THE BULLETIN OF OKAYAMA UNIVERSITY OF SCIENCE No.59 A pp. 101-108 (2023)

Insecticidal Effect of 4% (W/W) Permethrin Emulsions on Adults of the Housefly *Musca domestica*

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(Received October 31, 2023; accepted December 4, 2023)

Original and generic products of 4% (W/W) permethrin emulsion were evaluated for their insecticidal effects on housefly *Musca domestica* adults, which had been collected at a cattle house on Ishigakijima Island, of the Yaeyama Islands, Okinawa prefecture, Japan. Thirty 3–5-day-old adult female houseflies were put in rectangular parallelepiped containers of which the top cover was stainless mesh. Then 100-, 200-, 400-, and 800-fold diluted emulsions of respective drugs were sprayed in amounts of 25 mL/m^2, 50 mL/m^2, 100 mL/m^2, and 200 mL/m^2. When less-diluted drugs were sprayed in larger amounts, the times until 50% flies were knocked down (KT_{50}) were shorter and the knocked down rates were higher, reflecting dose-dependence. Among containers for which the same dosages of active ingredients were sprayed, KT_{50} were lower with larger amounts of drug solution, which demonstrated that quick-acting efficacy was achieved with larger spray amounts. Insecticidal effects of the two drugs, original and generic, were found to be equivalent from this study.

Keywords: housefly, insecticide, *Musca domestica*, permethrin, pyrethroid.

1. Introduction

Control of flies in and around cattle, swine, and poultry houses is an important issue for public health related to animal husbandry. Especially in situations where people who are not engaged in agriculture or animal husbandry reside near livestock or poultry houses, even a small number of flies are often capable of causing severe trouble.

Several compounds of pyrethroids, organophosphates, carbamates, and other classes have been formulated for ridding flies, as has insect growth regulator (IGR) (Yasutomi, 1980; Miyamoto, 2000). Nevertheless, flies have repeatedly developed drug resistance. Therefore, they have not always been exterminated favorably as they might have been long ago (Yasutomi, 1980; Motoyama, 1984; Shono, 1984; Funaki et al., 1986; Miyamoto, 2000).

Permethrin, a pyrethroid compound, has been used widely and for many years as an active ingredient in insecticidal and acaricidal drugs. It has been formulated as 4% (W/W) emulsions and combinations with other compounds such as fenitrothion for fly control. In the case of permethrin as well as other insecticides, flies have been known to be resistant against this compound (Motoyama, 1984). However, situations in which permethrin-resistant flies are dominant are not thought to be extremely common. Therefore, although permethrin remedies had been used for many years, their application in and around livestock and poultry houses will probably not be lost. When properly used, permethrin remedies will be regarded as providing satisfactory efficacy against flies, except for some strains which might develop remarkable resistance against the compound.

According to the recommended ‘dosage and administration’ described on the package insert, the 4% (W/W) permethrin emulsion should be diluted 200-fold to 400-fold; it should then be sprayed at an amount of 50–100 mL for m^2 when used for fly control in and around livestock and poultry houses.

Table 1 Knock down effects of 4% (W/W) permethrin emulsion applied with various dilution factors and spray volumes against adult houseflies

Case	Dilution factor	Spray volume (mL/m^2)	Dosage of active ingredient (mg/m^2)	Original product		Generic product	
				KT_{50} (min)	Ratio of knocked down flies (%)	KT_{50} (min)	Ratio of knocked down flies (%)
A	—	0	0	—	0	—	0
B	—	25	0	—	2	—	0
C	—	50	0	—	0	—	0
D	—	100	0	—	2	—	0
E	—	200	0	—	0	—	2
F	100	25	10	13.9	78	11.8	78
G	100	50	20	10.4	87	12.2	88
H	100	100	40	5.0	95	4.8	98
I	100	200	80	2.6	100	2.2	100
J	200	25	5	27.5	58	24.1	63
K	200	50	10	12.9	83	11.7	78
L	200	100	20	8.9	88	7.6	90
M	200	200	40	3.3	97	3.9	98
N	400	25	2.5	30.4	55	24.5	60
O	400	50	5	26.4	60	20.3	67
P	400	100	10	10.4	85	8.8	80
Q	400	200	20	7.2	90	5.3	90
R	800	25	1.25	51.7	42	61.8	40
S	800	50	2.5	29.7	57	30.6	57
T	800	100	5	19.1	62	16.0	68
U	800	200	10	7.9	87	7.3	83

