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Investigations on the Chemical Compositions and Biological Efficiencies of Some Public Health Pesticide Formulations under Different Storage Conditions*

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Seven public health insecticide formulations were stored at 4, 21ºC with 50-55 % relative humidity and 31ºC with 60-70% relative humidity in 1 L coex bottle during 12 months. The formulations contained two synthetic pyrethroids (deltamethrin and permethrin) and two organophosphates (chlorpyrifos-ethyl and chlorpyrifos-methyl) active ingredients. Changes of active ingredients were determined by gas chromatography equipped with flame ionization detector at intervals of 3 months. Biological efficacy of insecticides on house fly (*Musca domestica* L.) were investigated at intervals of 3 months and the residual efficiency of the insecticides which were applied to surface floor tiles were measured at intervals of 15 ± 3 d during 12 months. In spite of unacceptable changes of active ingredients in certain times, the biological efficacy test results of formulations stored in the original package showed that death and knock down effect percentages were above the effectiveness level (70%) in all conditions during 12 months. Although knock down effect percentages of all formulations, which were applied on surface floor tiles, were very low in the end of the study. Biological efficiency was determined longer than expected. Knock down effect of the synthetic pyrethroid formulations were determined longer than organophosphates. No statistical significance was determined between storage conditions ($p > 0.05$).

Key Words: Insecticide, Stability, Chemical composition, Biological efficacy.

INTRODUCTION

Chemical control, which includes the use of different alternatives of insecticides, is the most important element in the integrated approach to control of vectors and pests of public health importance[1,2]. However, these

*This article was summarized from author's doctoral thesis.

products must have been manufactured with high standards and certain pharmacological and toxicological principles. Good product quality is essential to the effectiveness of pesticides and to minimizing any risk involved in their use[3,4]. But, according to WHO, some 30 % of pesticides marketed in developing countries for agricultural and public health use, with an estimated annual market value of US$ 900 million, do not meet internationally accepted quality standards[5].

When stored improperly, pesticides can break down, especially under conditions of high temperature and humidity. Some pesticides can lose their active ingredients through chemical decomposition or volatilization. If the content of active ingredient in a product is less than the declared level, the results could be monetary loss and the application of sublethal doses-leading to ineffective control and potential development of resistance. Furthermore, some pesticides become more toxic, flammable, or explosive as they break down and they may cause unacceptable effects on non-target organisms[6-9].

The aim of this study is to investigate the chemical composition and biological efficacy changes of the some public health insecticide formulations under different storage conditions. Furthermore, the determination of residual efficiencies of the formulations on the application surface, under different conditions was also studied. The basic characteristics of the public health insecticides were evaluated with in the frame of the quality, market control and effectiveness.

EXPERIMENTAL

The study was performed according to the pesticide long term stability test procedure of The Ministry of Health of Turkey[10]. Public health insecticide samples were stored under three different storage conditions: +4ºC, +21ºC with 50-55 % relative humidity and +31ºC with 60-70 % relative humidity. A sample, taken from the formulations, was used in 1 L coex bottle for each condition. The samples were kept during 12 months and chemical analysis and biological efficacy tests were made with selected methods at the beginning and 3rd, 6th, 9th, 12th months. Furthermore, the insecticide formulations were applied on the surface floor tiles at the beginning of the study and they were stored same conditions for determination the residual efficacy. The residual efficacy tests were made at approximately intervals of 15 ± 3 d.

In all 7 public health insecticide formulations were used in the study. The formulations contained two synthetic pyrethroids (deltamethrin and permethrin) and two organophosphates (chlorpyrifos-ethyl and chlorpyrifos-methyl). Specifications and application doses of the formulations are shown in Table-1.

