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# Epicuticular Wax Content on Leaf Epidermis of Selected *Brassica* L. Taxa in Relation to Feeding Behaviour Against Diamondback Moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) Larvae

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### ABSTRACT

Many aspects of epicuticular waxes have been studied over the years. In plants, the epicuticular wax layer provides a protective barrier, which consists predominantly of long-chain hydrocarbon compounds, including alkanes, primary alcohols, aldehydes, secondary alcohols, ketones, esters, and other derived compounds. However, there is a limited understanding of the relationship between animal or insect-eating habits and plant micromorphological characteristics. This study investigated the impact of epicuticular wax content in five selected taxa of *Brassica* L. on the feeding behaviour of diamondback moth (DBM), *Plutella xylostella* L., using observation under the scanning electron microscope (SEM). The findings have revealed that the epicuticular waxes' morphology content was composed primarily of rodlets, flakes, thread-like, granular, and smooth film coating. The analysis was conducted using a one-way analysis of variance (ANOVA). Findings have shown that there was a significant difference regarding the time spent on feeding by the larvae of the 3rd instar of the DBM both in choice ( $F = 510.58$ ,  $df = 5 \text{ \& } 54$ ,  $P < 0.05$ ) and no-choice tests ( $F = 38.92$ ,  $df = 5 \text{ \& } 54$ ,  $P < 0.05$ ) to the epicuticular wax content in *Brassica* leaves. These insect pests showed a weak preference for leaves with high epicuticular wax. It was concluded that the variation in waxes present in the *Brassica* taxa studied has great significance as a mechanical barrier to the DBM larvae.

**Keywords:** epicuticular; Brassicaceae; leaf micromorphology; waxes; DBM larvae

## 1. Introduction

*Brassica* L. is Brassicaceae's most economically important genus (syn. Cruciferae). Several species or taxa of *Brassica* are significant oilseed crops, vegetables, and forage crops, and are used in the production of condiments, such as mustard [1][5]. In addition, they are widely used in the cuisine of many cultures and are recognised as a valuable source of dietary fibre. *Brassica* vegetables contain little fat, are sources of vitamins, minerals, and fibre, and have many phytochemicals, some of which protect against carcinogenesis [2][8][13][14].

In Malaysia, cultivating vegetables is an important agricultural activity, and more than 50 types of vegetables, comprising both tropical and temperate species, are grown here [7]. The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is the major insect pest of leafy

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*Brassica* vegetables in Malaysia, which was recorded in 1925. By 1941, it had become widely established all over Malaysia as a severe pest of *Brassica* spp. [12]. Therefore, the present research aimed to study the morphology of epicuticular waxes in kale or kailan (*B. oleracea* subsp. *alboglabra* Bailey), Chinese mustard (*B. juncea* L.), cauliflower (*B. oleracea* subsp. *botrytis* Miller), pak choi (*B. rapa* subsp. *chinensis* Hanelt), and broccoli (*B. oleracea* subsp. *italica* Plenck) with the feeding behaviour of DBM larva at the stage of 3rd instar using no-choice and choice tests.

## 2. Methodology

Seeds were collected from the Malaysian Agricultural Research and Development Institute (MARDI) and grown in flowerpots in a glasshouse at a constant temperature of  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$  under a 16h/8h photoperiod regime, with 25 replicates. Observations were conducted under a scanning microscope, following the method described by Noraini *et al.* [9]. In addition, tests on the feeding behavior of the diamondback moth *P. xylostella* on Brassica leaves were conducted using no-choice and choice tests, following Karcz *et al.*[5].

## 3. Results

### 3.1 SEM Analysis, Choice and No-choice Test

The cuticular wax varied from amorphous film layers to stacked platelets. A summary of the wax types and structures present in all the species studied is provided in Table 1, and selected micrograph images are shown in Figure 1. Cuticular wax is composed of a complex mixture of very-long-chain (VLC) aliphatic compounds and their oxygenated derivatives, including fatty acids, alkanes, alcohols, esters, aldehydes, ketones, and triterpenes [9]. Previous studies have suggested that the cuticular wax layer acts as the first protective barrier against pathogenic and insect attacks, as well as protection against mechanical damage [3][4][6].

