

The Toxin Components Study on Generic Products of Abamectin¹

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Abstract: Abamectin is the first microbial insecticide extracted from *Streptomyces avermitilis* and has been registered for diamondback moth control. Not long after abamectin was registered, the generic products of abamectin started to market, and the low-cost, un-refined generic products soon out-competed the registered abamectin by a volume of more than 9 to 1. This forced abamectin being withdrawn from its registration in 2001. Currently, the generic products have occupied the whole market without detailed chemical composition and toxicological information. In this study, we had collected 23 samples of generic products for components/composition analysis and compared those data with the standard abamectin on five aspects: (1) total avermectin content, (2) the B1 composition, (3) the ratios of B1a/B1b, (4) contents of avermectins, and (5) the avermectin composition spectrum. The results indicated the total avermectins of generic products is 2 to 6 times of the registered abamectin, but the generic products contained only two-thirds of registered B1. More than 81% of total avermectins in generic products are unregistered components i.e., A1a, A1b, A2a and B2a. The toxic components of generic products varied greatly from sample to sample. Only the averaged ratio of B1a/B1b is the same as abamectin, however, this ratio was also sample-dependent, which varied from 90:10 to 100:0. The biggest problem of generic products is the amount of their toxic impurity content is about 5.37%, that is 134 times higher than the legal limit. The result confirmed that the generic products are crude, low-cost and unrefined pesticides, which lack toxicological information and post possible health hazard of unregistered toxic residues on vegetables. The generic products of abamectin induced high resistance in the diamondback moth within five years and the resistance ratio had reached 2500- to 5000-fold in 2001. These generic products also cause significant problem for pesticide registration, regulation and application, which should be reevaluated and corrected.

Key words : Abamectin, Generic product, Impurities, Analysis, Pesticide regulation.

INTRODUCTION

Pesticides registration includes two parts: first, the toxicological and residue review; and the second, the

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field efficacy test for target pest. Once the pesticide was registered, its importation, manufacture and marketing are regulated by the Pesticide Regulation Acts.

Abamectin is the first commercialized insecticide with toxins extracted from *Streptomyces avermitilis*⁽³⁾. The producer of abamectin is very concerned about this biological-originated insecticide, hence published a monograph entitled "Ivermectin and Abamectin"⁽⁴⁾. The contents of 21 chapters include the biochemistry of abamectin, the producing organism, the mode of action, the toxicology and metabolism, etc.

The registration of abamectin in Taiwan had been processed seriously to meet the safety requirement even there's little information on those microbial toxins before. The registration had taken more than five years in order to fulfill the questions about antidote, environment impacts, degradation, mammalian toxicity, etc. The effectiveness of abamectin on the diamondback moth (*Plutella xylostella* L., DBM) is well known⁽⁴⁾, hence DBM is the first target pest for registration in Taiwan. Because DBM is also famous for its insecticide resistance problem, the manufacturer of abamectin had established a self-discipline marketing policy after its registration, and tried to slow down or minimize the resistance development.

Taiwan Agricultural Research Institute(TARI) started the resistance monitoring when abamectin became available in the market, however, there're two uncontrollable situations developed in the same time. Firstly, abamectin was smuggled by other pesticide retailers into Taiwan. Although it is illegal, these products were still the original abamectin except the marketing of abamectin became uncontrollable. The second interference was the generic abamectin also was smuggled into Taiwan about one year after the registration. These generic products were crude and un-refined, and their active ingredients and impurities are unknown.

In order to understand the toxic contents of these generic products of abamectin, TARI had collected samples island-wide for the composition analysis and hoped the result would reveal the fact of these products to help pesticide regulation as well as to estimate the effect of generic products on resistance.

MATERIALS AND METHODS

Sources of the generic products

Total of 37 generic products was collected from major vegetable production districts from April to May of 1998 through the help of farmers (Figure 1).



Fig. 1 Packing of the generic abamectin collected in Taiwan in 1998.

Analytic methods used

Through the assist of Novartis and working in conjunction with an UK consultancy, Renco Ltd., 23 samples were randomly selected from 37 collected products and subjected to the detailed composition analysis. The chemical composition was determined by UV diode array spectra, HPLC and LC/MS, respectively. The analytical method used by Renco Ltd. was summarized in below. Different avermectins found in a very high concentration have been confirmed by the LC/MS data.

The chromatographic analysis was performed on the Merck Hitachi 7200 HPLC equipped with Merck Hitachi 7400 detector and the wavelength was set at 254 nm. Nucleosil C18 column of 250 mm length and 4.0 mm i.d. was used and the column temperature was set at room temperature. Ten ml of reference/test solutions were used for analysis at the flow rate of 1.0 ml/minute. Duration of chromatographic analysis is approximately 60 minutes (Table 1).

