UNIVERSITI PUTRA MALAYSIA

RESISTANCE AND SYNERGISM OF INSECTICIDES IN DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA (LEPIDOPTERA : YPONOMEUTIDAE)

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By

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A leaf-dipped bioassay was conducted to evaluate the toxicity of insecticides cypermethrin, permethrin, fipronil, avermectin b_1 and emamectin benzoate against two lowland (Karak & Cheng strain) and a susceptible strains of diamondback moth (DBM). The synergistic effect of piperonyl butoxide (PBO), S,S,S-tributylphosphorotrithioate (DEF) and maleic acid diethyl ester (MADE) on the toxicity of the insecticides tested were also conducted by the combined leaf-dipped/topical bioassay. Both the lowland strains showed high LC_{50} values (> µg/mL) for cypermethrin and permethrin. Based on the LC_{50} values, toxicities of the insecticides tested in decreasing order for the Karak and Cheng strains were : emamectin benzoate > avermectin b_1 > fipronil > permethrin > cypermethrin. The most toxic insecticide was emamectin benzoate with LC_{50} value of 1.62 X 10^{-5} mg/L and 1.59 X 10^{-5} mg/L for Cheng and Karak strain respectively. The slope of the concentration-mortality line indicated that both field-collected strains gave homogenous response towards the cypermethrin and permethrin but not the newer

insecticides. The results also showed that the DBM developed high level of resistance toward cypermethrin and permethrin. Cheng strain showed a higher resistance ratio for fipronil compared with the Karak strain. In synergism study, cypermethrin was highly synergised by PBO compared to other insecticides tested. Cypermethrin was synergised 19.8-fold and 12.6-fold for Karak and Cheng strain respectively. Both DEF and MADE showed little synergistic effects to the insecticides tested with synergistic ratio of less than 3-fold for both Cheng and Karak strain respectively. The results suggested that microsomal monooxygenases played an important role in the detoxification metabolism of cypermethrin in both strains of DBM. Esterases and glutathione s-ttansferases, however, played a minor role in the metabolism of the insecticides for both strains of DBM.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat untuk Ijazah Master Sains Pertanian

KETOKSIKAN DAN SINERGISMA BAGI RACUN SERANGGA TERHADAP RAMA-RAMA INTAN, *PLUTELLA XYLOSTELLA* (LEPIDOPTERA : YPONOMEUTIDAE)

Oleh

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Satu teknik bioasai dengan kaedah celup-daun telah dijalankan bagi menilai ketoksikan cypermethrin, permethrin, fipronil, avermectin b_1 dan emamectin benzoate terhadap dua strain tanah rendah dan satu strain peka rama-rama intan (DBM). Kesan sinergis bagi piperonil butoksida (PBO), S,S,S-tributilfosforotrithioat (DEF) dan maleik asid diethil ester (MADE) ke atas ketoksikan racun serangga yang diuji juga dijalankan mengikut kaedah gabungan topikal/celup-daun. Kedua-dua strain tanah rendah menunjukkan nilai LC_{50} yang tinggi (>1000 mg/L) bagi cypermethrin dan permethrin. Berdasarkan kepada nilai LC_{50}, ketoksikan racun serangga bagi kedua-dua strain Karak dan Cheng yang berkurangan mengikut urutan ialah : emamectin benzoate > avermectin b_1 > fipronil > permethrin > cypermethrin. Racun serangga yang paling tosik ialah emamectin benzoate dengan nilai LC_{50} 1.62 X 10^{-5} mg/L dan 1.59 X 10^{-5} mg/L bagi Cheng dan Karak strain masing-masing. Kecerunan garis kepekatan-maut menunjukkan bahawa kedua-dua strain yang dikumpul adalah

