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The Fundamental Research to the Application of Systemic Insecticides (II). The Absorption and Translocation of Several Insecticides in Rice plant by Soil application. Takeo Ishiguro* and Tetsuo Saito (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Nagoya) Received November 2, 1970. *Botyu-Kagaku* 36, 17, 1971.

4. 浸透殺虫剤の施用法に関する基礎的研究 (II) 土壌処理による各種浸透殺虫剤の水稲における浸透および移行について. 石黒丈雄*, 斎藤哲夫 (名古屋大学農学部害虫学教室) 45. 11. 2. 受理

Vamidothion, Dimethoate, Disulfoton, Thiometon および Mecarbam の水稲における浸透移行性を ^{32}P-標識化合物を用いて検討した. すなわち, 上記薬剤の乳剤稀釈液を水稲の移植した土壌表面に施用し, 薬剤の浸透移行およびそれにともなう殺虫力を調べた.

その結果, Disulfoton は施用直後に水稲の地下部に薬量が多く, 地下部への薬剤浸透が早く, また Dimethoateは 地上部および地下部の薬量はほぼ同じであり, 植物体内の浸透および移行が極めて容易であると推察される.

これに対して Vamidothion の浸透移行性はゆるやかであり, Thiometon および Mecarbam のそれは弱い. 同じ施用法で行なったヒメトビウンカに対する殺虫試験による死虫率の増加は地上部の薬量の増加とほぼ一致した.

実験終了時(施用後6日目)においてVamidothionとMecarbamは土壌吸着物が多くDimethoateは少なかった.

Introduction

Since Schrader and Kükenthal first found the systemic action of organophosphorus insecticides in 1935, many experiments have been made on absorption, translocation and metabolism in plants with various application methods. At present, systemic insecticides occupy a very important position in pest control for agricultural crops.

It was proved by David (1952),[3] Iyatomi and Saito (1967)[7] and Harris (1967)[6] that insecticidal effect of the applied chemicals were influenced by the type of soil. Further, Ching *et al.* (1961),[2] Bennett (1949)[1] and Wedding *et al.* (1952)[18] and Teitz (1954)[16] studied absorption, translocation and persistence of the several insecticides respectively on each crops. They reported that the absorption site in crop varies according to the application methods of chemicals.

This report is concerned with the absorption and translocation of the following five ^{32}P-labelled systemic organophosphorus insecticides in rice plants: Vamidothion, Dimethoate, Disulfoton, Thiometon and Mecarbam.

Meterials and methods

Test insecticides: Sulfur powder, methanol and

* Present Address: Aburahi Laboratories, Shionogi & Co., Shiga Prefecture.
現在：塩野義製薬株式会社油日ラボラトリーズ

ammonia were added to 22.4mCi/g of ^{32}P-dimethyl phosphite, which was purchased from Sumitomo Atomic Energy Industries, and the mixture was stirred to make ammonium salt of ^{32}P-*O*, *O*-dimethyl thiophosphate.[8] Addition of equimolar 5-chloro 3-thia 2-*N*-methyl valeramide in aceton to the reaction mixture gave ^{32}P-Vamidothion,[13] *O*,*O*-dimethyl *S*-〔2-(1-methyl carbamoyl ethylthio) ethyl〕 phosphorothiolate.

57mCi/g of potassium salt of ^{32}P-dimethyl dithiophosphate was dissolved in aceton and added equimolar *N*-methyl 1-chloroacetoamide or ethylmercaptoethyl chloride to give ^{32}P-Dimethoate, *O*, *O*-dimethyl *S*-(*N*-methyl carbamoylmethyl) phosphorodithioate and ^{32}P-Thiometon, *O*, *O*-dimethyl *S*-〔(2-ethylthio)ethyl〕 phosphorodithioate.

54.5mCi/g of potassium salt of ^{32}P-diethyl dithiophosphate was dissolved in aceton and added equimolar ethylmercaptoethyl chloride or chloroacetyl *N*-methyl uretan to give ^{32}P-Disulfoton, *O*, *O*-diethyl *S*-(2-ethylmercapto) ethyl phosphorodithioate and ^{32}P-Mecarbam, *O*, *O*-diethyl *S*-(*N*-ethoxycarbonyl-*N*-methyl carbamoylmethyl) phosphorodithioate.

