

was used as enzyme solution, there is a lag period in the oxidation of monophenol, tyrosine. On the other hand, haemolymph prepared from the gregarious black larvae shows no lag phase in the oxidation of monophenols.

4. The gregarious black larvae show a higher phenoloxidase activity in both haemolymph and integument than the isolated pale larvae.

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References

- 1) Aruga, H., and S. Kawase: *Jap. J. Breed.*, **1**, 137 (1952).
- 2) Ikemoto, H.: *Botyu-Kagaku*, **36**, 59 (1971).
- 3) Ikemoto, H.: *Botyu-Kagaku*, **36**, 128 (1971).
- 4) Ito, T.: *Annot. Zool. Japon*, **26**, 176 (1953).
- 5) Ito, T.: *Jap. J. Zool.*, **11**, 253 (1954).
- 6) Iwao, S.: *Mem. Coll. Agr. Kyoto Univ.*, **84**, 1 (1960).
- 7) Kawase, S.: *J. Insect Physiol.*, **5**, 335 (1960).
- 8) Nakamura, T., and S. Sho: *J. Biochem.*, **55**, 510 (1964).
- 9) Nittono, Y.: *J. Seric. Sci.*, **21**, 29 (1952).
- 10) Nolte, D. J.: *South Africa J. Sci.*, **61**, 173 (1965).
- 11) Ohnishi, E.: *Annot. Zool. Japon*, **27**, 33 (1954).
- 12) Sato, Y.: *Ann. Rept. Takeda Res. Lab.*, **25**, 142 (1966).
- 13) Waku, Y., and S. Iwao: *Jap. J. appl. Entomol. Zool.*, **4**, 70 (1960).
- 14) Yamazaki, H. I.: *J. Insect Physiol.*, **15**, 2203 (1969).

Studies on Pyrethroidal Compounds* Part II. Comparative Activity of Pyrethrins I, Pyrethrins II and Other Synthetic Pyrethroidal Compounds. Kosuke TSUBA, Yasuo ABE and Yoshio FUJITA (Research Department, Pesticides Division, Sumitomo Chemical Co., Ltd., Takatsukasa, Takarazuka City, Hyogo, Japan.) Received February 22, 1972. *Botyu-Kagaku*, **37**, 48 1972.

8. ピレスロイド系化合物の研究 第2報 Pyrethrins I, II および数種合成ピレスロイドの効力比較 津田小亮, 安部八洲男, 藤田義雄 (住友化学工業株式会社宝塚研究所農薬事業部研究部, 兵庫県宝塚市高司4丁目) 47. 2. 22 受理.

ピレトリンエキスおよびそれから薄層クロマトグラフィーで分離精製した Pyrethrins I, II 並びに4種の合成ピレスロイド (アレスリン・テトラメスリン・レスメスリン・フラメスリン) の殺虫効力を比較し, ピレスロイド混合物の連合作用について検討した。その結果ピレトリンエキスの殺虫効力は Pyrethrins I と Pyrethrins II のみによるもので, 前者は主に致死剤として後者はノックダウン剤として作用し両者間に僅かの協力作用が認められた。しかし, Pyrethrins II は線香の殺虫効力にはほとんど寄与しない。オイルスプレーテストではテトラメスリンは優れたノックダウン速効効果を示し, レスメスリンは高い致死効果を示した。更に両者間には協力作用がみられた。アレスリンに少量のレスメスリンを添加した線香の希薄煙はノックダウン効果において速効性と同時に効果の持続性が認められた。また, 供試ピレスロイドに対して Lab-em-7-em 系 (感受性) および 203 d系 (ダイアジノン抵抗性) イエバエ間に明瞭な感受性差異は認められなかった。

Introduction

Pyrethrum extract has been widely used as an insecticide from ancient times due to its quick knock-down effect to insects and low toxicity to mammals. "Pyrethrins", the insecticidal principles, consist of six insecticidal esters; cinerin I (cin. I), jasmolin I (jas. I), pyrethrin I (pyr. I),

cinerin II (cin. II), jasmolin II (jas. II), and pyrethrin II (pyr. II). Pyrethrin I and II are more toxic than the corresponding cinerin I and II to houseflies¹⁻⁵⁾ and mustard beetles^{6,7)}. Pyrethrins I (a mixture of cin. I, jas. I, and pyr. I) are recovered in better yield than pyrethrins II (a mixture of cin. II, jas. II, and pyr. II) from smoke of mosquito coils⁸⁾. Cinerin I and II are more stable than pyrethrin I and II to sunlight⁹⁾.