Consequently, the recommended dosage of permethrin is very wide-ranging. However, the effects on fly control efficacy of using more or less of the amount of sprayed permethrin have not been clarified.

For this study, we evaluated the *in vitro* insecticidal effects of 4% (W/W) permethrin emulsions against *Musca domestica* housefly adults under various conditions of dilution factors and spray amounts. The effects of original and generic drugs were also compared because a generic product of this formulation has become available on the market.

2. Materials and Methods

2-1 Evaluated drugs

Original and generic products of permethrin emulsion formulations were evaluated. The original product was 'Kincho ETB Emulsion for Animals' (Dainihon Jochugiku Co., Ltd., Osaka, Japan). The generic one was 'Permethrin Emulsion [Fujita]' (Fujita Pharmaceutical Co., Ltd., Tokyo, Japan). Both remedies contain 4 g of permethrin as an active ingredient in 100 g of product. They also contain synergist(s) and repellent(s), although the specific names and contents of these compounds are not disclosed on their package inserts.

2-2 Insects

The *Musca domestica* houseflies were collected at a cattle house on Ishigakijima Island, of the Yaeyama Islands, Okinawa prefecture, Japan, and adult females of them were used for this study after breeding and rearing in a laboratory.

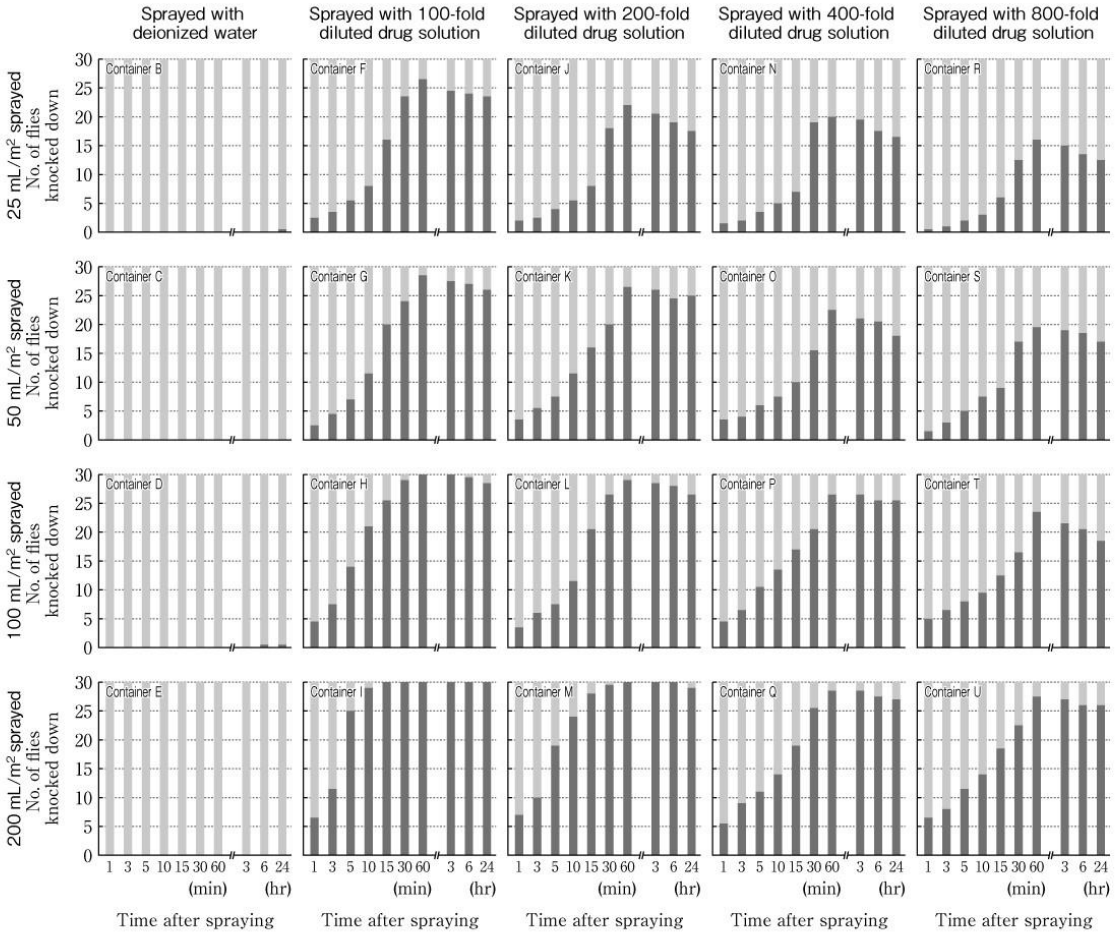

Fig. 1 Knock down effects of the original drug of 4% (W/W) permethrin emulsion 'Kincho ETB Emulsion for Animals' (Dainihon Jochugiku Co., Ltd., Osaka, Japan) against adult houseflies

