การทดสอบพิษของยาฆ่าแมลงประเภทต่าง ๆ ที่มีต่อหนอนกระทู้ข้าวโพด

Tests on Various Types of Insecticides to the Larvae of the Armyworm; Leucania separata Walker (Noctuidae : Lepidoptera)

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ABSTRACT

Thirty one compounds were applied on the 3rd-4th and 6th-instar larvae of the armyworm *L. separata* Walker by revolving spray tower in the screening test. Mostly, organophosphate compounds produced highly effective to the armyworm, chlorinated hydrocarbons only endrin and dieldrin gave the high toxicity while any toxicities did not find in carbamate group. Four compounds parathion, Elsan, Bayer 5621 and endrin were proposed to be the effective insecticides to the armyworm. Additionally, the results of topical application and injection method were obtained. Sumithion and diazinon were weaker in penetratibility into an insect body than Elsan and parathion.

Chemical control of the lepidopterous insects, including the armyworm *L. separata* Walker, a sporadic pest of cereal crops, has varied in effectiveness (9). This variation may be associated with the feeding habits of the species, or with the stage of larval development at which chemical control is directed. Differences in the susceptibility of insects at various stages of development to chemicals has been reported for contact insecticides in the past decade by Mukerjea (8). So far as *L. separata* Walker has become a serious pest, there is a lack of basic toxicological information about the effects of various types of insecticides. This study was undertaken at the laboratory of Applied Entomology and Nematology, Nagoya University, Japan to determine the susceptibility mainly in larval stages to typical chlorinated hydrocarbons, carbamates and organo phosphorus insecticides.

Materials and Methods

Preliminary screening test. When the majority of larvae reached the third instar, they were transferred from the original container to the new one and were kept without food for a few hours in order to standardize the condition of the batch. The fourth instar and fullgrown larvae were also selected from the colony for the other experiments. Thirty one insecticides were included in this experiment: Abate (0, 0-dimethyl phosphorothioate 0,0-diester with 4,4-thiodiphenol); Amiphos, DAEP (0,0-dimethyl-S-2 (acetylamino) ethyl-dithiophosphate); Anthio (0,0-dimethyl S-(N-methyl-N-formoylcarbomoyl methyl) phosphorodithioate; Bayer 5621, Bayer 77488 (Phenylglyoxylonitrile oxime 0,0-diethyl phosphorothioate); Bayer 5691, Bayer 78182 (0-chlorophenyl) glyoxylonitrile oxime 0,0-

diethyl phosphorothioate); Bayer 77049 (0,0-diethyl-0-(2-quinoxalyl)-thiophosphate); Cyanox CYAP (0,0-dimethyl 0-4-cyanophenyl phosphorothioate); DDVP, (2,2-dichlorovinyl dimethylphosphate); DDT (Dichloro diphenyl trichloroethane); Denapon, Sevin, NAC, Carbaryl (1-Naphthyl-N-methylcarbamate); Diazinon (O,O-diethyl 0(isopropyl-6-methyl-4-pyrimidyl) thiophosphate); Dieldrin (1,2,3,4,10,10-Hexachloro-6, 7-epoxyl-1,4,4a,5,6,7,8,8a-octahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene); Dimethoate (0,0-dimethyl S (N-methylcarbamoylmethyl) phosphorodithioate); Dilan (2-Nitro-1, 1-bis (p-chlorophenyl) propane + 2-Nitro-1, 1-bis (p-chlorophenyl) butane); Dursban (0,0-diethyl 0-(3,5,6,-trichloro-2-pyridyl) phosphorothioate); EPN (O-ethyl 0-p-nitrophenylphosphorothioate); Elsan PaP Papthion (Ethyl ester of 0,0-dimethyl dithiophosphoryl-1-phenylacetic-acid); Ethyl parathion (0,0-diethyl 0,-p-nitrophenylphosphorothioate); Endrin (1,2,3,4,10,10-Hexachloro-6, 7-epoxy-1, 4,4a,5,6,7,8,8a-octahydro-1, 4-endo-endo-5,8-dimethanonaphthalene); Heptachloro (1,4,5,6,7,8,8-Heptachloro-3a,4,6, 7a-tetrahydro-4, 7-methanoindene); Hidorol (4-Diallylamino-3, 5-dimethyl phenyl-N-methylcarbamate); Imidan, PMP (0,0-Dimethyl-S-phthalimidomethyl phosphorodithioate); Lebaycid (0,0-dimethyl 0-(4-(methylthio)-m-tolyl) phosphorothioate); Lindan (1,2,3,4,5,6-Hexachlorocyclohexane gamma isomer); Methyl parathion(0,0-dimethyl 0-p-nitrophenylphosphorothioate); MTMC (Metatolyl-n-methylcarbamate); NT5 (0,0-di-isopropyl S-(2-(1-hydroxy-2-trichloroethylamino) ethyl)phosphorodithioate);NT7(0,0-diethyl-S-(2-N-methylacetamidoethyl) phosphorodithioate); Sumithion (0,0-dimethyl 0-3-methyl-4-nitrophenyl phosphorothioate); Surecide, CYP (0-Ethyl 0-4-cyanophenyl phenylphosphonothioate); Trichlorfon, dipterex Dep (Dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate).

