rrelation coefficient is found to be 0.8901 in the male, and 0.8799 in the female and the correlation is significant under the level of five percent. From the above results, the writer is inclined to believe that the x^k transformation in constructing the time mortality curve which is adopted in the present study, is justified theoretically.

(4) The difference between the resistability of the male and female is distinctly. The male is more susceptible to heat than the female when compared with their median lethal time. But it is necessary to determine whether the similar result is expected or not within the limits of experimental and sampling error, According to the method of Bliss, the discrepancy between the time-mortality curves of the two sexes is tested. It has been found that the χ^2-value is significant for the range from 30°C～44°C while is not significant for 46° and 48°. The difference between the resistability of male and that of female, therefore, is not seen at high temperature.

(5) Applicability of the present method (the method of X^k tramsformation) to other cases has been examined in the case of the results obtained by some previous writers; i.e. with the azuki-bean weevil by Nishikawa, mosquito larva by the present writer, the rice weevil by Tuchiya and also by Kohno. The time mortality curves of those data differ slightly according to the intensity of heat. These asymmetrical mortality curves can be always transformed into a straight regression line by adopting the writers method and the correlation between the change of temperature coefficients b and that of k in x^k transformation is high.

It is suggested, therefore, that the variation of heat resis'ance of insects is represented by the normal distribution when x^k is taken as the time unit on the abscissa and k in x^k indicates different values with increasing temperature.

Insect Toxicological Studies on the Joint Toxic Action of Insecticides II. On the Joint Toxic Action between Contact Insecticides 1. Seiroku SAKAI, Minoru SATŌ and Ken'ichi KOZIMA (Agricultural Chemicals Inspection Station, Nishigahara, Tokyo, Japan) Received June 10, 1951. *Botyu-Kagaku* 16 : 130 (1951)

22. 殺虫剤の連合作用に関する昆虫毒物学的研究(第2報)接触殺虫剤相互の連合作用

酒井清六・佐藤稔・小島建一 (農林省農薬検査所) 26.6.10. 受理

接触殺虫剤相互の組合せを全般的に研究した報告はない。ここでは1), 接触殺虫剤相互の組合せによる効力の増進, 2), 連合作用概念の把握, 3), 殺虫機構の解明, 4), 混合殺虫剤の生物検定の確立の目的のために研究した。供試昆虫は 25°C で京大式処方で培養したキイロショウジョウバエの痕跡翅系統 *Drosophila melanogaster*, *Vestigial*, を総数5万匹用いた。実験は 20°C 恒温浸漬法で, 2 分間浸漬し, 処理後30分に生死の別を観察した。実験は単独薬剤と51の組合せの混合薬剤との2法を施行し, 混合は単独に用いた時の両剤の MLD の等毒量を定め, Horsfall(1945) 法に従つて, 100, 90+10, 80+20, 70+30, 60+40, 50+50, 40+60, 30+70, 20+80, 10+90, 100 (容量比) に混合した。その結果, 単独薬剤では, 毒力の強いものより, 1), Pyrethrins, 2), Allethrin, 3), Rotenone, 4), Gamma BHC, 5), TEPP, 6) PP' DDT, 8), Parathion, 9), o, o-diethyl-o-para-nitrop-henylphosphate, 10), Dieldrin, 11), Nicotine (石鹸添加せず) 12), Aldrin, 13) Toxaphene であつた。chlordane は殆んど毒力がなかつた。

混合薬剤の実験では, この MLD の等毒量を混合し, 再度実験を行い, 再実験の死亡率水準を求め, 更に各混合比の実験値を修正し, 各混合比の連合作用の力価を求め, 力価をグラフ上に作図した後, 100→50+50→100の力価を Planimeter で総計し, 総計値を対数値に変換し組合せ間の連合作用を評価した。

その結果, 協力, 拮抗の両作用が認められたが, 顕著な作用は少なく, 一方的連合作用で相互的連合作用はなかつた。接触殺虫剤の組合せによる連合作用には一定の法則を認め難かつた。この中, 協力作用の優れている組合せは, Toxaphene + Nicotine, Aldrin + Nicotine, BHC + Dieldrin, Toxaphene + TEPP, Rotenone + Nicotine, Dieldrin + Nicotine, OP' DDT + TEPP, BHC + Nicotine

の順序で，拮抗作用は大きなものから Rotenone + Toxaphene, Aldrin + PP'DDT, Rotenone + Aldrin, Nicotine + PP'DDT の順序であつた。

大観して接触殺虫剤相互の組合せを考察すると，*Drosophila* に対して，Dieldrin, Nicotin, TEPP, BHC の順に，これらの薬剤と他剤とを組合せると協力作用が発現し易く，その他の薬剤は殆んど意義なく，軽い拮抗作用をする。この中 Rotenone との組合せは一番悪い。結論的には軽い協力作用から軽い拮抗作用までありこれによつて強力な効果を期待することは困難である。

INTRODUCTION

The joint toxic action of insecticides is a fundamental problem in insect toxicology and their action is also important from a practical viewpoint. Today, a few insecticides are used singly, while they must be used in mixed together for various reasons. Interactions often occur between various components, sometimes by design, sometimes by accident. These interactions can be classified into two categories, synergism and antagonism. But, these categories are hazy and incomplete. Clark (1933, 37), Bliss (1939), Finney (1942, 47), Plackett & Hewlett (948, 50) and Wadley (1945, 49) have published papers on the statistical investigations dealing with joint toxic action. Horsfall (1945) has recently published a book on the toxicology of fungicide in which he proposed that the joint toxic action can be classified into four categries according to its action; I. Potentiated synergism, II. Supplementary synergism, III. Potentiated antagonism, and IV. Substractive antagonism.