2-3 Experiment procedures

Insecticidal effects of the drugs were evaluated by spraying them against adult houseflies that had been put in containers.

Thirty 3–5-day-old of adult housefly females were, respectively, put in 21 custom-made rectangular parallelepiped cardboard containers, 50 cm × 40 cm × 15cm (height), for evaluation of each of the original and generic drugs. The tops of the containers were covered by stainless steel wire gauze with 2 mm × 2 mm mesh.

One of the 21 containers was left as an untreated control. Deionized water was sprayed into four containers in the respective amounts of 25 mL/m^2, 50 mL/m^2, 100 mL/m^2, and 200 mL/m^2. For the other 16 containers, the drug solutions diluted 100-, 200-, 400-, and 800-fold by deionized water were sprayed respectively in the amounts of 25 mL/m^2, 50 mL/m^2, 100 mL/m^2, and 200 mL/m^2. The actual volumes of the drug solutions sprayed to each container were 5 mL, 10 mL, 20 mL, and 40 mL because the bottom area was 0.2 m^2. The spraying of the drug solutions was done from a position of about 20 cm distant from the top side of the container, using a trigger type sprayer (1.0±0.1 mL spray output per stroke, spray nozzle type, T-95 UDV; Canyon Corp., Tokyo, Japan).

Then the containers were left at a temperature of 25±2℃. Feeding of the flies was done by putting into each container some absorbent cotton into which 5% (W/V) sucrose aqueous solution had been impregnated. The number of flies knocked down was counted in each container at 1, 3, 5, 10, 15, 30, and 60 min, and also at 3, 6, and 24 hr after drug application.