* Part I of this series appeared in Reference 20).

The degree of synergism with piperonyl butoxide is higher in houseflies for the pyrethrins I than for the pyrethrins II^{9,10} and for the cinerin I and II than for the pyrethrin I and II^{9,10}. Comparatively wide insecticidal spectrum and the remarkable synergism of toxicity with piperonyl butoxide are considered to come from mixing effect of these various esters¹¹.

In recent years research on synthetic pyrethroidal compounds is rapidly advanced¹²⁻¹⁵ and there are some compounds which exceed natural pyrethrins regarding quick knock-down, high mortality to insects, harmlessness to mammals and chemical stability¹⁶.

This report deals with comparison between the insecticidal activities of four representative synthetic pyrethroids and those of pyrethrins I, pyrethrins II (two components of pyrethrins) and of pyrethrins in mosquito coils and oil sprays used as practical formulations. The combination effect of mixed pyrethroids on insecticidal activity is also discussed.

Materials and Methods

Preparation of Pyrethrins I and Pyrethrins II

Pyrethrins were extracted by agitation with nitromethane from pyrethrum extract. Its nitromethane extracts were decolorized with activated charcoal. The nitromethane was evaporated to yield a pyrethrins concentrate (purity ca. 60%)^{17,18}. The concentrate was dissolved in gracial acetic acid and shaken with petroleum ether. Addition of water caused the solution to separate into two layers, a petroleum ether layer ("pyrethrins I" fraction) and an acetic acid layer ("pyrethrins II" fraction)¹⁹. These two fractions

were separately subjected to silicic acid column chromatography (Mallinckrodt silicic acid, 100 mesh). Petroleum ether fraction on the column was eluted with *n*-hexane:ethyl acetate (95 : 5, v/v), acetic acid fraction with *n*-hexane : ethyl acetate (85 : 15, v/v) to give colorless viscous liquid, "pyrethrins I" and "pyrethrins II", respectively. "Pyrethrins I" and "pyrethrins II" were separately purified by thin layer chromatography (Silica Gel HF₂₅₄, Merk), in the solvent system, *n*-hexane : ethyl acetate (3 : 1, v/v).

Analytical Method

Quantitative determinations were made by gas-liquid chromatography in the way as described in the literatures²⁰⁻²⁴.

Preparation of Mosquito Coils

Blank coils prepared had the composition shown in Table 1. Toxicants were added to them by

Table 1. Composition of mosquito coils.

Pyrethrum marc	49.5% (w/w)
Tabu powder	30.0
Wood flour	20.0
Dye (Malachite green)	0.3
Fungistat (Benzoic acid)	0.2

pipetting the appropriate volume of solution in acetone as evenly as possible onto a side of the coil which was then air-dried.

Bioassay

Insecticidal materials used are shown in Table 2. Test insects were adults of houseflies (*Musca domestica domestica*) and mosquitoes (*Culex pipiens pallens*). The Lab-em-7-em strain of houseflies is the highly susceptible laboratory strain that was obtained from University of Kansas, Lawrence. The 203d strain of houseflies

Table 2. Pyrethroidal compounds.

Pyrethrins I	90.0%	a mixture of cinerin I (32.8%), jasmolin I (10.3%) and pyrethrin I (47.8%)
Pyrethrins II	99.2%	a mixture of cinerin II (26.2%), jasmolin II (3.2%) and pyrethrin II (69.8%)
Pyrethrins	17.3%	pyrethrum extract (pyrethrins I, 8.91%; pyrethrins II, 8.41%)
Allethrin	84.0%	allethronyl chrysanthemate (Pynamim®)*
Resmethrin	92.9%	5-benzyl-3-furylmethyl chrysanthemate (Chyrsron®)*
Tetramethrin	89.4%	<i>N</i> -(3, 4, 5, 6-tetrahydrophthalimido) methyl chrysanthemate (Neo-Pynamim®)*
Furamethrin	85.1%	5-propargyl-2-furylmethyl chrysanthemate (Pynamin-D®)*

*: Registered trade name by Sumitomo Chemical Co., Ltd.

is the diazinon resistant strain bred by Dr. J. Keiding, Denmark. The median lethal dose (LD_{50}) of diazinon to the former was $0.015 \mu\text{g}/\text{fly}$, and that of the latter was 1.0 to $1.5 \mu\text{g}/\text{fly}$ according to the results obtained in our laboratory. The mosquitoes were reared in successive generations in our laboratory. Those test insects were reared in the rearing room at $27 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ in relative humidity. These insects were tested by the following methods.