Table 1. Chromatographic program used for composition analysis

Time (min)	Acetonitrile (%)	Methanol (%)	Water (%)
0	60	-	40
40	60	30	10
50	90	-	10
51	60	-	40
60	60	-	40

Sample preparation

Reference solution: Weigh (to the nearest of 0.1 mg) 10 mg of abamectin reference substance into a 10 ml volumetric flask. Pipette 2 ml into a 100 ml volumetric flask and make up to volume with methanol.

Test solution: Weigh (to the nearest of 0.1mg) 2000 mg of technical abamectin or generic product formulation into a 20 ml volumetric flask. Make up to volume with methanol. In case of the out of specification content of the by-products, the amount of weighing has to be varied.

RESULTS AND DISCUSSION

Ever since the generic products of abamectin were illegally introduced into Taiwan, the market of abamectin has become uncontrollable. These un-refined, low-cost generic products hold bigger advantages in both the higher profit margin and the lower price of 3.9 vs. 8.8 NT dollars per ml compared to abamectin (Table 2), and outsell the registered abamectin by the volume of more than 9 to 1. Vegetable farmers are using a lot of these generic abamectin.

In an effort to understand the generic products of abamectin better, we had collected samples from vegetable production areas (Figure 2) for detailed analysis. All the collected samples were packed in different bottles with labeling (Figure 1), and none of them had specified the true content of the product. However, these generic products of abamectin can be easily identified, as their common appearances are viscous liquid with colors ranged from dark-brown to black (Figure 3) and bearing a distinguished abamectin odor. The contents of 23 generic products had been carefully analyzed by HPLC diodearray spectrum (Figure 4) and LC/MS spectrum (Figure 5), and compared with genuine abamectin (Figure 4, Figure 6 and Table 3). The results were discussed in the following aspects.

Table 2. Sampling locations, sample volume, packing and unit price of 37 generic products of abamectin

Sampling location	Package ²	ml/bottle	NT dollar/bottle	Unit price/ml
Hsichou	A	250	600	2.40
Ziguan	A	250	2000	8.00
Hsinkan	A	250	800	3.20
Hsihu-1	A	250	500	2.00
Lunbei	A	250	600	2.40
Hsihu-2	B	200	350	1.75
Erlung-1	B	200	600	3.00
Tziton-1	B	200	500	2.50
Erlung-2	B	200	500	2.50
Tounan	B	200	550	2.75
Hsilo	B	200	800	4.00
Luchu	B	200	900	4.50
Peitou	B	200	400	2.00
Huwei-1	B	200	800	4.00
Huwei-2	B	200	550	2.75
Huatan	C	200	400	2.00
Tenwei	C	200	650	3.25
Erlin	C	200	750	3.75
Yuanlin	D	100	250	2.50
Tziton-2	D	100	300	3.00
Tziton-3	D	100	280	2.80
Beitou-1	D	100	300	3.00
Beitou-2	D	100	300	3.00
Yongin	B	200	550	2.75
Pusin	B	100	300	3.00
Changhua	B	200	700	3.75
Kansun	D	120	1000	8.33
Hsinyi-1	D	100	300	3.00
Hsinyuan	D	100	250	2.50
Hsinyi-2	A	250	700	2.80
Liugiao	D	120	550	4.58
Tuku	D	100	300	3.00
Peikan	D	100	350	3.50
Sunsin	D	60	700	11.67
Ilan	D	100	700	7.00
Houlong	B	100	350	3.50
Tsubei	E	30	-	-

² Package of the containers: A: Aluminum can, 15cm height and 6cm in diameter; B: Glass bottle, 11cm height and 6cm in diameter; C: Glass bottle, 15cm height and 6cm in diameter; D: Glass bottle, 12cm height and 4.5cm in diameter; E: Plastic can

