Damage of Silkworms Caused by Pesticides and Preventive Measures

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Although pesticides contribute a great deal to control diseases, insect pests, and weeds in agricultural production including sericulture, the use of them in or around mulberry fields with carelessness causes contaminated mulberries. By feeding silkworms with such mulberry leaves, various damage such as poisoning to death of silkworms, deteriorated cocoon quality, reduced cocoon yield, and abnormal oviposition in silkworm egg production often occurs. The number of farm households which suffered the damage of silkworms by pesticides reached about 700, showing about 39 tons of estimated loss of cocoons in 1985, at least according to the official statistics.

Therefore it is very important to establish preventive measures to silkworm damage caused by pesticides. For that purpose, effects of many kinds of pesticides on silkworms were examined from various viewpoints. Based on the result obtained, how to prevent the damage by pesticides will be discussed.

Materials and methods

1) Toxicity of pesticides to silkworms

As the damage occurs by eating the mulberry leaves contaminated with a pesticide, by contacting with a pesticide, or by inhalation of a gasified pesticide, the following four methods were employed to examine pesticide toxicity: feeding, oral application, dermal application, and subcutaneous injection. In case of the feeding method, pesticides of liquid formulation were diluted stepwise, and spread on mulberry leaves at the rate of 100 *ml* per 1 kg of leaves. The treated leaves were supplied to silkworm larvae of the third instar, continuously for the whole period of that instar. From the result obtained, the relationship between the concentration of a pesticide and larval mortality was examined, and 50% and 5% lethal concentrations were calculated by the probit method of Finney[1]. Observations on toxic symptoms, cocoon quality, and oviposition were made according to the usual method[4-6,10], by continuous feeding at the fifth instar for the whole period of that instar. The oral application, dermal application, and subcutaneous injection were made on the second day of the fifth instar larvae, and relation of pesticide dosage to larval mortality was examined to estimate LD_{50} by the usual method[14].

2) Persistency of pesticides on mulberry leaves

As the silkworm damage is most frequently caused by eating the contaminated mulberry, pesticides were sprayed to the mulberry leaves in the field according to the standard way of spraying. The amount of pesticide deposits immediately after the spray, and its change with time were determined by chemical analysis[3] and bioassay[8]. The test of feeding silkworms was also carried out in parallel. Thus, the length of period required for the disappearance of toxicity on mulberry leaves was determined.

Result and discussion

1) Toxicity of pesticides to silkworms

The result of the experiment by the feeding method is shown in Table 1. Of the pesticides used, the most toxic one was cartap,

Table 1. Dosage probit-mortality regression equations, LC_{50}, toxicity index, LC_5 and permissible level of pesticides in continuous feedings of pesticide-sprayed mulberry leaves to the 3rd instar larvae of the silkworm