homogenus terhadap cypermethrin dan permethrin tetapi bukan terhadap racun serangga yang baru. Keputusan ini juga menunjukkan bahawa DBM telah resistan terhadap cypermethrin dan permethrin. Bagi kajian kesan sinergis, cypermethrin telah banyak disinergiskan oleh PBO berbanding racun serangga lain yang diuji. Cypermethrin telah disenergiskan sebanyak 19.8-kali dan 12.6-kali bagi Karak dan Cheng strain masing-masing. Kedua-dua DEF serta MADE menunjukkan sedikit kesan sinergis terhadap racun serangga yang diuji dengan nisbah keresistanan kurang dari 3-kali bagi kedua-dua Cheng dan Karak strain masing-masing. Keputusan ini mencadangkan bahawa mikrosomal monooksigena memainkan peranan penting dalam metabolisma nyahtoksik terhadap cypermethrin dalam kedua-dua strain *P. xylostella*. Esterase dan glutathion-s-tranferase, bagaimanapun, memainkan peranan minor dalam metabolisma racun serangga bagi kedua-dua strain DBM.

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CHAPTER 1

INTRODUCTION

Plutella xylostella (Lepidoptera : Yponomeutidae), commonly known as diamondback moth (DBM), is one of the most serious pests of cruciferous crops world-wide. The cost for controlling this notorious pest was reported to be around US$ 1 billion annually (Talekar, 1992). DBM is highly adaptable to different environment and has a shorter life-cycle in the tropics compared to the temperate regions (Ooi and Kelderman, 1979).

Farmers have since depended heavily on synthetic insecticides to control DBM. The quick action and relative ease in application of the synthetic insecticides have captured the hearts of many farmers. However, the problem of synthetic insecticides centred mainly on the extraordinary ability of DBM to rapidly develop resistance to most of the commercially available insecticides, including bioinsecticides such as *Bacillus thuringiensis* (Tabashnik *et al.*, 1990) and avermectin (Abro *et al.*, 1988). Several strategies have been adopted by farmers to cope with the problem of resistance. When a synthetic insecticide was found to be less effective, the farmer resorted to more frequent spaying with higher doses. Eventually, when the synthetic insecticide becomes ineffective, a new synthetic insecticide will replace the older one. Sometimes, farmers prepare insecticide cocktails as a last resort against DBM infestation. This process has been going on for decades with the farmers not having the slightest idea of the resistance mechanisms involved.

The escalating costs of developing an insecticide coupled with the pressure from the environmental groups for pesticide free vegetables have greatly limited the above mentioned strategies. The rate of new insecticides entering the market has been extremely slow. If chemical control is to continue, it seems that the next best option available to us is to prolong the shelf-life of newly introduced or the existing insecticides which are still effective against DBM. In this regard, one of the very important aspects needed to be elucidated is the insecticidal resistance mechanisms in the DBM.

Several mechanisms of resistance have been proposed for DBM. Three of the most frequently referred to are reduced penetration (Noppun, *et al*., 1989), increase metabolic detoxification (Sun, 1992), and insensitivity of the target sites (Hama *et al*., 1987). Amongst these, increased metabolic detoxification has been reported to play the major role in most cases of insecticide resistance. The role of reduced penetration, insensitivity of target sites as well as behavioural resistance (Casey & Franklin, 1993) are quite difficult to assess in the overall resistance phenomenon. However, when it involves two or more resistance mechanisms, the magnitude of resistance will be greatly enhanced.

Metabolic detoxification of insecticides involves several enzyme systems. DBM was shown to possess a very active and versatile microsomal monooxygenases system (Sun, 1992). In addition, esterases and glutathione s-transferases (Dauterman, 1985) also play an important part in some metabolic resistance cases. The *in vivo* studies of those enzyme systems were made possible with the use of synergists (Raffa and Priester, 1985) which effectively block the specific enzyme system. Further investigations of the role of these enzymes with newer insecticides are crucial in order to understand the metabolic mechanisms involved in the development of resistance.

In Malaysia, studies on the development of resistance in DBM have mainly been conducted on the Cameron Highland's strain (Syed, 1992). Fauziah *et al.* (1992) reported that microsomal monooxygenases and esterases were responsible for Insect Growth Regulator (IGR) resistance for the Cameron Highland's strain. Lowland cultivations of cruciferous crops have now become increasingly important with the introduction of heat-tolerant varieties. However, very little information is available on the development and mechanism of insecticide resistance in DBM in the lowlands (Omar and Edward, 1997).

