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ORIGINAL ARTICLE

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Effective period of volatiles from softwood veneers embedded in tatami mats on the activity of house dust mites

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Abstract To suppress the activity of house dust mites in tatami mats, where they tend to breed, tatami mats were prepared with embedded softwood veneers. The effective period of the volatiles from the veneers on the mites was then measured. To investigate the effective period of volatiles from hiba (*Thujopsis dolabrata* var. *hondai*) and hinoki (*Chamaecyparis obtusa*) veneers on house dust mites (*Dermatophagoides pteronyssinus*), 5-day exposure tests were conducted every few weeks for 54 weeks. In the exposure tests, the activity of the mites was observed after 5 days of exposure to the volatiles. Volatiles from hiba veneers strongly suppressed the activity of the mites for 15 weeks. The suppressive effect decreased gradually after that, but it was maintained for 54 weeks. Volatiles from hinoki veneers showed moderate to high suppression of mite activity for 11 weeks, but no suppressive effect was observed after that. In conclusion, embedding hiba or hinoki veneers in tatami mats is an effective method of suppressing the activity of mites for about a year or for slightly less than 3 months, respectively.

Key words Softwood veneer · House dust mite · *Dermatophagoides pteronyssinus* · Volatiles · Effective period

Introduction

In 1964, Voorhorst et al.[1] found that many of the allergic diseases that have recently become serious health problems are caused by house dust mites.[2] Since then, many reports have been published on the relation between allergic diseases and house dust mites. Sakamoto[3] reported that eliminating contact with the mite allergens is important in controlling allergic diseases caused by house dust mites, and he offered specific suggestions such as the daily cleaning of rooms and minimal use of carpeting.

Insecticides have been effectively used to kill mites, but they pose other health risks. For this reason, the effect of certain plant and wood oils on house dust mites has received much attention.[4–10] McDonald and Tovey[4] examined the effects of five essential plant oils (citronella, eucalyptus, spearmint, tea tree, and wintergreen oils) as laundry additives for killing house dust mites. They suggested that dilute solutions of essential oils were potentially an effective, acceptable, and inexpensive method of controlling mites. In addition, they reported that in laboratory experiments, more than 80% of house dust mites (*Dermatophagoides pteronyssinus*) were killed after immersion in 0.2% and 0.4% solutions of eucalyptus oil for 30 and 60 min, respectively, and that machine washing of woolen blankets in a 0.2% eucalyptus oil solution for 30 and 60 min killed 97% and 99% of mites.[5] Ottoboni et al.[6] evaluated ten essential oils and found the most effective ones for exterminating *D. pteronyssinus* were caraway, garlic, black pepper and Peru balsam. Takaoka et al.[7] kept *D. pteronyssinus* and *D. farinae* in a mixture of culture media and the sawdust of seven species of wood that are commonly used as construction materials, and they counted the number of the living mites every 10 days. As the number of living mites in the mixture decreased sharply, they found that the oils of hinoki (*Chamaecyparis obtusa*), sugi (*Cryptomeria japonica*), Douglas-fir (*Pseudotsuga menziesii*), eastern red cedar (*Juniperus virginiana*), and western red cedar (*Thuja plicata*) strongly suppressed the propagation of the mites. They also found that the oils of sitka spruce (*Picea sitchensis*) and western hemlock (*Tsuga heterophylla*) had small effects on the mites. (It was not mentioned in their study whether the activity of living mites was classified as walking or moving, as performed in this study.) Miyazaki et al.[8,9] reported that exposure to essential oils suppressed walking and moving of *D. pteronyssinus* and

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D. farinae, especially wood oils obtained from hinoki, Douglas-fir, western red cedar, and hiba (*Thujopsis dolabrata* var. *hondai*), but wood oils from sugi and western hemlock had only small effects on the mites. Chang et al.[10] reported that *Taiwania cryptomerioides* essential oil was effective in exterminating *D. pteronyssinus*.