Chemicals	Regression equation (Y=5+a (x−b))	LC_{50}*	Toxic index to LC_{50}	LC_5*	Permissible level
		ppm		ppm	ppm
cartap	5+4.786 (x −1.480)	0.030	830.0	0.014	0.001
allyxycarb	5+7.117 (x −1.645)	0.044	565.9	0.026	0.003
endosulfan	5+5.937 (x −1.695)	0.050	498.0	0.026	0.003
trichlorfon	5+5.522 (x −2.232)	0.170	146.5	0.086	0.008
isoxathion	5+4.951 (x −3.315)	0.206	120.9	0.096	0.01
fenitrothion	5+6.672 (x −2.396)	0.249	100.0	0.141	0.01
EPN	5+6.717 (x −2.494)	0.312	79.8	0.177	0.02
phenthoate	5+6.837 (x −2.662)	0.459	54.2	0.264	0.02
diazinon	5+3.939 (x −2.684)	0.483	51.6	0.185	0.02
dichlorvos	5+6.338 (x −2.646)	0.510	48.8	0.281	0.03
MAFA	5+6.226 (x −2.737)	0.546	45.6	0.298	0.02
blasticidin-S	5+5.868 (x −2.748)	0.560	44.5	0.293	0.02
nicotine sulfate	5+4.677 (x −2.793)	0.620	40.2	0.276	0.02
diflubenzuron	5+5.429 (x −3.193)	1.559	16.0	0.776	0.07
MNFA	5+5.046 (x −3.214)	1.636	15.2	0.773	0.07
IBP	5+6.071 (x −3.241)	1.742	14.3	0.934	0.1
naled	5+5.869 (x −3.266)	1.844	13.5	0.967	0.1
lead arsenate	5+5.951 (x −3.366)	2.322	10.7	1.229	0.1
dinocap	5+5.845 (x −3.388)	2.446	10.2	1.28	0.1
salithion	5+5.547 (x −3.450)	2.819	8.8	1.43	0.1
propoxur	5+5.427 (x −4.619)	4.162	6.0	2.07	0.3
calcium arsenate	5+7.773 (x −3.663)	4.605	5.4	2.83	0.3
edifenphos	5+6.290 (x −3.837)	6.870	3.6	3.77	0.4
carbaryl	5+6.857 (x −3.873)	7.466	3.3	4.30	0.4
MPMC	5+7.144 (x −3.892)	7.795	3.2	4.59	0.4
vamidothion	5+5.432 (x −4.165)	14.64	1.7	7.28	0.6
mecarbam	5+6.211 (x −4.169)	14.77	1.7	8.04	0.8
ESP	5+6.409 (x −4.277)	18.93	1.3	10.50	1.0
zineb	5+7.425 (x −4.320)	20.88	1.2	12.54	1.0
copper sulfate	5+6.295 (x −4.320)	20.91	1.2	11.45	1.0
fenthion	5+3.426 (x −4.379)	23.91	1.0	7.91	0.5
malathion	5+6.684 (x −4.465)	29.19	0.9	16.57	1.5
pentachlorophenol	5+5.696 (x −4.495)	31.27	0.8	16.08	1.5
amobam	5+7.385 (x −4.588)	38.68	0.6	20.15	2.0
dimethoate	5+6.724 (x −4.626)	42.26	0.6	24.05	2.0
sulfur	5+5.400 (x −4.949)	88.84	0.3	44.08	4.0

* LC_{50}, LC_5: Active ingredient of pesticide per fresh leaf which causes 50% or 5% mortality of the 3rd instar larvae.

which showed 0.03 ppm of LC_{50}, 830 of a toxicity index (that of fenitrothion was taken as 100), and 0.001 ppm for a permissible residual level. The lowest toxicity was shown by sulfur with LC_{50}: 88.8 ppm, toxicity index: 0.3, and permissible residual level: 4 ppm. The toxicity to silkworms of all other pesticides was between cartap and sulfur, and they were classified into three groups with LC_{50} 0.03–1 ppm, 1–10 ppm, and 10–90 ppm, respectively.

As the body weight of silkworms differs with different instars, the susceptibility to pesticides varys with the instar. However, the lethal dose per unit body weight is not so different, and particularly in the case of the feeding method the amount of contaminated leaves eaten by silkworms is almost proportional to the body weight. Thus, the difference of instars does not cause substan-

