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Summary

Houseflies were collected from 16 places in Indonesia and their resistance levels to DDT, Lindane (γ-BHC), Diazinon, Sumithion and Malathion were evaluated. The LC_{50} values of 16 colonies of houseflies larvae are shown in Table 1.

Most housefly larvae in Indonesia were less susceptible to the organophosphorus compounds than those of the Takatsuki strain. The highest resistance level for Diazinon was observed in Nos. 4, 13 and 2 colonies, showing the LC_{50} value of 194.56, 161.81 and 137.74 ppm respectively. Two colonies, collected from Java (No. 4) and Celebes (No. 9), were resistant to Sumithion, showing 176.68 and 154.32 ppm as LC_{50} value respectively.

Decrease in Residual Amounts of Diazinon in Cabbages and Soil after its Application

Satoshi Kono and Masakatu Yamasita (Hyogo Agricultural Experiment Station, Kitaoji, Akashi, Japan) Received September 14, 1974. *Botyu-Kagaku* 39, 119, 1974.

26. キャベツおよび土壌中におけるダイアジノンの消失について

河野 哲・山下優勝 (兵庫県立農業試験場, 明石市北王子町) 49. 9. 14 受理

ダイアジノン水和剤を, 圃場およびライシメーターに植付けたキャベツに散布し, キャベツならびに土壌中における残留と, 土壌の種類の違いによる残留量への影響について検討した. 散布回数が多く, 散布濃度が高いほど残留量は多いが, 最終散布後の経過日数に伴なって急速に減少し, 濃度1,000倍液の4回散布による1日後のキャベツのダイアジノン残留量は, 2.010, 5日後0.169, 15日後0.018 ppmであった. 土壌中での残留量の消失は, 土壌の種類によって異なり, 壌土では早く, グライ壌土では遅かったが, 供試した5土壌の1日後の残留量に対する, 5, 15, 35, 66, 170日後の平均残留量は, それぞれ51.2, 37.6, 22.6, 7.2%であった. 散布回数, 散布成分薬量 (g/10a), 最終散布後の経過日数を独立変数として多重回帰式を求め, キャベツにおける残留量の推定を試み, その計算値は, 実験値とよく一致した (r=0.8414***).

Diazinon [*O*, *O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphoro thioate] has been widely used for the control of the rice stem borer *Chilo suppressalis* in paddy fields and also various insect pests on other crops. By now, the national tolerances of diazinon have been determined for 20 crops including rice, vegetables, and fruits, and the holding periods of diazinon application for these crops before harvesting have also been established. The penetration of diazinon into rice plants when applied to paddy water and its residues in rice have been reported by Masuda *et al.*[1] and Sethunathan *et al.*[2]. Very few have been reported on the residues of diazinon in other crops with the exception of reports by Coffin[3] and Yakumaru *et al.*[4]

Nose[5] applied the equation $C=C_0^{-\lambda t}$ to the analysis of his data on residues of tetrachlorophthalide in rice straws and found that λ was 0.183, C_0 (residual amount at 0 time) was 1.384 ppm, and half life (=0.693/0.183) was 3.79. Kanazawa[6] also adapted the multiple regression analysis to his data on residues of BHC in rice and three independent variables: amounts of BHC applied, application frequencies, and periods from the final application to harvesting. These results suggest the possibility to presume the residual amount of a pesticide in a crop at harvesting time when we know how much, how many times the pesticide was applied and how many days have elapsed after the final application.

This paper reports the change in residual amounts of diazinon in cabbages when it was applied at different dosages, at different intervals, and cabbages were harvested at different days after the final application. Residues in soils of different types are also reported. Some attempts were made to express the residual amount of diazinon as a function of dosages of the pesticide and the time elapsed after its application. Such numerical expression may be useful to predict the residual amount of pesticide at harvesting time, consequently, to guide the safe use of the pesticide on cabbages.

Materials and Methods

Field experiments

Cabbage seeds "Haruhikari-1" were sown on October 4, 1972, small seedlings were transplanted to a nursery bed on October 25, and again transplanted in a field on November, 26. Thirty four % wettable powder of diazinon was diluted with water to 500-, 1,000-, and 2,000-folds, and 0.25 liters of each preparation was applied to each 1.5×2 m^2 plot in which cabbages were planted. Treatments were replicated three times in a randomized complete block design.