Each compound was dissolved in chloroform, and washed sufficiently with water and concentrated to dryness. Purity of each compound was determined by means of distribution of radioactivity in silica gel thin-layer chromatography and colorimetric determination with palladium chloride. Some of the compounds were purified further with thin-layer chromatography.

Each compound was added to aceton: benzene: Newcoal 863 (manufactured by Japan Emulsifier Co., Ltd.) at: 30:30:30 W/W and made 10% emulsifiable concentration.

Test plant: Two hundred gram of air-dried paddy field soil which was passed through a sieve of 5mm were put in a glass petri dish of 18cm in diameter and added water of prescribed quantity to it. Fifteen stands (3 plants per stand) of rice seedling (Aichi-Asahi) at three leaf stage were transplanted in glass petri dish.

Application of radioactive compounds to rice plants: Ten percent emulsifiable concentration was diluted at 0.05 percent with distilled water. After 2 days from transplanting of the rice plants, the diluted solution of each compound was dropped onto the surface of soil as evenly as possible with a pipette at the rate of 20m*l* (as active ingredient, 1mg/petri dish=3.6kg/10 ares in term of 5% granule). The plants were not directly contaminated with the solution. During the experiments, the rice plants were kept in a growth chamber that were set with a 10-W fluororecent lamp at 25°C, and supplied water to maintain the water surface at a constant level.

Sampling: At several time intervals after the application, three stands of the rice plants were sampled at randam in order to examine the distribution of radioactive metabolites in the rice plant. The roots were washed sufficiently with a running water to remove the unabsorbed insecticides. The treated rice plants were divided into the aerial and root parts, and weighed respectively after removing the moisture with a filter paper.

After mincing them, the materials were grinded with 2m*l* of distilled water in a glass homogenizer and shaked it vigorously with same amounts of chloroform. Homogenates of the aerial and root parts were centrifuged for 5 minutes at 2000 r.p.m. to separate chloroform layer from water layer.

On the other hand, all treated soils were collected from the petri dish respectively at 6 days after the application and extracted three times with 80% acetone in water. The extracts were concentrated with a rotary evaporator under reduced pressure, and then partitioned with equal volume of chloroform and distilled water. Chloroform, water extracts and soil-absorbed substances were separated by the above described operation. Each fraction was concentrated with a rotary evaporator under reduced pressure again.

Radioassay: Total amounts of chloroform extracts (undecomposed product), water extracts (decomposed product) and precipitates were put in sampling dishes for radioassay respectively, and poured 2% KOH-alcohol solution into them. And then, each fraction was dried under an infrared lamp. The precipitates were incinerated in an electric furnace at 500°C. The radioactivities of each fraction were measured by the Aloka gas-flow counter.

Bioassay for brown planthopper: Rice plants which were treated non-labelled compounds in the same

methods as the labelled ones were removed at 6, 24, 48, 72, 96, 120 and 144 hours after the application. Two plants of the treated rice plants were transferred into a transparent cylinder of 35mm in diameter and 20cm in height made by polyethylene film, and then fixed with sponge.

Ten females of adult smaller brown planthopper (*Delphacodes striatella* Fallen), which were reared for successive generations on rice plants in our laboratory, were released into the cylinder. The cylinders were kept in a growth chamber at 25°C. The number of fallen insects was counted after 24 and 48 hours, and the corrected percentage was caluculated by the Abbot's correction method. The insecticidal tests were replicated three times.

Results

In order to study absorption and translocation of systemic phosphorus insecticides in rice plants, ^{32}P-labelled compounds were applied onto soil surface. After the application, the rice plants were sampled at several intervals for the radioassay. The decrement of radioactivity was corrected and the amount of radioactive materials in the aerial and root parts of rice plant was calculated in term of initial compounds. The amounts of the radioactive materials in the rice plants between chloroform and water extracts are shown in Tables 1 and 2.

