Evaluation of the acaricidal efficacy of sixteen chemicals to three species of house dust mite, *Dermatophagoides farinae*, *Tyrophagus putrescentiae* and *Blomia tropicalis*, by filter paper contact method

Tomoyuki Hashimoto\(^1\), Naoki Motoyama\(^2\) and Kiyoshi Mizutani\(^1\)

\(^1\) Department of Environmental Biology, Japan Environmental Sanitation Center, Yotsuyakamicho 10-6, Kawasaki-ku, Kawasaki, 210-0828 Japan
\(^2\) Laboratory of Pesticide Toxicology, Faculty of Horticulture, Chiba University, Matsudo 648, Matsudo, 271-8510 Japan

(Received: 21 June 1999; Accepted: 6 November 1999)

Key words: acaricide, LC value, susceptibility, house dust mite, contact method

Abstract: Acaricidal efficacy brought about by contact to chemical residues was compared among 16 chemicals. Phenyl salicylate showed the lowest LC\(_{50}\) values, 9.59 mg (a.i.)/m\(^2\) for *Dermatophagoides farinae*, 12.2 mg/m\(^2\) for *Tyrophagus putrescentiae* and 6.38 mg/m\(^2\) for *Blomia tropicalis*. The LC\(_{50}\) values of synepirin 500, permethrin, cypermethrin and pp'-DDT were not obtained within a dosage of 4,000 mg/m\(^2\) against the 3 mite species. More than ten-fold differences in the LC\(_{50}\) values among the mite species were recognized in \(\gamma\)-BHC, IBTA, fenitrothion and dichlorvos. However, susceptibility of the 3 mite species in the LC\(_{50}\) values was similar in benzyl benzoate, phenyl salicylate and S421. *Blomia tropicalis* indicated the highest susceptibility in the LC\(_{50}\) values to 9 chemicals. Differences in concentration-response characteristics of some test chemicals were also observed.

INTRODUCTION

Some species of house dust mites (HDMs) are recognized as allergenic mites. They play medically important roles by producing mite allergens in their feces and bodies. In order to prevent outbreak of allergies, allergen avoidance and allergen removal are recommended. The use of acaricides has been considered as one mite allergen control strategy.

For the sake of mite control, chemical characteristics of the acaricide have to be understood accurately. The acaricidal efficacy of various chemicals against the HDMs have been assessed in several studies (Tanaka, 1974; Chisaka et al., 1985; Mizutani, 1988; Takahashi et al., 1990; Noda and Hirakoso, 1991; Kalpaklioglu et al., 1996; Tsutsumi et al., 1998). They employed various evaluation methods, e.g., medium-mixing method, contact method and filter paper mixing method, and reported differences in susceptibility among several mite species. It was also suggested that the evaluation of chemicals fluctuated depending upon the test methods used in those studies.

Although the medium-mixing method employed in many studies is appropriate for the evaluation of suppression efficacy of the population growth, it can not evaluate the acaricidal efficacy caused by contact to the chemical residue. For the evaluation of residual activity, the contact method is more suitable. However, little information regarding the residual activi-
ty of the chemicals has been available up to now.

In the current study the filter paper contact method was adopted to evaluate the acaricidal efficacy of 16 chemicals to 3 allergenic mites, *Dermatophagoides farinae* (Df), *Tyrophagus putrescentiae* (Tp) and *Blomia tropicalis* (Bt), which often dominate the HDM fauna.

**MATERIALS AND METHOD**

**Test mites**

All the test mites were laboratory colonies. Both Df and Tp were colonies of the Tokyo Women's Medical College (TWMC). Bt was provided by the Laboratory of Medical Zoology, University of the Ryukyus, in 1998. All colonies were reared on powder food for laboratory mice (Oriental Yeast Co., Ltd.; MF) conditioned at a moisture rate of 12% (W/W) for Df and Bt, and 15% for Tp in glass containers. Individuals clinging to the wall and/or lid of the glass containers were used for the experiments.

**Test chemicals**

The sixteen chemicals examined were: two organophosphates (fenitrothion and dichlorvos), three pyrethroids (permethrin, emmethrin and cypermethrin), three organochlorines (pp'-DDT, dicofol and γ-BHC), an organotin (cyhexatin), five synergists of insecticide (IBTA, S421, pyrethrin 222 and synepirin 500) and benzyl benzoate and its derivative (phenyl salicylate). All chemicals were technical grade or reagent grade. They were dissolved in acetone except for cyhexatin which was dissolved in chloroform.

**Test method**

Filter paper sized to 5 x 10 cm (Toyo; No. 131) was treated with 0.5 ml of each test chemical solution so that the amount of the active ingredient gave the desired concentration. The filter papers were dried for 3-5 h at room temperature.