Hence, the objectives of the present studies are :

1. To establish the toxicity reference of several insecticides on lowland strains of *Plutella xylostella.*
2. To determine the status of resistance of field collected lowland strains to these insecticides.
3. To investigate the roles of metabolism in the detoxification of insecticides.

CHAPTER 2

LITERATURE REVIEW

Plutella xylostella

Biology

The life-cycle of DBM varies considerably (Sarnthoy *et al.*, 1989) and is greatly influenced by temperature (Shigekazu *et al.*, 1992). In Malaysia, for example, in the lowlands, the egg would hatch in 2-3 days while the larvae and pupae stages lasted for 6-7 days and 1-2 days, respectively (Ho, 1965; Wan, 1970). The time required to complete its life-cycle almost doubled in the highlands (Ooi and Kelderman, 1979).

Several characteristics can be used to differentiate between the sexes of DBM. Normally, the male moth has a clearer diamond pattern on the back and a shorter wing span (Ho, 1965), and with a slender abdomen than the female (Biever and Boldt, 1971).

There are many different reports regarding the number of egg lay by female DBM. Ho (1965) reported that each female could lay between 81 and 379 eggs while a total of between 124 to 414 with an average of 288 eggs per female was reported by Ooi and Kelderman (1979).

Laboratory studies by Ooi (1986) revealed that adult male DBM could survive for 8 to 27 days with a mean of 13 days while female could survive for 6 to 26 days with a mean of 16 days when fed with diluted honey solution.

Distribution

DBM is the most widely distributed lepidopteran due to its high ability to adapt extreme climatic conditions (Chen & Su, 1986). In addition, DBM possesses a strong migratory ability (Mackenzie, 1958). In Malaysia, DBM was first recorded in 1925 and was believed to be an introduced pest (Ho, 1965).

DBM was suggested to be originated from the Mediterranean due to the presence of complex natural enemies and effective natural control in that regions (Hardy, 1938). It is believed to have spread throughout the world including New Zealand (Beck & Cameron, 1992), North America (Shelton *et al.*, 1996), Southeast Asia (Cheng, 1988) and Japan (Ken-ichiro, 1992) through international trade and exports of cruciferous crops.

Host Plants

DBM is an oligophagous insect that feed on plants that contain mustard glucoside (Thorsteinson, 1953). One of the economically important plant groups that fall into this category is the cruciferae family. Crucifers are grown world-wide and are believed to be the most common vegetables in Asian diet.

The major crucifers that DBM feeds on include cabbage (*Brassica oleracea* var. capitata), cauliflower (*B. oleracea* var. botrytis), Chinese cabbage (*B. rapa* cv. gr. pekinensis), and mustard (*B. juncea*). Apart from that, DBM was reported to feed on many other cruciferous plants which are considered as weeds such as *Barbarea stricta*, *Beta vulgaris*, *Galinsoga ciliata*, *Rorippa alba* and *Sisymbrium officinale* (Lauda, 1986).

Certain allelochemicals that are present in crucifers such as sulphur containing glucosinolate or its metabolites, allyl-isothiocyanates, act as oviposition stimulants (Reed *et al.*, 1989). In addition, many characteristics of the leaf also influence the oviposition activity of DBM (Tabashnik, 1985; Uematsu and Sakanishita, 1989).

Symptom of Damage

The DBM larva is the only damaging stage. Upon hatching, the first instar larva mines into the leaf and feeds on the spongy mesophyll. Then the larva will feed on the abaxial surface by scraping the epidermis leaving the wax layer on the leaf surface. This causes a transparent 'window' on the leaf, a distinctive characteristic of DBM damage.

When feeding activities are completed, the fourth and last instar larva constructs an open-network cocoon on the leaf surface usually along the vein and enters the pupal stage. The younger seedlings were observed to be more vulnerable than the mature plants (Ho, 1965). Usually, when a large number of larvae feed on the leaves, the plant will be skeletonised and would not survive.