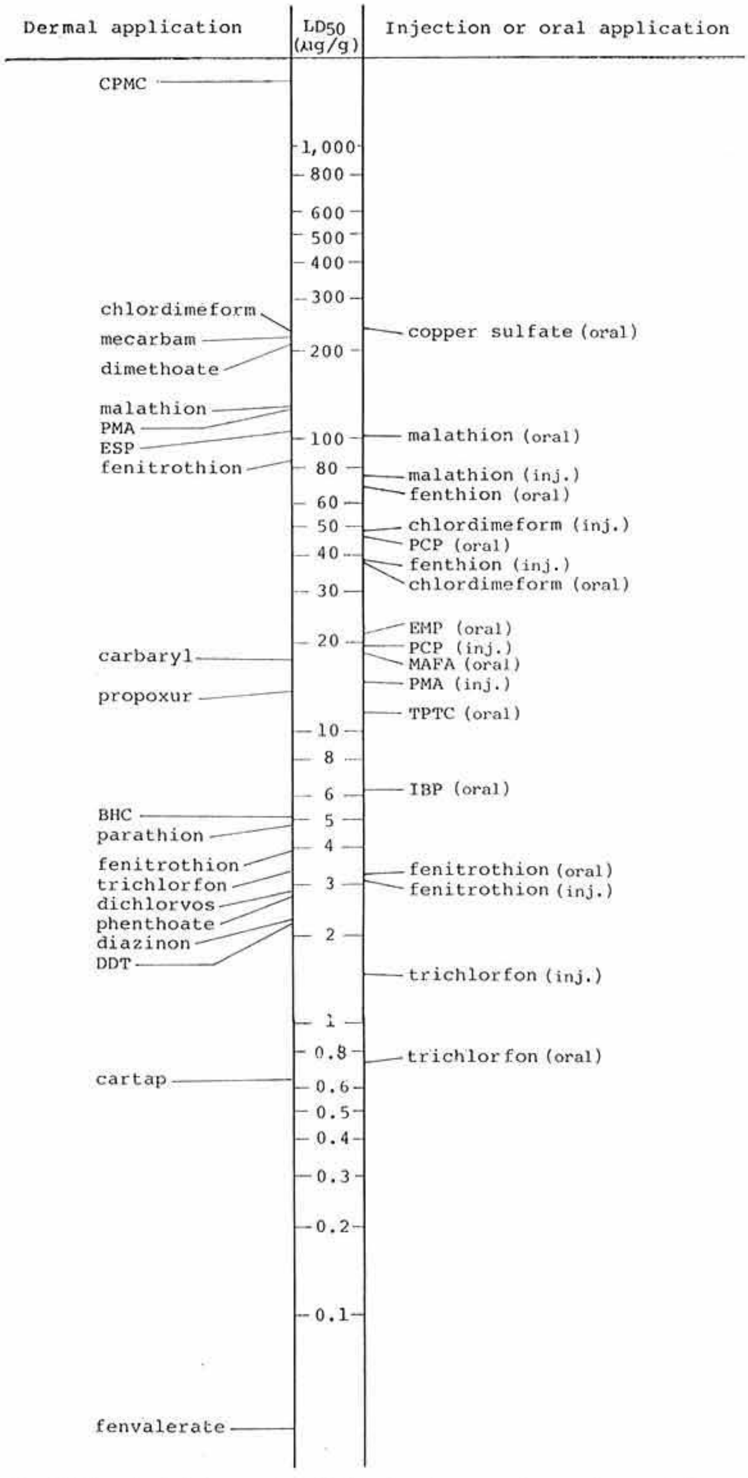


Fig. 1. Median lethal dose of pesticides to silkworm larvae (per g body weight, the 2nd day of the 5th instar)