Thirty adult mites were placed onto the filter paper with a soft writing brush. The filter paper was folded along the center line of the long side and was firmly closed with 3 paper clips to prevent the mites from escaping (Fig. 1).

The filter paper containing the mites was kept in an air-tight container at 25°C for 24 h with a wet paper to maintain high humidity (80-90% RH). Mortality was observed with a binocular, stimulating the adult mites lightly with an insect pin. Only mites that did not move were counted as dead. The mortality rates obtained from each treated plot were corrected with the rates of the control (the solvent only) plots. The 50% and 90% lethal concentration (LC50 and LC90) values were calculated by the Litchfield-Wilcoxon method (Takahashi and Okuda, 1975).

Each chemical was tested at 4-8 dosages and each test was replicated 2-6 times. The data in which the mortality rate of the control plot was more than 10% were omitted from the analysis.

**RESULTS**

The LC values of the 16 chemicals are presented in Table 1. Phenyl salicylate showed the lowest LC50 values against all 3 mite species. On the contrary, the LC50 values of synepirin 500, permethrin, cypermethrin and pp'-DDT were more than...
Table 1. Acaricidal efficacies of 16 chemicals against 3 species of house dust mites by the filter paper contact method.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th><em>Dermatophagoides farinae</em></th>
<th><em>Tyrophagus putrescentiae</em></th>
<th><em>Blomia tropicalis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC₅₀ (95% C.L.)</td>
<td>LC₉₀</td>
<td>LC₅₀ (95% C.L.)</td>
</tr>
<tr>
<td>IBTA</td>
<td>15.2 mg/m²</td>
<td>24.7</td>
<td>518 (452-593)</td>
</tr>
<tr>
<td>Benzyl benzoate</td>
<td>23.5 (21.7-25.5)</td>
<td>41.5</td>
<td>25.3 (22.1-29.0)</td>
</tr>
<tr>
<td>Phenyl salicylate</td>
<td>9.59 (8.72-10.5)</td>
<td>13.1</td>
<td>12.2 (10.3-14.4)</td>
</tr>
<tr>
<td>S42I</td>
<td>43.8 (34.4-55.8)</td>
<td>640</td>
<td>44.9 (41.1-49.0)</td>
</tr>
<tr>
<td>Pyreonyl butoxide</td>
<td>4,000 (3,010-5,330)</td>
<td>21,100</td>
<td>&gt;4,000</td>
</tr>
<tr>
<td>Synepirin 222</td>
<td>90.4 (83.6-97.8)</td>
<td>156</td>
<td>311 (265-363)</td>
</tr>
<tr>
<td>Synepirin 500</td>
<td>&gt;4,000</td>
<td>&gt;4,000</td>
<td>&gt;4,000</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>261 (214-318)</td>
<td>930</td>
<td>43.4 (31.9-59.0)</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>179 (153-210)</td>
<td>515</td>
<td>140 (108-180)</td>
</tr>
<tr>
<td>Permethrin</td>
<td>&gt;4,000</td>
<td>&gt;4,000</td>
<td>&gt;4,000</td>
</tr>
<tr>
<td>Emphenthrin</td>
<td>&gt;4,000</td>
<td>&gt;4,000</td>
<td>&gt;257 (199-330)</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>&gt;4,000</td>
<td>&gt;4,000</td>
<td>&gt;4,000</td>
</tr>
<tr>
<td>Dicofol</td>
<td>29.8 (25.4-35.0)</td>
<td>75.8</td>
<td>53.0 (47.7-58.9)</td>
</tr>
<tr>
<td>pp'-DDT</td>
<td>&gt;4,000</td>
<td>&gt;4,000</td>
<td>&gt;4,000</td>
</tr>
<tr>
<td>γ-BHC</td>
<td>629</td>
<td>1,100</td>
<td>86.3</td>
</tr>
<tr>
<td>Cyhexatin</td>
<td>33.5 (30.7-36.6)</td>
<td>49.9</td>
<td>89.1 (78.7-101)</td>
</tr>
</tbody>
</table>

Continuous contact for 24 h.
Values are shown in mg (a.i.)/m².
LC₅₀, 50% lethal concentration; LC₉₀, 90% lethal concentration; 95% C.L., 95% confidence limit.
☆: The ratio of LC₉₀/LC₅₀ is less than 2.
4,000 mg/m² in 3 mite species. Increases in the mortality rates in a concentration-dependent manner were not observed for these 4 chemicals. Empenthrin for Bt showed the lowest LC value among the 3 test pyrethroids. In spite of a similarity in the chemical structure between dicofol and pp'-DDT, the LC values of pp'-DDT were greater than those of dicofol for all 3 mite species.