After the experiment described above was repeated

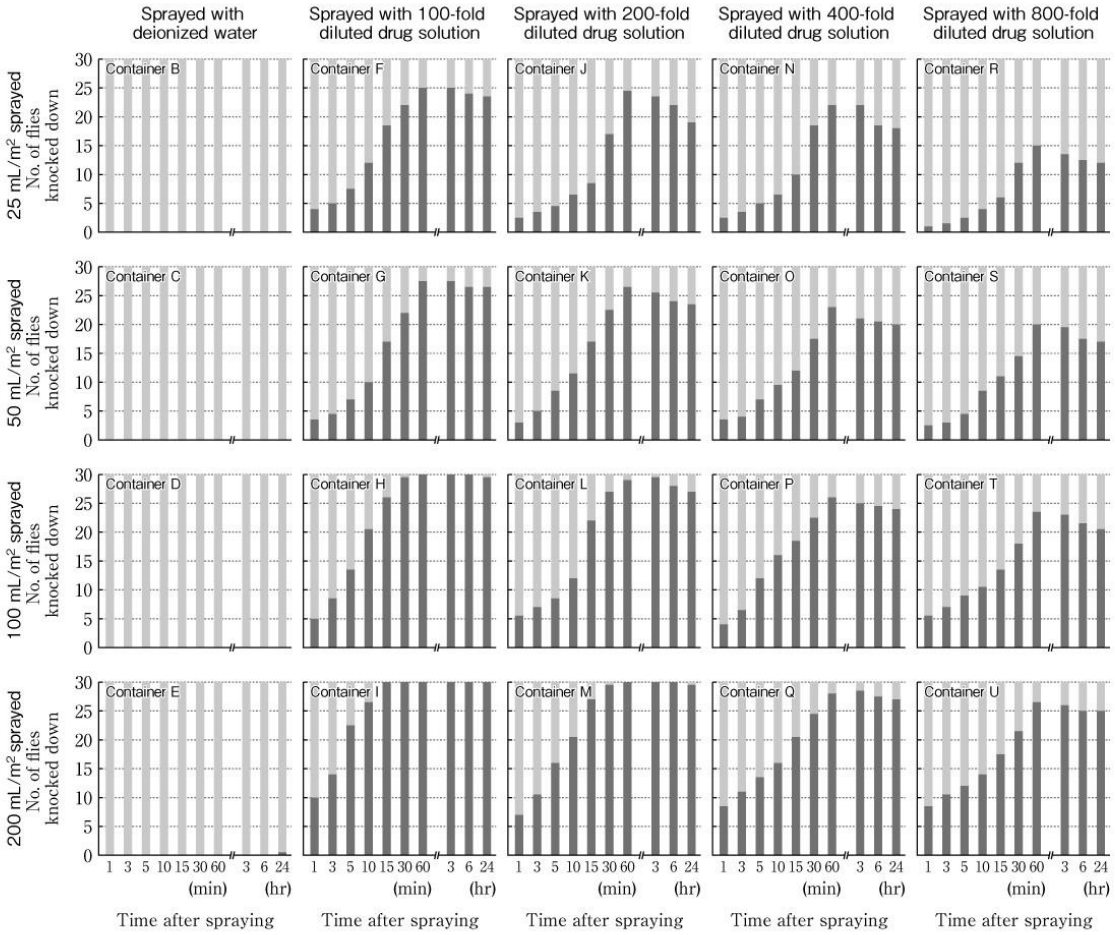

Fig. 2 Knock down effects of the generic drug of 4% (W/W) permethrin emulsion 'Permethrin Emulsion [Fujita]' (Fujita Pharmaceutical Co., Ltd., Tokyo, Japan) against adult houseflies

twice for each drug, the mean value of the number of knocked flies was calculated. Next, based on the time course of the mean value of the knocked down flies, the times until 50% flies were knocked down (KT_{50}) and the times until 95% flies were knocked down (KT_{95}) were calculated using probit analysis. The ratio of flies knocked down after 24 hr was also calculated using the following formula.

Ratio of knocked down flies at 24 hr after drug application (%)
= (Number of flies knocked down at 24 hr / Number of flies used) × 100

3. Results

In the untreated containers, no flies were knocked down during the 24 hr observation period. When deionized water alone was sprayed, just one fly (0.5 as a mean value of repeated experiments) was knocked down in some containers (Table 1, Figs. 1 and 2).

