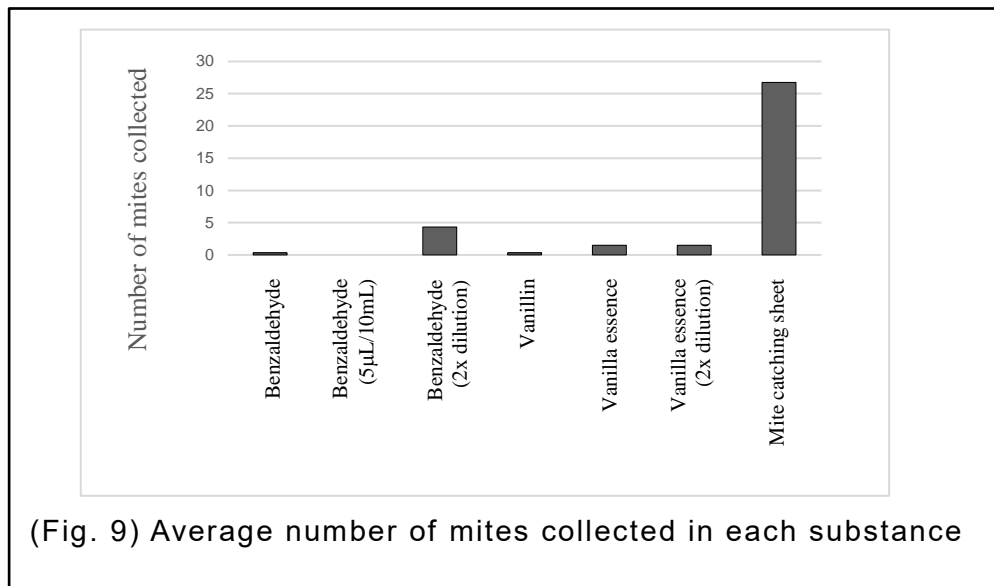
- (2) Place the filter paper from (1) between two double-sided tapes and bond them together, and attach them to the edge of the container (14 cm x 21 cm x 7 cm) with the side with the double-sided tape with holes facing up. If the sample is a solution, soak the filter paper with 5 μL of the solution. If the sample is powder, place the powder on filter paper. If the sample is solid and not powder, place it on the tapper as is.
- (3) Stick a wire mesh on the filter paper from (2) (do not stick it on the solid that was left as it is), and put 0.01 g of mites together with the medium 5 cm from the filter paper. Then close the lid.
- (4) Keep the mites at a suitable temperature and humidity and leave it for 24 hours until the mite's taxis is observed.
- (5) After 24 hours, check the number of mites on the filter paper or sample. (Fig. 11) Structure of tick trap (Photo 4) Inside the tapper (Left: Sample center powder: Tick medium)

[Substances to be examined]

- Benzaldehyde
- Vanilla essence
- Vanillin (solid) (Fig. 8)
- Mite catching sheet



4-2. Experimental results and discussion (Fig.9)



Clear taxis against mite catching sheet was observed, but other than that, there was no clear taxis other than the slightly diluted benzaldehyde (Fig. 2). From this, it is considered that the method using the Petri dish in Experiment 1 could not measure the appropriate chemical taxis. On the other hand, the fact that the mite taxis could be observed on the mite catching sheet confirms the effectiveness of this implementation method. Next, in order to find the reason why mites are attracted to the mite catching sheet, the components contained in the mite catching sheet were investigated, and it was thought that they might be concentrated in the fruit extract used as an attractant. Therefore, we next decided to investigate the taxis of fruits.