Cabbage Plant

One of the most economically important cruciferous crops attacked by DBM is the head cabbage (*Brassica oleracea* var. capitata L.). In Malaysia, head cabbage is grown on a large scale in the highlands such as the Cameron Highlands. However, heat-tolerant varieties are now available for cultivation in the lowlands. Some of the most popular heat-tolerant varieties are the K.K. Cross, the K.Y. Cross, the Eiyu and the U.S. Tropical-hybrid. Yusoh (1982) reported that these varieties gave high yields. Most of the heat-tolerant varieties grown in Malaysia are imported mainly from Japan and the United States.

Pests of Cabbage

A total of at least 31 species of insects have been recorded to feed on cabbages in Malaysia (Yunus and Ho, 1979). Of that total, 17 species are from the

Cameron Highlands (Ooi,

lepidopterous pests of cabbage include the tobacco cutworm, *Spodoptera litura* F.,

The cabbage webworm,

Rott., and cabbage heartworm, *Hellula undalis* F. (Ibrahim and Khoo,

The larvae of *A. ipsilon* are active at night and usually feed on the base of the stem. This insect use to be controlled by spraying with trichlorphon at the soil around the seedling (Yunus and Subramaniam,

from its seedling stages. Normally,

leaves.

Both *C. binotalis* and *H. undalis* can cause serious damage to cabbage when outbreak occurs. *C. binotalis* causes severe damage to the cabbage head while *H. undalis* feeds on the terminal bud and resulted in the formation of small multiple cabbage heads (Ibrahim and Khoo,

permethrin, fenvalerate and trichlorphon (Syed *et al.*, 1987).

Control Methods

Cultural Control

Cultural practices are considered as important measures to suppress pest population (Brader,

affected by the changing weather which is a density independent factor (Harcourt, 1963). DBM infestation was observed to be lower in the wet season compared to drier season (Ooi, 1979; Sivapragasam *et al.*, 1988). Rainfall was the major mortality factor in the population dynamic of DBM (Chin, 1974; Talekar & Lee, 1985). The use of overhead sprinkler was shown to significantly reduced DBM infestation (Talekar *et al.*, 1986). This may be due to the distraction of adult flying, mating and ovipositioning activities (AVDRC, 1988). However, other environmental factors such as temperature and wind condition may also play an important part in the total martality of DBM (Muckenfuss *et al.*, 1992).

Resistant Cultivar

In recent years, much effort have been geared toward finding commercially viable cruciferous plants which also possess resistance characteristics to DBM. The thickness of the wax layer on cruciferous plants was found to effect DBM infestation (Eckenrode *et al.*, 1986; Uematsu & Sakanoshita, 1989). This was attributed to the decreased rate of release of mustard oil from the leaves of crucifer plants which could have reduced the oviposition activities preference of DBM (Gupta & Thorsteinson, 1960). A long-season cauliflower from Australia (PI234599) which possesses glossy leaves was found to be more resistant to DBM in the field (Dickson & Eckenrode, 1980) but not in the greenhouse (Lin *et al.*, 1983). Therefore, the relationship between the thickness of the wax layer and the resistance is still unclear. Nevertheless, its potential cannot be ignored and further research is needed in this area.

Intercropping

Intercropping of cabbage with tomato has been shown to significantly reduce the infestation of DBM (Buranday & Raros, 1973; Sivapragasam *et al.*, 1982; Othman, 1986). However, results have not been as observed by Srinivasan (1984) who reported that the planting of tomato and cabbage together did not effectivelyreduce infestation, but when tomato was planted first followed by cabbage 30 days later resulted in a significantly effective control of DBM. The author attributed this to the repellent effect of volatile substances released from mature tomato plants.

Trap Crops

Trap crops have been used in controlling agricultural insect pests (Riechardt, 1919; Ghesquiere, 1939) long before the introduction of synthetic chemical insecticides. Trap crops planted were usually of an economically less important plant but highly preferred by DBM (Metcalf & Luckman, 1975). For example, planting of Indian mustards together with cabbage was found to be successful in reducing DBM infestation in India (Srinivasan & Krishna, 1991). In recent years, this control tactic has been given increasing emphasis as an alternative control measure in view of the many problems arising from heavy dependence on synthetic chemical insecticides.