1) Topical application test

Adult houseflies, which were 3 or 4 days old after emergence, were narcotized by solid carbon dioxide (dry ice), and treated with $0.5 \mu\text{l}$ of acetone solution containing test chemicals at the dorsum prothorax using a microsyringe. The mortality was observed twenty four hours later.

2) Oil spray by turntable

This method was the same as that described by Campbell and Sullivan²⁰⁾. A petri dish (14 cm in diameter and 7 cm in height) covered with wire gauze and containing susceptible or resistant houseflies (about 100 adults), was placed beneath a cylinder with a sliding shutter at the bottom. Five ml of deobase solution of the chemicals was sprayed with an atomizer at a pressure of 10 psi. in about eight seconds. Thirty seconds later, the shutter was opened to allow the test insects to be exposed to the insecticidal mist. Ten minutes after the shutter opened, the treated insects were transferred into a clean cage to observe the mortality after twenty four hours, and the LC_{50} was calibrated.

3) Oil spray test in glass chamber

A glass chamber (70 cm cube) was used in the following tests. Twenty susceptible houseflies (ten each of females and males) or twenty mosquitoes (females) were released into the glass chamber. And 0.7 ml of deobase solution containing 0.2% of active ingredient was sprayed into glass chamber with an atomizer at the pressure of 20 psi. The knocked down insects were counted at indicated intervals up to 10 minutes. The mortality was observed after twenty four hours.

4) Test method of mosquito coil

4-1) High concentration method

This method was the same in principle as the standard method established by Japan Environ-

mental Sanitation Center. Half a gram of coil containing 0.2% of active ingredient was fixed on a stand and ignited at the both ends in the chamber. After the coil was burnt out, about twenty adult mosquitoes were released into the chamber. Knocked down mosquitoes were observed at indicated intervals up to 17 minutes. The mortality was observed after twenty four hours. Knocked down mosquitoes were collected and the mortality was based on the whole number of initially released insects, respectively.

4-2) Low concentration method

One end of the test coils containing 0.5% of active ingredient was burnt in the glass chamber for precisely one minutes. Then about twenty adult mosquitoes (females) were released into the chamber. Knocked down mosquitoes were counted at indicated intervals up to 120 minutes. The mortality was observed after twenty four hours.

Results and Discussion

1) Topical application test

The results on susceptible houseflies (Lab-em-7-em strain) and diazinon resistant houseflies (203d strain) are given in Table 3. The activity of pyrs. I was comparable to that of pyrethrins

Table 3. Twenty four hours LD_{50} values from topical application tests of pyrethroids against houseflies.

Pyrethroids	LD_{50} values ($\mu\text{g}/\text{fly}$)	
	Lab-em-7-em strain	203d strain
Pyrethrins I	0.62 (1.1)	0.44 (2.0)
Pyrethrins II	3.1 (0.2)	1.6 (0.6)
Allethrin	0.5 (1.4)	1.3 (0.7)
Resmethrin	0.034 (20.0)	0.013 (70.0)
Tetramethrin	1.0 (0.7)	0.55 (1.6)
Furamethrin	0.19 (3.5)	0.32 (2.8)
Pyrethrins	0.68 (1.0)	0.89 (1.0)

(): Relative toxicity of pyrethrins
(pyrethrins=1.0)

against Lab-em-7-em strain, and 1.9 times stronger than that of pyrethrins against 203d strain. It is likely that pyrs. II is 0.3 times toxic than pyrs. I against both strains. Pyrs. I and pyrs. II were a mixture of cinerin I, jasmolin I, and pyrethrin I, and a mixture of cinerin II,

jasmolin II, and pyrethrin II, respectively. Therefore, these results cannot be directly compared with the data of the reconstituted pyrethrin I and pyrethrin II²⁰). Resmethrin was superior in mortal activity to other pyrethroids. This result is consistent with those given in Okuno *et al.*'s report²⁷). The efficacies of tetramethrin and furamethrin were comparable or superior to those of pyrethrins against both strains. The difference in susceptibility of both strains to pyrethroids was not clearly observed.