Thus, some studies have reported the effect of certain plant and wood oils on house dust mites, but there are few studies on the effect of volatiles directly obtained from wood. Enomoto et al.[11] used eastern red cedar balls (diameter 7 mm) and chips and showed that their volatiles repelled *D. farinae*. Hiramatsu and Miyazaki[12] showed that volatiles from hiba, hinoki, and kusunoki (*Cinnamomum camphora*) chips strongly suppressed walking and moving of *D. pteronyssinus*. For practical use of volatiles from wood, Mori and Miyazaki[13] prepared tatami mats embedded with softwood veneers and examined the suppressive effect of volatiles from the veneers on *D. pteronyssinus*. They found that the volatiles from the hiba and hinoki veneers strongly suppressed walking and moving of the mites, and that sugi caused moderate suppression. They suggested that tatami mats embedded with softwood veneers was useful for suppressing the activity of mites.

In the experiments, volatiles obtained directly from wood had an effect on mites for a short time. However, it is important to examine the effective period of volatiles for their practical use. Therefore, in this study, the effective period of volatiles from softwood veneers embedded in tatami mats on *D. pteronyssinus* was measured, using tatami mats of the same specifications as those used in the study of Mori and Miyazaki.[13]

Materials and methods

Adult female house dust mites, *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae), were cultivated in a mixture of powdered animal food (CE-2; Clea, Japan) (50%) and dry yeast (Ebios, Asahi Beer Pharmaceutical) (50%) culture media.

The exposure chambers (Fig. 1), which were modified versions of the rearing containers used by Matsumoto et al.,[14] were made as follows: a filter cloth (Toray; Axtar, H306-10, 15 × 15 mm) was placed on a polyacrylate resin plate (75 × 25 mm, thickness 1 mm) with a 10-mm diameter hole in the center. A polyacrylate plate of the same size (thickness 3 mm) was placed on the filter cloth. About ten mites were put into the hole and on the filter cloth. The hole of the polyacrylate plate was covered with a glass plate of the same size (thickness 1 mm). The plates were held with double clips at both ends. The mites were exposed to the volatiles in the chambers.

Tatami mats (450 × 450 mm, thickness 95 mm), in which three sheets of softwood veneers (150 × 450 mm, thickness 2 mm), hiba (*Thujopsis dolabrata* var. *hondai*) or hinoki (*Chamaecyparis obtusa*), were embedded, were used for the exposure test. These softwood veneers had been kept for a month in a room without air conditioning after being manufactured. The tatami mats were separable into the upper part (thickness 20 mm) that had 6 cavities for arranging the chambers in their regular positions and the lower part (thickness 75 mm) in which softwood veneers were fixed, so that the chambers could be placed on the veneers directly. In order to diminish contact with the allergen, it is important to reduce the mites that live in the upper levels of tatami mats. Therefore the tatami mats were divided into a 20-mm upper part, and a 75-mm lower part.

To investigate the suppressive effect of volatiles from two softwood veneers on the activity of the mites, 5-day exposure tests were conducted. Six exposure chambers were placed on each of the two species of softwood veneers embedded in the tatami mats (Fig. 2). Tatami mat without embedded veneers was used as a control sample. The degree of activity of the mites was classified into three categories: (1) walking, (2) moving (legs, chelicerae, pedipalpi, etc.), and (3) immobilized. The activity of the mites was