TABLE-1
SPECIFICATIONS AND APPLICATION DOSES OF THE USED INSECTICIDE FORMULATIONS

Formulation	Insecticide	Chemical type[a]	Formulation type[b]	a.i.[c] in the formulation ($g\ L^{-1}$)	Dosage of a.i. recommended by WHO (g/m^2)[d]	Dosage of a.i. used in the study (g/m^2)
1	Deltamethrin	PY	SC	50	0.075-0.015	0.011
2	Deltamethrin	PY	SC	50	0.075-0.015	0.011
3	Permethrin	PY	EC	250	0.0625	0.0625
4	Permethrin	PY	EC	250	0.0625	0.0625
5	Chlorpyrifos-methyl	OP	EC	250	0.4-0.6	0.5000
6	Chlorpyrifos-methyl	OP	EC	225	0.4-0.6	0.5000
7	Chlorpyrifos-ethyl	OP	EC	250	0.2	0.2000

[a]PY: Synthetic pyrethroid, OP: Organophosphate
[b]SC: Suspension concentrate, EC: Emulsifiable concentrate
[c]a.i.: active ingredient
[d]WHO recommended residual treatment doses for fly control.[11]

Certified insecticide standards were used for the chemical analysis. Chlorpyrifos-ethyl (99.1 %) and chlorpyrifos-methyl (99.8 %) were purchased from Dow Chemicals (Indianapolis, USA), deltamethrin (99.6 %) were purchased from Aventis CropScience (Lyon, France) and permethrin (96 %) were purchased from Changzhou Ltd. (Changzhou, China). Acetone, ethyl acetate and chloroform were obtained from Merck (Darmstadt, Germany) and methanol was obtained from Riedel-deHaen (Hanover, Germany).

Gas chromatography was performed on a Shimadzu GC-7A gas chromatograph with flame ionization detector (FID). A glass column (OV-210, 1 mL diameters) was used. Two microliter of sample was injected in the splitless mode. Shimadzu C-R1B integrator was used as the data system. The detector temperature was 250-280ºC, the injector temperature was 250-280ºC and the column oven temperature was 200-280ºC. Carrier gas was nitrogen carrier at 50 mL/min.

A refrigerator (Arcelik, Ankara, Turkey) was used for +4ºC condition, a climatic cabinet (Elektro Mag, Istanbul, Turkey) was used for +21ºC with 50-55 % relative humidity RH condition and an incubator (Elektro Mag, Istanbul, Turkey) was used for +31ºC with 60-70 % relative humidity condition.

Susceptible WHO housefly (*Musca domestica* L.) population was used as the experiment organism in the biological efficiency tests. The population was reared at 26 ± 1ºC with 55 ± 5 % relative humidity with a 12 h photoperiod in Hacettepe University Insecticide Test and Production Laboratory. A generation was completed in 15 ± 2 d and adult house flies were

used for bioassays in 4-6 d. Biological efficiency tests were performed according to the WHO standard residual method (jar methods) with some deviations[12-14]. Application solutions were prepared according to the WHO recommended dosage (Table-1) and the solutions sprayed to surface floor tiles[15]. Then, glass lanterns were closed on these surfaces and counted houseflies were put. The houseflies were waited during 15 min and knock down effected fly counts were determined. After that, house flies were put in labelled clear jars for 24 h. After 24 h alive and dead organism counts were determined. Thus, test data, which are based on insecticidal activity of insecticides, were obtained. To determination of the residual efficacy, the insecticide formulations were applied on the surface floor tiles with likewise method at the beginning of the study and they were stored in the test conditions. Same biological efficiency tests were performed after intervals of 15 ± 3 d during 12 months.

Tolerance limits on content of active ingredients of WHO and FAO were accepted in the chemical analysis[11,16]. According to the these, ± 10 % tolerance limit was used for the formulations, contained 50 g L^{-1} active ingredients and ± 6 % tolerance limit was used for the formulations, contained 225 and 250 g L^{-1} active ingredients. In the biological efficiency tests, 70 % mortality and knock down effect values were accepted efficient[12-14]. Variance analysis in the repeated measures was used to compare the obtained data and The Duncan Test was used to determination of the significance between groups ($p < 0.05$)[17-19].

RESULTS AND DISCUSSION

The results of chemical analysis during 12 months are shown in Table-2. Most of the variety values (%) were found under the declared active ingredients. But, due to decrease of water or other solvents of formulations in the stored conditions, some of the variety values were found above the declared counts. Death and knock down effect percentages of the formulations, stored in the original package, on house fly are shown in Table-3. Total 20 biological efficiency test results were made at approximately intervals 15 ± 3 d (related to the life period of house flies) during 12 months are shown in Table-3.