Lysi-meter experiments

In order to elucidate the effects of different kinds of soil on residual amounts of diazinon in cabbages and in soil, the following 5 kinds of soil were used: sandy loam, loam, glei clay loam, gray-brown clay loam, and conglomeratic clay loam. These were placed in 1×2 m^2 lysi-meters and experiments were performed with two replications for each treatment designed in Fig. 1.

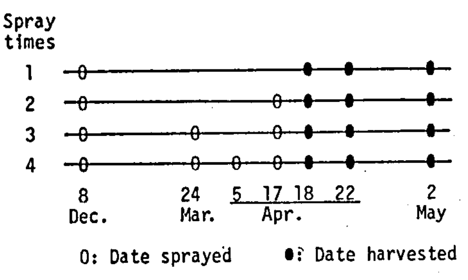

Fig. 1. Programs for residue analyses of diazinon.

Determination of diazinon in cabbages

Cabbages were chopped finely with a knife, 50 g were taken in a mixer, and homogenized, with the addition of 200 m*l* of acetonitrile, for 5 minutes. The homogenate was transferred to a 1 liter Vidrex blender cup, added with 500 m*l* of distilled water and 15 g of NaCl, and agitated vigorously for a few minutes. From this mixture diazinon was extracted twice with each 120 m*l* of *n*-hexane, shaking vigorously for 10 minutes. The extracts were combined, concentrated in a rotary evaporator at 45°C and dehydrated with anhydrous sodium sulfate. The concentrated extract was charged on a 1.5cm×9cm column consisting of 7.4 g of activated Florisil and 2 g of anhydrous sodium sulfate, and the column was washed with 200 m*l* of *n*-hexane-ethyl ether (70 : 6, v/v) mixture. The effluent was concentrated exactly to 2 m*l* and was subjected to gas chromatography.

Recovery of diazinon from cabbages

Adding known amounts of diazinon to homogenized cabbage, recovery of diazinon from the cabbage was 89 to 94%.

Determination of diazinon in soil

Soills, 0-10 cm from the surface, were sampled from each plot at 1, 5, 15, 35, 66, and 170 days after the last spray, and then air-dried. Each 30g of the soils was used for analysis. The soil sample was extracted with 100 m*l* of chloroform on a water bath at 80°C for 8 hours. Concentration and clean-up of the extract was done as described above.

Gas Chromatography

Analyses of diazinon were performed by using a gas chromatograph, Model Varian 1700, equipped with an alkali flame ionization detector. The column was 1.8 m long, 2 mm in inner diameter, and filled with 2% P.E.G.A./Chromosolb W.aw (60-80 mesh). The temperature of the column was 180°C, and those of injector and detector were 250°C. Flow rates of nitrogen, hydrogen and air are 45 m*l*/min., 35 m*l*/min., and 240 m*l*/min. respectively.

Results and Discussion

Field experiments

Diazinon was applied to cabbages grown in the field at definite dates and time intervals as shown in Fig. 1. Cabbages were harvested 1, 5 and 15 days after the last application and analyzed for diazinon residues. Results are shown in Table 1.

It is evident from the results that the more the times of spray, and the higher the concentration of diazinon, the larger the amount of residues, and that the amounts are decreased as the period after the last spray become longer. Coffin *et al.*[1] reported that residues of diazinon

Table 1. Residues of diazinon in cabbages grown in the field.

