## Control of Tea Pests with Bacillus thuringiensis

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Chemical pesticides have played an important role in pest control, but too much dependence on them has caused serious problems such as the emergence of pesticide resistance, changes in fauna and flora, and environmental pollution. To solve these problems, the integrated pest control is being under study. The microbial control is one of its measures.

The bacillus pesticide (BT pesticide), making use of a toxin produced by *Bacillus thuringiensis* as an active ingredient, is a so-called microbial insecticide, and it has a long history of study in Europe and U.S.A.<sup>2)</sup> In Japan, although some basic researches have been carried out, the study on the practical application of microbial insecticide has been insufficient because there exist problems to be considered in relation to sericulture. However, since about 1970, studies on the utilization of BT pesticide to each kind of crops were initiated.

Pest control is essential to stabilize tea production, because there are many kinds of pests with great population that cause serious damage to tea plants. Being a national drink, tea is consumed daily, so that consumers have a strong concern about its dietary safetyness. Therefore, to reduce the use of chemical pesticides as far as possible, the author examined the utilization of BT pesticide as one of the measures of integrated pest control.

B. thuringiensis produces a toxin, which shows its toxicity in the body of larvae of lepidopterous insects and kills them. The killing effect differs with different strains of the bacillus<sup>1,5)</sup> and with different kinds of insect<sup>6)</sup>. Therefore, various BT pesticides (with different strains but it was not clear which strains

Table 1. Cumulative per cent mortality of 1st instar larvae of the tea tortrix exposed continuously to tea leaves treated with BT products

Name of	Dilution	mo	mortality		ve per cent at indicated		
products	(times)	2	lays of	treatment 6 8			
		%	%	%	%		
SB-471	1000	85.0	95.0	95.0	95. (		
	4000	65.0	75.0	80.0	80. 0		
	16000	30.0	50.0	60.0	60.0		
SBI-0721	1000	65.0	80.0	90.0	90. 0		
	4000	60.0	75.0	80.0	85. (		
	16000	45.0	55.0	65.0	70.0		
SBI-0722	1000	35.0	75.0	90.0	90.0		
	4000	35.0	70.0	75.0	80.0		
	16000	5.0	20.0	25.0	30.0		
Thuricide-A	1000	60.0	65.0	75.0	90.0		
	4000	60.0	65.0	75.0	90. (		
	16000	30.0	50.0	55.0	55. (		
Thuricide-B	250	60.0	75.0	80.0	80.0		
	1000	20.0	55.0	55.0	55.0		
	4000	25.0	30.0	30, 0	30. 0		
	16000	5.0	5.0	5.0	5. (		
Arrow BT 601	1000	65.0	100.0	100.0	100.0		
	2000	60.0	85.0	85.0	85. (		
	4000	30.0	65.0	70.0	75.0		
	16000	10.0	35.0	45.0	45. (		
Arrow BT 101	1000	5.0	75.0	80.0	85. (		
	4000	15.0	35.0	40.0	45. (		
	16000	10,0	20.0	25.0	25.0		
CI-712M-W	250	60.0	80.0	80.0	85. 0		
	1000	20.0	35.0	40.0	45.0		
	4000	5.0	10.0	10.0	10.0		
	16000	0.0	0.0	5.0	5. (		
Biotrol	1000	25.0	85.0	90.0	90. (		
	4000	20.0	55, 0	60.0	60. (		
	16000	5.0	25, 0	40.0	40. 0		
NNI-714	250	30.0	30.0	30.0	30.0		
	1000	40.0	40.0	40.0	40.0		
	4000	30.0	40.0	40.0	45.0		
	16000	40.0	45.0	45.0	45.0		

were used) were tested with major lepidopterous insect pests of tea, tea tortrix (*Homona* magnanima Diakonoff), smaller tea tortorix (*Adoxophyes* sp) and tea leaf miner (*Calop*tilia theivora Walshingham).