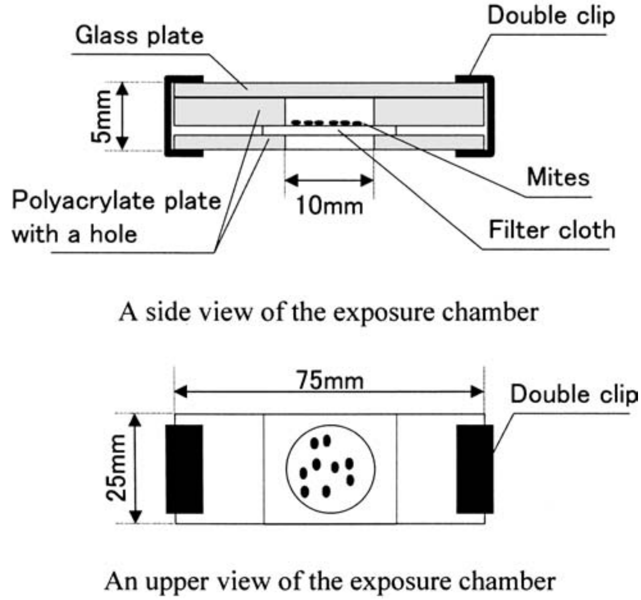

Fig. 1. Schematic illustrations of the exposure chamber for testing the effect of volatiles from softwood veneers embedded in tatami mats on house dust mites

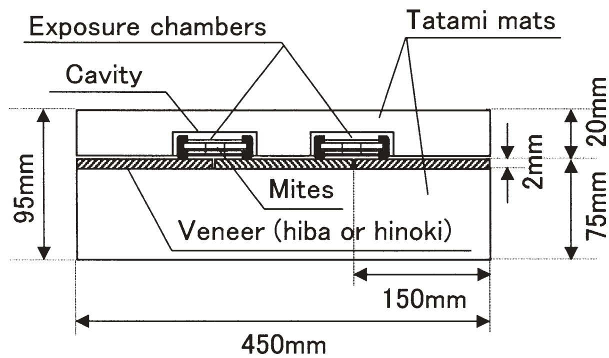

Fig. 2. Schematic illustration of the cross section of the tatami mats embedded with softwood veneers

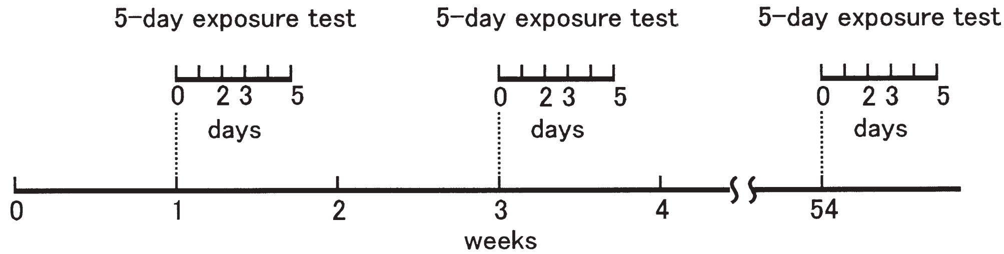

Fig. 3. The method of investigating the effective period of volatiles from softwood veneers on the activity of mites. In each 5-day exposure test, the activity of mites was measured after 2, 3, and 5 days of exposure. Exposure tests were conducted every several weeks for 54 weeks

measured by microscope after 2, 3, and 5 days of exposure. Difference between values was analyzed by the Student's *t*-test, and was considered significant when the *P* value was less than 0.05 compared with the control. The tests were conducted in desiccators (500 × 500 × 500 mm). The cavities in the upper part of tatami mats where the chambers were placed were conditioned to about 25°C and 60% relative humidity (RH).

To investigate the effective period of the suppressive effect of the volatiles from the veneers, 5-day exposure tests were conducted every several weeks for 54 weeks (Fig. 3). New exposure chambers were prepared at the start of each 5-day exposure test, and the mites in the culture media were placed in them. The same tatami mats and veneers were used. They were kept in a controlled room at a temperature of 23°C and 60% RH, except during the exposure test period. Difference between values was analyzed by the Student's *t*-test, and was considered significant when the *P* value was less than 0.05 compared with the control. The exposure tests were conducted from July 2000 to August 2001.