2) Oil spray by turntable method

The results on Lab-em-7-em and 203d strains are given in Table 4. The efficacy of pyrs. II

Table 4. Twenty four hours LC₅₀ values from turntable method of pyrethroids against houseflies.

Pyrethroids	LC ₅₀ values (mg/100ml deobase)	
	Lab-em-7-em strain	203d strain
Pyrethrins I	145 (1.2)	119 (1.3)
Pyrethrins II	168 (1.1)	285 (0.5)
Allethrin	186 (1.0)	139 (1.0)
Resmethrin	16.6(10.8)	10.3(13.3)
Tetramethrin	214 (0.8)	112 (1.2)
Furamethrin	82 (2.2)	66 (2.1)
Pyrethrins	180 (1.0)	138 (1.0)

(): Relative toxicity of pyrethrins (pyrethrins=1.0)

was 0.4-0.9 times toxic against both strains than that of pyrs. I. And pyrs. I showed the same efficacy as pyrethrins against both strains. Resmethrin had more excellent activity than the other pyrethroids. Allethrin and tetramethrin showed the same effectiveness as pyrethrins, and

furamethrin was about 2.0 times more effective than pyrethrins. The difference in susceptibility of both strains to tested pyrethroids was not clearly observed.

3) Oil spray test in glass chamber

The results on houseflies (Lab-em-7-em strain) are given in Table 5. It appears that the knock-down effect of pyrs. II seems to be about twice that of pyrs. I. Tetramethrin showed extremely strong initial knock-down effect, while resmethrin showed excellent mortal effect. A mixture of tetramethrin and a small amount of resmethrin had excellent knock-down and mortal effects. And, then the results on mosquitoes are given in Table 6. Although we did not test mortal effect, it is likely that the same tendency as houseflies might be observed on mosquitoes.

4) Mosquito Coil

The results of toxicity test against mosquitoes with smoke from various coils are in Tables 7, 8 and Figure 1. They indicate that pyrs. I was in knock-down effect superior to pyrs. II and pyrethrins as tested by high concentration method (Table 7). The mortal activity of pyrs. I was comparable to that of pyrethrins. Allethrin and furamethrin had more rapid knock-down effect than pyrethrins, and the latter and resmethrin showed excellent mortal activity. Also in such a low concentration as that in the practical use (Table 8, Figure 1), it is found that the each coil tested shows the same tendency in efficacy as that in high concentration. Pyrs. I was superior to pyrethrins in the persistence of knock-down effect, and it was comparable to pyrethrins in the mortal activity (Table 8, Figure 1-A).

Table 5. Comparative effectiveness of several pyrethroids against houseflies (Lab-em-7-em strain) by oil spray test in glass chamber.

Insecticide in deobase (0.2% of a. i.)	Percent knock-down in minutes									KT ₅₀ (sec.)	Percent mortality after 1 day
	38"	53"	1'15"	1'45"	2'30"	3'30"	5'00"	7'00"	10'00"		
Pyrethrins I		3	10	18	35	53	68	80	90	211"	20
Pyrethrins II	8	15	35	58	68	88	93	95	98	103"	10
Pyrethrins	3	10	33	43	65	80	80	93	93	127"	23
Allethrin	3	5	13	20	40	58	88	100	100	169"	38
Resmethrin					5	20	53	80	98	292"	95
Tetramethrin		13	43	63	85	93	100	100	100	89"	20
Tetramethrin+ Resmethrin (8:2)	5	23	35	55	75	85	98	100	100	96"	95
Furamethrin		3	5	23	60	78	98	98	100	143"	68

Table 6. Comparative effectiveness of several pyrethroids against mosquitoes by oil spray test in glass chamber.

Insecticide in deobase (0.2% of a. i.)	Percent knock-down in minutes									KT ₅₀ (sec.)
	38"	53"	1'15"	1'45"	2'30"	3'30"	5'00"	7'00"	10'00"	
Pyrethrins I	5	8	15	40	43	70	83	98	100	145"
Pyrethrins II	10	13	28	39	62	80	87	95	95	121"
Pyrethrins	8	11	24	32	40	53	84	97	97	150"
Allethrin				10	30	55	73	95	100	201"
Resmethrin			3	5	13	30	55	78	90	279"
Tetramethrin	21	35	42	58	90	97	100	100	100	75"
Tetramethrin+ Resmethrin(8:2)	13	15	38	50	75	88	95	100	100	97"
Furamethrin			3	15	33	72	92	100	100	169"

Table 7. Comparative effectiveness of smokes from coils (0.5 gram containing 0.2% of active ingredient) against free-flying mosquitoes by high concentration method.