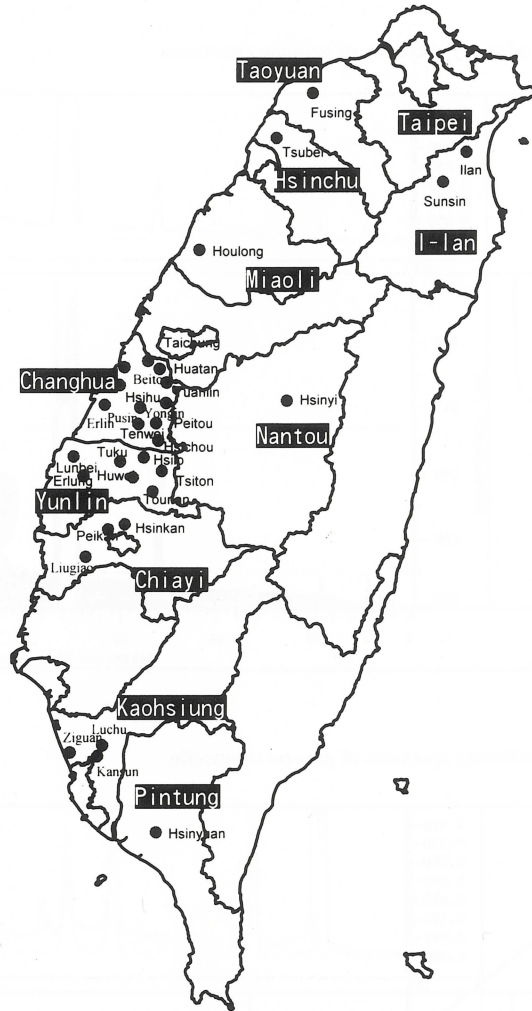


Fig. 2 Sampling locations of generic abamectin in Taiwan.

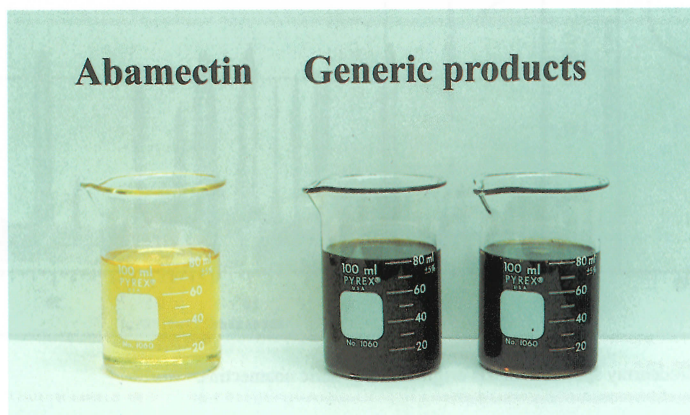
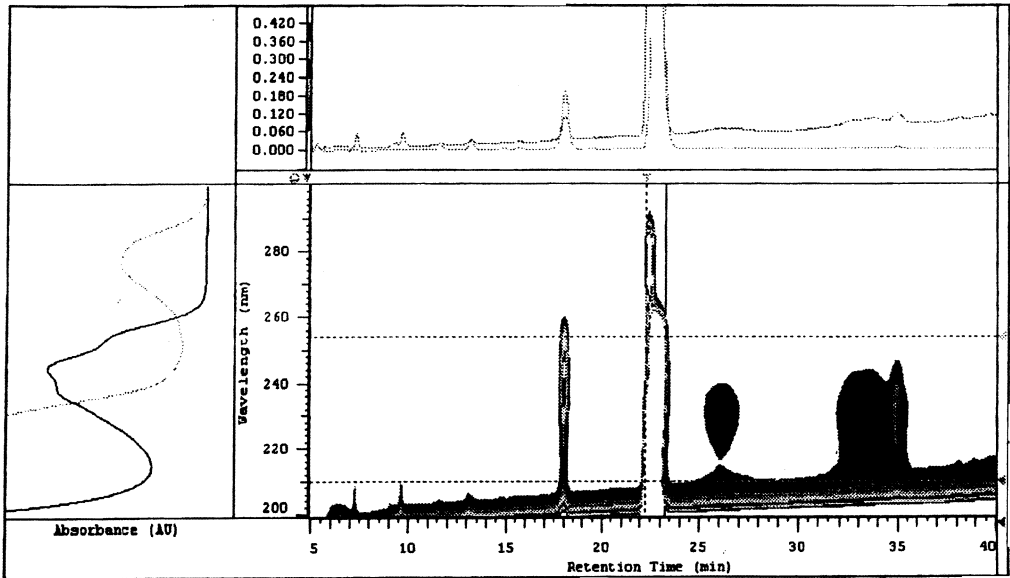


Fig. 3 Color variation of abamectin and the generic products.