#### Effect on tea tortrix

Larvae hatched from egg masses, sampled in fields, were used. Tea leaves were immersed in pesticide solutions of given concentrations and dried. The leaves were inoculated with the larvae immediately after hatching, and placed in test tubes, which were kept in a constant temperature room at 25°C. Mortality of the larvae was examined (Table 1). Remarkable difference in the effectiveness of insecticides was observed: SB-471, Arrow BT-101, SBI-0721 etc. were highly effective, while NNI-714, Thuricide-B, CI-712M-W etc. were less effective. The effectiveness was expressed slowly with the elapse of days, but its difference was observed clearly at 4 days after the treatment.

Intermediate instar larvae were tested with SB-471 by the same method as above. They showed a lowered sensitivity as compared to that of 1st instar larvae (Table 2).

Based on above results, a field experiment was conducted with SB-471 at 500 times dilution. The result showed that SB-471 was highly effective as compared with methomyl pesticide, indicating a practical applicability<sup>9)</sup>.

Table 2. Cumulative per cent mortality of 3rd instar larvae of the tea tortrix exposed continuously to tea leaves treated with BT product, SB-471

Name of product	Dilution (times)		morta	lity at	per cer indicat	ed
• 11 m 1 m 2 m 2 m 2 m 2 m 2 m 2 m 2 m 2		0	1	2	4	7
SB-471	62. 5	0.0	% 55, 0	80. 0	100.0	100.0
	250	5. 0	5.0	25, 0	50, 0	85. 0
	1000	0.0	10.0	15.0	30.0	70.0
	4000	5.0	5. 0	5.0	20.0	65.0

The use at 500 times dilution is expensive, but some results showed an effectiveness at 1,000 times dilution, suggesting an economic feasibility.

#### Effect on smaller tea tortrix

Larvae used were taken from the stock culture fed with artificial diet. Methods of treatment were same as in the case of tea tortorix.

The 1st instar larvae of smaller tea tortrix were found highly sensitive as compared to tea tortrix, being killed at fairly low concentrations. Effectiveness varied with kinds of pesticides: SB-471, Thuricide-A, Arrow BT-601 were highly effective while Biotrol, SBI-0721 etc. were less effective. This trend is almost the same as observed with tea tortorix (Table 3). As seen from the result,

Table 3. Per cent mortality of 1st instar larvae of the smaller tea tortrix at 4 days after treatment

% mortality at indicated dilutions

v v v	2007	600 X	1200 X	2400 X	4800 X	9600 X	19200 X	38400 X	76800 X
Name of products	300 X	000 X	1200 A	Z400 X	4000 A	3000 A	13200 A	00400A	100007
	%	%	%	%	%	%	%	%	90
SBI-0721	100.0	100.0	100.0	87.5	62. 5	15.0	62. 5	25. 0	25.
SBI-0722	90.0	85. 0	85.0	90.0	60. 0		-	-	-
Arrow BT 101	95.0	95. 0	73. 7	57.9	68. 4	1	-		-
Arrow BT 601	100.0	100.0	94. 7	73.7	94.7	==	_	7-2	-
Thuricide-A	100.0	100.0	100.0	100.0	85.7	100.0	78, 5	50. 0	14.
CI-712M-W	90.0	90.0	90.0	95.0	80, 0	- Table	(7,000)	===	
Biotrol	_	-	-	42.9	57. 1	35. 7	21.4	50. 0	42.
SB-471	100.0	100.0	100.0	100.0	100.0	100.0	87. 5	62.5	37.