Insecticide in coil	Burnt time (sec.)	Percent knock-down in minutes						KT ₅₀ (sec.)	Percent mortality after 1 day
		3'00"	4'15"	6'00"	8'30"	12'00"	17'00"		
Pyrethrins I	10'19"	8	30	67	90	100	100	5'12"	70
Pyrethrins II	9'42"	6	6	11	12	18	36	—	58
Pyrethrins	10'07"	14	21	38	66	89	100	7'00"	81
Allethrin	10'23"	11	29	55	72	90	97	5'48"	50
Resmethrin	10'27"	7	12	28	59	89	100	7'30"	84
Allethrin+ Resmethrin (9:1)	10'14"	6	17	40	70	97	100	6'30"	64
Furamethrin	10'08"	20	59	84	97	100	100	4'00"	84
Blank	10'28"	5	5	5	8	8	16	—	23

Table 8. Comparative effectiveness of low concentration smokes from coils against free flying mosquitoes.

Insecticide in coil	Percent knock-down in minutes*									KT ₅₀ (sec.)	Percent mortality after 1 day
	3'	6'	12'	24'	34'	48'	60'	84'	120'		
Pyrethrins I 0.5%		17	75	95	96	98	97	99	95	8'24"	47
Pyrethrins II 0.5%				3	4	4	10	11	16	—	9
Pyrethrins 0.5%	1	3	31	67	74	84	88	88	89	18'20"	44
Allethrin 0.5%	5	26	62	66	59	53	48	54	54	9'15"	38
Resmethrin 0.5%			15	72	88	93	95	96	96	17'20"	64
Furamethrin 0.5%	36	90	100	100	100	100	100	100	100	3'25"	87
Allethrin 0.35%	2	13	50	64	52	44	40	44	47	11'48"	30
Pyrethrins 0.35%	1	7	23	57	65	69	72	68	68	21'12"	44
Allethrin + Resmethrin 0.30%	2	15	43	69	73	75	75	72	69	14'12"	37

*: More detailed data is shown in Figure 1.

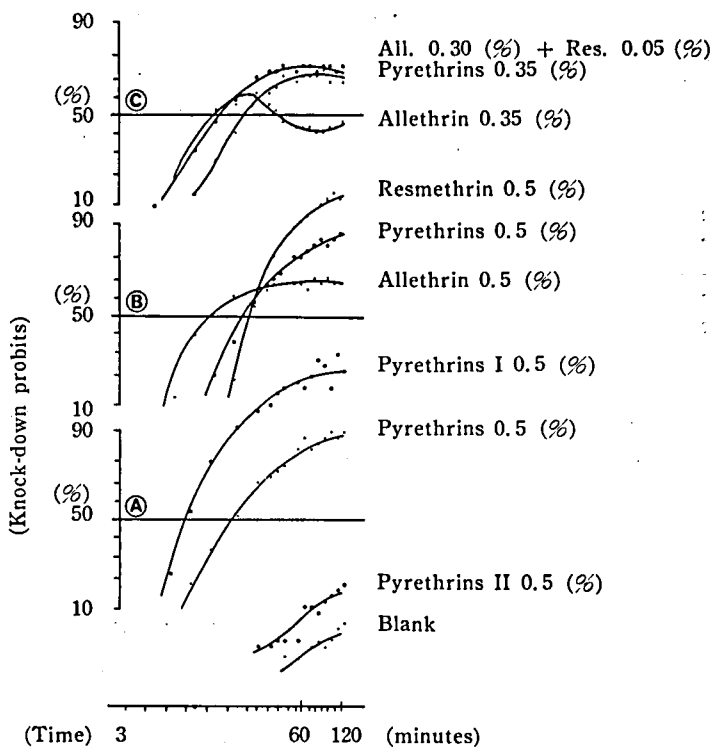


Fig. 1. The knock-down effect of coil against mosquitoes at indicated time.