(A) HPLC Diodearray spectrum of genuine abamectin



(B) HPLC Diodearray spectrum of generic abamectin

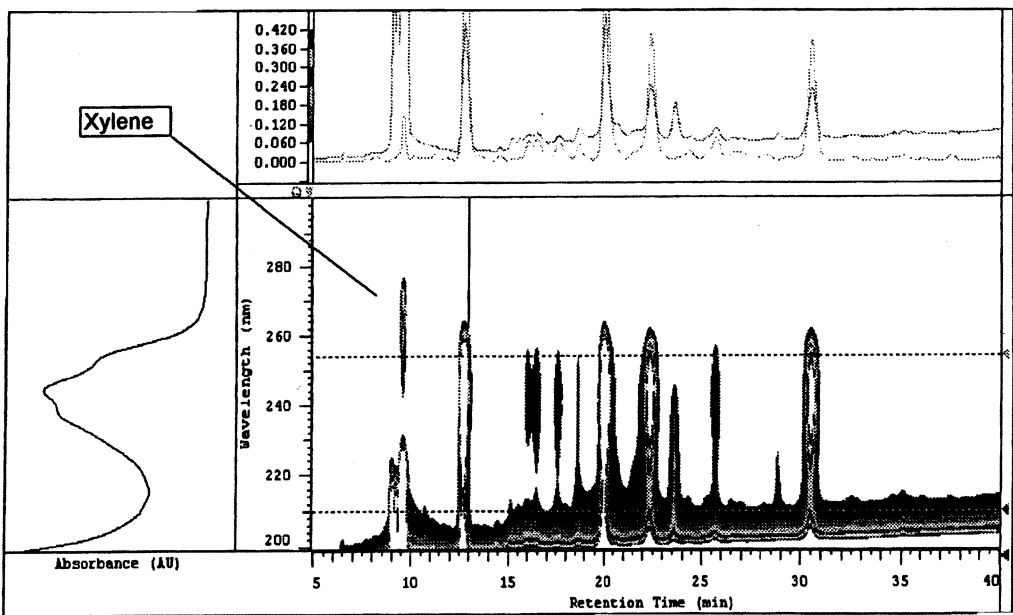


Fig. 4 The HPLC diodearray spectrum of the original and generic abamectins.

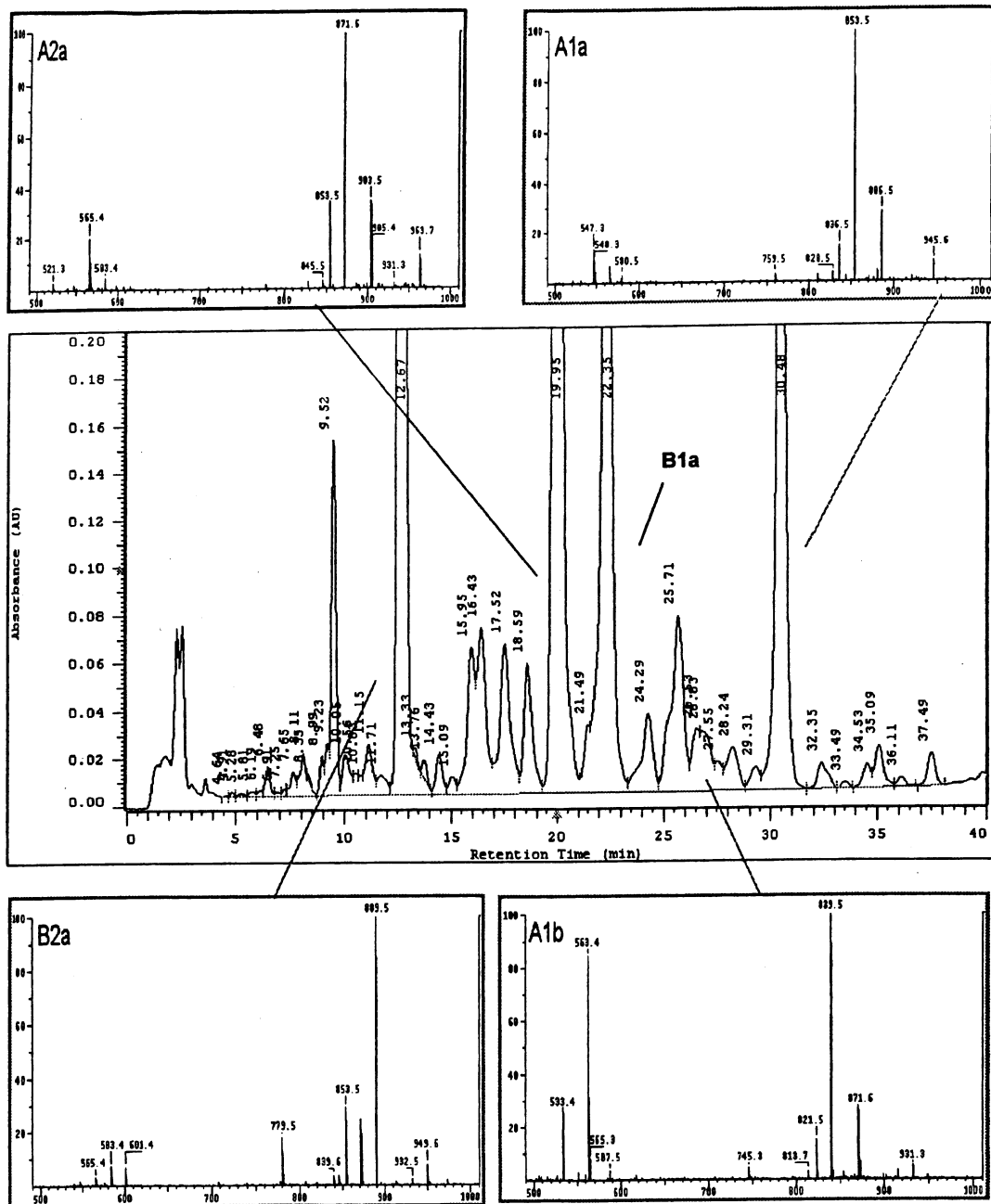


Fig. 5 The HPLC chromatogram and LC/MS spectra of unregistered avermectin impurities (A1a, A1b, A2a and B2a)