Results

The percentages of walking and moving *Dermatophagoides pteronyssinus* after 5 days of exposure for each 5-day exposure test are shown in Fig. 4. The activity of the mites was not suppressed in the control (tatami mat without veneers), and the percentage of walking and moving mites was 68%–78% in each exposure test. Volatiles from the hiba veneers strongly suppressed the activity of *D. pteronyssinus* in the tests of the 1st to 15th weeks, and significant difference from the control ($P < 0.05$) was seen. However, their suppressive effect gradually decreased with time (Fig. 4a), and the effect was moderate in the tests of the 31st and 54th weeks. The suppressive effect of the volatiles from hinoki veneers on *D. pteronyssinus* also gradually decreased as time passed (Fig. 4b). In the 5-day exposure test of the 1st week, hinoki strongly suppressed the activity of the mites and significant difference from the control ($P < 0.05$) was seen. However, in the tests of the 3rd to 15th weeks, the effect was moderate, and in the tests of the 31st and 54th weeks, it was low and significant difference from the control ($P < 0.05$) was not observed.

The percentages of walking mites are shown in Fig. 5. For the control (tatami mat without veneers), the percent-

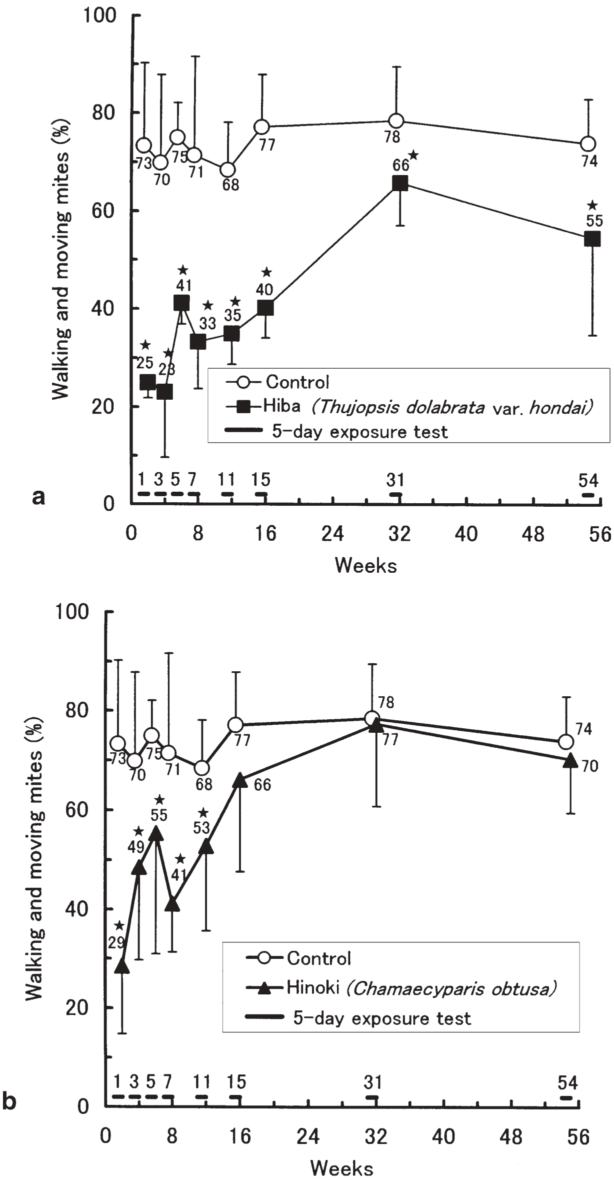

Fig. 4. The percentage of walking and moving mites after 5 days of exposure to volatiles from hiba (**a**) and hinoki (**b**) veneers embedded in tatami mats. Significant difference from control is marked with a *star* ($P < 0.05$ by Student's *t*-test). Standard deviation is marked with a *bar*