The temperature range normally recommended for liquid insecticides is 40 to 100ºF (*ca.* 4 to 40ºC) and stability tests make in these conditions[8,11]. In the present study, all formulations were found suitable according to declared content of the active ingredients at the beginning chemical analysis. However active ingredients of some formulations (4 from 7) were found out of the tolerance limits in some conditions at certain times. Generally, unexpected chemical analysis results were found in the 31ºC with 60-70 % relative humidity condition. But, these findings were not determined statistically important ($p > 0.05$).

TABLE-2
RESULTS OF CHEMICAL ANALYSIS*

Form.	Active ingredient and tolerance limit (TL)	Condition (°C/RH%)	Beginning		3rd month		6th month		9th month		12th month	
			Found g L^{-1}	Variety %	Found G L^{-1}	Variety %	Found g L^{-1}	Variety %	Found g L^{-1}	Variety %	Found g L^{-1}	Variety %
1	Deltamethrin 50 g L^{-1} TL.: ±10%	+4	50.8	1.68	51.8	3.60	45.8	8.40	49.9	0.20	40.9	**18.20**
		+21/50-55	50.8	1.68	51.8	3.60	48.6	2.80	51.1	2.20	44.2	**11.60**
		+31/60-70	50.8	1.68	48.8	2.40	45.6	8.80	41.5	**17.00**	25.4	**49.20**
2	Deltamethrin 50 g L^{-1} TL: ±10%	+4	53.3	6.60	48.9	2.20	49.3	1.40	53.5	7.00	46.9	6.20
		+21/50-55	53.3	6.60	45.8	8.40	46.1	7.80	49.4	1.20	46.5	70
		+31/60-70	53.3	6.60	47.6	4.80	45.8	8.40	53.6	7.20	47.0	6.00
3	Permethrin 250 g L^{-1} TL: ±6%	+4	244.7	0.52	251.2	0.48	261.5	4.60	258.1	3.24	253.2	1.28
		+21/50-55	244.7	0.52	241.1	0.44	255.1	2.04	262.1	4.84	279.0	**11.60**
		+31/60-70	244.7	0.52	251.8	0.72	258.3	3.32	262.6	5.04	268.0	**7.20**
4	Permethrin 250 g L^{-1} TL: ±6%	+4	242.3	3.08	250.1	0.04	242.9	2.84	242.8	2.88	211.2	**15.52**
		+21/50-55	242.3	3.08	238.3	4.68	246.1	1.56	257.9	3.16	244.3	2.28
		+31/60-70	242.3	3.08	241.8	3.28	248.5	0.60	238.7	4.52	249.2	0.32
5	Chlorpyrifos metyl 250 g L^{-1} TL: ±6%	+4	244.2	2.32	232.3	**7.08**	227.5	**9.00**	223.4	**10.64**	204.3	**16.00**
		+21/50-55	244.2	2.32	253.5	1.40	220.5	**11.80**	234.4	**6.24**	212.1	**15.16**
		+31/60-70	244.2	2.32	223.8	**10.48**	231.4	**7.44**	229.9	**8.04**	201.2	**19.52**
6	Chlorpyrifos metyl 225 g L^{-1} TL: ±6%	+4	237.8	0.97	221.6	1.51	212.4	5.60	230.0	2.22	214.7	4.57
		+21/50-55	237.8	0.97	221.1	1.73	213.2	5.20	221.2	1.68	236.5	5.11
		+31/60-70	237.8	0.97	227.0	0.88	221.8	1.40	218.6	2.84	214.0	4.88
7	Chlorpyrifos etyl 250 g L^{-1} TL: ±6%	+4	252.8	1.12	243.5	2.60	247.5	1.00	254.3	1.72	255.5	2.20
		+21/50-55	252.8	1.12	255.5	1.80	238.1	4.76	244.0	2.40	241.1	3.56
		+31/60-70	252.8	1.12	247.5	1.00	254.4	1.76	255.6	2.24	246.8	1.28

*Bold typed values are out of the tolerance limits; RH = Relative humidity.