The low activity of pyrs. II smoke may come from its low vapourization from coil⁸². Allelethrin showed quicker knock-down effect than pyrethrins, and resmethrin could continuously keep high knock-down level even after 24 minutes, being superior to pyrethrins (Figure 1-B). When small part of allelethrin was substituted by resmethrin, the persistence of knock-down effect exceeded that of allelethrin alone and become like that of pyrethrins (Figure 1-C). It is significant to mention that the mixed synthetic pyrethroids could have the same effectiveness properties as pyrethrins. Furamethrin also showed excellent knock-down and mortal activities in this case.

5) Joint action of natural pyrethroids and of synthetic pyrethroids

The intensity of joint action in mixtures was measured by comparing theoretical LD₅₀ values with experimental ones. The co-toxicity coefficients of mixtures were calculated by using the Sun's method²⁹. The results obtained from various mixtures of natural pyrethrins and synthetic pyrethroids, are given in Tables 9 to 11.

Mixtures of pyrs. I and II compounds in ratio of 51.4 to 48.6, which is believed to be the same as that found in natural pyrethrum extract, gave almost the same co-toxicity coefficient as natural pyrethrins. The value of natural pyrethrins was calculated from the consisting ratio of pyrs. I and II in them. Incho *et al.* reported that no one of the components of pyrethrum contributed the major portion of the synergism found with combination of piperonyl butoxide and pyrethrins using turntable methods against houseflies⁵⁷. From this series of experiments using topical application, however, it may be suggested that the combinations in several ratios of pyrs. I and II show the additive action or slight synergism (Table 9). And it is possible that the other components (82.7%) of natural pyrethrins except pyrs. I and II do not contribute to insecticidal activity. Tables 10 and 11 indicate the efficacy-mixing ratio relationships between resmethrin and tetramethrin or between resmethrin and allelethrin. It is likely that the synergism is observed at the ratio of tetramethrin to resme-

Table 9. Comparative effectiveness of joint action between pyrethrins I and pyrethrins II against houseflies (Lab-em-7-em strain).

Mixing ratio of pyrethroids	LD ₅₀ ($\mu\text{g}/\text{fly}$)		Co-toxicity coefficient
	Exp. value*	Theor. value**	
Pyrethrins I : Pyrethrins II			
100 : 0	0.49		
80 : 20	0.47	0.56	119
60 : 40	0.80	0.64	81
51.4 : 48.6	0.54	0.70	129
40 : 20	0.87	0.93	114
20 : 80	1.25	0.96	77
0 : 100	1.26		
Pyrethrins	0.58	0.70***	121

*: Experimental value

**: Theoretical value

***: This value was calculated from the consisting ratio of pyrs. I and II in natural pyrethrins.

Table 10. Comparative effectiveness of joint action between tetramethrin and resmethrin against houseflies (Lab-em-7-em strain).

Mixing ratio of pyrethroids	LD ₅₀ ($\mu\text{g}/\text{fly}$)		Co-toxicity coefficient
	Exp. value*	Theor. value**	
Tetramethrin : Resmethrin			
100 : 0	1.1		
80 : 20	0.12	0.21	173
60 : 40	0.09	0.12	128
40 : 60	0.065	0.079	122
20 : 80	0.055	0.061	110
0 : 100	0.049		

*: Experimental value

**: Theoretical value

Table 11. Comparative effectiveness of joint action between allethrin and resmethrin against houseflies (Lab-em-7-em strain).

Mixing ratio of pyrethroids	LD ₅₀ ($\mu\text{g}/\text{fly}$)		Co-toxicity coefficient
	Exp. value*	Theor. value**	
Allethrin : Resmethrin			
100 : 0	0.63		
80 : 20	0.125	0.15	120
60 : 40	0.057	0.085	143
40 : 60	0.044	0.059	135
20 : 80	0.042	0.046	109
0 : 100	0.037		

*: Experimental value

**: Theoretical value

thrin (80/20), allethrin to resmethrin (60/40), and allethrin to resmethrin (40/60). Fujimoto *et al.* reported a synergism in combination of resmethrin and tetramethrin at various ratios using turntable method¹⁶⁾. Hayashi also found synergism against houseflies (Takatsuki strain), using topical application²⁹⁾. He reported remarkable synergism with allethrin (80%) plus resmethrin (20%) system. The difference in degree of synergism between Hayashi's results and those in this report may be ascribed to the difference of houseflies strains.

The joint actions found and confirmed in the present investigation are of quite interest. Properly selected and proportioned synthetic pyrethroids system can be substituted for natural pyrethrins in practical applications.