Table 4. Cumulative per cent mortality of 3rd instar larvae of the smaller tea tortrix exposed continuously to tea leaves treated with BT products

Name of products	Dilution (times)	mo	rtality	ve per cent at indicated treatment		
products	(chines)	4 7		10	13	
		%	%	%	%	
Thuricide-A	500	50.0	70.0	85.0	90. (	
	1000	15.0	50.0	80.0	90. (	
	2000	5. 0	15.0	45.0	60.	
	4000	20.0	45.0	45.0	55. (	
	8000	10.0	15. 0	20.0	40. 0	
Thuricide-B	250	0.0	10.0	45.0	75. (	
	500	0.0	15.0	50.0	55.	
	1000	0.0	5. 0	10.0	15.	
SBI-0722	500	20.0	45. 0	65. 0	90.	
The state of the s	1000	0.0	0.0	30. 0	65.	
	2000	5. 0	15. 0	25. 0	65.	
	4000	10.0	10.0	50.0	75.	
	8000	15. 0	25.0	25.0	35.	
SBI-0721	300	45. 0	90.0	95.0	100.	
	600	25. 0	70.0	75. 0	95.	
	1200	25. 0	55.0	60.0	95.	
	2400	20.0	45.0	50.0	70.	
SB-471	300	75. 0	100.0	100.0	100.	
	600	70.0	100.0	100.0	100.	
	1200	40.0	70.0	85.0	100.	
	2400	35.0	65.0	75.0	95.	
Arrow BT 101	62. 5	25. 0	64.0	88.0	-	
	250	16. 0	72. 0	92.0		
	500	5. 0	5. 0	70.0	80.	
	1000	8. 0	68.0	88.0	<del></del> ).	
	4000	12.0	60.0	80.0	=	
Arrow BT 601	62. 5	80.0	100.0	100, 0	100.	
	250	48.0	92. 0	92.0	\$100 to	
	500	0.0	40.0	55. 0	100.	
	1000	28.0	72.0	96.0		
	4000	32.0	72.0	84.0	-	

the effect on the 1st instar larvae was unstable, because the larvae were small in size and hence some of them were killed during a procedure of inoculation. However, if they are handled carefully, they may possibly be used as the material for testing pesticide activity, due to their high sensitivity<sup>3,4</sup>).

To test the effect on intermediate instar larvae, the 3rd instar larvae at 10 days after hatching were treated by the same method used for tea tortorix. The sensitivity was found remarkably lowered at the 3rd instar.

Difference in effectiveness of pesticides, however, was similar to the case of 1st instar (Table 4). Such a marked decrease in sensitivity at the intermediate instar seems to cause a problem in practices, but there is a report<sup>7)</sup> that the effectiveness of pesticides is similar to that of chemical pesticides, when viewed from the rate of dilution alone.

Through both experiments with the 1st instar and intermediate instar larvae, it was observed that survived larvae of treated plots were very poor in growth as compared to the larvae of untreated plots. To know whether the growth retardation was caused by toxicity or malnutrition due to diet-repelling, the following experiment was carried out. The 3rd instar larvae were fed on leaves treated with pesticides or starved for different periods from 1 to 5 days; and growth and mortality were examined. In each plots, after the given days of treatment the larvae were transferred to untreated leaves.

Mortality was increased with the increase of treatment period<sup>4)</sup>, but the mortality after the transfer to untreated leaves was lower after the longer period of treatment (Fig. 1). Number of faecal pellets per individual larva during the treatment period showed no change with the duration of the treatment, indicating that the larvae did not feed the treated leaves. The number of faecal pellets returned to normal at 3-4 days after the transfer to un-

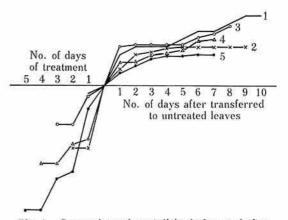


Fig. 1. Comparison of mortalities before and after transferred to untreated leaves from treated leaves.

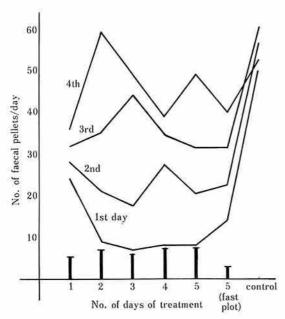


Fig. 2. Recovery of faecal pellets number after transferred to untreated leaves from treated leaves.