The commercial formulations of emulsified concentration (EC) and wettable power (WP) of these materials were dissolved in and diluted with distilled water to arrange the concentration by percentage of active ingredient. As direct contact toxicity, these diluted toxicants were sprayed on larvae by Kawano revolving spray tower apparatus (6) at the pressure 250 mmHg with the amount of liquid applied at 4 ml, of each concentration, the insects were divided into 3 replicated groups of 5. In control, distilled water was sprayed at the same volume as in the treated larvae. After treated, then, larvae were held in the clean container, a polyethylene cups of 9.8 cm. in diameter, 4.5 cm. in height, with fresh corn leaves under constant room temperature of 25 C. Mortality was recorded at 24 hours intervals for 72 hours. Effectiveness of all compounds were tabulated orderly. The data were corrected for natural mortality by Abbott's formula (1). According to the indication from the results, the available effective compounds were further tested and performed the following result by median lethal concentration (LC50). In this test, insecticidal concentrations were provided to 0.05, 0.0125, 0.025 and 0.05 percent. The 10 4th-instar larvae were used for each concentration with three replicates. After the mortality data were corrected by Abbott's formula, median lethal concentrations were calculated by the method described by Finney (3).

Susceptibility of different instars to Elsan. The test was done based on the assessed result, as shown in Table 3 and 4. The 2nd-through the 5th instar larvae were treated in the same procedure as that of the screening test. A 0.05 percent concentration of Elsan was directly sprayed to determine the variation in susceptibility of the different instar (5,8).

Topical application and injection to the 4th-instar larvae. To reveal the mechanism of insecticidal activity, an attempt was made. Elsan and Methyl parathion were selected as representative of effective compounds, while Sumithion and diazinon represented on less effective to the armyworm. The 3rd-instar larvae were not used since they were too small to be treated topically or to inject by micropipette. 3 replicates each, containing of 10 4th-instar larvae were treated with each dosage. Tentative tests were made to provide the range of dosage required. Technical grade of 4 materials, dissolved in acetone were prepared, and dilution, with acetone of these stock solutions were used in all experiments. Prior to treatment, despite the fact that the insects were prepared similar to the procedure used in the experiment of LC50, but somewhat different in the way in which they were weighed, then, the average weight per individual was used to calculate the dosage of insecticide applied. Toxicants were topically applied to the dorsum of the abdomen by means of the microsyringe and injected by micropipette, glassneedle which was developed by the author to serve as a more convenient technic and to adequately reduce the mortality by mechanical effect (Fig. 1). The volume of inseticidal solution per insect was arbitrarily adjusted at 0.0007 ml. In control. insects were treated with the same volume by acetone.

Fig. 1. Glassneedle used for micro-injection.

As soon as the acetone evaporated, the insects were transferred into container with fresh food, and then, kept under 25 C. constant temperature for observing the mortality. All mortality data were taken 72 hr. after treatment and justified insect abnormality as similar to that of Ando and Sherman (2), the badly affected and moribund larvae were recorded as dead although actual deadth did not necessarily occurs until a later period.

The median-lethal dosages (LD50) and ninety five percent-lethal dosages (LD95) of the insecticides based on the mean body weight of the insects were calculated by the method described by Finney (3). The comparison of the LD50 level between the topical application and injection method was made for the purpose of an interpretation of the effect so-called "Phamacological action" of insecticides to the insects (11, 14).

Results and Discussion

Regarding the initial screening test (Table 1), the toxicity level of a compound was considered effective at the given dose that produced 100 percent mortality within 72 hr. of the chlorinated hydrocarbon insecticides, endrin and dieldrin were the only two compounds which gave high larvicidal toxicity, while organo phosphorus insecticides primarily produced the high effectiveness and acute toxicity to larvae of the armyworm. Both methyl and ethyl parathion, Elsan Bayer's compounds, DDVP, Cyanox and Dursban were slightly toxic more than Sumithion, Surecide, trichlorfon and EPN but more strongly toxic than the remaining organo phosphorus compounds, particularly, dimethoate which was not toxic to this species. A similar result was obtained in the pale cutworm, *Agrotis orthogonia* Morr. control (10).

With most of the carbamate insecticides used in the experiment there was no appreciable effect to the armyworm. Carbaryl, a well-known carbamate compound, did not exhibit any toxic symptoms. In 1969 McDonald (10) stated that recently treated larvae which regurgitated were, in most instances, able to resume feeding after 72 hr. It probably Suggests that larvae readily detoxify carbamate insecticides.