Recently, the problem of the joint toxic action of insecticides has very extensively been reviewed by Sakai (1949, 1950), one of the present authors. He studied the joint toxic action between Monochlorbenzen and Chloroform and proposed the idea opposing to the Läuger's theory of DDT from the standpoint of their combination. Recently, Sakai and his co-workers (1951) studied experimentally the joint toxic action of some soaps for Pyrethrins using the adult of the rice weevil, *Sitophilus oryzae* L. The potencies of the joint toxic action to each mixture were expressed in the order of effectiveness as follows: 1) 60% Soap plus 40% Sodium carbonate, 2) 60% Soap plus 40% Casein, 3) 60% Soap plus 40% Bentonite, 4) Soap alone, and 5) 60% Soap plus 40% Sodium sulphate. In the case of the 60% Soap plus 40% Sodium carbonate, the highest potency of joint toxic action was shown at the point of equal proportions of two agents.

The present investigation attempts to demonstrate the joint toxic action between some of the contact insecticides. Much efforts have been done by many insect toxicologists for discovering the theory on the combinations of contact insecticides. Toxicological investigations on several combinations between contact insecticides have been reported by Gnadinger & Corl (1932), Lepelly & Sullivan (1936), Lilley (1943), Morrison (1943). Turner & Saunders (1947), Siegler & Bowen (1947), Medler & Chamberlin (1948), Sun (1948), Fransen (1948), Medles & Thompson (1949) and Mayer and his coworkers (1950). In these investigations, however only limited combinations of contact insecticides were made. The present work has following purposes: to extend the effectiveness for practical application, to establish the method of biological assays of combined insecticides, to find the mode of action of insecticide.

The authors wish to express their gratitude to Doctor S. Utida, Profesor of entomology, Kyoto University, for his help and advice given them during the course of the present investigation. The authors are also indebted to Director A. Kamito, Agricultural Chemicals Inspection Station, Ministry of Agriculture & Forestry and to Mr. Toshioka, 406th Med. Gen. Lab.. Without Mr N. Hatai's kindness this paper could not been published in English language.

MATERIALS AND METHODS.

The insect used is the adult of the vestigial form of *Drosophila melanogaster* which is reared with artificial culture media in a milk bottle 180 cc under constant temperature of 25° C. The culture medium per bottle consists of water, 30 cc; potato, 15gr.; sucrose, 2gr.; agar-agar, 0.45 gr.; and pepton, 0.3gr. The vestigial form is convenient as test insect, because the form can not fly during observation. The total number of tested flies used about 50,000.

The following insecticides, which are having the following percentages of active ingredients, were tested: 0.5% Pyrethrins 2.0% Rotenone, 40.9% Nicotine, 0.5% Allethrin, 10% Gamma BHC, 10% PP'-DDT, 10% OP'-DDT, 21.8% Aldrin, 20.4% Dieldrin, 50% Toxaphene and 37.6% TEPP. Each of these insecticides is emulsified or is made in water soluble form, is diluted more than 10 times for using the experiments. The immersion method is used for the present investigation. The immersion procedure is as follows. Fifty insects are placed in a glass cylinder of 13.3 cc wire netting on the both ends are fastened with rubber bands. The diluted drug is poured in the test tubes of 1.7 cm in diameter and of 17 cm in length. The drug is maintained at constant temperature of 20°c in a water bath. The immersion time is 2 minutes. After the treatment, the insects are placed quickly on filter paper. Then, the insects are at once transferred in an incubater under the constant temperature of 30°C. The percentages of mortality are examined 30 minutes after the treatment.

In the case of experiment on various mixtures of each, the combination of two insecticides is based on the median lethal dose of each, which is the point of an equitoxic dose. In practice, the experimenter prepares insecticidal fluid of the median lethal dose of each. Then he takes the test mixtures as the following formulations; one insecticide alone, 90% of one plus 10% of the other insecticide, 80% plus 20%, 70% plus 30%, 60% plus 40%, 50% plus 50%, 40% plus 60%, 30% plus 70%, 20% plus 80%, 10% plus 90% and another insecticide alone (ratio by volume).

The other points for the test mixtures are similar to those of alone. After experiments, the results obtained are analysed by a mathematical examination concerning the joint toxic action.

RESULTS OF TESTS

The results of experiments on a insecticide alone, are given in table 1. Data of the experiment in dosage-mortality relation for each insecticide is calculated by the following equation, $Y=5+b(X-\bar{x})$, where Y is the mortality in Probit, X is the logarithm of the concentration, b is the slope of line and $\bar{x}$ is the median lethal dose in logarithm.

Table 1. Comparative toxicity of thirteen contact insecticides against the pomace fly.