5. Experiment 3 (Experiment on fruits)

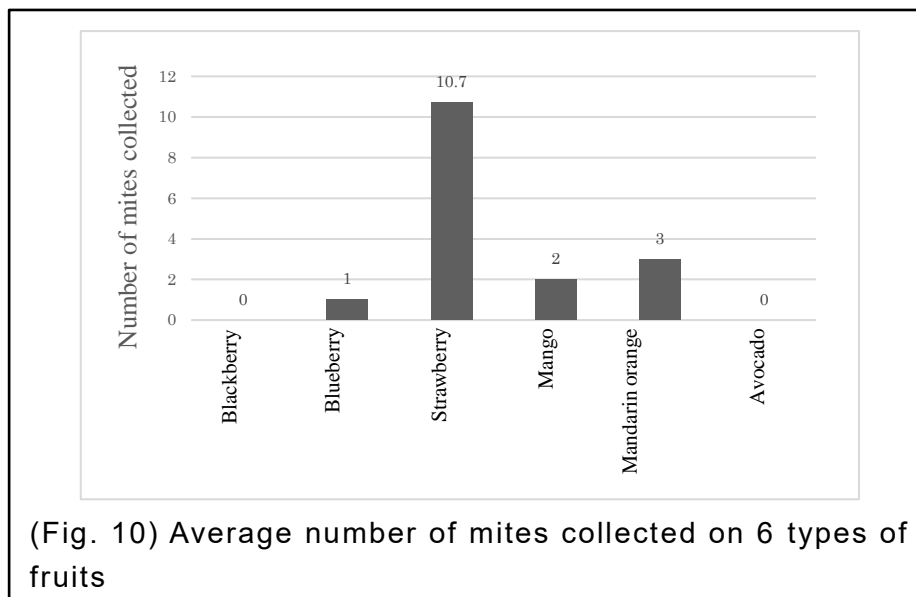
5-1. Method

Experiments were conducted three times each on strawberries, blackberries, blueberries, mandarins, avocados, and mangoes.

[Fruits used]

- Strawberry
- Blackberry
- Blueberry
- Mandarin orange
- Avocado
- Mango

5-2. Experimental results and discussion (Fig. 10)



The taxis of the experiment was evaluated by the average number of individuals three times each. The positive taxis for strawberries was greater than that of other fruits (Fig. 13). The fact that the fruit extract on the mite catching sheet is an attractant suggests that the fruit scented substance may be attracting mites rather than the fruit itself. Therefore, instead of fruit extract, we conducted a taxis experiment using fruit candy with a scent of fruits such as strawberries

6. Experiment 4 (Experiment I for fragrances)

6-1. Method

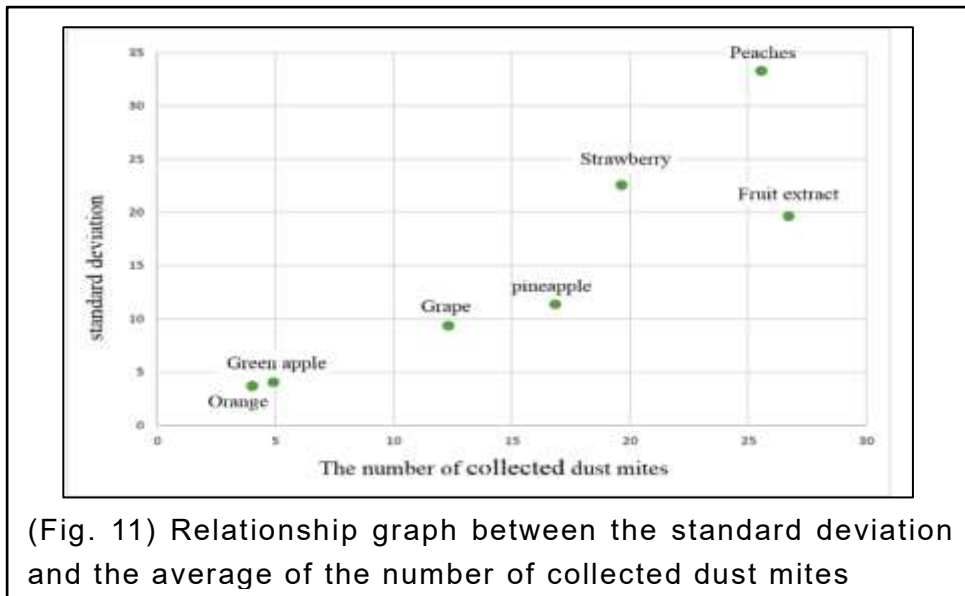
The method was the same as in Experiment 3. Experiments were conducted eight times each.

[Substances used in experiment]

- Candy (Grape, Strawberry, Peach, Pine, Orange, Green Apple)
- Fruit extract of sheet catching mites
- Glucose

As a comparison of the experiments, we also examined the attractant of the mite catching sheet and glucose in the same manner.

6-2.Result and discussion (Fig.11)



The runnability of the experiment was evaluated from the relationship between the standard deviation and the mean. From the relationship graph between the standard deviation and the average (Fig. 11), it is considered that the candy has a taxis for peach candy, strawberry candy, pine candy, and grape candy.

7. Experiment 5 (Experiments II for fragrances)

7-1. Method

Experiment 2 was modified as follows to simplify the preparation of the attractant side.