In addition to the comparative toxicity, the test of Elsan, parathion and Bayer 5621 was taken

Table 1. Laboratory screening test of 31 insecticides by means of direct contact spraying on the 3-rd instar larvae of the armyworm at the rate of 0.05 percents concentration.

Insecticide	%Mortality checked at the indicated time (hr)			Insecticide	%Mortality checked at the indicated time (hr)		
	24	48	72		24	48	72
Methyl-parathion	100......................			trichlorfon	67	75	75
parathion	93.3	100		Heptachlor	17.1	67	75
Elsan	100......................			Abate	57.1	72.6	72.6
Bayer 5621	100......................			diazinon	25	42.6	42.6
Bayer 5691	100......................			Amiphos	8.2	17.1	25
Bayer 77049	100......................			Hirodol	8.2	17.1	17.1
Dursban	100......................			Lindane	0	8.2	8.2
endrin	46.6	93.3	100	Lebaycid	0	0	8.2
DDVP	100......................			NT5	7.1	7.1	7.1
Cyanox	100......................			Carbaryl	0	0	0
dieldrin	13.2	86.6	100	dimethoate	0	0	0
Dilan	92.8	92.8	92.8	Anthio	0	0	0
EPN	9.17	91.7	91.7	Imidan	0	0	0
Surecide	83.3	91.7	91.7	MTMC	0	0	0
Sumithion	67	75	91.7	NT7	0	0	0
DDT	50	83.3	83.3	Control	.... 0		

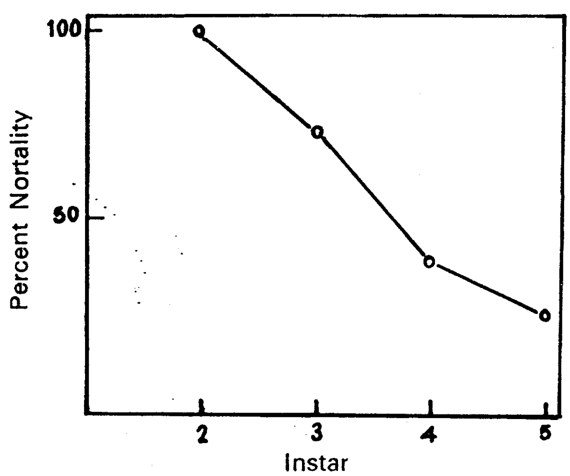

Fig. 2. Direct contact toxicity (by revolving spray tower) of Elsan at 0.05 percent to the 2nd-through the 5th instar larvae of the armyworm.

Table 2. The effectiveness of insecticides to the 3 rd-instar larvae of the armyworm at various concentrations.

Insecticide	Concentration (%)	%Cumulative mortality after treated at the indicated time (hr) 24	48	72
Methyl parathion	0.05	100..		
	0.005	50	58.2	58.2
	0.0005	0	0	0
	0.00005	0	0	0
Sumithion	0.05	58.2	67	67
	0.005	0	0	0
	0.0005	0	0	0
	0.00005	0	0	0
endrin	0.05	67	83.3	83.3
	0.005	0	0	0
	0.0005	0	0	0
	0.00005	0	0	0
EPN	0.05	100..		
	0.005	19.8	19.8	19.8
	0.0005	0	13.2	13.2
	0.00005	0	13.2	13.2
DDT	0.05	53.3	53.3	60
	0.005	26.6	26.6	33.3
	0.0005	13.2	13.2	20
	0.00005	0	0	0
DDVP	0.05	100..		
	0.005	0	6.6	6.6
	0.0005	0	6.6	6.6
	0.00005	0	0	0
Control		....	0	....

with full-grown larvae of the armyworm, pupation percentage and mortality were observed (Table 3). Bayer 5621 gave the highest effectiveness followed by parathion and Elsan in the same rate of application.

Data in Table 4,5 are the results of the toxicity tested with Elsan, parathion and endrin which were analysed by the standard probit technic of Finney (3). The LC50 value of each compound favored the last results but it appeared somewhat different from that of the effectiveness of endrin which seemed to be the highest. This test, the result with parathion, however, was in general agreement with the work of other investigators who have tested this compound against lepidopterous larvae (13). Elsan, a new compound, with low mammalian toxicity (mouse 350-400 mg/kg) also gave promise for the control of the armyworm.

Table 3. The relative toxicity of 3 effective insecticides to fullgrown larvae of the armyworm at 0.05 percent.

Insecticide	% pupation	% mortality
parathion	35.88	64
Elsan	8.96	91
Bayer 5621	0	100
Control	100	0

Table 4. Direct contact toxicity of 3 effective insecticides to 4th-instar larvae of the armyworm.