Insecticide	Percent of active ingredient	MLD of sample	Provisional regression equation*
Pyrethrins	0.5	0.00068	$Y=5+4.00(X+0.17)$
Rotenone	2.0	0.00340	$Y=5+6.67(X-0.63)$
Nicotine	40.9	0.68160	$Y=5+1.54(X-2.80)$
Allethrin	0.5	0.00176	$Y=5+4.17(X-0.24)$
γ BHC	10.0	0.01380	$Y=5+2.94(X-1.14)$
PP'-DDT	10.0	0.01960	$Y=5+4.76(X-1.36)$
OP'-DDT	10.0	0.02659	$Y=5+5.00(X-1.43)$
Aldrin	21.8	0.72500	$Y=5+3.85(X-2.86)$
Dieldrin	20.4	0.52500	$Y=5+10.00(X-2.72)$
Toxaphene	50.0	1.99500	$Y=5+2.94(X-3.30)$
TEPP	37.9	0.01480	$Y=5+5.88(X-1.17)$
Parathion	10.0	0.03630	$Y=5+4.17(X-1.56)$
Paraoxon	10.0	0.28900	$Y=5+4.55(X-2.46)$

Paraoxon is O, O-diethyl-O-para-nitrophenylphosphate.

* Y is the mortality in Probit, X is the logarithm of [(Concentration) $\times 10^3$]

As shown in table 1, Pyrethrins were markedly more toxic than the other insecticides. It is interesting to note that Allethrin is more toxic than the other synthetic insecticides as regard to MLD values. On the basis of the MLD values, Rotenone, Gamma BHC, TEPP, PP'-DDT, OP'-DDT and Parathion were generally more toxic than the others. Although Chlordane was tested in the present investigation, it was not toxic against the pomace fly. The toxicities to each contact insecticide in descending order of effectiveness are expressed as follows. 1) Pyrethrins 2) Allethrin, 3) Rotenone, 4) Gamma BHC, 5) TEPP, 6) PP'-DDT, 7) OP'-DDT, 8) Parathion, 9) Paraoxon, 10) Dieldrin, 11) Nicotine, 12) Aldrin, and 13) Toxaphene.

When effectiveness is examined by the following equation, the order of effectiveness differs from the above.

$$\text{Practical MLD} = \frac{\text{Percent of active ingredient of sample}}{\text{Median lethal dose } (\%)}$$

According to the practical MLD in this paper, the order of effectiveness becomes as follows, 1) TEPP, 2) Pyrethrins, 3) Gamma BHC, 4) Rotenone, 5) PP'-DDT, 6) OP'-DDT, 7) Allethrin, 8) Parathion, 9) Nicotine, 10) Dieldrin, 11) O,O-diethyl-O-para-nitrophenylphosphate, 12) Aldrin, and 13) Toxaphene. Generally speaking, it is shown that such residual insecticides as Chlordane, Aldrin, Dieldrin are not expected to be more toxic in rapidity and they are also considerably less residually toxic than the well known synthetic insecticides such as DDT and BHC. Using the results described above, the following experiments on combined insecticides were carried out.

MATHEMATICAL EXAMINATION

In the experiments on mixed insecticides the results obtained were analysed by the following examinations. When we come to analyse the interaction of two insecticides acting jointly, we must know the dosage-mortality curve for each insecticide separately then LD-50 can be calculated from this curve.

In next, we prepare various test mixtures from above results and obtain the mortality for the test mixtures of each. If insecticides act alone without experimental errors, the mortality must theoretically be the mortality of 50 percent. In practice, it is rare that the result does not include various experimental errors. In the second experiment, the mortality usualy fluctuates around the level of 50 percent. The deta from various mixtures of each can best be appraised graphically. The Y-axis represents the mortalities and the X-axis the proportions of insecticides-one insecticide at the left, the other insecticide at the right. The straight line connecting the two points of the mortality of each insecticide alone is to be used as a level of mortality. If the experiment does not include any error, the level of mortality will not have the slope of line. As the level of mortality has the slope in practical test, the equation of level is calculated as follows.

$Y=aX\pm b$, where Y is the mortality, X is the proportion of one insecticide and the zero point on the graph is at the right of X axis. The assumption that the variation of the mortality for each insecticide alone is directly proportional to the previous data seems relatively reasonable if the slope of the dosage-mortality line of the first line is parallel to the second line in the case of same insecticide. If the assumption is recognized, the slope of the level of mortality may be transferred by the following calculation. $ER=\frac{50}{LM}$, where 50 is the theoretical mortality when the second experiment has not any experimental error and any slope of the level of mortality, LM is the theoretical value of the test mixtures of each on the level of mortality and ER is the experimental error. $Pc=\frac{ER\times MO}{50}$ where MO is the experimental velue of the test mixtures of each and Pc is the potency of the joint toxic action. From above equations, $Pc=\frac{MO}{LM}$. If the value of Pc is 2, the interaction between each is the maximum synergism. If the value is 1, the interaction occurs without synergism of antagonism. If value is zero, the interaction is maximum antagonism.

In the third stage, the Pc values of the test mixtures are plotted on another graphic paper. A series of Pc values are connected with straight line. The Y-axis on the graph represents the potencies of the joint toxic action and the X-axis the proportions of insecticides. The polygonal area of connecting a series of Pc value is measured with a planimeter. An arbitrary measurement of a planimeter is used to indicate the degree of joint toxic action as determined by inspection. The polygonal area indicates the sum of all potencies of the test mixtures of each. The measurement of a planimeter is transformed to the theoretical value of the sum.