As regards to the penetration of the compounds into the root of rice plants, the amounts of chloroform extractable metabolites of Disulfoton, Vamidothion, Thiometon and Mecarbam attained maximum within only 6 hours after the application, and did not increase afterwards. On the contrary, chloroform extractable metabolites of Dimethoate increased gradually with the lapse of time. Dimethoate was proved to have a nature of the strongest penetration among the test compounds. Vamidothion showed rapid increase in the acumulated amount of chloroform extractable metabolites in the aerial parts of rice plants from 48 hours after the application. The radioactive metabolites partitioned in aqueous phase were increased with the lapse of time, and the large amounts of its metabolites were found in all test compounds at 48 hours after the application.

Table 3 shows the concentration of chloroform partitioned metabolites in the rice plants at certain time intervals after the application, and the data

Table 1. The amounts of radioactive materials found in the root parts of three rice plants (1 stand) after the application of ^{32}P-labelled insecticides.

Test compounds	Fraction	Time after application			
		6 hrs.	24 hrs.	48 hrs.	72 hrs.
Vamidothion	chloroform extracts	0.22γ	0.20γ	0.21γ	0.12γ
	water extracts	0.10	0.14	0.55	0.12
	unextractable	0.02	0.05	0.20	—*
Dimethoate	chloroform extracts	0.37	0.86	1.07	1.33
	water extracts	0.14	0.52	3.31	1.79
	unextractable	0.06	0.02	0.33	—
Disulfoton	chloroform extracts	0.69	0.59	0.69	0.58
	water extracts	0.58	0.89	1.05	0.86
	unextractable	0.23	0.64	1.61	—
Thiometon	chloroform extracts	0.34	0.26	0.20	0.17
	water extracts	1.02	0.82	1.05	0.87
	unextractable	0.25	0.34	0.49	—
Mecarbam	chloroform extracts	0.05	0.07	0.12	0.13
	water extracts	0.10	0.10	0.15	0.09
	unextractable	0.00	0.01	0.03	—

* Undetectable by the gas-flow counter.

Table 2. The amounts of radioactive materials found in the aerial parts of three rice plants (1 stand) after application of ^{32}P-labelled insecticides.

Test compounds	Fraction	Time after application			
		6 hrs.	24 hrs.	48 hrs.	72 hrs.
Vamidothion	chloroform extracts	0.05γ	0.09γ	0.60γ	0.40γ
	water extracts	0.04	0.07	0.49	0.29
	unextractable	0.01	0.03	0.06	—*
Dimethoate	chloroform extracts	0.36	0.86	1.07	1.37
	water extracts	0.14	0.52	3.31	1.79
	unextractable	0.01	0.07	0.19	—
Disulfoton	chloroform extracts	0.14	0.35	0.91	1.38
	water extracts	0.15	0.32	1.20	1.34
	unextractable	0.01	0.12	0.34	—
Thiometon	chloroform extracts	0.30	0.26	0.30	0.30
	water extracts	0.77	0.83	0.78	1.09
	unextractable	0.01	0.07	0.06	—
Mecarbam	chloroform extracts	0.01	0.02	0.07	0.08
	water extracts	0.02	0.10	0.18	0.22
	unextractable	0.00	0.01	0.02	—

* Undetectable by the gas-flow counter.

Table 3. The concentration of chloroform partitioned metabolites in rice plants after application of ^{32}P-labelled insecticides.

Test compounds	Part of extraction	Time after application			
		6 hrs.	24 hrs.	48 hrs.	72 hrs.
Vamidothion	aerial	0.36ppm	0.84	4.08	2.28
	root	1.87	1.14	1.18	0.62
Dimethoate	aerial	3.02	6.18	7.33	6.40
	root	2.64	6.17	7.27	7.50
Disulfoton	aerial	1.11	2.79	5.59	6.36
	root	4.80	4.72	4.16	3.36
Thiometon	aerial	2.45	1.70	1.92	1.55
	root	2.18	1.69	1.23	1.17
Mecarbam	aerial	0.09	0.15	0.44	0.39
	root	1.02	0.46	0.63	0.84

Data expressed as initial compounds per fresh plant tissue.

were expressed as initial compounds per fresh plant tissues. Dimethoate exhibited the highest concentration in both parts, and followed by Disulfoton. Concentration of chloroform partitioned metabolites in the aerial parts tended to be the highest at 48 hours after the application and gradually lowered afterwards. Concentration of its metabolites in the root parts lowered with the lapse of time after the application of Disulfoton, Vamidothion, Thiometon and Mecarbam except Dimethoate.