Summary

The above mentioned results may be summarized as follows;

- 1) It seems probable that insecticidal activity of natural pyrethrum extract depends only on pyrethrins I and pyrethrins II. The former mainly shows the mortal activity and the latter does the quick knock-down effect, and the both show slight synergism. But pyrethrins II does not contribute to the effect of mosquito coils.
- 2) Tetramethrin has a quicker knock-down effect and resmethrin has a higher mortality than natural pyrethrins as tested by oil spray. Furthermore, synergism is observed in the mixture of them.
- 3) It is suggested that coils, which contain allethrin and a small amount of resmethrin, exhibit quick knock-down effect and has excellent effect to delay the recovery of the knocked down insect.
- 4) The difference between Lab-em-7-em and 203d strain of houseflies in susceptibility to pyrethroids is not clearly recognized in the pyrethroids tested.

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References

- 1) Sawicki, R. M., M. Elliott, J. C. Gower, M. Snarey and E. M. Thain: *J. Sci. Food Agric.*, **13**, 172 (1962).
- 2) Chang, S. C. and C. W. Kearns: *J. Econ. Entomol.*, **55**, 919 (1962).
- 3) Sawicki, R. M.: *J. Sci. Food Agric.*, **13**, 260 (1962).
- 4) Gersdorff, W. A.: *J. Econ. Entomol.*, **40**, 878 (1947).
- 5) Incho, H. H. and H. W. Greenberg: *J. Econ. Entomol.*, **45**, 794 (1952).
- 6) Ward, J.: *Chem. Ind.*, 1953, 586.
- 7) Elliott, M., P. H. Needham and C. Potter: *J. Sci. Food Agric.*, **20**, 561 (1969).
- 8) Webley, D. J.: *Pyrethrum Post*, **9** (4), 4 (1969).
- 9) Head, S. W., N. K. Sylvester and S. K. Chalinor: *Pyrethrum Post*, **9** (3), 14 (1968).
- 10) Sawicki, R. M.: *J. Sci. Food Agric.*, **13**, 591 (1962).
- 11) Casida, J. E.: *J. Agric. Food Chem.*, **18**, 753 (1970).
- 12) Elliott, M., A. W. Farnham, N. F. Janen, P. H. Needham and B. C. Pearsum: *Nature*, **213**, 493 (1967).
- 13) Kato, T., K. Ueda and K. Fujimoto: *Agr. Biol. Chem.*, **28**, 914 (1964).
- 14) Katsuda, Y., T. Chikamoto, H. Ogami, H. Hirobe and T. Kunishige: *Agr. Biol. Chem.*, **33**, 1361 (1969).
- 15) Nakanishi, M., T. Mukai, S. Inamasu, A. Tsuda and K. Abe: *Botyu-Kagaku*, **35**, 76 (1970).
- 16) Fujimoto, K., T. Kadota, Y. Fujita, Y. Okuno, and H. Koda: *International Aerosol Congress Report*, 1970, Tokyo.
- 17) Barthel, W. F., H. L. Haller and F. B. LaForge: *Soap and Sanit. Chemicals*, **20**, 121 (1944).
- 18) Sawicki, R. M. and E. M. Thain: *J. Sci. Food Agric.*, **12**, 137 (1961).
- 19) LaForge, F. B. and H. L. Haller: *J. Am. Chem. Soc.*, **57**, 1893 (1935).
- 20) Abe, Y. and Y. Fujita: *J. Agric. Chem. Soc. Japan*, **45**, 22 (1971).

- 21) Baba, N., A. Nagayasu and M. Ohno: *Agr. Biol. Chem.*, 34, 343 (1970).
- 22) Murano, A., S. Fujiwara, M. Horiba and J. Miyamoto: *Agr. Biol. Chem.*, 35, 1200 (1971).
- 23) Kato, T., J. Hattori, S. Kuramoto and N. Ooi: *Sumitomo Kagaku*, 1965-II, 18 (1965).
- 24) Nakanishi, M., T. Kuriyama and A. Kudo: *Botyu-Kagaku*, 35, 96 (1970).
- 25) Campbell, F. L. and W. N. Sullivan: *Soap and Sanit. Chemicals*, 14, 119 (1938).
- 26) Sawicki, R. M. and M. Elliott: *J. Sci. Food Agric.*, 16, 85 (1965).
- 27) Okuno, Y., K. Fujimoto, T. Kadota, J. Miyamoto and K. Hamuro: *Botyu-Kagaku*, 34, 157 (1969).
- 28) Sun, Y. P. and E. R. Johnson: *J. Econ. Entomol.*, 53, 887 (1960).
- 29) Hayashi, A.: *Japanese J. of Sanit. Zoology*, 20, 261 (1970).