When X-axis represents the proportion of one insecticide alone and the interaction between each insecticide occurs without synergism of antagonism, 1×100, viz., (potency of joint toxicaction) × (all combinations between each), the theoretical value of the sum is 100.

From above reason,

$$Cc = \frac{100}{\text{a measurement of an arbitrary planimetry when the interaction occurs without synergism or antagonism}}$$

where Cc is a corrected coefficient, the measurement of planimetry between two insecticides is corrected to the theoretical value by the corrected coefficient and then transformed to logarithm

$$\text{Log}\left(\frac{\text{(the measurement of a planimetry between each)} \times Cc}{100}\right) - \text{Log } 100 = SPc,$$

where SPc is the logarithm of the sum of all potencies of the mixtures between two insecticides. If synergism has occurred, SPc represents a plus value, but if antagonism has occurred, SPc represents a minus value. If the interaction has occurred without synergism or antagonism, SPc represents zero value.

NUMERICAL EXAMPLE

The above procedures may be applied to the data on the interaction between Gamma BHC and Nicotine. Two provisional regression equations for BHC and Nicotine were calculated as follows

BHC : $Y=5+2.94\ (X-1.14)$

Nicotine : $Y=5+1.54\ (X-2.30)$

The MLD of BHC was 0.01380% and the MLD of Nicotine was 0.6816%. On the basis of those MLD values, the experimenter prepared the test mixtures of each. The results obtained are given in table 2.

The straight line connecting 66% (BHC alone) and 52.8% (Nicotine alone) is the level of mortality 66% and 52.8% must be 50% in the theoretical values. The level of mortality was $Y=0.132X+52.8$ where X is the proportion of BHC and Y is the mortality. The theoretical values of the test mixtures on the level of mortality are given in table 2. From the equation of $Pc=\frac{MO}{LM}$, the potencies is calculated as given in table 2. A series of potencies were plottd on the graphic paper as shown in Fig. 3.

The polygonal area measured with a planimeter was 6550 mm². When the interaction occurs without synergism or antagonism, the measurement is 5000mm² From above reason, the corrected coefficient is $\frac{1}{50}$ viz. $\frac{100}{5000}$. The theoretical value obtained was 131 and then $\text{Log}\left(\frac{131}{100}\right) = \text{Log } 131 - \text{Log } 100 = +0.11727$.

In conclusion, the value of joint toxic action between BHC and Nicotine was +0.11727 which is a synergistic value

RESULTS FOR COMBINED TOXICANTS

This investigation attempted to demonstrate the joint toxic action of 51 combinations between contact insecticides. The potencies are illustrated in Fig. 1 to Fig. 15.

As discussed by Horsfall (1945), the difficulty with all this theory is that seldomly a simple sag or a simple bulge is obtained. In our experiments, the mixtures acted effectively as synergism in one case or antagonism in

Table 2 Various values applied to the calculation on the interaction between Gamma BHC and Nicotine.

Formulation of the tested mixtures	B H C / Nicotine	100 / 0	90 / 10	80 / 20	70 / 30	60 / 40	50 / 50	40 / 60	30 / 70	20 / 80	10 / 90	0 / 100
Mortality (MO)		66	76	73	61	89	89	79	83	83	66	53
Theoretical value on the level of mortality (LM)		66	65	63	62	61	50	58	57	55	54	53
Potency of the joint toxic action (Pc)		1.00	1.17	1.15	0.98	1.47	1.50	1.36	1.45	1.49	1.22	1.00

Table 3. The equation of the level of mortlity connecting the two points of the mortality at each insecticide alone.

Combination		Equation of the level of mortality *	Combination		Equation of the level of mortality*
B H C	Pyrethrins	Y=62.7−0.033X	Rotenone	PP' DDT	Y=92.4−0.462X
	Rotenone	Y=42.9−0.033X	Toxaphene	OP' DDT	Y=0.020X+30.0
	Toxaphene	Y=0.43X+3.3		TEPP	Y=90.0−0.760X
	OP'DDT	Y=36.3−0.030X		Allethrin	Y=0.040X+16.0
	TEPP	Y=100−0.604X		Nicotine	Y=0.100X+4.0
	Allethrin	Y=0.264X+26.4		TEPP	Y=0.180X+48.0
	Aldrin	Y=0.165X+19.8	OP'DDT	Allethrin	Y=0.420X+56.0
	Dieldrin	Y=72.6−0.528X		Aldrin	Y=0.198X+9.9
	Nicotine	Y=0.132X+52.8		Dieldrin	Y=0.297X+29.7
	PP'DDT	Y=92.4−0.564X		Nic tine	Y=0.132X+46.2
Pyrethrins	Rotenone	Y=0.132X+9.9		PP'DDT	Y=42.9−0.132X
	Toxaphene	Y=62.7−0.429X	TEPP	Allethrin	Y=0.396X+52.8
	OP'DDT	Y=95.7−0.561X		Aldrin	Y=0.638X+36.2
	TEPP	Y=100−0.208X		Dieldrin	Y=66.0−0.340X
	Allethrin	Y=100−0.901X		Nicotine	Y=19.8
	Aldrin	Y=72.6−0.264X		PP'DDT	Y=0.138X+13.2
	Dieldrin	Y=85.8−0.198X	Allethrin	Aldrin	Y=3.3
	Nicotine	Y=0.627X+26.4		Dieldrin	Y=39.6−0.604X
	PP'DDT	Y=0.165X+13.2		Nicotine	Y=0.429X+13.2
Rotenone	Toxaphene	Y=85.8−0.099X		PP'DDT	Y=39.6−0.099X
	OP'DDT	Y=80.2−0.302X	Aldrin	Dieldrin	Y=92.4−0.730X
	TEPP	Y=0.099X+82.5		Nicotine	Y=0.264X+13.2
	Allethrin	Y=0.03X+16.5		PP'DDT	Y=0.13 X+72.6
	Aldrin	Y=0.066X+33.0	Dieldrin	Nicotine	Y=0.396X+23.1
	Dieldrin	Y=85.8−0.660X		PP'DDT	Y=0.455X+39.6
	Nicotine	Y=42.9−0.198X	Nicotine	PP'DDT	Y=66.0−0.090X