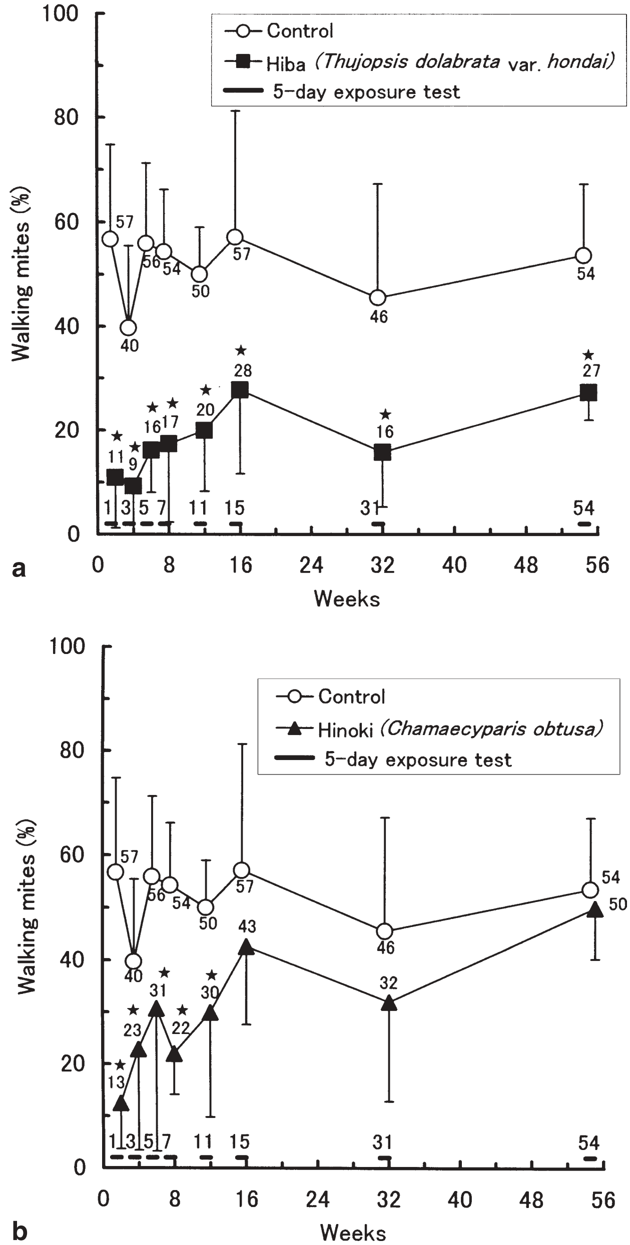

Fig. 5. The percentage of walking mites after 5 days of exposure to volatiles from hiba (**a**) and hinoki (**b**) veneers embedded in tatami mats. Significant difference from control is marked with a *star* ($P < 0.05$ by Student's *t*-test). Standard deviation is marked with a *bar*

age of walking mites was 40%–57% after 5 days of exposure in each test. For tests with hiba, the percentage of walking mites was 11% in the test of the 1st week and 27% in the test of the 54th week (Fig. 5a). For 54 weeks, hiba strongly suppressed walking activity, and significant difference from the control ($P < 0.05$) was seen. The results in which the number of moving mites increased as time passed, but there were few walking mites in each exposure test, indicate that the volatiles from hiba strongly suppressed the activity of the mites, especially their walking, and it was maintained for 54 weeks. Volatiles from hinoki suppressed walking activity in the tests of the 1st to 11th weeks (Fig. 5b); the percentage of walking mites was 13%–31%, and significant difference from the control ($P < 0.05$) was seen. However, in the tests after the 15th week, the suppressive effect decreased, and significant difference from the control ($P < 0.05$) was not observed. The number of walking mites increased with time and it appeared that volatiles from hinoki did not suppress walking activity to a great degree.

