

Insecticide Resistance in Diamondback Moth in Malaysia

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Abstract

The main problems faced by vegetable growers in Malaysia are pests and diseases. Among the major pests of vegetables in Malaysia, diamondback moth, *Plutella xylostella* (L.) is the most serious. The main method of control has been the use of insecticides. However, outbreaks of this pest occur every 2-3 years. New and old insecticides, with regular use, were found to be ineffective in controlling this pest because of the development of insecticide resistance. Studies were initiated to monitor resistance to the major insecticides used by the growers. Field-collected larvae were reared in the laboratory and tested against selected insecticides using the leaf dip method and the spray tower technique. The LC₅₀ values were calculated using probit analysis. The resistance ratio (RR) values were calculated by dividing LC₅₀ values for resistant DBM strain and LC₅₀ values of a known susceptible strain. Diamondback moth populations in the highland and lowland vegetable-growing areas showed resistance to methamidophos, cypermethrin, teflubenzuron, chlorfluazuron and diflubenzuron. Resistance was also recorded to *Bacillus thuringiensis* var. *kurstaki* in one population of this pest in Cameron Highlands. No resistance was recorded to avermectin and *Bacillus thuringiensis* var. *aizawai*.

Introduction

The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Yponomeutidae), is the most important pest of crucifers in Malaysia. Since it was recorded in 1925 (Ho 1965) the main method of control has been the use of insecticides. The first comprehensive study on detection of resistance to insecticides was conducted by Sudderuddin and Kok (1978). High levels of resistance were detected to malathion, DDT, chlorpyrifos-methyl, lindane and dichlorvos. Insecticide resistance in DBM was found to be lower towards cartap, methomyl, methamidophos, carbaryl and resmethrin.

In the 1980s, studies on DBM were mainly focused on biological control (Lim 1986; Chua and Ooi 1986; Lim et al. 1986) and integrated pest management (IPM) (Syed et al. 1990; Lim 1986). However, several new insecticides were introduced to control DBM, due to the frequent outbreaks of this pest and the failure of the other control methods. In Malaysia, common insecticides used for DBM control in the last decade were a wide range of synthetic pyrethroids, methamidophos, and various formulations of *Bacillus thuringiensis* Berliner. In the mid 1980s several formulations of insect growth regulators (IGRs) were used by the vegetable growers in the highland and lowland areas.

In 1988, serious outbreaks of DBM were recorded in the Cameron Highlands and in the lowland areas of Malaysia. We suspected resistance development to the IGRs which had been used extensively since 1986. To confirm this, insecticide resistance studies were initiated. Furthermore, it was felt that resistance monitoring in DBM would provide useful information to the existing IPM program in Malaysia.

Materials and Methods

Sites

Three locations were selected in the Cameron Highlands: Tringkap (CTT), Kea Farm (CKF) and Sungai Palas (CSP) and two locations in the lowlands: Jalan Kebun (LJK) and Karak Highway (LKH) around Kuala Lumpur. The DBM populations in the highlands and in Jalan Kebun were collected from cabbage plots while in Karak Highway from Chinese mustard. The growers were interviewed to obtain information on types of insecticides used and frequency of application (Table 1).

Insects

During 1988-89, about 500-1000 DBM larvae were collected from each field. The larvae were reared in the laboratory in the Cameron Highlands on 40-45-day-old potted cabbage (cv. KY Cross) plants in screen cages (42 cm × 43 cm × 55 cm), at 18-28°C with a photoperiod of 12L:12D. With the exception of the susceptible strain (SS), larvae used in bioassays were F₁, F₂ or F₃ offspring of field-collected individuals. The SS colony was obtained from Taiwan (Sun. C. N. National Chung Hsing University, Taichung), and was maintained without insecticide treatments for more than 20 generations before it was bioassayed.