Table 4. The logarithmic value of joint toxic action between contact insecticides.

Combination	Log. value	Combination	Log. value
Nicotine Toxaphene	+0.27875	Rotenone TEPP	−0.01773
Nicotine Aldrin	+0.23553	Pyrethrins BHC	−0.01954
Dieldrin BHC	+0.23045	Rotenone Pyrethrins	−0.03152
Toxaphene TEPP	+0.21484	Nicotine OP'DDT	−0.04576
Nicotine Rotenone	+0.19590	PP'DDT OP'DDT	−0.05355
Nicotine Dieldrin	+0.19866	OP'DDT Pyrethrins	−0.05552
OP'DDT TEPP	+0.14922	Allethrin TEPP	−0.05849
Nicotine BHC	+0.11727	Pyrethrins TEPP	−0.07058
Allethrin BHC	+0.11060	Toxaphene BHC	−0.07935
Aldrin BHC	+0.10721	Dieldrin Allethrin	−0.15490
PP'DDT Allethrin	+0.10721	BHC TEPP	−0.17783
Dieldrin PP'DDT	+0.10721	Toxaphene OP'DDT	−0.18046
Dieldrin TEPP	+0.10037	OP'DDT Allethrin	−0.20761
Dieldrin OP'DDT	+0.10037	Rotenone BHC	−0.23768
Nicotine Allethrin	+0.09342	Toxaphene Pyrethrins	−0.28400
PP'DDT TEPP	+0.08991	Nicotine TEPP	−0.31336
Aldrin Allethrin	+0.08636	PP'DDT BHC	−0.32057
Dieldrin Pyrethrins	+0.07555	Aldrin Pyrethrins	−0.32790
Rotenone Dieldrin	+0.07555	Rotenone PP'DDT	−0.34486
Dieldrin Aldrin	+0.01703	Pyrethrins Allethrin	−0.35655
Nicotine Pyrethrins	+0.00860	Rotenone Allethrin	−0.37675
Rotenone OP'DDT	+0.00860	Nicotine PP'DDT	−0.42022
PP'DDT Pyrethrins	−0.00436	Rotenone Aldrin	−0.44370
Toxaphene Allethrin	−0.00877	Aldrin PP'DDT	−0.60555
Aldrin OP'DDT	−0.00877	Toxaphene Rotenone	−0.67778
OP'DDT BHC	−0.01144		

* X of the equation represents the proportion of the combined insecticide at the left side.

the other case. This means that synergism may occur at some relative proportions of the insecticides and antagonism at other proportions. The logarithmic values of 51 combinations between contact insecticides are given in table 4 and illustrated in Fig.1 and Fig. 15. Fig. 16 illustrates synergism and Fig. 17 illustrates antagonism. In Fig. 16 and 17, the Y-axis represents the logarithmic values and the X-axis the molecular weight of insecticide. The upper axis of X expresses the combined insecticide of the higher molecular weight and the lower axis that of the lower molecular weight.

In our experiments, the logarithmic values of a given insecticide which combined various insecticides, varied from minus values to plus values. As shown in table 4, the synergistic values were 22 combinations and the antagonistic values were 29 combinations in all the tested combination. Although antagonism has occurred more than synergism, the interactions indicated neither marked synergism nor marked antagonism. On the basis of the logarithmic value, the synergism to each combination were expressd in the order of effectiveness as follows:

Toxaphene plus Nicotine, Aldrin plus Nicotine, BHC plus Dieldrin, TEPP plus Toxaphene, Rotenone, plus Nicotine, Dieldrin plus Nicotine, TEPP plus OP' DDT, BHC plus Nicotine, BHC plus Allethrin and BHC plus Aldrin.

The antagonism expressed in descending order as follows:

Rotenone plus Toxaphene, PP' DDT plus Aldrin, Aldrin plus Rotenone, PP' DDT plus Nicotine, Allethrin plus Rotenone, Allethrin plus Pyrethrins, PP' DDT plus Rotenone, Pyrethrins plus Aldrin, BHC plus PP' DDT ande TEPP plus Nicotine. At a glance of the results, the interaction may be expected to show

Figure I to Figure 15. Potency of the joint toxic action between contact insecticides. Y-axis represents the potency of the joint toxic action and X-axis represents the proportion of insecticide. The upper proportion of X-axis represents the proportion of the insecticide on left side and the lower proportion the proportion of the insecticide on right side.