Bar graphs indicate average no of

Bar graphs indicate average no. of faecal pellets per survived larva during indicated days of treatment

treated leaves. Although the longer the treatment period the more time was required to return to normal, the same was observed with the starvation treatment (Fig. 2). Larvae fed on treated leaves appear not showing toxicity symptoms. Only the number of days to pupation was increased with the increase of treatment period, but no difference was observed in pupa weight between treated and untreated plots. These results suggest that the mortality of smaller tea tortorix caused by BT pesticides is largely due to starvation and malnutrition induced by the diet-repelling, in addition to the death by pesticidal toxicity.

A field experiment was carried out to test the practical applicability to smaller tea tortrix. To know the right time of application, a BT-pesticide was sprayed at 4 times from 5 days to 20 days after the peak of moth emergence (Table 5). Effect was high at 15 days, i.e., slightly later than the proper time of application of Cidial, a control pesticide. The effect was 3 times > 2 times > single ap-

Table 5. Field evaluations of the effectiveness of BT product SBI-0721 to control smaller tea tortrix

No. of sprays applied	Spra	ying	dat	e	Dilution (times)	% control
1	Aug.	15			500	56.5%
		21			500	87.0
		21			1000	83.0
		25			500	93.0
		30			500	90.0
2	Aug.	15,	21		500	94.5
		21,	25		500	90.5
		25,	30		500	89.5
3	Aug.	15,	21,	25	500	98.0
		21,	25,	30	500	96.0
1 (Cidial)*	Aug.	21			1000	94.5

Note: Quantity of solution: 400 litres/10 a.

\* Organophosphate insecticide.

plication, but the difference was only little. If properly timed, even a single application gave a similar effectiveness as the chemical pesticide. As BT-pesticides are stomach poison, and are effective only when they are given to plant parts where insect pests live and feed, it is considered that the quantity applied exerts a great influence on the effectiveness. However, spray of 400 1/10a was found to be enough to give sufficient effect.

#### Effect on tea leaf miner

Effect on tea leaf miner was examined in the laboratory using mature larvae. Methods were same as used for tea tortrix. As given in Table 6, some larvae survived. But by taking into account that no feed is taken up immediately before pupation it can be considered that all pesticides used have a killing effect.

Based on the result of laboratory experiment, a field experiment was conducted. Observations at 8 days after the spray showed that some pesticides were effective but the effect was far lower than that of the control pesticide. At 12 days after the spray, only 2 times application of Thuricide-A and SB-471 showed a little effect while other pesticides

Table 6. Effectiveness of BT products to control intermadiate instar larvae of the tea leaf miner

Name of	Cur	nulative ndicate	per ce	nt morta of treatn	lity at nent
products	1	2	3	4	5
Thuricide-A	10.0	% 75. 0	% 80. 0	90. 0	100.0
The second secon	700000	100000000000000000000000000000000000000		(36)35/40/36/11	O-60-60-60-60-60
Arrow BT 101	25.0	60.0	85.0	100.0	100.0
Arrow BT 601	20.0	80.0	90.0	100.0	100.0
CI-712 M-W	20.0	50.0	70.0	85.0	85.0
SBI-0721	35.0	75.0	95.0	95.0	95.0
SBI-0722	20.0	50.0	60.0	80.0	90.0
SB-471	25.0	75, 0	80.0	100.0	100.0

Note: Dilution; 500X

Table 7. Effectiveness of BT products in controlling tea leaf miner in field

Name of products	Dilution	% control at indicate days after treatment			
	(times)	8	12		
Thuricide-A	500	% 41.7	% 0		
Thuricide-A	1000	13.7	0		
Thuricide-A*1	500	83.5	76. 1		
SBI-0721	500	57.6	0		
SBI-0722	500	35, 3	16.2		
SB-471	250	73.4	67.8		
CI-712M-W	500	61.9	0		
Arrow BT 101	500	27. 3	0		
Arrow BT 601	500	35, 3	0		
Vinyphate*2	1000	98.6	99.8		

<sup>\*1: 2</sup> times application.

were completely uneffective (Table 7).