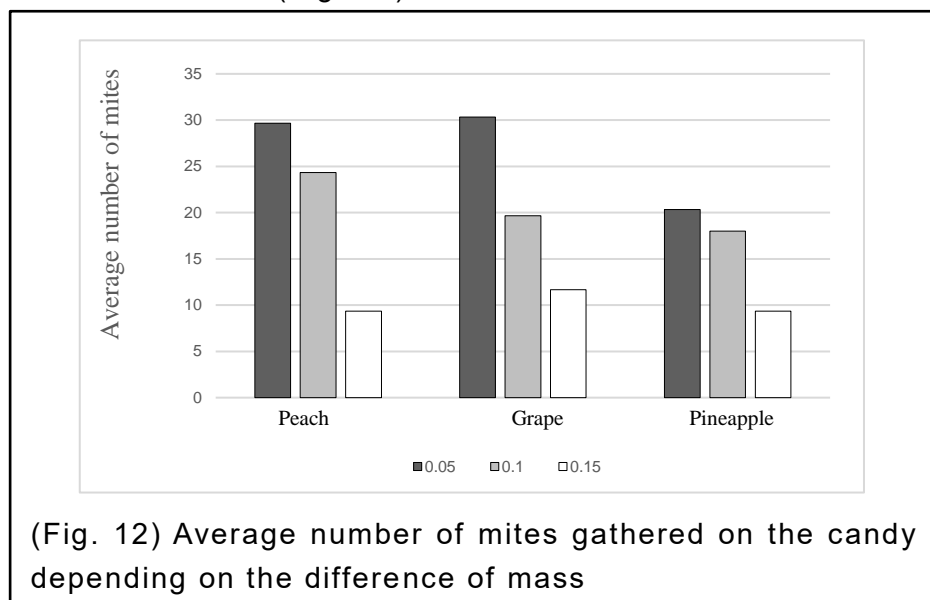
- (1) Stick a piece of 8 mm square double-sided tape on the edge of the container and put the substance to be examined on the tape. If the substance to be tested is a solution, place an 8 mm square piece of filter paper on the tape and soak the paper with 5 μ L of the solution.
- (2) Place 0.01g of mites with the medium at 5cm from the filter paper and close the lid.
- (3) Keep the container at the suitable temperature and humidity for mites.
- (4) After 24 hours, count the number of mites on the filter paper or on the sample.

[Fragrance-emitting substances used]

Candy (grape, peach, pineapple)

7-2. Experimental results and discussion

For the peach, grape and pineapple candies, three experiments were conducted, each measuring 0.05g, 0.01g and 0.15g, respectively, using the method described in Experiment 5, and the results were evaluated by the average number of mites. (Fig. 12)



(Fig. 12) Average number of mites gathered on the candy depending on the difference of mass

Since the difference of chemical taxis between the different masses was small, the data of the result of 6-2 is reasonable. In other words, mites are considered to show chemical taxis to the peach, pineapple and grape candies.

There is no difference in the chemical taxis to the three candies depending on the concentration or mass. Moreover, the standard deviation increases with the increase of the chemical taxis, as shown in Fig. 11. These facts suggest that the mites might have been attracted to the candies because they happened to encounter the sugar and not the scent of the candies. In order to rule out the possibility that the mites were attracted to the candies by accidental encounters with the sugar and not by the scent of the candies, experiments were carried out with salt water, salt, glucose and with nothing. The results showed that the mites did not come close to these substances.

It was reconfirmed that the modified and simplified method of experiment 5 itself is significant.

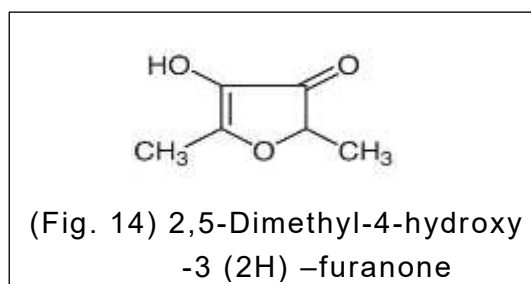
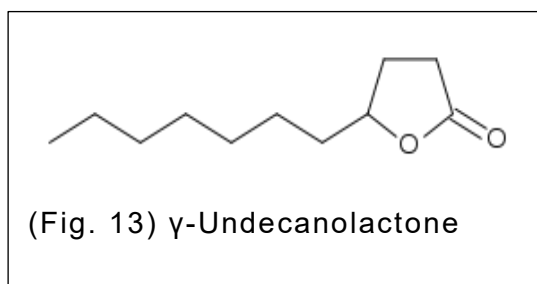
8. Experiment 6 (Search for attractant III)

8-1. Method

The method of Experiment 5 was evaluated to be significant, so we conducted the experiment using this method.