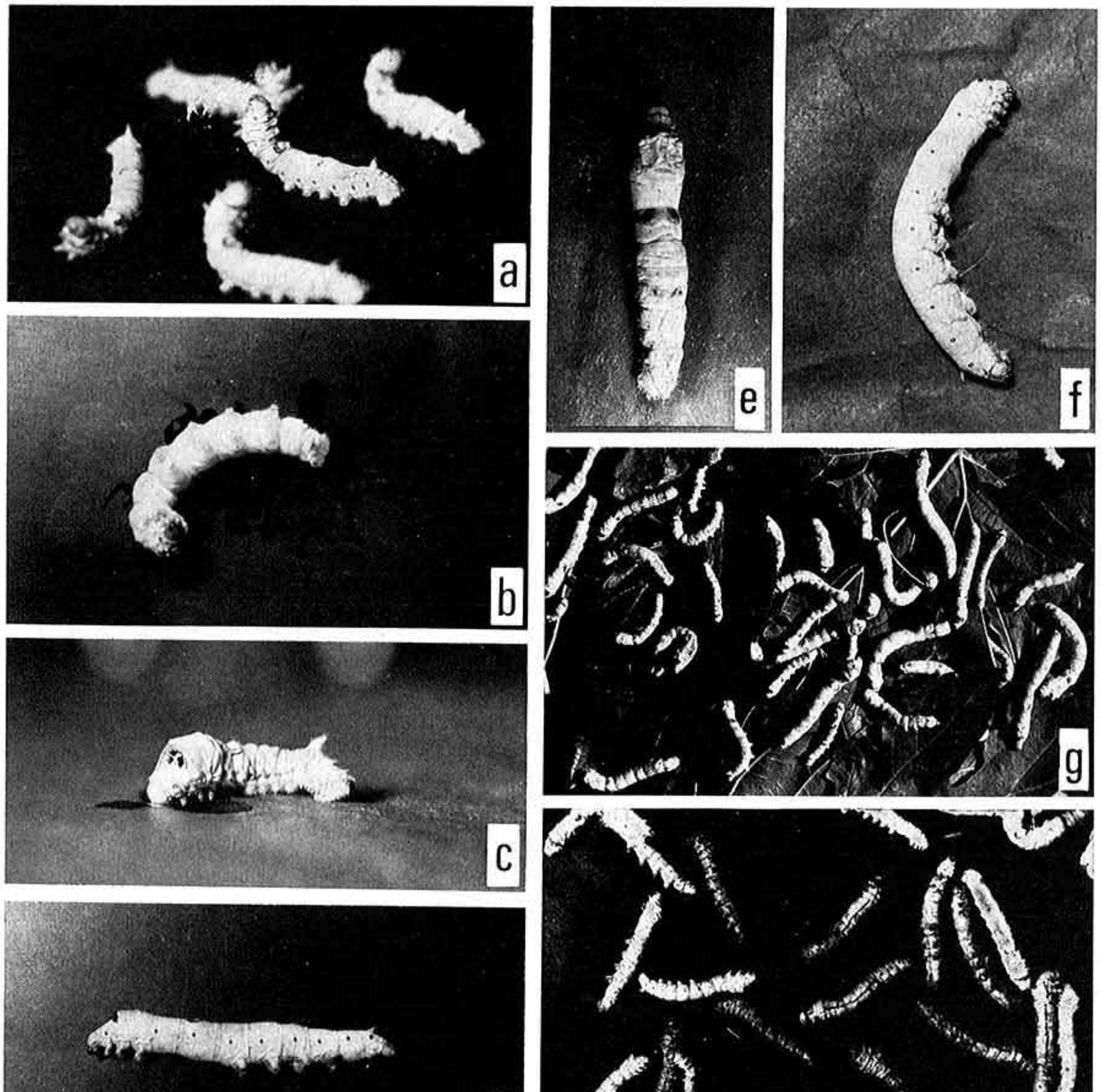


Plate 1. Symptoms of silkworm larvae poisoned by pesticides

a. Bending the front part of the body upward, and swinging furiously (fenitrothion poisoning).
b. Twisting the body in agony and vomitting (pyrethrins poisoning).
c. Body shrinks due to vomitting (trichlorfon poisoning).
d. No eating, inactiveness, slower growth, legs lose clasping power, softening of body and paralysis (cartap poisoning).
e. Atony. The larva looks inactive, doesn't feed or move about, stretches the body and is prostrate (copper sulfate poisoning).
f. Inactiveness, followed by inelasticity of the body, which is usually lying on its side (zineb poisoning).
g. Retarded and irregular growth at the molting stage (sulfur poisoning)
h. Non-exuviated (TPTC poisoning).

cial difference in poisoning. Therefore, the result given in Table 1 is applicable to other instars.

The result of the dermal application, subcutaneous injection, and oral application is given in Fig. 1. The median lethal dose (LD_{50} value) showed great difference depending on kinds of pesticides and methods of application. The smallest LD_{50}, i.e. the highest toxicity was shown by the dermal application of fenvalerate (0.02 μg per g body weight), while the lowest toxicity was shown by the dermal application of CPMC (LD_{50}=1.7 mg). The other pesticides ranged 1–250 μg of LD_{50}. As to the method of application, the subcutaneous injection showed the highest toxicity, followed by the oral application, and dermal application in that order. This result indicates that many pesticides show high toxicity by entering into the silkworm body through the skin, not only by entering orally.