Tea leaf miner is a leaf miner until intermediate instar stage, but at the old instar stage it comes out of the mine to move to a new leaf and roll it up. If there is enough amount of pesticide sticked on new leaves, it may be effective to control the insect, but enough covering of pesticide is not easy with successively developing new leaves. There are many other experimental results showing unstable effect, suggestive of the difficulty in controlling tea leaf miner by BT pesticides. For the practical use of BT pesticides to tea leaf miner, further studies to increase an

effectiveness by the combination of low concentration of chemical pesticides will be needed.

# Effect on Hymenopterous parasite

It is regarded as an advantage of BT pesticide that it is non-toxic to human being and animals as well as natural enemies other than lepidopterous insects. According to the survey in tea gardens, it was made clear that the spray of BT pesticides reduced the activity of Hymenopterous parasite, but its effect was less than that of chemical pesticides8). As a laboratory experiment in which the parasite was kept in a vessel coated with BT pesticide did not show the killing effect of the pesticide, the inhibition of parasite activity observed in fields may presumably be caused by a repelling effect of the pesticide. It is not known whether the substance derived from the bacillus or additives contained in the pesticide formulation is an active substance for the repelling effect.

#### Conclusive remarks

BT pesticides are effective to control not only tea tortrix, smaller tea tortrix and tea leaf miner, but also tea geometrid and tea tussock moth. Thus, all of the lepidopterous insect pests can be controlled by the BT pesticides. However, there are many insect pests other than lepidopterous insects: Kanzawa spidermite, yellow tea thrips, tea green leafhopper, black citrus aphid etc in tea gardens.

Usually they are controlled by chemical pesticides together with lepidopterous insects. Therefore, pesticides effective to only lepidopterous insects are not being used. Such a situation makes it very difficult to use BT pesticides right now, because simultaneous application of chemical pesticides is still required, and which reduces merit of the use of BT pesticides and increases expense for

<sup>\*2:</sup> Organophosphate insecticide.

pest control. For the time being, combined use of pyrethrins and that of chemical pesticides at low concentrations have to be studies to make use of BT pesticides even a little, and in future the full use of characteristics of BT pesticides in an integrated pest control has to be studied.

#### References

- Ebihara, T. & Tomita, T.: Comparative tests on toxicity (Part 1). Results of experiments on BT pesticides 1. 5-9 (1972) [In Japanese].
- Heimpel, A. M.: A critical review of Bacillus thuringiensis var. thuringiensis Berliner and other crystalliferous bacteria. Ann. Rev. Entomol., 12, 287-322 (1967).
- Kariya, A.: Studies on BT pesticides. Results of experiments on BT pesticides 1.

- 59-63 (1972) [In Japanese].
- Kariya, A.: Studies on BT pesticides. Results of experiments on BT pesticides 2, 99-104 (1973) [In Japanese].
- Kusuno, M. & Morita, Y.: Comparative tests on toxicity of BT pesticides on slikworm. Results of experiments on BT pesticides 2, 45-47 (1973) [In Japanese].
- Kusuno, M. & Morita, Y.: Studies on BT pesticides. Results of experiments on BT pesticides 3, 27-40 (1975) [In Japanese].
- Oho, N. et al.: Studies on BT pesticides. Results of experiments on BT pesticides, 3, 69-71 (1974) [In Japanese].
- Takagi, K.: Monitoring of Hymenopterous parasite in tea field. Bull. National Res. Inst. Tea, 10, 91-131 (1974) [In Japanese with English summary].
- Takagi, K. & Kariya, A.: Effect of BT pesticides on Hymenopterous parasite of tea insect pests. Results of experiments on BT pesticides, 4, 35-42 (1975) [In Japanese].