Plot No.	Diazinon concentration	Spray times	Day elapsed after the final spray (Days)		
			1	5	15
			ppm	ppm	ppm
1	1/500	2	5.300(100)	0.421(7.9)	0.031(0.6)
2		3	6.424	0.541	0.050
3		4	9.856	1.091	0.065
4	1/1,000	2	0.538(100)	0.074(13.7)	0.006(1.1)
5		3	0.771	0.154	0.009
6		4	2.010	0.169	0.018
7	1/2,000	2	0.210(100)	0.025(11.9)	0.005(2.4)
8		3	0.717	0.032	0.004
9		4	0.915	0.096	0.005

Wettable powder of diazinon containing 34% active ingredient was diluted to 1/500, 1/1,000, 1/2,000.

sprayed on lettuce were 8.1 ppm immediately after the spray and reduced to 0.3 ppm after 7 days. This was also the case with cabbages, *i.e.*, in plots 1, 4, and 7, residues were reduced to 8-14/100 at 5 days and 0.6-2.4/100 at 15 days after the last spray when compared with those at one day after the last spray.

Residues of diazinon were decreased exponentially with lapse of days after its application. When diazinon diluted to 1/1,000 was sprayed 2 and 3 times (plots 4 and 5) diazinon residues were 0.006 and 0.009 ppm respectively at 15 days after the last spray. These values are almost equal to those of plots 10, 11, and 12 where diazinon preparations of 1/500, 1/1,000 and 1/2,000 were sprayed only once, and 132-146 days have already elapsed after the sprays (Table 2). This may be due to the fact that, as reported by Miyamoto[7], diazinon deposited on plant surface disappears rapidly by evaporation and washing-off by rain, and then gradually by degradation, however, residues at a low level will be retained for a long time because plants, while they are young, absorb diazinon through their roots from the soil.

Lysi-meter experiments

Diazinon preparations diluted to 1/500, 1/1,000 and 1/2,000 were sprayed on cabbages grown in lysi-meters containing different kinds of soil. The pesticide was sprayed 2, 3, and 4 times at definite dates as shown in Fig. 1. Cabbages were harvested 1, 5, and 15 days after the last spray and analyzed for diazinon residues. Results are shown in Table 3. Similar trends as shown in Table 1 in diazinon residues are observed in relation to the concentrations of the pesticide, times of application and the lapse of days after the final spray.

Just as it has already been known that the residues of pesticide in crops were influenced by the kind of soil, our data indicated that much more residues were retained in conglomeratic and glei clay loam than in loam.

Results of Table 1 and 3 show that residues resulted from 2 sprays of 1/500-diluted diazinon are almost equal or a little larger than those

Table 2. Residues of diazinon in cabbages grown in the field.

Plot No.	Diazinon concentration	Spray times	Day elapsed after the final spray (Days)		
			132	136	146
			ppm	ppm	ppm
10	1/500	1	0.006	0.006	0.005
11	1/1,000	1	0.004	0.006	0.003
12	1/2,000	1	0.003	0.003	0.002

Table 3. Residues of diazinon in cabbages grown in Lysi-meters.

Plot No.	Diazinon concentration	Spray times	Kind of soil	Day elapsed after the final spray		
				1	5	15
				ppm	ppm	ppm
13	1/500	3	Glei clay loam	1.584	0.703	0.062
14		3	Gray-brown clay loam	4.819	1.286	0.030
15		4	Sandy loam	10.015	1.049	0.029
16		4	Loam	1.880	0.653	0.006
17	1/1,000	2	Conglomeratic clay loam	2.110	0.544	0.006
18		3	Conglomeratic clay loam	3.815	0.792	0.021
19		4	Sandy loam	9.724	0.822	0.015
20		4	Loam	1.264	0.280	0.013
21		4	Conglomeratic clay loam	4.632	0.549	0.047
22	1/2,000	3	Gray-brown clay loam	0.736	0.118	0.005
23		3	Glei clay loam	0.825	0.184	0.010
24		4	Conglomeratic clay loam	1.642	0.622	0.014

resulted from 4 sprays of 1/1,000-diluted diazinon. Similar relationship can be seen between the results of 2 sprays of 1/1,000-diluted and 4 sprays of 1/2,000-diluted ones. Because of rapid disappearance of diazinon, the residues in cabbages were much more influenced by the concentration of the last spray than the frequencies of spray. These results suggest that more frequent sprays of diazinon at a low concentration are more desirable thanfewer sprays at higher concentrations from the view point of safe use of pesticides.