Residual amounts of the insecticides and their metabolites in soil at 6 days after the application are described in Table 4. The residual amounts

Table 4. The amounts of radioactive materials found in treated soils (200g.) at 6 days after application.

Test compounds	Chloroform extracts	Water extracts	Soil-absorbed substance
Vamidothion	149.7γ	149.7	532.4
Dimethoate	456.5	303.1	253.2
Disulfoton	524.4	232.6	336.2
Thiometon	331.8	274.2	309.5
Mecarbam	329.1	51.2	512.6

Table 5. Insecticidal tests of systemic organophosphorus insecticides to the smaller brown planthopper (*Delphacodes striatella* F.) fed on rice plants treated with the insecticides.

Time after application	Vamidothion			Dimethoate		
	No. of insects	mortality		No. of insects	mortality	
		24 hrs.	48 hrs.		24 hrs.	48 hrs.
6 hrs.	22	68.2%	95.5%	25	84.0%	100.0%
24	22	95.5	100.0	22	100.0	100.0
48	30	76.6	100.0	28	92.9	100.0
72	28	96.4	100.0	25	92.0	100.0
96	36	91.7	100.0	24	100.0	100.0
120	28	82.2	96.4	32	96.7	100.0
144	21	41.8	90.2	20	70.0	100.0

Time after application	Disulfoton			Thiometon		
	No. of insects	mortality		No. of insects	mortality	
		24 hrs.	48 hrs.		24 hrs.	48 hrs.
6 hrs.	23	56.6	87.0	23	4.4	17.4
24	24	46.8	91.7	27	22.2	48.1
48	27	70.4	100.0	27	11.1	63.0
72	36	27.8	94.4	23	13.0	78.7
96	27	29.6	96.3	26	30.8	92.3
120	25	36.0	52.0	24	12.5	52.0
144	—	—	—	—	—	—

Time after application	Mecarbam		
	No. of insects	mortality	
		24 hrs.	48 hrs.
6 hrs.	23	17.4	26.1
24	23	4.3	30.4
48	33	9.2	36.4
72	31	6.5	54.8
96	28	3.6	42.7
120	28	7.1	14.3
144	—	—	—

of chloroform partitioned metabolites of Disulfoton and Dimethoate were very large, and followed by Thiometon, Mecarbam and Vamidothion in decreasing order. On the contrary, soil-absorbed substances of Vamidothion was the largest, and followed by Mecarbam.

Adults of the smaller brown planthopper were fed on the rice plant treated with non-labelled same insecticides. Table 5 shows the percentage of fallen insects at 24 and 48 hours after the

application. The rice plants treated Dimethoate exhibited the best insecticidal effect, and followed Vamidothion and Disulfoton. However, Thiometon and Mecarbam had almost no insecticidal effect for the smaller brown planthopper.

Discussion

It has been said that all outer surface of plant body can absorb various kinds of compounds under certain conditions. Therefore, as for the penetration of systemic insecticides into the plant body, it should be taken into consideration whether the surface of the plant body has adaptability for absorption of the compounds.

It is considered that insecticides penetrate plant body from the root system *via* the same route as water or organic materials.

When the insecticides are applied on water surface of a paddy field, the insecticides are not only absorbed *via* roots but also moves upward along surface and interstices of stem or leaf sheath by capillarity, and arrives in the stem or leaves under such a special environment of flooding (Isii and Hirano,[6] 1962, Tomizawa,[17] 1963). Therefore, the systemic insecticides should be contacted directly with the plant body in order to expect the highest effect for noxious insects.

Metcalf (1956)[9] reported that one of the factors to control the penetration of chemicals into the plant tissue was its water-solubility, and that any chemical should have the solubility enough for enabling it to move freely in plant tissues. It was reported also by Metcalf *et al.* (1957),[10] David (1952)[3] and Ridgway *et al.*[14] (1956) that translocation of chemicals into leaves and stem depend upon the liposolubility and that substance having water-solubility readily penetrate the plant tissue *via* the root.