In the 5-day exposure test, the activity of *D. pteronyssinus* was measured after 2, 3, and 5 days of exposure. The results of the 5-day exposure tests of the 3rd, 11th, 31st, and 54th weeks are shown in Fig. 6. The suppressive effect of hiba on the activity of the mites was strong in the tests of the 3rd and 11th, weeks, and it was moderate in the tests of the 31st and 54th weeks. In the test of the 3rd, 11th, and 31st weeks, the percentages of walking and moving mites decreased to about 60% after 2 days of exposure, and after 3 days of exposure, they did not change as much. However, after 5 days of exposure in the test of the 3rd and 11th weeks, the percentages of the walking and moving mites decreased to 23% and 35%, respectively. On the other hand, in the test of the 31st week, the percentage of the walking and moving mites did not decrease as much. The suppressive effect of hinoki on the activity of the mites was moderate in the tests of the 3rd and 11th weeks. After 2 days of exposure, the percentages of the walking and moving mites decreased, but after that they did not decrease as much. In the tests of the 31st and 54th weeks, a suppressive effect was not observed.

Discussion

In the test of the first week, the volatiles from the hiba and hinoki veneers embedded in the tatami mats strongly suppressed the activity of *Dermatophagoides pteronyssinus*, and these results are similar to those of other studies. Mori and Miyazaki[13] embedded hiba and hinoki veneers in tatami mats of the same specifications as used in this study to examine the suppressive effect of volatiles from these veneers on *D. pteronyssinus* in a 5-day exposure test. They showed that the volatiles from hiba and hinoki veneers strongly suppressed the activity of the mites, and embedding these veneers in tatami mats is useful in controlling them. They used a tatami mat without veneers and no tatami mat as control samples, and showed that volatiles from the tatami mat had no effect on mites. Miyazaki[9] used hiba wood oil of various concentrations, and examined the suppressive effect on the activity of *D. pteronyssinus*. He showed that the activity of the mites was strongly suppressed by a wood oil concentration that is 12.5% of that found in hiba. Hiramatsu and Miyazaki[12] used wood chips and showed that the volatiles from hiba and hinoki chips strongly suppressed the activity of *D. pteronyssinus*.

Volatiles from hiba and hinoki veneers embedded in tatami mats strongly suppressed mite activity, but with time, their suppressive effect decreased, and a difference between hiba and hinoki was observed. The suppressive effect of hiba on the mites was maintained for 54 weeks, but the