Decrease of diazinon residues in soil

Cabbages were grown in Lysi-meters containing different kinds of soils and diazinon preparations diluted to 1/500, 1/1,000, and 1/2,000 were sprayed on cabbages 2, 3, and 4 times according to the program shown in Fig. 1. The soils were sampled from each plot and analyzed for their diazinon residues. Results are shown in Table 4.

As shown in the table, the differences in residues among three concentrations of diazinon, and among the times of application were not so significant as compared with those in the cab-

Table 4. Residues of diazinon in several kinds of soils. (ppm)

Plot No.	Diazinon concentration	Spray times	Kind of soil	Day elapsed after the final spray					
				1	5	15	35	66	170
25	1/500	3	Glei clay loam	0.063	0.061	0.059	0.056	0.031	0.010
26		3	Gray-brown clay loam	0.390	0.248	0.101	0.069	0.055	0.022
27		4	Sandy loam	0.367	0.109	0.075	0.054	0.053	0.057
28		4	Loam	0.631	0.405	0.109	0.058	0.053	0.018
29	1/1,000	2	Conglomeratic clay loam	0.064	0.063	0.058	0.058	0.052	0.003
30		3	Conglomeratic clay loam	0.122	0.067	0.066	0.060	0.057	0.010
31		4	Sandy loam	0.098	0.063	0.055	0.051	0.027	0.016
32		4	Loam	0.128	0.064	0.048	0.024	0.010	0.005
33		4	Conglomeratic clay loam	0.552	0.105	0.072	0.035	0.018	0.012
34	1/2,000	3	Gray-brown clay loam	0.531	0.071	0.050	0.015	0.012	0.014
35		3	Glei clay loam	0.176	0.077	0.044	0.026	0.021	0.013
36		4	Conglomeratic clay loam	0.513	0.082	0.042	0.038	0.023	0.009
Average of the amount a day after the final spray (%)				100.0	51.2	37.6	31.0	22.6	7.2
Control (without spray)				—	—	0.007	0.010	0.007	0.003

bages. This may be due to the fact that the variance of residues in the soils is larger than that of the cabbages, because sprayed diazinon fall directly on the cabbages but indirectly on the soils, and the pesticide is much more evenly applied to the cabbages than to the soils.

Residues decrease rapidly soon after the final spray toward 15th day (in average 37.6% of the amount a day after the final spray), then gradually toward 66th (22.6%) and 170th day (7.2%) (Fig. 2).

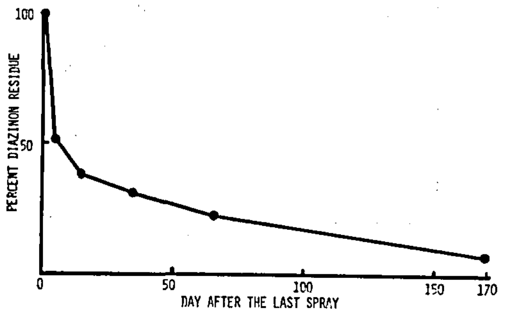

Fig. 2. Decrease of diazinon residues in soil after its last spray.

The rate of decrease of residues in the soils during the first 15 days agrees well with that obtained by Yakumaru *et al.*[3] who applied diazinon dust at 6 kg/10*are* and those after a week and two weeks were 0.744 (65.7%) and 0.435 (38.4%) respectively. There are slight differences in the rate of decrease in the later period between Yakumaru's and our results. It seems possibly because of the diference in the type of formulation of the pesticide and soil used. Generally, suspension of wettable powder of a pesticide is liable to be evaporated more rapidly than dust and granules during the early period after application. In some kinds of soils, i.e., in loam and conglomeratic clay, percentage of residues during the later period agreed well with that of Yakumaru's.

Residues in glei clay loam and sandy loam at 170th day were comparatively high, 15-16%, whereas those in loam decreased rapidly during the first 15 days, then gradually throughout the whole period, and those in gray clay loam decreased rapidly during the first 35 days then remained almost constant toward 170th day. Days required for 90% disappearance were 170 days in sandy loam, 108 days in glei clay loam. Average half life period was 5 days and the period for 90% disappearance was 147 days.