The toxic symptoms are specific to the kind of many pesticides. This knowledge is helpful in identifying causal pesticides[7]. The general features of the toxic symptoms caused by insecticides (Plate 1, from a to c) are as follows: The front part of the body bends upward, and swings. The body twists in agony, followed by severe vomitting. The body shrinks due to vomitting and lays on its side, etc. Those symptoms can certainly be regarded as poisoning of insecticides. On the other hand, in case of fungicides or herbicides, apparent abnormal symptoms are not

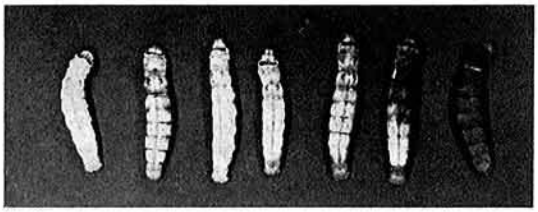


Plate 2. Non-cocooning larvae
Matured silkworm larvae which appear normal, but die without making cocoon after mounting (lead arsenate poisoning).

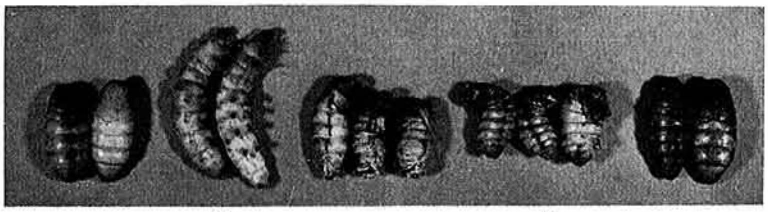
shown at the early stage of the poisoning, but gradually reduced feed intake and inactivity occur, followed by inelasticity of the body laying on its side or in prostrate form. These symptoms, shown in Plate 1, d–f, resemble those of silkworm diseases like flacherie in appearance. Larvae poisoned by these chemicals show delayed growth, non-uniform growth, and inability of ecdysis, resulting in frequent death (Plate 1, g and h).

Among the silkworms affected by pesticides, some ones grow almost normally during the larval period, but die in cocooning frames due to lack of cocooning ability (Plate 2), or some ones carry out cocooning, but die inside the cocoons (Plate 3). Furthermore, abnormal cocoons with abnormal cocoon shells (Plate 4), normal cocooning but giving poor silk reeling (Table 2), and abnormal eggs laid after almost normal eclosion (Plate 5) are also observed[2,10-13]. To prevent the damage

Table 2. Effects of pesticides on cocoon quality of silkworms

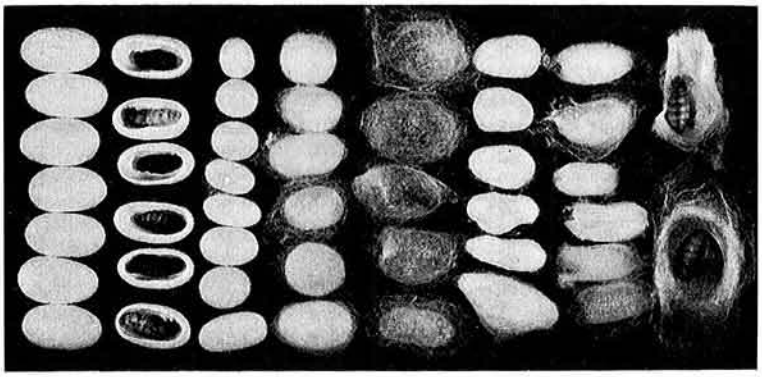
Chemicals	Dilution	Duration of 5th instar	Pupation rate	Cocoon weight	Cocoon shell weight	Weight of cocoon filament	Length of cocoon filament	Reelability	Size of cocoon filament	Raw silk percentage of cocoon
	×	day	%	g	cg	cg	m	%	d	%
malathion	500	7.8	86	1.62	37.3	32	1,060	79	2.76	19.3
fenthion	2,000	8.2	84	1.14	25.2	15	660	54	2.07	12.7
fenitrothion	40,000	8.7	61	1.17	25.7	20	810	82	2.30	17.6
EMP	32,000	8.7	64	1.60	33.7	24	781	52	2.61	15.1
control (water treatment)	—	7.8	97	1.72	42.5	35	1,134	90	2.78	19.9