Water-solubility of the compounds used for this experiment is as follows: Vamidothion 20g, Disulfoton 2.5mg, Dimethoate 250mg per 100m*l* of water respectively. Thiometon is practically insoluble and Mecarbam is very slightly soluble in water. Thus, it is presumed that Vamidothion having the highest water-solubility among test compounds is greatly absorbed and translocated *via* root system, based on the reports by Metcalf *et al.*[10] and David.[3]

As seen in Tables 1 and 2, however, the penetration and translocation of Vamidothion is rather slower as compared with Dimethoate and Disulfoton.

According to the observation of Miyata (1965)[11] on the penetration of various kinds of organophosphorus insecticides into the plant tissue, when the insecticides were applied on rice plants with the root dipping method, some insecticides penetrated only a little into the plant tissue in spite of its high water-solubility, and stability of chemicals and penetration mechanism greatly influenced on the penetration of the chemicals into the plant tissue besides the water-solubility.

On the other hand, Disulfoton penetrated and translocated readily into the plant tissue although it was a little soluble in water. As evidenced from the above result, the translocating rate of the chemicals could not be judged from only its water-solubility.

The amount of radioactive metabolites in Dimethoate was the largest at the aerial and root parts of rice plants, and the amount gradually increased with the lapse of time after the application. From the result, it is presumed that Dimethoate is a fast-acting insecticide owing to readily penetration and translocation in the plant tissues. However, it is suggested that Dimethoate causes readily phytotoxicity to some plants since it penetrates and translocates very rapidly in the plant tissue.

Sato and Miyamoto (1967)[15] reported the following results of the fate of Dimethoate in soil: Dimethoate translocates in plant tissue with soil moisture, but the translocating rate of Dimethoate is rather small in the case of immovability of water even when the soil contains sufficient water. Afterwards Dimethoate is accumulated without being decomposed and so Dimethoate caused phytotoxicity.

Tomizawa (1963)[17] reported that dimethyl *S*-isopropyl-2-sulfinyl phosphorothiolate is absorbed *via* leaf sheath and translocate into leaf blade, but it also moves to root in a fairly amount, when applied on water surface of pot in which rice plant had been transplanted. In general, organophosphorus insecticides appited in paddy field almost remain on the soil surface without diffusing

into the lower layer of soil.

In this experiment, under the condition that water was excessively saturated, radioactive metabolites of Dimethoate moved to the aerial parts of rice plant very rapidly, and were accumulated in the leaf sheath and leaf blade in a large amount. From these result, it seems probable that Dimethoate was not only absorbed through roots but also penetrated through leaf sheath and translocated in the plant tissues.

A large amount of chloroform partitioned metabolites of Dimethoate was detected in the aerial and root parts as compared with other test compounds. Therefore, it is presumed that Dimethoate was decomposed in the plant tissue. However, Morikawa and Saito (1966)[12] and Sato and Miyamoto (1967)[15] reported, as a result of study on metabolism of Dimethoate, that the compound can not be readily decomposed in the leaves of rice plant and soil.

The amount of radioactive materials of Disulfoton, Vamidothion, Thiometon and Mecarbam in the root parts tended to be kept almost constant or slightly lower with the lapse of time after the application. From those results, it seems probable that the root absorb further the chemicals to supplement the decreased amount after the movement of chemicals into the aerial parts, since amount of the chemicals uptaken from root is almost invariable.

According to the investigation of residue in soil at 6 days after the application, chloroform and water partitioned metabolites of Dimethoate and Disulfoton still remained in soil in a large amount. On the contrary, the amount of chloroform partitioned metabolites in Vamidothion was small but the amount of soil-absorbed substance was large.