TABLE-3
DEATH AND KNOCK DOWN EFFECT PERCENTAGES OF THE FORMULATIONS STORED IN THE ORIGINAL PACKAGE AND APPLIED ON SURFACE FLOOR TILES ON HOUSE FLY[d]

Formulation and active ingredient	Conditions	Stored in original package						Applied on surface floor tiles					
		B		12th month		Mean*		B		12th month		Mean**	
		dx%[e]	KD%[f]	dx%	KD%	dx%	KD%	dx%	KD%	dx%	KD%	dx%	KD%
1	+4	100	100	97.05	100	99.4[a]	100[a]	100	100	81.08	**14.5**	90.5[a]	**51.3[a]**
Deltamethrin	+21/50-55	100	100	100	100	99.8[a]	98.4[a]	100	100	85.0	**60.0**	96.0[a]	83.3[a]
50 g L^{-1}	+31/60-70	100	100	95.4	100	99.1[a]	100[a]	100	100	92.5	**52.5**	95.5[a]	84.9[a]
2	+4	100	99.4	100	100	100[a]	99.9[a]	100	100	78.1	**66.8**	95.1[a]	83.0[a]
Deltamethrin	+21/50-55	100	99.4	100	100	100[a]	98.5[a]	100	100	79.1	**68.3**	96.9[a]	88.2[a]
50 g L^{-1}	+31/60-70	100	99.4	100	100	100[a]	99.2[a]	100	100	93.75	**53.8**	97.6[a]	92.6[a]
3	+4	100	96.8	96.4	100	98.9[b]	96.8[b]	100	100	70.5	**31.1**	82.5[b]	**65.6[b]**
Permethrin	+21/50-55	100	96.8	89.4	100	97.7[b]	92.7[b]	100	100	90.9	**25.3**	90.9[b]	**68.6[b]**
250 g L^{-1}	+31/60-70	100	96.8	96.2	100	99.0[b]	98.8[b]	100	100	83.8	**50.0**	90.3[b]	77.8[b]
4	+4	100	89.1	78.3	**61.6**	88.5[b]	88.6[b]	100	89.1	77.2	**25.6**	78.3[b]	**49.8[b]**
Permethrin	+21/50-55	100	89.1	70.6	71.03	93.4[b]	83.7[b]	100	89.1	72.1	**36.3**	85.8[b]	**67.7[b]**
250 g L^{-1}	+31/60-70	100	89.1	93.7	**60.6**	98.3[b]	82.9[b]	100	89.1	78.6	**24.1**	82.2[b]	**59.1[b]**
5	+4	100	97.5	100	100	100[b]	95.1[c]	100	97.5	80.0	**6.7**	81.1[b]	**21.1[c]**
Chlorpy. metyl	+21/50-55	100	97.5	86.2	93.1	97.2[b]	97.7[c]	100	97.5	81.3	**8.2**	84.03[b]	**28.0[c]**
250 g L^{-1}	+31/60-70	100	97.5	91.6	87.5	98.3[b]	94.0[c]	100	97.5	89.6	**6.2**	81.6[b]	**18.7[c]**
6	+4	100	98.5	100	100	99.8[b]	97.5[c]	100	100	78.5	**2.8**	86.3[b]	**29.0[c]**
Chlorpy. metyl	+21/50-55	100	98.5	96.2	100	99.2[b]	96.9[c]	100	100	93.02	**6.0**	88.3[b]	**28.4[c]**
225 g L^{-1}	+31/60-70	100	98.5	100	100	100[b]	95.2[c]	100	100	87.2	**3.3**	85.6[b]	**21.9[c]**
7	+4	100	97.5	100	77.2	100[b]	82.3[c]	100	100	80.0	**5.5**	83.2[b]	**22.7[c]**
Chlorpy. etyl	+21/50-55	100	97.5	100	100	100[b]	93.5[c]	100	100	70.0	**12.8**	83.5[b]	**27.2[c]**
250 g L^{-1}	+31/60-70	100	97.5	100	100	100[b]	92.5[c]	100	100	82.8	**22.3**	82.3[b]	**27.7[c]**

*Average of totally 5 biological tests which were made intervals of 3 months. **Average of totally 20 biological tests which were made intervals of 15±3 days. [a,b,c]Means with different superscript in the same column differ significant ($p < 0.05$). [d]Bold typed values are under the effectiveness level (70 %), [e]dx%: Death percentage, [f]KD%: Knock down effect percentage.