Leaves sprayed with the diluted chemicals were prepared every day, and kept for one day before feeding; feeding of larvae was carried out with these leaves throughout the whole 5th instar.



a b c d e

Plate 3. Effects of pesticides on pupation of silkworms
a. Normal pupa b. Unpupated larva
c. Incompletely exuviated pupa d. Malformed pupa
e. Dead pupa



a b c d e f g h

Plate 4. Effects of pesticides on cocooning of silkworms
a. Normal cocoon b. Dead worm and inside-soiled cocoon
c. Light and small cocoon d. Loose-shelled cocoon
e. Thin-shelled cocoon f. Malformed cocoon
g. Thin-end cocoon h. Plate shape cocoon

a b c d e

Plate 5. Effects of pesticides on reproduction of silkworms
a. Normal eggs after hatching b. Non-fertilized eggs (metepa poisoning)
c. Death at an early stage (EMP poisoning) d. Death prior to hatching (fenthion poisoning) e. Death after hatching (EPN poisoning)

of silkworms including such adverse influence shown above, which is hardly recognized, it is necessary to set up the permissible residual levels of pesticides on mulberry leaves, and leaves contaminated beyond that levels should not be supplied to silkworms.

2) *Pesticide deposit and its persistence on mulberry leaves*

Pesticides sprayed to mulberry according to the customary practice gave the average of the deposit quantity shown in Table 3. Even when the same pesticide is used, its deposition is known to vary depending on differences in formulation, dilution rate, and the

Table 3. Log residue-time regression equations and no. of days to reach certain residual levels of pesticides on and in mulberry leaves

Chemicals	Formulation*	Active ingredient	Dilution	Initial deposit	Regression equation (log Y=a−bx)	No. of days to reach		
						1/2 level	1/10 level	Permissible level
		%	×	ppm		day	day	day
dichlorvos	emul.	50	1,000	57.5	2.055−1.135x	0.31	0.93	3
thiophanate-methyl	wett.	70	3,200	30.8	2.006−0.298x	1.03	3.38	5
menazone	〃	70	2,000	49.4	1.999−0.213x	1.41	4.69	5
vamidothion	emul.	40	1,000	46.0	2.001−0.304x	1.00	3.30	6
malathion	dust	1.5	—	72.2	2.008−0.276x	1.05	3.82	7
phenkapton	wett.	45	1,000	60.8	2.001−0.279x	1.08	3.59	7
ESP	emul.	45	1,000	54.9	2.003−0.235x	1.29	4.26	7
malathion	〃	50	1,000	55.5	2.000−0.217x	1.39	4.60	7
naled	〃	50	1,000	22.0	2.009−0.337x	0.92	3.00	8
MAFA	liq.	6.5	1,000	9.0	2.003−0.314x	0.97	3.20	8
mecarbam	emul.	25	1,000	28.8	2.001−0.187x	1.62	5.37	8
MPMC	wett.	50	1,000	67.5	2.001−0.233x	1.30	4.29	9
IBP	dust	1.5	—	91.4	1.981−0.305x	0.88	3.34	10
salithion	emul.	25	1,000	28.8	2.004−0.211x	1.45	4.77	10
IBP	〃	48	1,000	55.2	2.009−0.224x	1.38	4.50	12
edfenphos	〃	40	1,000	46.0	1.998−0.166x	1.80	6.00	12
diazinon	wett.	34	1,000	45.9	2.007−0.232x	1.33	4.35	14
zineb	〃	65	1,000	87.8	2.000−0.151x	1.99	6.62	14
dimethoate	emul.	43	1,000	49.5	1.997−0.095x	3.14	10.50	14
diazinon	dust	3.0	—	164.9	1.995−0.275x	0.87	3.18	15
fenitrothion	〃	2.0	—	91.4	1.988−0.280x	0.95	3.80	15
phenthoate	〃	2.0	—	110.3	2.001−0.256x	1.10	3.95	15
calcium arsenate	wett.	40	300	173.3	2.003−0.177x	1.72	5.68	15
fenthion	dust	3.0	—	172.8	1.991−0.146x	1.85	6.30	16
trichlorfon	emul.	50	1,000	61.0	2.010−0.254x	1.23	3.98	17
fenitrothion	〃	50	1,000	54.0	2.000−0.218x	1.38	4.59	17
phenthoate	〃	50	1,000	57.5	2.001−0.200x	1.51	5.00	17
carbaryl	dust	1.5	—	125.0	2.004−0.176x	1.52	7.62	18
fenthion	emul.	50	1,000	66.0	2.000−0.112x	2.69	8.95	18
endosulfan	〃	35	500	73.5	2.007−0.219x	1.41	4.60	20
carbaryl	wett.	50	500	77.8	2.002−0.105x	2.89	9.55	20
propoxur	〃	50	1,000	70.5	2.001−0.106x	2.85	9.43	21
allyxycarb	〃	50	1,000	67.5	2.004−0.211x	1.44	4.75	24
lead arsenate	〃	32	500	92.8	2.003−0.112x	2.71	8.94	25
EPN	dust	1.5	—	71.4	1.991−0.134x	2.22	7.24	26
EPN	emul.	45	1,000	51.8	2.002−0.112x	2.71	8.95	30
nicotine sulfate	liq.	40	800	65.2	1.997−0.062x	4.85	16.20	60
cartap	wat.	50	1,000	67.5	2.000−0.052x	5.82	19.31	110