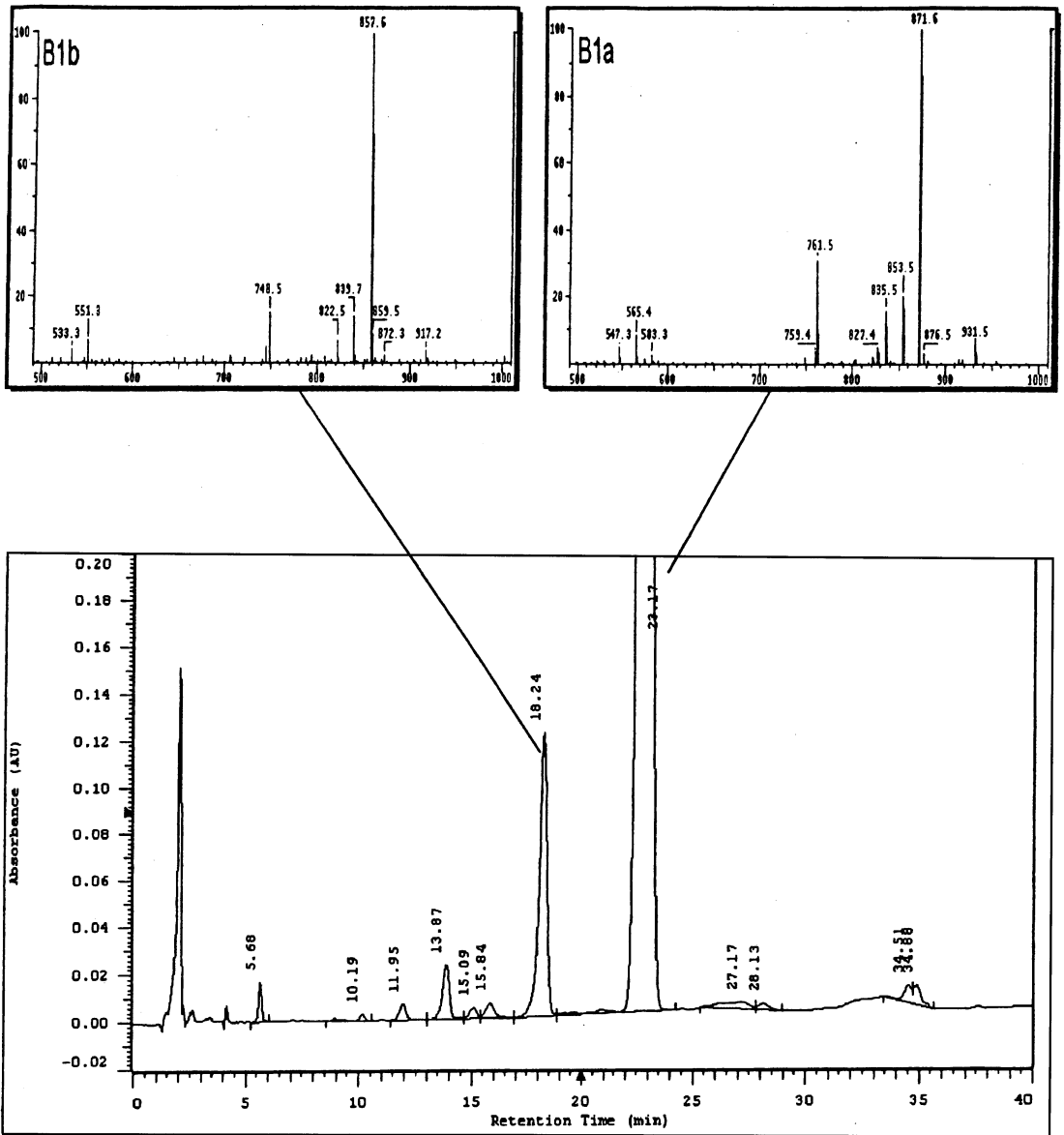


Fig. 6 The HPLC chromatogram and LC/MS spectra of the genuine abamectin (avermectins B1a and B1b)