Chemicals

Eight formulated insecticides were used: three IGRs viz., teflubenzuron (Nomolt 15% SC; Hoechst (M) Co. Ltd.) chlorfluazuron (Atabron 1.12% EC; ICI (M) Co. Ltd.), diflubenzuron (Dimilin 25% WP; Serba Kimia (M) Co. Ltd.); one macrocyclic lactone viz., avermectin (Avermectin 1.8% EC; Hoechst (M) Co. Ltd.) two *Bacillus thuringiensis* var.: *aizawai* (Florbac FC, 8500 IU/mg; Zeenex (M) Co. Ltd.) and *kurstaki* (Dipel WP, 16 000 IU/mg; Oriental Agricultural Products (M) Co. Ltd.); one organophosphorus, methamidophos (Tamaron 600 48.4% LC; Bayer (M) Co.) and one pyrethroid, cypermethrin (Ripcord 505 5.66% EC; Thiram Kimia (M) Co. Ltd.).

Bioassays

The leaf dip method (Tabashnik and Cushing 1987) was used for the IGRs and *B. thuringiensis*, while the spray tower (Potter 1952) technique was used for methamidophos and cypermethrin. For the leaf dip method, leaf disks (5 cm diam) were cut from fully expanded cabbage leaves grown from seeds in the greenhouse. Disks were dipped for 10 sec in distilled water solutions of formulated insecticide with wetter/spreader (Extravon; Ciba-Geigy (M) Co. Ltd; Rate 0.5 ml/l) and air-dried at 23°C for 2 hours. Each disk was then placed inside disposable plastic cups (6.5 cm × 6.0 cm × 4.5 cm). Ten to 15 DBM larvae (4-day-old, early 3rd instar) were placed on the disk (one replicate) and allowed to feed for 48 hours at 23°C:15°C (D:N). Larval mortality was checked daily.

For the spray tower method, 10-15 larvae were anesthetized with CO₂ for 1 min in a petri dish and placed on the spray tower holding stage. The anesthetized larvae were sprayed with 3 ml of the insecticide and placed on untreated cabbage disk in plastic cups as described above.

At least five insecticide concentrations plus a control (distilled water with spreader/sticker) was included in each test. Each test was replicated at least four times. The overall control mortality was <0.1% (range 0-1.5% per test). For the IGRs, *B. thuringiensis* and avermectin analysis was conducted on mortality after 5 days, while for cypermethrin and methamidophos analysis on mortality was done after 3 days.

Analysis

Data were analyzed by the probit procedure (SAS Institute 1985), using the 'C =' option for control mortality. Resistance ratios (RR) were calculated by dividing the LC₅₀ of each field population by the LC₅₀ of the SS population.

Results and Discussion

Results of the probit analysis are shown in Tables 2-3. The RRs for CSP, LJK and LKH populations were 4.96, 16.99 and 7.22 respectively. The population in Jalan Kebun showed resistance to cypermethrin even though only permethrin and deltamethrin were used in this area. This is probably due to development of cross-resistance. Cross-resistance in pyrethroids is well documented (Sudderuddin and Kok 1978; Liu et al. 1981; Tabashnik 1986; Tabashnik et al. 1987). The lower RR in the Cameron Highlands population is probably due to the shift in pattern in insecticide usage among the growers. The growers in the Cameron Highlands began using mainly teflubenzuron and chlorfluazuron in 1986, 1987 and early 1988. Other compounds were used only 3-4 times in each crop cycle (Table 1).

The RRs for methamidophos in CSP, LJK and LKH populations were 5.3, 123.2, 307.7 respectively. The lowland populations showed high levels of resistance to methamidophos, which was ineffective against DBM. The lower RR value in the highlands was expected due to the rare usage of methamidophos during this period.

The two common IGRs used in most of the crucifer-growing areas were chlorfluazuron and teflubenzuron. These insecticides were considered 'miracle' compounds in 1986 and 1987, when production of cabbage almost doubled in most of the cultivated areas. However in early 1988 these compounds were ineffective and serious outbreaks of DBM became apparent. Resistance was found in all the populations tested both in the highlands and the lowlands. However, the RR was significantly higher in the lowlands, even though these compounds were also extensively used in the highlands. The reason for this is unknown. One of the factors that may contribute to this phenomenon could be the generation time of DBM in the highlands is longer

Table 1. Background information on DBM field sites (1986-87).