* emul.: emulsifiable concentrate, wett.: wettable powder, liq.: liquid formulation, wat.: water soluble concentrare.

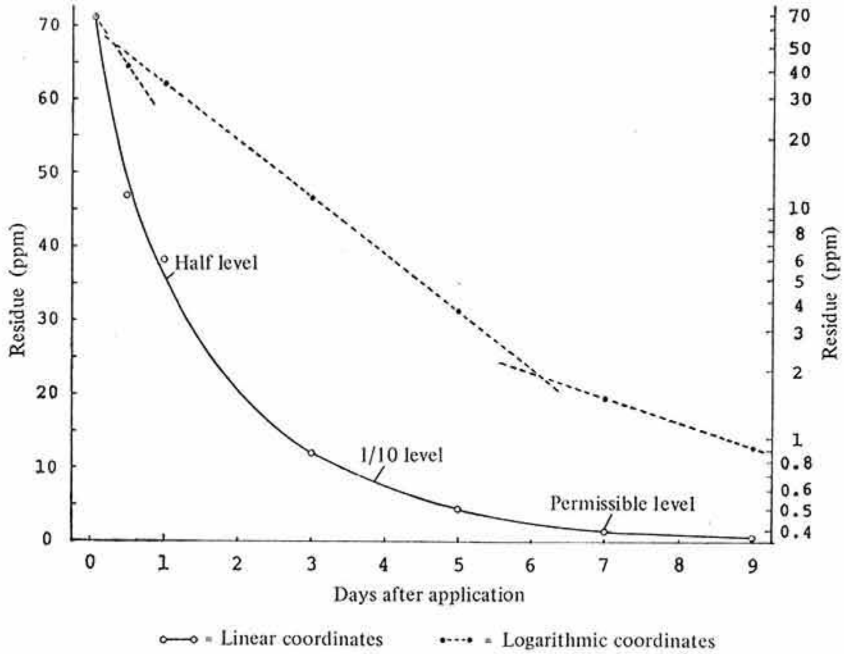


Fig. 2. Residual behavior of malathion on and in mulberry leaves

quantity or method of spraying[9]. In the present experiment, the deposition was 30–90 ppm in case of wettable power or emulsifiable concentrate and 70–170 ppm in case of dust.