The mistakes of manufacturing, formulating and packaging of products may be cause of unacceptable changes of chemical compositions[8,20]. Also, the formulation type, the types of stabilizers and emulsifiers used in the product, the type of container and its closure are effect insecticide shelf life[9]. In this study, formulation types of all formulation were EC and SC, also all formulations were stored same coex bottles. Different manufacturing date of the formulations, types and impurities of the active and other ingredients used in the products and the mistakes of manufacturing and formulating of products were considered the cause of changes of chemical composition[16,20].

Although unacceptable changes of active ingredients, except a permethrin formulation (formulation 4), the biological efficacy test results of the formulations stored in the original package showed that, death and knock down effect percentages were above the effectiveness level (70 %) in all conditions during 12 months. Only, knock down effects of this formulation were found under the effectiveness level in the 4 and 31ºC with 60-70 % relative humidity conditions at the end of the study. No statistical significance was determined between conditions and formulations ($p > 0.05$). These findings may show that, changes of the active ingredients are not adequate for decreasing of the biological efficacy. It is also reported that in spite of chemical decomposition, some insecticide formulations can effective on the target organisms in the end of the shelf life[7,9]. Furthermore, usage of the susceptible WHO housefly population as experiment organism is another important factor of the high biological efficacy[15,21].

The death percentages of formulations, applied on surface floor tiles were determined very high at the beginning (100 % for all formulations). The death percentages were decreased from time to time, but they were found above the accepted effective level (70 %) in all conditions end of the study. Deltamethrin formulations were observed statistically different from other formulations ($p < 0.05$) and no statistical significance was determined between conditions ($p > 0.05$).

The biological activity of the insecticides on the house fly depends on insecticide dose, climate, surface and resistance level of house fly population[15,21,22]. Also the efficiency duration of the various insecticides on the house fly normally occur between 1-2 weeks to 1-2 months[15]. But, in the present study, as an interesting result, death percentages were determined high in all storage conditions during 12 months. Although this finding depend on usage of the susceptible WHO housefly population in the study, other factors, such as true dose, suitable application, not to cleaning the surface, may be prolonged the biological effect of the insecticide formulations on a large scale[15,21,22].

Knock down effect percentages of formulations, applied on surface floor tiles, were high in the beginning (89.1-100 %), but these values decreased (sometimes occurred 0) in time. Although the knock down effect percentages of all formulations were very low in the end of the study, they were determined longer than expected. Decrease of knock down effect occurred in organic phosphate formulations earlier. Knock down effect percentages of organophosphate formulations generally was observed under the effective level on 32 d and afterwards. However, synthetic pyrethroid formulations showed the long activity. Deltamethrin formulations were observed statistically different from other formulations and permethrin formulations were observed statistically different from organophosphate formulations ($p < 0.05$). No statistical significance was determined between storage conditions again ($p > 0.05$).

Synthetic pyrethroids causes a rapid and strong knock down effect and this effect is longer than other insecticide groups (organophosphates, organochlorins and carbamates)[1,2,22]. Knock down effects of deltamethrin and permethrin were found until 16 weeks in various studies[21,23,24]. Similarly, synthetic pyrethroid formulations showed the higher and longer knock down activity in present study.

As a result, it was concluded that suitable storage conditions, application practice and application period are very important for effective insect control and public health. Governments have to do market control frequently and registration authorities have to require straight real time stability tests from insecticide manufacturers. Furthermore, advanced studies should have been done about this matter and detailed information should have been given to manufacturers, applicators, other concerned persons and public.

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