When the 100-fold diluted drug solution was sprayed in amounts of 25–200 mL/m^2 (10–80 mg active ingredient/m^2), on the other hand, many flies were knocked down. Especially, all individuals were knocked down in containers spayed with 100 mL/m^2 and 200 mL/m^2 for both original and generic products. However, some flies that had been once knocked down revived in all containers other than the container of 200 mL/m^2. The ratios of the knocked down flies after 24 hr when the 100-fold diluted drug solution was sprayed were 78–100%, demonstrating dose-dependency for both the original and generic drugs. The KT_{50} values were 2.6–13.9 min for the original drug and 2.2–12.2 min for the generic drug, showing

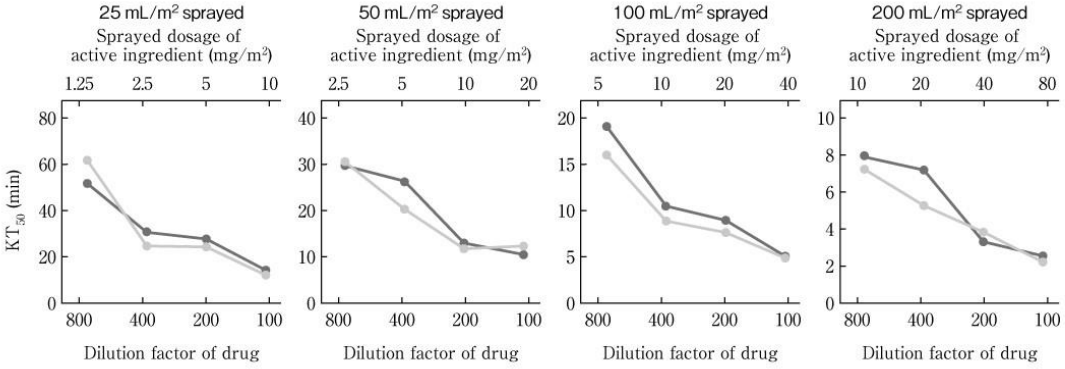

Fig. 3 Comparison of knock down effects (KT_{50}) of 4% (W/W) permethrin emulsions based on the sprayed volume of the drug
Original drug of 4% (W/W) permethrin emulsion 'Kincho ETB Emulsion for Animals', Generic drug of 4% (W/W) permethrin emulsion 'Permethrin Emulsion [Fujita]'

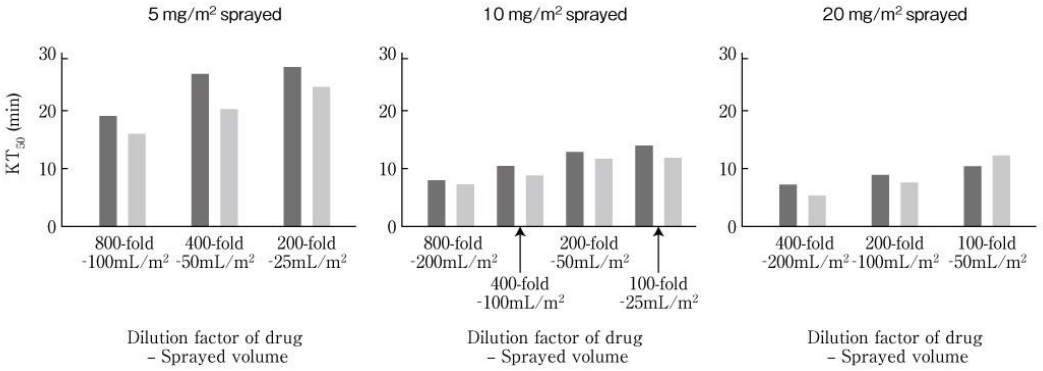

Fig. 4 Comparison of knock down effects (KT_{50}) of 4% (W/W) permethrin emulsions based on the sprayed dosage of the active ingredient
Original drug of 4% (W/W) permethrin emulsion 'Kincho ETB Emulsion for Animals', Generic drug of 4% (W/W) permethrin emulsion 'Permethrin Emulsion [Fujita]'

lower values when higher dosages were sprayed for both drugs (Table 1, Figs. 1 and 2). The KT_{95} values were calculated as 34.5 min and 11.3 min, respectively, when sprayed in amounts of 100 mL/m^2 and 200 mL/m^2 for the original drug. They were 34.1 min and 22.7 min, respectively, when sprayed in amounts of 100 mL/m^2 and 200 mL/m^2 for the generic drug. The KT_{95} values of the other containers were not calculated because of the low knocked down ratios.