Table 3. Comparison of toxin contents of genuine abamectin and its generic products toxin contents

Toxin content	% Content in Abamectin	Generic products (samples 1~12), % contents of individual toxin											
		1	2	3	4	5	6	7	8	9	10	11	12
		Hsichou	Ziguan	Hsinkan	Hsihu-1	Lunbei	Hsihu-2	Erlung-1	Tziton-1	Erlung-2	Hsilo	Luchu	Peitou
B1a	1.87	1.27	2.95	0.52	1.11	0.63	0.85	1.79	1.31	1.31	0.24	1.75	1.39
B1b	0.12	0.04	0.32	0.02	0.08	0.0	0.0	0.10	0.12	0.11	0.02	0.09	0.12
Abamectin(B1a+B1b)	1.99	1.31	3.27	0.54	1.19	0.63	0.85	1.89	1.43	1.42	0.26	1.84	1.51
B1a/B1b	94/6	97/3	90/10	96/4	93/7	100/0	100/0	95/5	92/8	92/8	92/8	95/5	92/8
A1a	0.01	2.39	2.68	0.83	0.60	0.65	1.27	1.29	1.66	1.47	0.23	0.75	1.54
A1b	0.003	0.09	0.17	0.04	0.03	0.03	0.08	0.09	0.08	0.07	0.01	0.09	0.07
A2a	0.004	4.13	3.98	2.16	1.23	1.44	2.91	2.28	3.03	2.80	0.44	2.25	3.19
B2a	0.02	2.09	2.76	1.86	0.79	1.25	0.08	2.44	2.69	2.44	0.85	2.45	2.89
Total avermectin	2.03	10.01	12.86	5.43	3.84	4.00	5.19	7.99	8.89	8.20	1.79	7.38	9.20
Active Ingredient	(%)												
B1a	92.2	12.7	22.9	9.6	28.9	15.8	16.4	22.4	14.7	16.0	13.4	23.7	15.1
B1b	6.0	0.4	2.5	0.4	2.1	0.0	0.0	1.3	1.3	1.3	1.1	1.2	1.3
Abamectin(B1a+B1b)	98.2	13.1	25.4	9.9	31.0	15.8	16.4	23.7	16.1	17.3	14.5	24.9	16.4
A1a	0.5	23.9	20.8	15.3	15.6	16.3	24.5	16.1	18.7	17.9	12.8	10.2	16.7
A1b	0.1	0.9	1.3	0.7	0.8	0.8	1.5	1.1	0.9	0.9	0.6	1.2	0.8
A2a	0.2	41.3	30.9	39.8	32.0	36.0	56.1	28.5	34.1	34.1	24.6	30.5	34.7
B2a	1.0	20.9	21.5	34.3	20.6	31.3	1.5	30.5	30.3	29.8	47.5	33.2	31.4

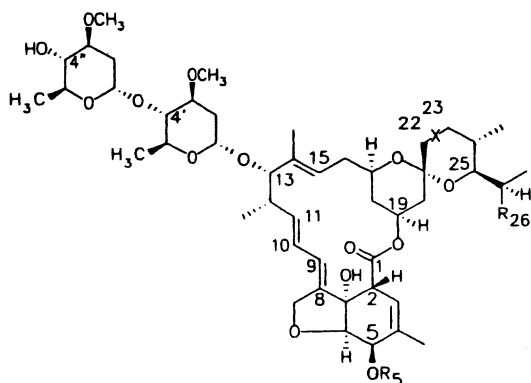
Toxin content	% Content in Abamectin	Generic products (samples 13~23), % contents of individual toxin											
		13	14	15	16	17	18	19	20	21	22	23	
		Huwei-1	Huwei-2	Huatan	Tenwei	Yuanlin	Tziton-2	Tziton-3	Beitou-1	Beitou-2	Yongin	Pusin	
B1a	1.87	0.55	0.92	0.69	1.17	0.88	1.57	0.93	1.19	1.65	0.71	1.71	
B1b	0.12	0.01	0.09	0.05	0.10	0.05	0.08	0.05	0.10	0.14	0.07	0.08	
Abamectin(B1a+B1b)	1.99	0.56	1.01	0.74	1.27	0.93	1.65	0.98	1.29	1.79	0.78	1.79	
B1a/B1b	94/6	98/2	91/9	93/7	92/8	95/5	95/5	95/5	92/8	92/8	91/9	96/4	
A1a	0.01	0.82	1.97	0.30	1.49	0.88	1.60	0.45	0.69	1.49	1.78	2.13	
A1b	0.003	0.05	0.04	0.0	0.09	0.0	0.05	0.05	0.04	0.08	0.03	0.08	
A2a	0.004	2.12	2.05	0.65	2.84	1.56	2.88	0.96	1.65	2.74	1.40	3.83	
B2a	0.02	1.86	1.74	1.02	2.50	1.20	2.02	0.72	1.21	2.28	1.19	2.49	
Total avermectin	2.03	5.41	6.81	2.71	8.19	4.57	8.20	3.16	4.88	8.38	5.18	10.32	
Active Ingredient	(%)												
B1a	92.2	10.2	13.5	25.5	14.3	19.3	19.1	29.4	24.4	19.7	13.7	16.6	
B1b	6.0	0.2	1.3	1.8	1.2	1.1	1.0	1.6	2.0	1.7	1.4	0.8	
Abamectin(B1a+B1b)	98.2	10.4	14.8	27.3	15.5	20.4	20.1	31.0	26.4	21.4	15.1	17.3	
A1a	0.5	15.2	28.9	11.1	18.2	19.3	19.5	14.2	14.1	17.8	34.4	20.6	
A1b	0.1	0.9	0.6	0.0	1.1	0.0	0.6	1.6	0.8	1.0	0.6	0.8	
A2a	0.2	39.2	30.1	24.0	34.7	34.1	35.1	30.4	33.8	32.7	27.0	37.1	
B2a	1.0	34.4	25.6	37.6	30.5	26.3	24.6	22.8	24.8	27.2	23.0	24.1	