A-line:——, B-line: - · - · -, C-line: —·—·—, D-line:——, E-line: - - - - -, F-line: —·—·—,

Fig.1, A : BHC-Pyrethrins, B : BHC-Rotenone, C : BHC-Toxaphene

Fig.2, A : BHC-OP' DDT, B : BHC-TEPP, C : BHC-Allethrin, D : BHC-PP' DDT

Fig.3, A:BHC-Aldrin, B : BHC-Dieldrin, C : BHC-Nicotine, D : Pyrethrins-Toxaphene E : Pyrethrin-TEPP, F : Pyrethrins-Aldrin

Fig.4, A:Pyrethrins-Rotenone, B : Pyrethrins-OP' DDT, C : Pyrethrins-Allethrin

Fig.5, A : Pyrethrins-Dieldrin, B : Pyrethrins-Nicotine, C : Pyrethrins- PP' DDT

Fig.6, A : Rotenone-Toxaphene, B: Rotenone-OP' DDT, C:Rotenone-TEPP

Fig.7, A : Rotenone-Dieldrin, B:Rotenone-Nicotine, C : Rotenone-PP' DDT

Fig.8, A : Toxaphene-TEPP, B : Rotenone-Aldrin, C : Toxaphene-Allethrin, D : Rotenone-Allethrin

Fig.9, A: OP' DDT-TEPP, B: Toxaphene-Nicotine, C : OP' DDT-Nicotine, D: Toxaphene-OP' DDT, E : OP' DDT-Allethrin

Fig.10, A : OP' DDT-PP' DDT, B : OP' DDT-Aldrin, C : OP' DDT-Dieldrin

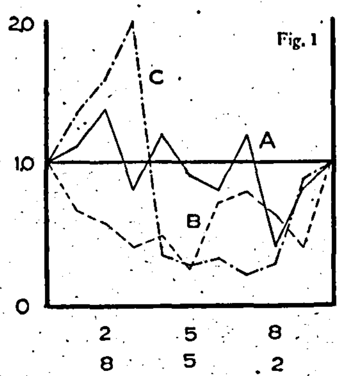
Fig.11, A : TEPP-Allethrin, B:TEPP-Aldrin, C: TEPP-Dieldrin

Fig.12, A : TEPP-Nicotine, B : TEPP-PP' DDT, C : Dieldrin-PP' DDT

Fig.13, A : Allethrin-Aldrin, B : Allethrin-Dieldrin, C: Aldrin-Dieldrin

Fig.14, A: Dieldrin-Nicotine, B : Aldrin-Nicotine, C : Nicotine-PP' DDT, D: Aldrin-PP' DDT

Fig.15, A : Allethrin-Nicotine, B : Allethrin-PP' DDT



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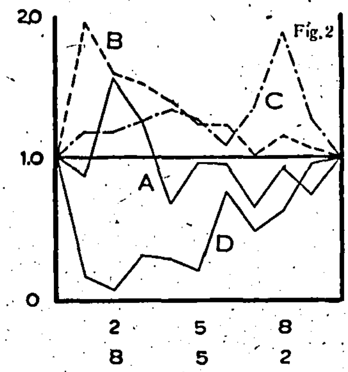

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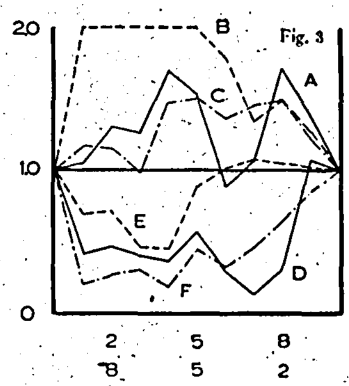

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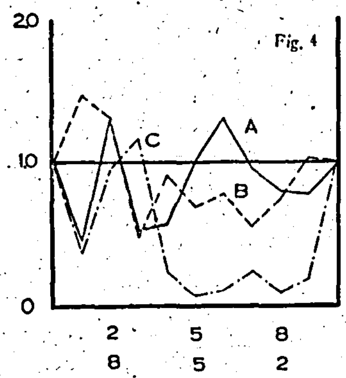

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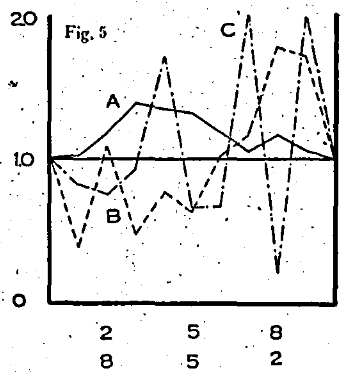

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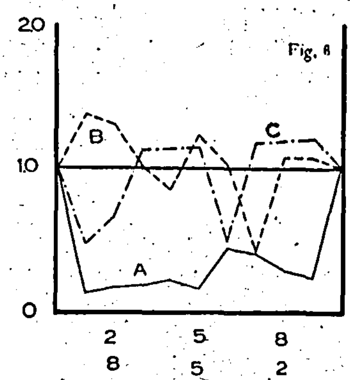