When the 200-fold diluted drug solution was sprayed in amounts of 25–200 mL/m^2 (5–40 mg active ingredient/m^2), many flies were knocked down. Especially, all individuals were knocked down in containers which had been sprayed with 200 mL/m^2 for both original and generic products. Revivals of the flies once they had been knocked down were observed in all the containers. The ratios of the knocked down flies after 24 hr were 58–97% for the original drug and 63–98% for the generic drug. Also, the KT_{50} values were 3.3–27.5 min and 3.9–24.1 min, respectively, for the original and generic drugs. The KT_{95} values could not be calculated for either drug (Table 1, Figs. 1 and 2).

When the 400-fold diluted drug solution was sprayed

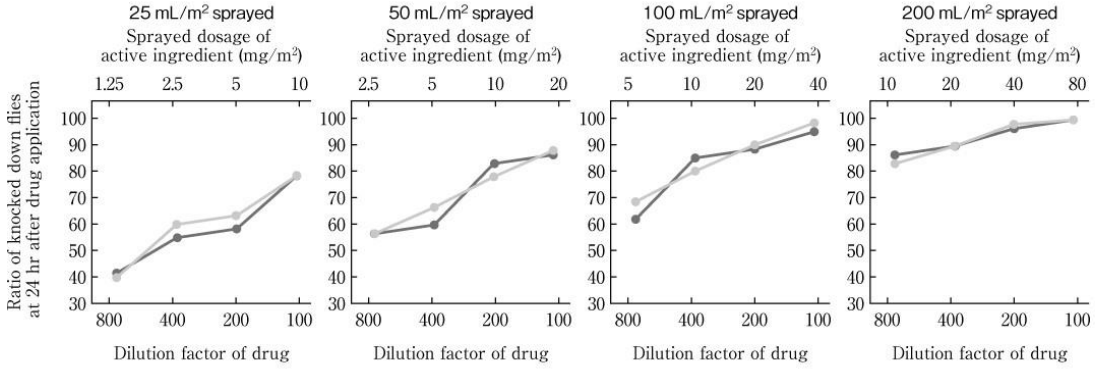

Fig. 5 Comparison of knock down effects (ratio of knock down houseflies at 24 hr after drug application) of 4% (W/W) permethrin emulsions based on the sprayed volume of the drug
Original drug of 4% (W/W) permethrin emulsion 'Kincho ETB Emulsion for Animals', Generic drug of 4% (W/W) permethrin emulsion 'Permethrin Emulsion [Fujita]'

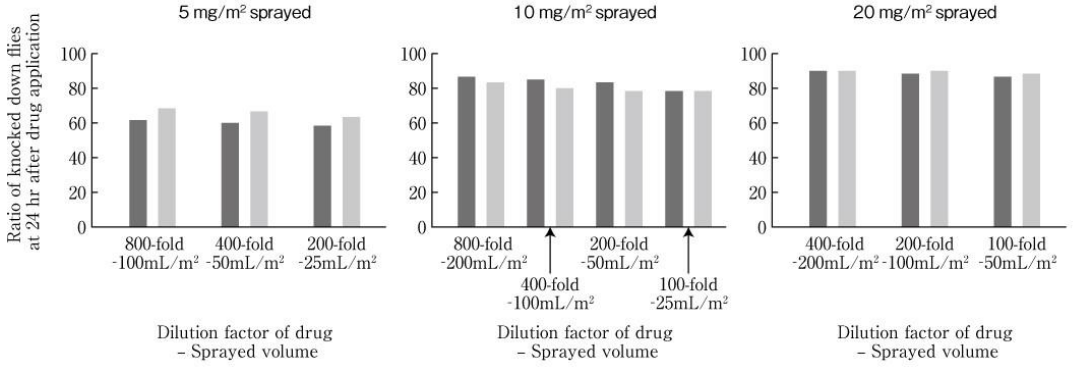

Fig. 6 Comparison of knock down effects (ratio of knock down houseflies at 24 hr after drug application) of 4% (W/W) permethrin emulsions based on the sprayed dosage of the active ingredient
Original drug of 4% (W/W) permethrin emulsion 'Kincho ETB Emulsion for Animals', Generic drug of 4% (W/W) permethrin emulsion 'Permethrin Emulsion [Fujita]'