As it was already stated by Getzin[8,9] that the rate of disappearance of diazinon is more closely correlated with contents of organic matters than clay contents, results of the present study suggest that the rate would be influenced by some factors other than clay contents, details of which remain to be solved in future.

Numerical expression of the results obtained

When a definite amount of a pesticide is applied to plants at a definite date, the residue C (ppb) in plants at t days after the application will be given by

$$C=C_0e^{-\lambda t}$$

where C_0 is the residue immediately after the application and λ the coefficient of decrease. When equal amounts of diazinon are sprayed at t_1, t_2, t_3 and t_4 days before harvesting, the residue at harvesting time is given by the following equation:

$$C=C_0\,(e^{-\lambda t_1}+e^{-\lambda t_2}+e^{-\lambda t_3}+e^{-\lambda t_4})$$

where t_1 is days from final spray to harvesting, hence $t_4>t_3>t_2>t_1$. When the residue immediately after the final spray is expressed as C_{A0} the residue at t will be given by the following simple equation:

$$C=C_{A0}e^{-\lambda t} \qquad (1)$$

where t is the number of days after the final spray.

Since t_4 is enough large to consider that $e^{-\lambda t}4$ is negligible, the residue C_{A0} will be determined by the last 3 sprays, hence

$$C_{A0}=aX_1^{b1}\cdot X_2^{b2} \qquad (2)$$

where X_1 is the amount of diazinon applied at t_1, t_2 and t_3, X_2 the times of spraying (=1, 2, 3) and b_1 and b_2 are the index numbers for correcting the residue (C_{A0}), because this is not simply proportional to the total amount of diazinon applied to plants but is determined mainly by the residues of final and to less extents by previous sprays. When C_{A0} in (1) is replaced by (2)

$$C=aX_1^{b1}\cdot X_2^{b2}\cdot e^{-\lambda t} \qquad (3)$$

Replace $-\lambda t$ with b_3X_3, then equation (3) is rewritten as

$$C=aX_1^{b_1}\cdot X_2^{b_2}\cdot e^{b_3X_3}$$

or $\log C=\log a+b_1\log X_1+b_2\log X_2+b_3\log e$ (4)
where b_3 is the reciprocal of $-\lambda$ (decreasing coefficient) and X_3 days elapsed from the final spray to harvesting.

Variables in equation (4) (C, X_1, X_2, X_3) were substituted with corresponding values in Tables 1, 3 and a, b_1, b_2, and b_3 were calculated by using the multiple regression analysis method proposed by Snedecor *et al.*[10]

Results are as follows:

$a=3.077$ $b_1=1.726$ $b_2=1.054$ $b_3=0.339$

Then equation (4) is

$$C=3.077X_1^{1.726}\cdot X_2^{1.054}\cdot e^{-0.339X_3} \quad (5)$$

The half life period is

0.693/0.339=2.04 days

In the above analysis, data of plots 10, 11, and 12 in Table 2 were omitted because the residues resulting from the first spray (December the 8th) are too small as compared with those resulting from other sprays. Calculations were made thinking that the residue resulting from 2 sprays was that from one spray.

The values found agreed well with those calculated with a high correlation coefficient, 0.8414*** (Fig. 3). 90 and 95% confidence intervals of 3 sprays at 1/1,000 dilution are shown in Fig. 4.

Equation (5) enables us to predict the level of diazinon residue in cabbages at any harvesting

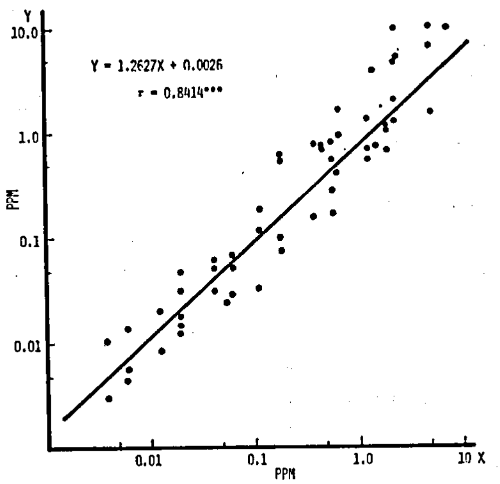

Fig. 3. Correlation between analyzed (Y) and calculated (X) values of diazinon residues in cabbages.