Site (elevation)	Crop (duration)	Insecticides	
		Type	no.spray/crop cycle
Highlands			
Tringkap (1220 m)	Cabbage (90 days)	Pyrethroids	3-4
Kea Farm (1372 m)		IGRs (Teflubenzuron, Chlorfluazuron)	4-6
Sungai Palas (1830 m)		<i>Bacillus thuringiensis</i>	0-3
		Methamidophos	3-4
Lowlands			
Jalan Kebun (50 m)	Cabbage (90 days)	Pyrethroids	8-10
Karak Highway (76 m)		(Permethrin, Deltamethrin)	
		IGRs (Teflubenzuron, Diflubenzuron)	3-4
		<i>Bacillus thuringiensis</i>	8-10
	Methamidophos	8-10	
	Chinese mustard (35 days)	Pyrethroids	6-8
		IGRs (Teflubenzuron)	6-8
		<i>Bacillus thuringiensis</i>	6-8
		Methamidophos	6-8

(28 days) than in the lowlands (14 days). Among other factors, high temperatures are also conducive to insecticide resistance development in DBM (Yamada and Koshihara 1978).

Development of resistance to diflubenzuron was not surprising since this compound was used in the vegetable-growing areas for almost 12 years. In the early 1980s diflubenzuron was found to be effective to control DBM in the lowlands, but was not effective in the highlands areas. This is probably due to the development of resistance in the highlands much earlier than in the lowlands.

No resistance was recorded to avermectin (Table 2) in populations tested in the Cameron Highlands. This compound is not registered for use in Malaysia. However, avermectin was found to be very effective in controlling DBM (Syed and Hee 1989).

Bacillus thuringiensis has been used by the growers since 1965 for the control of DBM. However, it was not popular among the vegetable growers due to the slowness in its activity in killing DBM. The serotype generally used in the 1960s was *B. thuringiensis*, then *B.*

Table 2. Susceptibility of DBM to various insecticides.

Population	n	LC ₅₀ µg (AI)/ml	RR ^a
Cypermethrin			
SS	240	9.43	1
CSP	280	46.81	4.96
LJK	280	160.25	16.99
LKH	280	68.12	7.22
Methamidophos			
SS	280	21.95	1
CSP	240	116.35	5.30
LJK	320	2703.50	123.16
LKH	240	6754.85	307.73
Chlorfluazuron			
SS	240	0.057	1
CSP	280	0.56	9.82
CTK	280	0.54	9.47
CKF	240	1.62	28.42
LJK	200	58.46	1025.61
LKH	280	16.63	291.75
Teflubenzuron			
SS	280	0.46	1
CSP	480	6.73	14.63
CTK	240	6.45	14.02
CKF	240	8.22	17.86
LJK	480	1440.00	3130.43
LKH	200	1361.14	2959.00
Diflubenzuron			
SS	200	60.98	1
CTK	280	2171.82	35.61
LJK	480	1907.00	31.27
Avermectin			
SS	240	0.013	1
CKF	240	0.024	1.84
CSP	280	0.042	3.23

^aRR = LC₅₀ Field/LC₅₀ SS.

thuringiensis var. *kurstaki* was introduced in the 1980s. Recently, *B. thuringiensis* var. *aizawai* was registered for the control of DBM in Malaysia. This serotype gave effective control of DBM (Syed and Hee 1989). The serotype *kurstaki* showed high levels of resistance in Kea Farm populations in the Cameron Highlands (Table 3). This formulation was found to be ineffective in several areas in the Cameron Highlands, and today *B. thuringiensis* var. *azawai* is mainly used. No resistance was found in the DBM population tested with *B. thuringiensis* var. *aizawai*.

Current levels of resistance in Malaysia are high especially for the IGRs, and these chemicals are not recommended for use in the IPM program. Therefore usage of IGRs should be reduced drastically with increased emphasis on biological control. This is in line with the IPM approach adopted in Malaysia in 1988, where biological control was given priority. Therefore constant monitoring for insecticide resistance should be undertaken to provide information in formulating new strategies in the management of DBM.

Table 3. Susceptibility of DBM larvae to strains of *Bacillus thuringiensis*.

Population	<i>B. thuringiensis</i> var. <i>kurstaki</i>			<i>B. thuringiensis</i> var. <i>aizawai</i>		
	n	LC ₅₀ µg (AI)/ml	RR ^a	n	LC ₅₀ µg (AI)/ml	RR ^a
SS	320	0.04	1	320	0.21	1
CKF	280	4.50	112.50	240	0.70	3.33

^aRR = LC₅₀ Field/LC₅₀ SS.

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