in amounts of 25–200 mL/m^2 (2.5–20 mg active ingredient/m^2), there was no container in which all the flies had been knocked down. Revivals of once knocked down flies were observed in all containers. The ratios of the knocked down flies after 24 hr were 55–90% for the original drug and 60–90% for the generic drug. The KT_{50} values were 7.2–30.4 min and 5.3–24.5 min, respectively, for the original and generic drugs. The KT_{95} values could not be calculated for either drug (Table 1, Figs. 1 and 2).

In all containers to which the 800-fold diluted drug solution was sprayed in amounts of 25–200 mL/m^2 (1.25–10 mg active ingredient/m^2), some flies were not knocked down. Revival of once knocked down flies was observed in all containers. The ratios of the knocked down flies after 24 hr were 42–87% for the original drug and 40–83% for the generic drug. The KT_{50} values were 7.9–51.7 min and 7.3–61.8 min, respectively, for the original and generic drugs. The KT_{95} values could not be calculated for either drug (Table 1, Figs. 1 and 2).

For the data described above, the KT_{50} values were lower when less-diluted drug solutions were sprayed (i.e., when higher dosages of the active ingredient

were sprayed) among the containers to which the same volume of the drug solution had been sprayed, for both the original and generic drugs (Fig. 3). By contrast, comparison of the efficacies among the containers to which the same dosage of the active ingredient had been sprayed demonstrated that the KT_{50} values were lower when the highly diluted drug solution was sprayed in larger amounts (Fig. 4).

Ratios of the knocked down flies after 24 hr were higher when less-diluted drug solutions were sprayed (i.e., when higher dosages of the active ingredient were sprayed) among the containers to which the same volume of the drug solution was sprayed, for both the original and generic drugs (Fig. 5). However, comparison of efficacies among the containers to which the same dosage of the active ingredient was sprayed demonstrated that these ratio values were almost identical irrespective of the dilution factors and spray volumes (Fig. 6).

4. Discussion

Permethrin, a pyrethroid class insecticide, has been used widely as an active ingredient of insecticidal and acaricidal drugs for many years. For veterinary uses, it has been, for example, formulated for control of flies occurring in and around livestock and poultry houses. However, the existence of houseflies which have developed resistance against permethrin has been reported (Motoyama, 1984). Additionally, the insecticidal efficacy of permethrin is less than that of cyfluthrin, which is also a pyrethroid, against houseflies (Fukase et al., 1991). Nevertheless, we have inferred that permethrin will be effective against flies which have not developed drug resistance. The lower price of permethrin products than those of other insecticides such as cyfluthrin products can provide benefits from a practical viewpoint.

The original and generic products of 4% (W/W) permethrin emulsion formulation have been launched as veterinary drugs in Japan for the control of flies in and around livestock and poultry houses. These drugs are recommended for use by spraying against fly habitats in amounts of 50–100 mL/m^2 after 200–400-fold dilution when sprayed in livestock and poultry houses. For this study, we compared the insecticidal effects of the permethrin products against houseflies with different conditions of application: dilution factors and spray volumes.

From results of the study, it was confirmed that larger volume of sprayed drug solution caused higher insecticidal effects with dose-dependence for the respective dilution factors. To the degree that the dilution factors are equal, a larger spray volume means a larger amount of the active ingredient. Therefore, development of higher insecticidal efficacy was an expected consequence. However, among the containers to which the same amount of the active ingredient was sprayed, the KT_{50} values were shorter (i.e., the quick-acting efficacy was higher) when a larger amount of the drug solution was sprayed. A larger amount of drug solution is thought to bring an evenly and widespread distribution of the solution in the area so that the quick-acting effect of the drug will be achieved. In the situation of actual use of the drugs in and around livestock and poultry houses, spraying with a larger amount of the drug solution will be successful when a low dose of the active ingredient is required for some reasons, such as economic conditions. However, it is not preferable to spray large amounts of watery materials in livestock and poultry houses because of reduced work efficiency. We suggest here that a larger amount of the drug solution should be sprayed within the allowable range.