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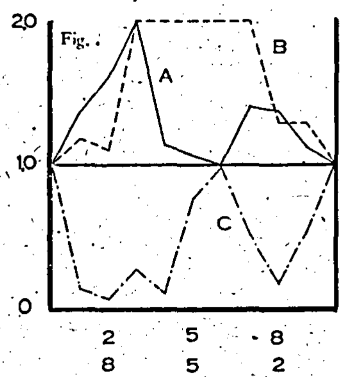

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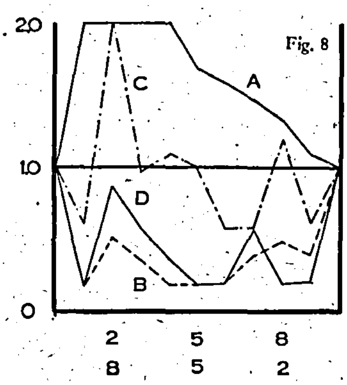

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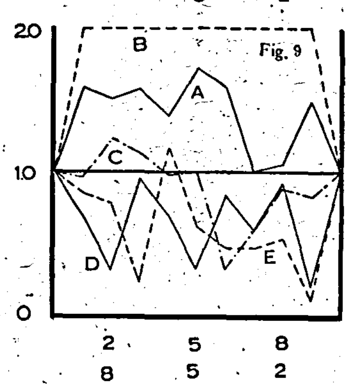


Fig. 9

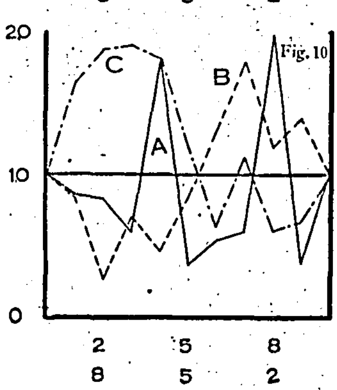


Fig. 10

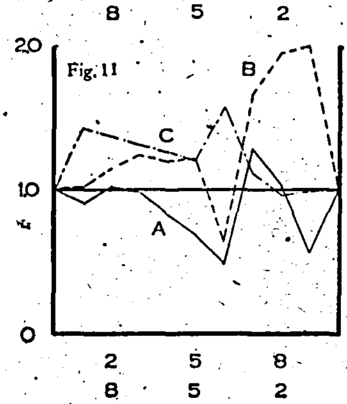


Fig. 11

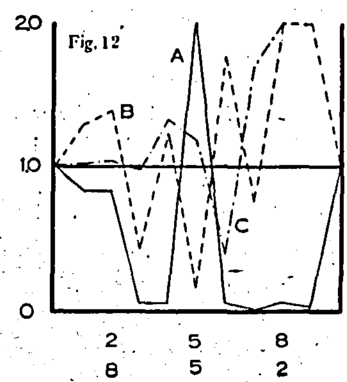
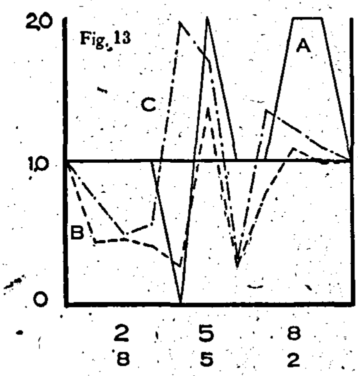
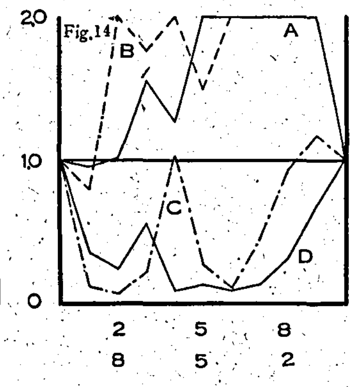
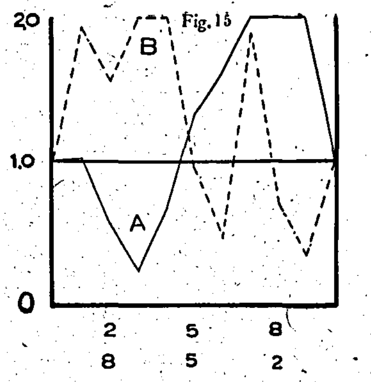


Fig. 12







a slight synergism if one insecticide combines Dieldrin, Nicotine, TEPP or BHC. In conclusion it may be stated that the joint toxic action between contact insecticides is from a slight synergism to a slight antagonism and that the combinations between each do not indicate the expected marked increase of effectiveness.

DISCUSSION

In the writer's opinion, the joint toxic action is limited to the phenomenon between two or more insecticides which act physiologically in the internal locus of insect body and which do not react chemically out of the insect body. In the present case, we do not consider the chemical reactions between two insecticides because our investigation is in a preliminary stage. We also regard a insecticide containing various carriers and one toxicant. It is clear, in practice, that the joint toxic action occurs between an active ingredient and various carriers. If we find a striking interaction, we should study these questions.