We considered which substance contained in each candy attracts mites and examined the movement of mites to γ -Undecanolactone (Fig. 13) and 2, 5-Dimethyl-4-hydroxy-3 (2H) -furanone (Fig. 14). γ -Undecanolactone is a

component peculiar to the fragrance of pine candy, and the fragrance of strawberry candy and pine candy. 2, 5-Dimethyl-4-hydroxy-3 (2H) –furanone is a component of the fragrance of strawberry candy and pine candy.



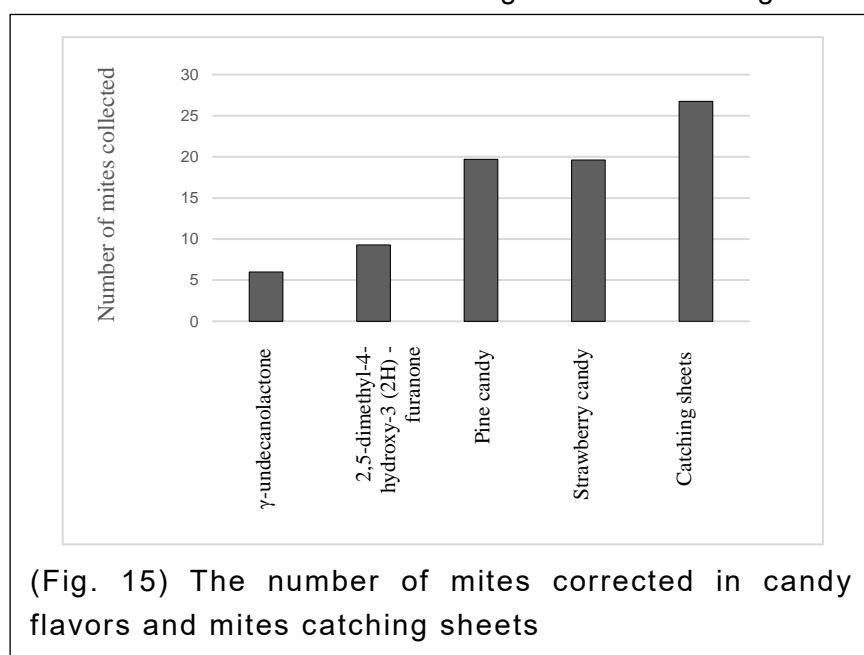
[Substances to be examined]

- Candy(pine, strawberry)
- Attractant of mites catching sheet
- γ -Undecanolactone
- 2,5-Dimethyl-4-hydroxy-3 (2H) –furanone

Experiments were conducted 7 times each.

8-2. Experimental results and discussion

From Fig. 15, γ -undecanolactone and 2, 5-dimethyl-4-hydroxy-3 (2H) -furanone showed some taxis, although the number of mites attracted was smaller than that of candy. Therefore, it is considered to be one of the substances that attract mites. Since the number of dust mites attracted to pine candy and strawberry candy is larger than that of these components, it is considered that the combined flavors have higher taxis. It is also considered that the result is that the value of the tick catching sheet is the largest.



9. Conclusion

Mites have shown taxis in pineapple candy, peach candy, strawberry candy, and grape candy, but at this time no substance has been found that is superior to the mite catching sheet. It was found that the chemical taxis of mites is enhanced by the combination of each material component. Among them, although there are variations in the results, since peach candy attracts a comparable number of mites compared to mites catching sheets, we think that it is possible to establish a cheaper trap that attracts mites using peach candy. In addition, under observation, it was observed that the ticks gathered on the candies were unable to move due to the stickiness of the candies, which suggests that inexpensive and sticky candies may be a practical method of tick removal. In the future, we would like to develop a simple device to capture ticks using candy.

10. Future prospects

In this study, an experiment was conducted without separating the medium and mites so it was difficult to distinguish between the medium, okonomiyaki flour and mites. Therefore, we would like to establish a method for separating the mites and the medium. In addition, it is thought that mites show stronger chemical taxis depending on the composition of scents, so we would like to consider the best combination to attract dust mites. Furthermore, in this research, only the number of mites collected in the sample was counted, but in order to obtain more accurate data, we would like to carry out the research to show the distance mites have moved.

11. References

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12. Key words

Dermatophagoides farinae

chemical taxis

2-Hydroxy-6-methylbenzaldehyde

Benzaldehyde

vanilla essence

fruit extract

mite catching sheet

γ -Undecanolactone

2, 5-dimethyl-4-hydroxy-3(2H)-furanone