Degradation of pesticides deposited on mulberry leaves proceeded fast immediately after the deposition, but it gradually slowed down with time, showing a hyperbolic change (a solid line in Fig. 2), although the degradation velocity differed greatly with different pesticides. The log value of residual quantity of pesticides plotted against time showed a steep declining line immediately after the spray, followed by a moderately sloping line, and lastly a much less sloping line, such as broken lines in Fig. 2, in many cases. This result indicates that the degradation proceeds approximately in proportion to the existing quantity of residual pesticides. At the initial phase of degradation the pesticide on the leaf surface is directly exposed to the degrading effect of climatic factors. At the next phase, the pesticide gradually enters into leaf tissues, so that it evades the effect of climatic factors, but it is subjected to the effect of plant factors such as enzymatic actions. This explains the moderate rate of degradation in the second phase. At the final phase, the pesticide residue remaining in a portion where plant factors or climatic factors hardly reach, continues to degrade only naturally, showing the slow degradation at that phase.

Using the data of degradation vs. time, the following regression equation was obtained for each of pesticides tested: $\log y = a + bx$, where y = the amount of residual pesticides, and x = no. of days after the spray. The regression equations, and no. of days required for each pesticide to decrease to 1/2 and 1/10 of the initial quantity on mulberry leaves as well as to the permissible level of the residue are shown in Table 3. Mulberry leaves with this permissible level of pesticide residue are used to feed silkworms without adverse effects. The no. of days to reach that level ranged from 3 to 110 days. The pesticides tested are classified into four groups: showing less than 7 days, 8–14 days, 15–30 days, and more than 31 days, respectively, to reach the permissible level. It is relatively easy to use the pesticide of the first group by avoiding the silkworm-rearing season. The second group is within the range of possibility of the use in silkworm-rearing areas by adjusting the time of its spray. The use of the 4th group in silkworm-rearing areas must be restricted, because the use by avoiding the silkworm-rearing season is fairly difficult, and furthermore, once contamination of mulberry leaves occurs, the leaves can not be used for a long time. Pesticides showing more than 60 days to reach the permissible level should not be used in silkworm-rearing areas.

As shown above, pesticides, presently in use, exhibit fairly high oral and dermal toxicity to silkworms. Their toxicity lasts for a long period, such as 30–110 days, when they are deposited on mulberry leaves. To prevent the silkworm damage caused by pesticide-contamianted mulberry the following caution is needed.

(1) Before the use of pesticides in mulberry fields or neighboring areas, a pesticide with the lowest toxicity to silkworms is selected by consulting Table 1 and Fig. 1 from the pesticides known to be effective to the target organism.

(2) In the neighboring areas, drifting of pesticides toward the mulberry field should be avoided.

(3) It is most important that, in areas where the possibility of pesticidal contamination of mulberry exists, the spray should be done by taking into account the length of period required for the disappearance of insecticidal toxicity to the silkworm, which must be finished before the anticipated time of harvesting mulberry.

(4) When mulberry is contaminated with pesticides, its leaves are not used for feeding, until the disappearance of toxicity is confirmed.

When pesticide-poisoning has occurred, the affected silkworms should not be discarded in a hurry, but try to promote their early recovery by supplying clean and good quality mulberry leaves and circulating clean air stream in the rearing-room, because the possibility of recovering from very severe poisoning exists in some cases.

Conclusion

The pesticidal poisoning of silkworms causes various types of damage to silkworms, depending on kinds and quantity of pesticides. The severe case is fatal. Even in the mild case, the deterioration of important useful characteristics occurs, such as delayed and uneven growth, weak body, light and small, or uneven cocoons, shortened cocoon filament and uneven thickness of the filament, poor reelability of cocoons, reduced number of eggs, production of abnormal eggs, etc. All of them result in economic loss.

The most frequent case of the silkworm damage by pesticides occurs by feeding silkworms with pesticide-contaminated mulberry leaves. The toxicity of the pesticides deposited on mulberry leaves decreases with time and finally disappears, although the toxicity is very high immediately after the deposition. Therefore, to increase pesticidal effectiveness without causing damage to silkworms, an appropriate pesticide for the purpose of pesticidal control should be used by a proper method at the time when no risk of causing residual toxicity to silkworms is anticipated.

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