The total avermectin content

Six avermectins, B1a, B1b, A1a, A1b, A2a and B2a (Figure 7) were found and analyzed. B1a and B1b are the registered components of original abamectin, while A1a, A1b, A2a and B2a are classified as avermectin impurities. In the registered formulation, the total content of B1a and B1b is limited to 2%, and total avermectin impurities should be 0.04% or less. The analysis result of generic products indicated that only sample 10 contained 1.79 % total avermectin, and the rest 22 samples contained 2.7~12.9% total avermectin as presented in Table 3.

The B1 composition analysis

Although the generic products contained 2 to 6 times more total avermectin than the registered 2% B1a/B1b active ingredient, 81% avermectins found in the generic products were A1a, A1b, A2a and B2a impurities (Table 4). The registered formulation contained 1.99% of B1 avermectin. In generic products, the B1 content is only 1.26%, or two-thirds of that of genuine abamectin (Table 4). In generic products, the B1a content ranged from 0.24% to 2.95% i.e., more than 10-fold difference was found between the lowest and the highest samples, and the content of B1b also varied significantly from 0% to 0.32%.



Avermectin	R ₅	R ₂₆	C ₂₂ -X-C ₂₃
A _{1a}	CH ₃	C ₂ H ₅	-CH=CH-
A _{1b}	CH ₃	CH ₃	-CH=CH-
B _{1a}	H	C ₂ H ₅	-CH=CH-
B _{1b}	H	CH ₃	-CH=CH- OH
A _{2a}	CH ₃	C ₂ H ₅	-CH ₂ -CH- OH
A _{2b}	CH ₃	CH ₃	-CH ₂ -CH- OH
B _{2a}	H	C ₂ H ₅	-CH ₂ -CH- OH
B _{2b}	H	CH ₃	-CH ₂ -CH-
Ivermectin	H	>80% C ₂ H ₅ <20% CH ₃	-CH ₂ -CH ₂ -

Fig. 7 Chemical structures of different avermectins (from Campbell, 1989)

Table 4. Toxin contents of abamectin and its generic products

Avermectins	Abamectin	% Content	
		Generic products	
		Ave. of 23 samples	Range
B1a	1.87	1.18	0.24~2.95
B1b	0.12	0.08	0.00~0.32
Total B1	1.99	1.26	0.26~3.27
Ratio of B1a/B1b	94:6	94:6	90:10~100:0
A1a	0.010	1.26	0.23~2.68
A1b	0.003	0.06	0.00~0.17
A2a	0.004	2.28	0.44~4.13
B2a	0.020	1.77	0.08~2.89
Total impurities	0.037	5.37	1.53~9.59
Total impurities/total avermectin	1.8	81.0	
Total impurities/B1	1.86%	426.2%	222%~906%

The ratios of B1a/B1b

In registered abamectin, the ratio of B1a/B1b is 94:6, and the averaged B1a/B1b ratio of 23 generic products is the same as the standard value, which stands at 94:6 (Table 4). However, for individual samples, the ratios varied from 90:10 to 100:0.

Contents of avermectin impurities

The biggest problem of unregistered avermectin impurities is they lack the toxicological information. When we inspected the unregistered avermectin impurities, significant difference was discovered between abamectin and its generic products. In abamectin, the total content of A1a, A1b, A2a and B2a is 0.037%, which is below the legal limit of 0.04%; but the total avermectin impurity in generic products is in average of 5.37% (Table 4) i.e., 145-fold more than the registered abamectin, or 134 times over the legal limit. In addition, the total impurities/B1 ratio of generic products varied from 222% to 906%, indicating the unpredictability of impurity content in each sample. The highest impurity content found in the samples is 9.5%, which is 240-fold higher than the legal allowable level.