More than ten-fold differences in LC₅₀ values among the mite species were recognized in γ-BHC (Df/Bt = 38.6), IBTA (Tp/Df = 34.1), fenitrothion (Df/Bt = 29.9) and dichlorvos (Df/Bt = 18.0). On the other hand, the LC₅₀ values for 3 mite species were similar in benzyl benzoate, phenyl salicylate and S421. However, the LC₉₀ value of S421 was much higher. Bt demonstrated the highest susceptibility to 9 chemicals. Particularly, the LC₅₀ values of the organophosphates including fenitrothion and dichlorvos for Bt were as low as that for phenyl salicylate.

The values marked with (☆) represent those with an LC₉₀/LC₅₀ ratio of less than 2. Five of these chemicals were common to Df and Bt, whereas for Tp only phenyl salicylate showed a ratio of less than 2.

**Discussion**

The LC values assessed by the contact method for the HDM have been unknown. Almost all the studies which used the contact method estimated the lethal efficacy at only 1 or 2 dosages. A part of the mortality rates for fenitrothion, permethrin, phenyl salicylate and benzyl benzoate in our experiments approximated the results from other studies (Mizutani, 1988; Tsutsumi et al., 1998).

Phenyl salicylate showed the highest acaricidal efficacy against the 3 species in this study. Ninety percent mortalities, however, were not obtained at a dosage of 1,000 mg/m² for Cheyletus malaccensis (TWMC colony), Chelacaropsis moorei (TWMC colony) and Ornythonyssus bacoti (Teikyo University colony) (Hashimoto, unpublished). Although it is considered that phenyl salicylate can acquire a good control effect against most HDM fauna, these results suggest that this chemical has a specificity in the acaricidal efficacy the same as other chemicals.

The values marked with (☆☆) mean that regression lines on the probit have high slope values. On the contrary, the ratios of LC₉₀/LC₅₀ were more than 10 in S421 for Df, dichlorvos for Tp and γ-BHC for Bt. As a result, the LC₅₀ value for S421 was half of that for synepirin 222 in Df. However, the LC₉₀ value of S421 was more than 4 times higher than that of synepirin 222. Thus, the order of efficacy orders evaluated by the mortality rates fluctuated, because of differences in the concentration-response characteristics of the chemicals.

Conventional insecticides, such as fenitrothion, dichlorvos and permethrin, indicated suppression efficacy by the medium-mixing method. Mizutani (1988) reported the order of suppression efficacy was fenitrothion > permethrin = IBTA > S421 for Tp and IBTA > permethrin > fenitrothion > S421 for Df. In addition, Noda and Hirakoso (1991) also demonstrated the order of 50% inhibition concentration (IC₅₀) values for various chemicals by the medium mixing method. According to their study, the order of efficacy was dichlorvos (IC₅₀ = 1.3 ppm) > fenitrothion (18) > permethrin (27) > γ-BHC (58) > dicofol (230) > S421 (640) for Tp, and γ-BHC (75) > fenitrothion (110) > permethrin (200) > dichlorvos (230) > dicofol (300) > S421 (>10,000) for Df. In those studies, the efficacy of S421 was always less than that of permethrin. And permethrin sometimes showed higher efficacy than the organophosphates including fenitrothion and dichlorvos. These tendencies were not found in our study. Although the LC₅₀ values of both test organophosphates were less than 300 mg/m², those of permethrin were greater than 4,000 mg/m² in spite of 24 h contact. In practical use, such a high dosage as 4,000 mg/m² is not treated, and 24 h continuous contact is not
impossible. With regard to this, the authors concluded that the agent of which the LC₅₀ was more than 1,000 mg/m² with this method was not effective by contact. Furthermore, Tanaka (1974) contacted Tp to the chemical residue (2,500 mg/m²) for 1–60 minutes. Fifty percent mortalities with fenitrothion and dichlorvos were obtained with 10-minute and 1-minute contact, respectively. It is considered that extended contact is required to obtain high mortality with low dosage.

Evaluation with the medium-mixing method requires more than 1 week to confirm the efficacy, and it is affected by several factors, e.g., ovicidal effect, chemical disintegration, oral intake. It is considered that the mechanism of chemical exposure to the test mites with the medium-mixing method is different from that with the contact method. In other words, the differences in the evaluation of acaricidal efficacy are due to the test method. Some chemicals examined in this study have already been formulated as aerosols, paper underlays and smoking agents for house-use acaricides. These formulations have separate usages. Therefore, the acaricidal efficacy of each chemical should be evaluated by adjusting the usage accordingly.

Acknowledgements

We are deeply grateful to Dr. De Mar Taylor of University of Tsukuba for the review of our manuscript and Dr. Fumiko Takeda of University of the Ryukyus for providing the test mites. We also thank the staff of Department of Environmental Biology, Japan Environmental Sanitation Center, for helpful suggestions.

References


酸ベンジル、サリチル酸フェニル、S421ではダニ3種に対して LC50値がほぼ近似した値を示し、LC値で見た薬量－反応の特性には、薬剤とダニ種の組み合わせによって差異が見られた。