In the field of pharmacology, Bürgi has said that potentiated action can occur when the poisons react with each other to act a

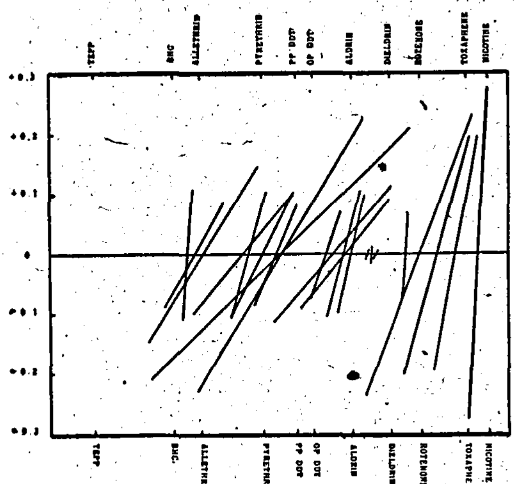


Figure 16. The logarithmic values of synergism of 22 combinations between contact insecticides.

Y-axis represents the logarithmic value and X-axis the molecular weight of insecticide. The upper axis of X represents the combined insecticide of the higher molecular weight and the lower axis that of the lower molecular weight.

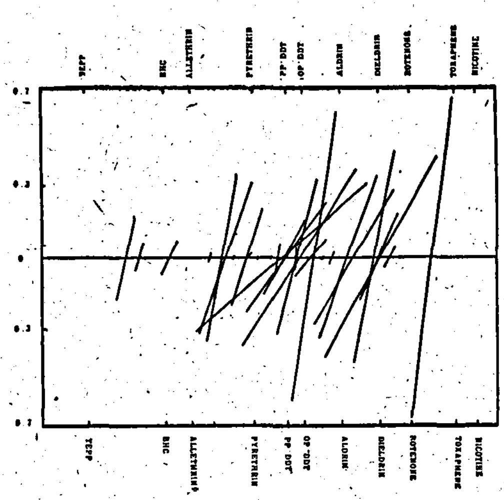


Figure 17. The logarithmic values of antagonism of 29 combinations between contact insecticides.

The Y and X-axis are similar to Figure 16.

different type of recepter and that additive action (that supplementary and substractive action are called by Horsfall) can occur, when the poisons react together to act a similar type of recepter.

The important point of the present paper is to discuss whether the interactions between contact insecticides are supplemental or potentiating, while we avoid discussing the above questions.

SUMMARY

The present authors attempted to demonstrate the joint toxic action between several contact insecticides. The insect used for test was the vestigial form of the pomace fly, *Drosophila melanogaster*, which was reared with artificial media in a milk bottle under constant temperature of 25°C. The numbers of flies tested were about 50,000 in total.

0.5% Pyrethrins, 2.0% Rotenone, 40.9% Nicotine, 0.5% Allethrin, 10% Gamma BHC, 10% PP' DDT, 10% OP' DDT, 21.8% Aldrin, 20.4% Dieldrin, 50% Toxaphene, and 37.9% TEPP, each of these insecticides is emulsified or made soluble to water. The immersion method was used in the present investigation. The immersion time was 2 minutes under the constant temperature of 20°C. The percentages of mortality were examined 30 minutes after the treatment. The combination of two insecticides was based on the median lethal dose which is the point of a equitoxic dose. The test mixtures were as follows: one insecticide alone, 90% of one plus 10% of the other insecticide, 80% plus 20%, 70% plus 30%, 60% plus 40%, 50% plus 50%, 40% plus 60%, 30% plus 70%, 20% plus 80%, 10% plus 90% and then the other insecticide alone. (ratio by volume).

In experiments on insecticide alone, Pyrethrins were markedly more toxic than the other insecticides. It is interesting to note that Allethrin was more toxic than the other synthetic insecticides as regard to MLD values. On the basis of the MLD, the toxicities to each contact insecticide were expressed in order of effectiveness as follows. 1)Pyrethrins, 2)Allethrin, 3) Rotenone, 4) Gamma BHC, 5)TEPP, 6)PP' DDT, 7)OP' DDT, 8)Parathion, 9) O, O-diethyl-O-para-nitrophenylphosphate, 10, Dieldrin, 11)Nicotine, 12)Aldrin and 13)Toxaphene. Although Chlordane was tested in the present investigation, it was not toxic against the pomace fly.

After experiments on the test mixtures, the results obtained were analysed by a shortcut mathematical examination. The interaction of a given insecticide combined other insecticides varied from minus values to plus values. The synergistic values were 22 combinations and the antagonistic values were 29 combinations in all the test mixtures. Antagonism has occurred more than synergism, while the interactions indicated neither marked synergism nor marked antagonism. The synergism to each combination were expressed in the order of effectiveness as follows: Toxaphene plus Nicotine, Aldrin plus Nicotine, BHC plus Dieldrin, TEPP plus Toxaphene, Rotenone plus Nicotine, Dieldrin plus Nicotine, TEPP plus OP' DDT, BHC plus Nicotine, BHC plus Allethrin and BHC plus Aldrin.

The antagonism were expressed in the order of high value as follows: Rotenone plue Toxaphene, PP' DDT plus Aldrin, Aldrin plus Rotenone, PP' DDT plus Nicotine, Allethrin plus Rotenone, Allethrin plus Pyrethrins, PP' DDT plus Rotenone, Pyrethrins plus Aldrin, BHC plus PP' DDT and TEPP plus Nicotine.

At a glance of the results, an interaction showing a slight synergism if one insecticide combines Dieldrin, Nicotine, TEPP or BHC may be expected.

In conclusion it may be stated that the joint toxic action between contact insecticides is from a slight synergism to a slight antagonism and that the combinations between each do not indicate the expected marked increase of effectiveness.

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