Insecticide	% Mortality after 48 hr-treated at indicated concentrations			
	0.005	0.0125	0.025	0.05
parathion	6.7	23.3	80	96.7
Elsan	10	10	36.7	63.3
endrin	20	33.3	83.3	93.3

Table 5. LC50 and LC95 of 3 effective insecticides calculated at 48 hr treatment at holding temperature, 25 C.

Insecticide	LC50	LC95	Regression equation Y = Probit mortality, X = log conc.
parathion	0.018	0.047	Y = 5.207 + 3.811 (x - 0.303)
Elsan	0.036	0.064	Y = 4.890 + 2.520 (x - 0.517)
endrin	0.016	0.034	Y = 5.192 + 5.236 (x - 0.254)

The result of the experiment on the 2nd through the 5th-instar of larvae of the armyworm was found with Elsan 0.05 percent. As shown in Fig. 2 the older larval stages are more resistant to the insecticides than the earlier larval stages. Similar results have been obtained with other lepidopterous insect (7,8,9). Gast (4) suggested on this point that changes in the integument and physiology of larvae elevate the tolerence.

Table 6 and 7 represented the results of the topical application and the injection methods. LD50 and LD95 values were expressed as micrograms of toxicant per gram of mean body weight, consequently, the toxicity was established in toxic index (12) for comparative purposes on which parathion was given as the standard. Elsan was about 3.5 times less toxic than parathion while the lesser effective compounds, diazinon and Sumithion were 30 and 50 times respectively less toxic to the standard.

Micro-injection data (Table 8 and 9) indicated the relative toxicity as compared with the data of topical application at LD50 level that Elsan and parathion gave less than one. Such phenomenon was also found in rice stem borer, *Chilo sup-*

Table 6. Mortality percentage of the armyworm larvae topically treated with 4 organo phosphorus insecticides.

Insecticide	Dosages of active ingredient (mg)							
	8	4	2	1	0.5	0.25	0.125	0.0625
Methyl parathion	-	-	-	-	-	73.6	46.2	19.8
Elsan	-	-	-	59.4	26.4	-	-	-
diazinon	85.8	19.8	13.2	-	-	-	-	-
Sumithion	98	33	19.8	13.2	6.6	-	-	-

Table 7. LD50, LD95, the linear relationship of dosage-mortality and the relative toxicity in 4 organophosphorus insecticides topically treated on 4th-instar larvae of the armyworm.

Insecticide	LD50 mg/g	LD95 mg/g	Regression equation	Toxic index(LD50)
Methyl parathion	1.02	8.61	Y = 5.1581 + 1.7779(x-1.0961)	100
Elsan	3.90	23.45	Y = 5.5427 + 2.0897(x-1.8391)	26
diazinon	37.25	109.70	Y = 5.1325 + 3.5395(x-1.6091)	3
Sumithion	46.17	87.50	Y = 4.2904 + 1.2906(x-2.1145)	2

Table 8. Mortality percentage of the armyworm larvae injected with 4 organophosphorus insecticides.

Insecticide	Dosage of active ingredient (mg)										
	8	7.5	5	4	2.5	2	1.25	1	0.625	0.5	0.3125
Methlyl parathion	-	-	-	-	80	-	60	-	46.6	-	26.6
Elsan	80	-	-	66.7	-	53.3	-	33.3	-	20	-
diazinon	-	86.6	53.3	-	26.6	-	-	13.3	-	-	-
Sumithion	80	-	-	53.3	-	40	-	20	-	-	-

Table 9. LD50, LD95, the linear relationship of dosage-mortality and the relative toxicity in 4 organophosphorus insecticides treated on 4th-instar larvae of the armyworm by micro-injection method, and the comparative toxicity with topical application at LD50 level.

Insecticide	LD50 mg/g	LD95 mg/g	Regression equation	Toxic index (LD50)	Topical LD50 injection LD50
Methyl-parathion	2.30	25.10	Y = 5.2876 + 1.4417(x-1.0243)	100	0.44
Elsan	6.70	91.80	Y = 5.2626 + 1.5692(x-1.5229)	34	0.58
diazinon	10.65	44.55	Y = 5.1895 + 1.7850(x-1.0516)	26	3.50
Sumithion	8.82	73.15	Y = 4.9518 + 2.6395(x-1.0094)	22	5.23

pressalis Walker with guthion which is still unknown. On the contrary, the toxicity of diazion and Sumithion remarkably increased to 3.5 times and 5 times respectively. By this finding, it is possibly to suppose that the penetratibility through the insect integument may play an important role in the mechanism of effectivity of insecticides. From this respect, it may conclude that Elsan and parathion could effectively penetrate the larval cuticle while diazinon and Sumithion are lacking this property.

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