The avermectin composition spectrum

Significant difference in composition spectrum was shown between the genuine abamectin and the generic products (Table 5). When compared the composition of avermectins, the ratio of B1a: B1b: impurities is 92.2: 6.0: 1.9, while the ratio is 17.8: 1.2: 81.0 for the generic products. The unregistered avermectin impurities i.e., A1a, A1b, A2a and B2a, constituents the major part of active ingredient in generic products with the A2a stands as the highest component, and the ratio of four impurity components are 0.6: 0.1: 0.2: 1.0 vs. 19.0: 0.9: 34.4: 26.7 for genuine and generic abamectin, respectively.

In all five aspects, significant differences were found between the genuine and the generic abamectin as we can conclude that the so-called generic products of abamectin should be considered as the significantly varied products from abamectin.

Table 5. Toxin compositions of abamectin and generic products

Avermectins	% Content	
	Abamectin	Generic products (Ave. of 23 samples)
B1a	92.2	17.8
B1b	6.0	1.2
A1a	0.6	19.0
A1b	0.1	0.9
A2a	0.2	34.4
B2a	1.0	26.7
Total	100.1	100.0

The generic products of abamectin have created several problems. First, the dominance of generic products in the market has eventually forced the abamectin manufacturer to withdraw the registration on DBM in 2001, hence in respect of abamectin, all farmers now have to use the illegal products with lower active ingredient and higher unregistered toxic impurities.

Second, the generic products introduced huge quantities of unregistered toxic residues on vegetables, and there is no toxicological information on avermectin impurities to assess the risk of consumers, hence render the pesticide residue regulation meaningless.

Third, the original-planned resistance management program had to be abandoned. When TARI detected the abamectin resistance in DBM, there's nothing could be done to slow down the resistance because the chaos of market. The monitoring results in Lu-chu and Hsi-hu areas from 1996 to 2001 indicated that the resistance in DBM continuously rose from 140~200-fold in 1996 to 2500~5000-fold in 2001⁽²⁾. The development of resistance in pest definitely will pushed farmers to apply higher dosage of generic abamectin and consequently resulted in even more unregistered toxic residues on vegetables.

This study has confirmed that the currently marketed generic product of abamectin in Taiwan cannot be considered as the copy of abamectin, and rather is another complete different illegal pesticide with no regulation to control its safety.

The episode of abamectin demonstrated that the poor quality generic products could easily destroy the efforts of pesticide registration and regulation. Unfortunately, generic products usually are the synonym of poor quality, high impurities and other unstable characters. Its cheap price out-competes the name brand products, shortens the market longevity of a product because the producers do not care about the resistance management. Eventually, the outcome will discourage the research and development ambition of pesticide industry and result in a "bad-replace-good" effect. If we do not pay attention to this matter, there will be future crisis that renders the pesticide registration, regulation and application system to nothing.

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類阿巴汀產品之成份研究¹

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摘要

自從阿巴汀在本省登記以來，市面上經常可購得非法進口的類阿巴汀產品，即所謂的「黑藥水」，供農民防治蔬菜害蟲使用。此類產品低價且未經精煉，所含毒性成份究竟為何，迄今仍不為人知。本研究針對此一問題於田間取得37件樣品，並逢機取樣其中23件進行成份分析，於五方面進行比較：(1)總毒性成份及含量、(2)具毒理資料之成份(B1a及B1b)分析、(3)B1a及B1b成份及比率分析、(4)不具毒理資料成份(A1a、A1b、A2a及B2a)之分析及(5)各成份間比率分佈分析。結果顯示，類阿巴汀成品之毒性成份含量超過登記之阿巴汀2~6倍，但其中有81%為未具毒理資料之毒性不純物(A1a、A1b、A2a及B2a)，而所含具毒理資料之B1a及B1b較阿巴汀低37%。23個類阿巴汀樣品之B1a/B1b平均值與阿巴汀相同，但樣品間之變動極大，在90:10至100:0之間。不具毒理資料之毒性成份超過法訂標準134倍，且因小菜蛾對此類毒物已產生2500~5000倍之抗藥性，在合法之阿巴汀撤銷其登記後，超量使用類阿巴汀產品將造成蔬菜上殘留大量未具毒理資料之殘毒，對消費者安全造成潛在之威脅。

關鍵詞：阿巴汀、類阿巴汀、毒性不純物、成份分析、農藥管理。

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2. 本所應用動物組研究員、副研究員及助理研究員。臺灣省 臺中縣 霧峰鄉。

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