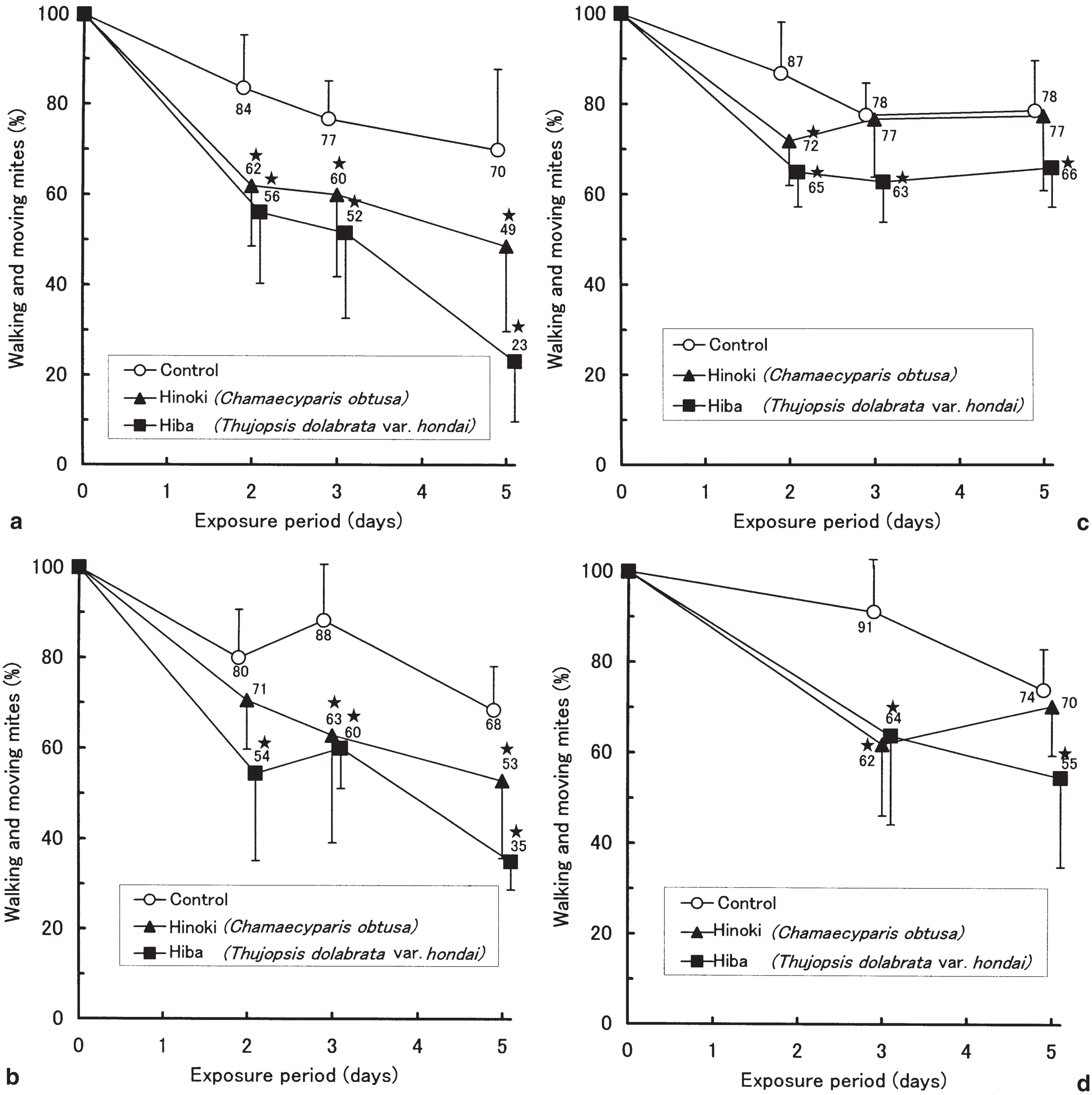

Fig. 6. Changes in the activity of mites during 5-day exposure tests of the 3rd (**a**), 11th (**b**), 31st (**c**), and 54th (**d**) week to volatiles from softwood veneers embedded in tatami mats. Significant difference from control is marked with a *star* ($P < 0.05$ by Student's *t*-test). Standard deviation is marked with a *bar*

effect of hinoki continued for only 11 weeks and was not seen after the test of the 15th week. Thus, the effect of hiba veneers was maintained for longer than that of hinoki. However, both of them were effective for suppressing the activity of the mites.

To use wood volatiles in daily life, not only their effect on mites but also their influence on human comfort should be considered. In this study, the influence of volatiles from hiba and hinoki veneers on humans was not examined, but in other studies, the influence of wood oils and volatiles on humans were investigated. Miyazaki et al.[15] and Yamamoto et al.[16] conducted sensory evaluations to investigate the psychological effect of certain wood oils and showed that the smells of hiba and hinoki wood oils were considered natural. Hiramatsu and Miyazaki[12] conducted sensory evaluations using wood chips and showed that the smell of hiba chips was considered natural, unexciting, and refreshing and that of hinoki was considered natural and refreshing. It appeared that the smell of hiba and hinoki was comforting to human beings.

In conclusion, volatiles from hiba and hinoki veneers embedded in tatami mats suppressed the activity of house dust mites for 54 weeks and 11 weeks, respectively. This suggests that embedding hiba or hinoki veneers in tatami mats where mites tend to breed is effective in controlling mites for about a year or a little less than 3 months, respectively, and the suppressive effect of veneers can be maintained for longer periods by adjusting their shapes and sizes.

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