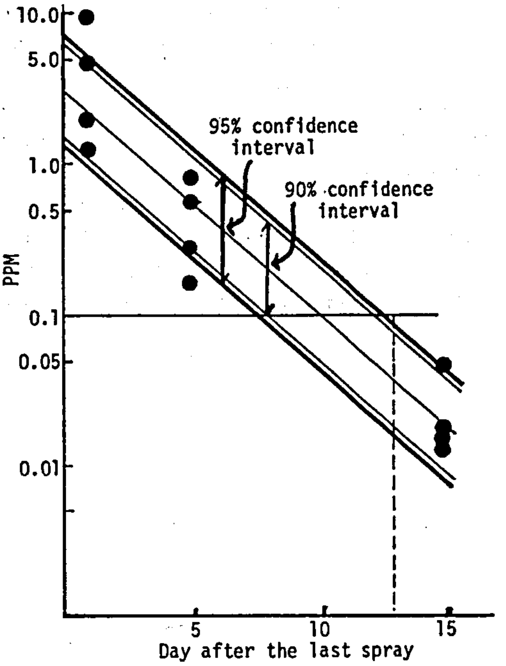

Fig. 4. 90% and 95% confidence intervals for values obtained from 3 sprays of 1/1,000-diluted diazinon. Center line indicate the residue calculated by equation (5).

time when times of spraying, amount of the pesticide applied for each spray (g/10a), and days elapsed after the final spray are given. If cabbages are sprayed with diazinon at 1/1,000 dilution 3 times and harvested later than 13 days after the final spray, the residue level will be below 0.1 ppm at a critical ratio (δ) below 5% (Fig. 4). If diazinon was sprayed at 1/500 or 1/2,000 dilution 3 times, 16 ($5<\delta<10$) or 9 days ($5<\delta<10$), respectively, are required to keep the residue level below 0.1 ppm.

Acknowledgement

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Laboratory Evaluation of Effectiveness of Some Insecticide Emulsifiable Concentrates for the Immature Stage of the Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann)

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27. チチュウカイミバエの幼虫に対する殺虫乳剤有効度の評価法 長沢純夫 (FAO 在ブラジルサンパウロ生物学研究所) 49. 9. 20 受理

コーヒーの実を加害して，成長，脱出するチチユウカイミバエの幼虫の分布は，切れたポアソン系列に近似できた．この知見にもとづいて，殺虫乳剤に浸漬処理した実から脱出する幼虫数と，薬液の濃度の関係を Wadley の方法によって解析し，その適用の可能性を証明した．併せて数種市販乳剤の有効度の検定結果を例示した．

Laying aside the economical problems, insecticide spray tests in the field are undoubtedly a big task for experimenters. In spite of their hard operations, the results of experiments sometimes leave much to be desired, largely due to the complex environmental factors and technical problems involved. If it were possible to bring the test materials into the laboratory under the same condition of infestation in field, the field evaluation test of insecticide formulations could be satisfactorily replaced with laboratory scale test with no big difference in results.

For example, the control test of immature stage of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), growing on coffee berry usually would be carried out by spraying or dusting on the infested berries following an appropriate experimental design. After several days the counts would be made of the numbers of flies which survived and developed from randomly sampled berries. But the total number of flies treated could be discovered only by laborious counts on the dissected berries. If the infested berries are at first randomly collected from the field and assigned to some levels of dosage of insecticide, and then spraying, dusting or dipping treatment is made in the laboratory, the effectiveness of test insecticide could more precisely be determined statistically from counts of surviving flies only by the standard probit method of parameters of tolerance distribution (Wadley 1949, Finney 1949). A method of effectiveness evaluation of insecticide emulsifiable concentrates for the immature stage of the Mediterranean fruit fly will be discussed in the present paper.

Spatial distribution of the Mediterranean fruit fly on coffee berries

The fitting of an appropriate mathematical model to the spatial distribution of insects per unit of area, host or time is essential for

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