Regarding the ratios of knocked down flies at 24 hr after the drug application, on the other hand, these ratios were almost identical among various volumes of sprayed solutions as far as the same dosage of active ingredient was applied. This finding might appear to be inconsistent with the KT_{50} findings. However, the present experiments were conducted in small, semi-closed containers. Therefore, the flies were unable to dodge the drug: the efficacy will be obtained finally, corresponding to the sprayed doses of the active ingredient.

The present research evaluated dosages of more than and less than the recommended dosage (100-fold and 800-fold dilutions, respectively) of the products for use against adult flies, and clarified that higher doses than the recommended dosages develop a stronger effect. However, because of risks such as drug residues in animal bodies, extra-label use of the drug should not be done. The drugs evaluated here, simultaneously, are approved to be sprayed also in a use of 100-fold dilution against fly larvae. When the drugs are sprayed to materials other than live animals, such as compost, 100-fold dilution will produce higher efficacy.

The present experiments used bred and reared houseflies after they had been collected in a field. Although the sensitivity of the flies against insecticides was not examined, the insecticidal effects of permethrin against this fly isolate corresponded closely to that observed in an earlier experiment (Fukase et al., 1991). For that earlier experiment, the insecticidal effect of the original product was evaluated against houseflies collected at a swine house; the flies had been regarded as having developed remarkable pyrethroid-resistance (Fukase et al., 1991). Accordingly, the houseflies used in the present experiments are inferred to have developed a certain degree of pyrethroid resistance.

The present study also compared the insecticidal effects of the two products, original and generic, of 4% (W/W) permethrin emulsion. No significant difference was found between the effects of the two drugs. Therefore, it was concluded that the generic products develop equivalent effects to those of the original one. Generic remedies are launched after the expiration of patent rights of the original product, based on the system which approves them with a smaller amount of data than for the original products. Although definitions of generic drugs differ among countries (Alfonso-Cristancho et al., 2015), it is described as follows in Japan. Generic drugs contain the same active ingredient(s) at the same amount(s), and are administered by the same route(s) as the original drug. The 'indications' and 'dosage and administration' of generic drugs are also fundamentally the same as those of the original. Generic drugs can develop efficacy equivalent to the original (Shiragami, 2002). For the development of generic drugs, their equivalencies are evaluated pharmacokinetically by measuring the blood concentrations of the drug in time in the case of drugs for which the active ingredient(s) are absorbed into the animal body. By contrast, in the cases of drugs for which the active ingredient(s) are not absorbed into the animal body (e.g., 4% (W/W) permethrin emulsion), the blood concentration cannot be measured. Therefore, the equivalence of these drugs is usually evaluated by comparing the efficacy, especially the *in vitro* efficacy between original and generic drugs. The assessment of equivalency by comparing the effects is apparently much more difficult than by using pharmacokinetic methods with measurement of blood concentrations.

Active ingredient(s) and its (or their) content(s) are essentially the same between original and generic products, but the kinds and contents of the other components (that is, pharmaceutical excipients) are not always the same. Regarding the evaluated drugs in the present study, components described obligatorily in package inserts according to the 'Fire Service Act' (Act No. 186 of 1948, last version: Act No. 41 of 2008) mutually differ, so that it is readily apparent that differences exist in pharmaceutical excipients between the two drugs. The possibility exists that these differences in pharmaceutical excipients cause the different efficacies of the drugs. Nevertheless, as judged based on the results of the present study, the insecticidal efficacies of the original and the generic products of 4% (W/W) permethrin emulsion are mutually equivalent against adult houseflies.

Results showed that 4% (W/W) permethrin emulsion can be used successfully for house fly control, even today, under many situations.

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