Studies on Sex Pheromone of Pyralididae IV. The Male Response to the Female Sex Pheromone of the Almond Moth, *Cadra cautella* Walker (Phycitinae). Fumiki TAKAHASHI, Akio MASUI, Yasumasa KUWAHARA, Shoziro ISHII and Hiroshi FUKAMI (College of Agriculture, Kyoto University, Kyoto) Received April 20, 1972. *Botyu-Kagaku*, 37, 56 1972. (with English Summary 60).

9. メイガ科の性誘引物質に関する研究 第IV報 スジマダラメイガの性フェロモンに対する雄成虫の行動 高橋史樹, 樹井昭夫, 桑原保正, 石井象二郎, 深海 浩 (京都大学農学部) 47. 4. 20 受理

スジマダラメイガ雌の性フェロモンに対する雄成虫の応答行動を2種の生物試験法によって比較した。生物試験法-1は100×150×160 cm (2.4 m³) の箱の中で、生物試験法-2は径3 cm, 長さ30 cm のガラス管内で一定流量の空気を流した中で行なわれた。雌成虫の粗抽出物はいずれの試験法によっても、供試量が大いほど誘引性は高くなる。一方、精製フェロモン (*cis*-9, *trans*-12-tetradecadienyl acetate) は生物試験法-2では粗抽出物の場合と同じ傾向の結果を示すが、生物試験法-1においては、供試量が一定の限度を越すと、誘引性を示さなくなる結果となった。

さきに、スジマダラメイガ *Cadra cautella* Walker, ノシメダラメイガ *Plodia interpunctella* Hübner, スジコナダラメイガ *Anagasta kuhniella* Zeller の3種の未交尾雌から性フェロモンを抽出単離し、いずれも同じ化合物で、構造は *cis*-9, *trans*-12-tetradecadienyl acetate であることを報告した^{1,2,3,4}。引続いて、チャダラメイガ *Ephesia elutella* Hübner の雌の性フェロモンもまた同一化合物であることが明かにされた⁵。このように、近縁の蛾が同じ性フェロモンを分泌発散している事実が明らかになったことから考えて、これらの蛾が自然の条件下で配偶に際して、たがいに種の識別をしている機構の解明に興味を覚えざるをえない。Brady らはノシメダラメイガの雌成虫あるいはその粗抽出物を誘引源として試験する場合にはノシメダラメイガ雄成虫に比してスジマダラメイガ雄成虫が誘引される度合が極端に低い事実を明かにし、ノシメダラメイガ雌成虫にはスジマダラメイガ雄成虫に対する性フェロモンの活性を阻害する物質の存在を推定している^{2,6}。このような事実はマダラメイガ類の配偶における種の識別の要因の1つを示唆しているが、いまのところでは、これが要因のすべてと切り切るには問題がある。ここではマダラメイ

ガ類の配偶における種の識別の機構を探る目的で、まず性フェロモン活性の生物試験法を検討した。マダラメイガ類の性フェロモンの単離においては、カイコガの場合の Karlson-Butenandt の方法⁷ に準じて、小さな空間での試料に対して雄成虫が翅を小刻みに振動させる行動 (以下、バツキ行動と略す) を性フェロモン活性の有無の指標とした⁸。今回はさらに大きな空間での生物試験や空気の流れの中での試験を行ない、性フェロモンに対する雄成虫の反応を比較した結果について報告する。

材料と方法

供試雄成虫: 30°C で長日条件下 (16時間明期: 8時間暗期) で腰高シャーレ (径11 cm×高7 cm) 内で、米ぬか20 g を飼料として卵約200 を用いて継代飼育し、得られたスジマダラメイガ (FT-系統⁹) 雄成虫の羽化後3日目の未交尾のものを試験に供した。通常、羽化は夕刻に始まり、暗期開始2時間以内にほとんどが集中する。羽化後1時間以内に雌雄を分けることによって未交尾雄を集めた。雄成虫の性フェロモンに対する感受性は羽化後2日間に急激に増大し、その後死亡するまで徐々に増え続けるが、余り日数が経過すると