Getzin *et al.* (1970)[4] reported Phorate applied in soil showed good persistence, and that sulfoxide and sulfone of Phorate were strongly absorbed by soil components. Dimethoate having natures of good penetration and translocation was best in the insecticidal effect against the smaller brown planthopper and it was followed by Vamidothion and Disulfoton. Thiometon and Mecarbam proved to be poor effect. This is considered to be due to the insecticidal effect itself of each compound against the smaller brown planthopper not to be due to the accumulated amount of each chemicals at the aerial parts of the rice plants. In general, the insecticide having natures of strong penetration and translocation has also the highest insecticidal potency. Insecticidal efficacy of the chemicals was in accord with the residual amount of the chemicals in the aerial parts of the plant.

Summary

The absorption and translocation of five systemic insecticides, Vamidothion, Disulfoton, Dimethoate, Thiometon and Mecarbam in rice plants were quantitatively and qualitatively studied by using ^{32}P-labelled insecticides. Rice seedlings were cultured in the paddy field soil contained in the glass petri dish, and the labelled insecticides were applied onto the soil surface.

Results of the experiments showed that Disulfoton was absorbed rapidly by the roots of the rice seedling, and large amounts of Dimethoate was absorbed by the aerial parts as well as by the roots.

It was assumed that absorption and translocation of Dimethoate in rice plants were extremely easy. While translocation of Vamidothion which has a nature of high water-solubility was found to be slow.

The translocation of the chemicals to the aerial parts were associated with the insecticidal effects to the brown planthopper which attacks rice plants.

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The Comparative Pheromone Activity of Acetates of Unsaturated Alcohol to Males of the Almond Moth. Shozo TAKAHASHI, Chikayoshi KITAMURA and Yasumasa KUWAHARA (Pesticide Research Institute, College of Agriculture, Kyoto University, Kyoto Japan) Received January 20, 1971. *Botyu-Kagaku* 36, 24, 1971. (with English summary 26)

5. スジマダラメイガに対する性フェロモン類縁化合物の活性について*

高橋正三，北村実彬，桑原保正（京都大学 農学部，農薬研究施設）46. 1. 20. 受理

スジマダラメイガの雄は性フェロモン類縁化合物に対して，フェロモンの場合とは比較にならないほどの高濃度ではあるがフェロモンに対する反応と同様の挙動を示す．それらの化合物の中では特に，*cis*-7-tridecen-1-ol acetate と *cis*-9-tetradecen-1-ol acetate に対する反応が顕著であった．

既に報告したように，スジマダラメイガ (the almond moth, *Cadra cautella* Walker) の性誘引物質は *cis*-9, *trans*-12-tetradecadien-1-ol acetate であることが明らかとなった[1]．

今回，われわれは性フェロモン類縁化合物である高級不飽和アルコールおよびエポキシアルコールの酢酸エステル36種を用いて生物検定を行ない，それぞれのフェロモン活性を調べ，あわせて化学構造と活性との関係を解明することを目的として以下の実験を行なった．

実験材料および方法

供試化合物：検定に供した不飽和アルコールおよびエポキシアルコールの酢酸エステルは当研究室で合成されたもの，および M. Jacobson 博士 (U. S. D. A.) より提供されたもの（表1において化合物の肩に＊印をつけた）を用いた．

生物検定：1) 第一次検定：供試化合物が多く，フェロモン活性の低い化合物も含まれているため第一次検定として，Butenandt らの方法に準じて定性的な生物検定を以下のように行なった[2]．羽化後3日目のスジマダラメイガ未交尾雄10～25匹を腰高シャーレ（直径 11cm，高さ 7cm）に入れ室温 28°±1°C 条件下で検定を行なった．供試化合物を細いガラス棒につけ（約 300～500μg）ふたにあいた穴からシャーレの中に挿入した．15～30秒以内に翅を特徴的に振動させて動きまわる反応を示す虫数を数えた．

2) 第二次検定：第一次検定で活性を示した化合物について，以前報告したフェロモン検定装置を使用して一定量（100μg あるいは 10μg）の化合物に対する雄の "per cent response" を求めた[3]．

実験結果および考察

表1に示す36種の化合物を第一次検定に供した．ガラス棒の先に付着した 300～500μg の試料に対してスジマダラメイガ雄が反応した化合物には○印をつけた．それぞれについて活性の強度を表2に示した．分

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