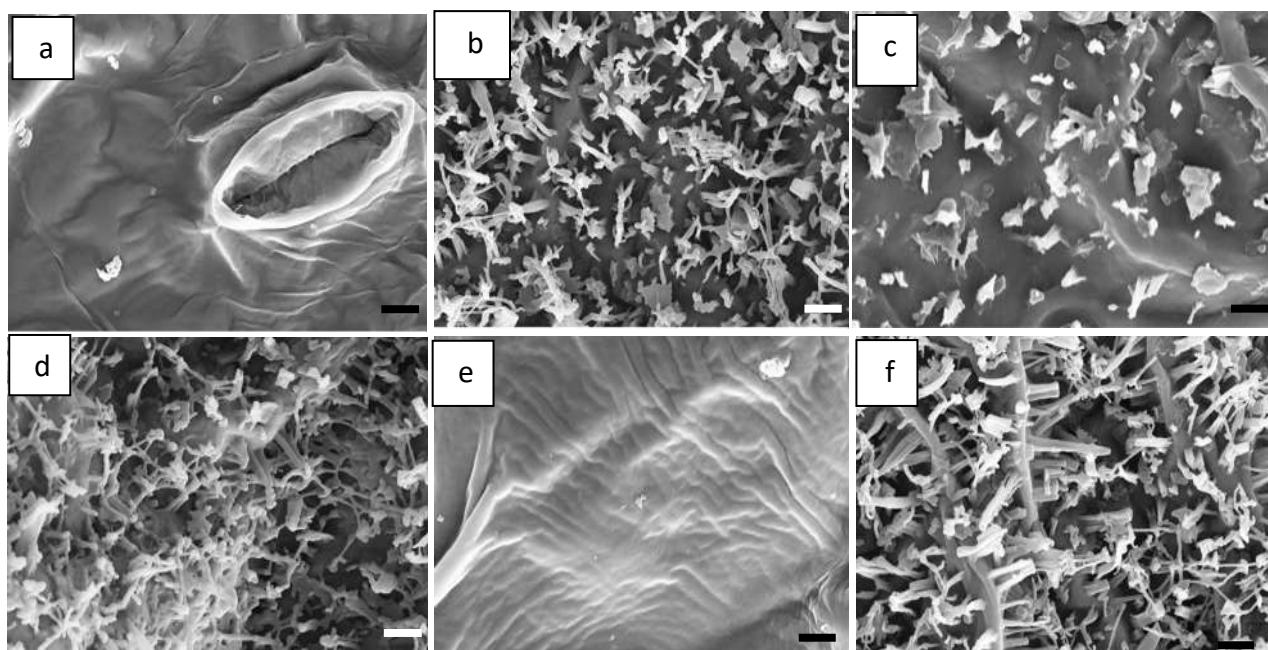
**Table 1**

Superficial waxes in *Brassica* taxa studied

Wax coating	Description of wax structures	Taxa	Thickness ( $\mu\text{m}$ )	No-choice test	Choice test
Film layers and granular coatings	A single layer of sparsely scattered granular or spherical masses. The individual elements reached or exceeded $1\mu\text{m}$ in diameter.	<i>B. juncea</i> , <i>B. rapa</i> subsp. <i>chinensis</i>	9.0-10.0	$52.04 \pm 1.25\text{a}$	$20.13 \pm 0.69\text{a}$
			1.8-2.0	$34.14 \pm 1.25\text{b}$	$13.21 \pm 0.69\text{b}$
Rodlets and threads	Straight, curved or coiled which project from the epidermis. The lower portions of the rods were straight and the upper portions curled into various shapes which interlaced with one another.	<i>B. oleracea</i> subsp. <i>italica</i> , <i>B. oleracea</i> subsp. <i>botrytis</i>	0.7-1.0	$21.64 \pm 1.25\text{c}$	-
			0.5-6.3	$47.58 \pm 1.25\text{a}$	$16.65 \pm 0.69\text{ab}$
Layers and crusts	It may be smooth or show rod-like or other projections. It varied between $1\mu\text{m}$ in thickness up to $70\mu\text{m}$ .	<i>B. oleracea</i> subsp. <i>albogabra</i>	1.0-1.5	$21.65 \pm 1.25\text{c}$	-

There was a significant difference regarding the time spent on feeding by the larvae of the DBM in choice and no-choice tests. In choice test  $F = 510.58$ ,  $df = 5 \ \& \ 54$ ,  $P < 0.05$ ) and in no-choice tests  $F = 38.92$ ,  $df = 5 \ \& \ 54$ ,  $P < 0.05$  were obtained (Table 1). The DBM larvae spent significantly longer time

( $P < 0.05$ ) feeding on the Chinese mustard, pak choi, and cauliflower compared to others. However, in the case of pak choi and cauliflower, the time spent was not significantly different ( $P > 0.05$ ) in the no-choice test.



**Fig. 1:** SEM of adaxial surface (x 5,000): (a) Pak choi, (b) Cauliflower, (c) Kailan, (d) Broccoli. SEM of abaxial surface (x 5,000): (e) Pak choi, (f) Cauliflower. Scale bar: 2 $\mu$ m.

Noraini *et al.*, [9] stated that waxes differ in their chemical compositions, including long-chain hydrocarbons, acids, alcohols, aldehydes, and esters found on the leaf surface. These compounds may render the leaves unpalatable or difficult for DBM larvae to feed on (Table 1). The DBM larvae took a shorter time to feed on broccoli and kailan, indicating that the waxy leaf surface of other *Brassica* species may have been more complex for the larvae to grip or initiate leaf scraping due to the increased epicuticular wax content. In the choice test, DBM larvae restricted their feeding to only three *Brassica* species (Chinese mustard, pak choi, and cauliflower). They took significantly longer ( $P < 0.05$ ) to feed on Chinese mustard compared to the other species. This finding aligns with the study by Žnidarčič *et al.* [15] and Julio *et al.* [4], which reported that insect pests showed a weak feeding preference toward cabbages with high epicuticular wax levels. Leaf wax chemistry and its physical attributes, along with other plant characteristics, act collectively to influence diamondback moth behaviour on cabbage plants [1][10][11]. The larvae were observed to spend significantly more time walking or moving faster on leaves of wax-resistant cabbage than on susceptible varieties. Therefore, it is suggested that Chinese mustard, pak choi, and cauliflower be incorporated into ecologically engineered cropping systems to help reduce DBM populations and minimise economic damage.

Macroscopically, cuticular wax appears thick, visible, glossy, and glaucous. On *Brassica* leaves, the wax is observed as a bluish-white coating (glaucousness or waxy bloom), consisting primarily of film layers and granular coatings, rodlets, threads, layers, and crusts. These epicuticular wax crystalloids, which contribute to the waxy bloom on plant surfaces, can make the leaves slippery and more challenging for insects to adhere to.

## 4. Conclusions

The study overviewed the micromorphological characteristics of cuticular wax found in the selected *Brassica* species. Their wax structures could influence the feeding behaviour of the DBM. Therefore, this observation could positively impact the formulation of integrated DBM management strategies.

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