THE EFFECTS OF CHRYSANTHEMUM BIOACTIVE EXTRACTS ON
THE ACTIVITY OF DIAMONDBACK MOTH
(Plutella Xylostella) ON
MUSTARD CROP

MUHAMMAD BAIHAQI BIN HJ. BAKRI

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Disahkan Oleh:

(TANDATANGAN PENULIS)

(Untuk Penyelidikan atau Disertasi

Alamat Tetap: 

(Rosmah Murdad)

286 89058 KUBAT, SABAH

Pengarah Penyelidikan Akademik

Universiti Malaysia Sabah

Tarikh: 9/5/2011

(TANDATANGAN PENYELIDIK)

(TARNAH MURDAD

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DECLARATION

I hereby declare that this dissertation is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that no part of this dissertation has been previously or concurrently submitted for a degree at this or any other universities.

MUHAMMAD BAIHAQI BAKRI
BR07110071
12 APRIL 2011
I would like to express my sincere thanks to God for giving me strength to finish up this dissertation successfully. It is a kind of new experience for me during the period of conducting the experiment and the writing. It involves so many effort include time and finance to fulfil this dissertation. I also would like to express my deepest thanks for my supervisor, Pn. Rosmah Bt. Murdad and my Co-supervisor, Associate Prof. Dr. Harpal Singh Saini because of their kindness in guide and helping me to fulfil this dissertation. I also want to express my thanks for my parents who always stand behind me to support all the time whenever I facing good and bad time. For our lab assistant, especially Madam Christina, thank you for the good co-operation. To all my colleagues who always support me and giving me idea, thank you and May God bless you all.

Thank you.
ABSTRACT

This research was conducted to evaluate the effect of natural bioactive constituents derived from *Chrysanthemum species*. The objectives of this study were to evaluate the effectiveness of active ingredients of *Chrysanthemum sp.* toward the mortality of *Plutella xylostella*, a major pest of *Crucifers* family and to determine the effectiveness of *Chrysanthemum sp.* extract obtained from flowers and leaves part. This study was conducted in a farm owned by local farmer at Kundasang that applied semi control environment agriculture practice. *Chrysanthemum sp.* was purchased from commercial nursery. The extracts were prepared from flower and leaves of *Chrysanthemum species*. The extracts obtained were evaluated for their effectiveness against the *P. xylostella* that attack mustard crop. The concentration applied for the spraying is 2.5% w/v concentration and distilled water act as a control. The extracts were then tested over a time period of 24 to 72 hours. The treatment was replicated twenty times and each replicate contains average of two larvae of *P. xylostella*. The result revealed that spraying by using *Chrysanthemum* flower extract was more effective compare to the leaves extract in causing 100% mortality at the first 48 hours of exposure. One-way ANOVA showed that there was significant different between different extracts (p<0.05) on the mortality of *P. xylostella*. In conclusion, this study has clearly demonstrated the usefulness of *Chrysanthemum sp.* as a control *P. xylostella* on mustard crop in real field condition.
ABSTRAK

KESAN EKSTRAK BIOAKTIF CHRYSANTHEMUM TERHADAP AKTIVITI DIAMONDBACK MOTH (Plutella xylostella) PADA TANAMAN SAWI

Kajian ini dilakukan untuk mengkaji kesan unsur bioaktif alami yang berasal dari spesies Chrysanthemum. Tujuan kajian ini adalah untuk menilai keberkesanan bahan aktif Chrysanthemum sp. terhadap kematian Plutella xylostella, perosak utama keluarga Cruciferaceae dan untuk menentukan keberkesanan ekstrak Chrysanthemum sp. yang diperolehi dari bahagian bunga dan daun. Penelitian telah dilakukan di sebuah ladang yang dimiliki oleh petani tempatan di Kundasang yang menerapkan amalan pertanian dibawah sistem pertanian terkawal. Chrysanthemum sp. dibeli dari tapak semai komersil. Ekstrak dikumpul dari bunga dan daun spesies Chrysanthemum. Ekstrak yang diperolehi dinilai untuk keberkesanan mereka terhadap P. xylostella yang menyerang tanaman sawi. Konsentrasi yang digunakan untuk semburan adalah 2.5% berat/isipadu dan air suling bertindak sebagai kawalan. Ekstrak itu kemudian diuji selama tempoh masa dari 24 hingga 72 jam. Rawatan diulang dua puluh kali dan setiap ulangan mengandungi dua larva P. xylostella. Keputusan kajian menunjukkan bahawa semburan dengan menggunakan ekstrak bunga Chrysanthemum lebih berkesan berbanding dengan ekstrak daun dalam menyebabkan 100% kematian serangga P. xylostella pada 48 jam pertama selepas penyemburan dijalankan. Ujian ANOVA 1-hala menunjukkan bahawa ada perbezaan yang signifikan antara ekstrak yang berbeza (p<0.05) terhadap kematian P. xylostella. Sebagai kesimpulan, kajian ini telah secara jelas menunjukkan kegunaan Chrysanthemum sp. sebagai sumber kawalan terhadap P. xylostella pada tanaman sawi dalam keadaan persekitaran yang sebenar.
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CHAPTER 1

INTRODUCTION

1.1 Introduction

Cruciferous vegetables are economically important cole crops (Lim et al., 1996) for the Asian population as they form an essential part of diet (Shelton, 2001; Schuler et al., 2001). On a worldwide basis, the extent cultivation of these vegetables are about 2.2 million ha with fifty percent (50%) of the production came from Asia (Shelton, 2001). Despite the advancement in pest control technologies, pest such as the diamondback moth (DBM) still poses major constraints for growing cruciferous vegetables in many countries (Schuler et al., 2001).

DBM or mostly known as Plutella xylostella has become the most destructive insect pest of crucifer vegetables worldwide, and annual costs for managing it are estimated at USD 1 billion (Talekar 1992, Talekar and Shelton 1993). The absence of effective natural enemies and insecticide resistance are believed to be the major cause of the P. xylostella pest status in most parts of the world (Lim, 1986; Talekar and Shelton, 1993). The infestation of this pest now occurs wherever crucifers are grown and now it is believed to be the most universally distributed of all Lepidoptera (Shelton, 2001). Despite of various attempts made to control this pest, outbreaks and poor control still being reported in many countries (Shamsudin et al., 2010).

In Malaysia, insecticides are commonly used to control this type of pest. However, higher dosage of insecticides consumption had cause cross resistancy. This in turn make the cultivation of cruciferous crops become economically impossible (Chong et al., 1991; Ooi, 1994). As reported by Chong (1991) and Shamsudin (2010), the attack of this pest generally result in reduction of quality and quantity of vegetable production and have been estimated to reach up to 52% of losses.
Moreover, the application of synthetic insecticides that contain chemical such as organophosphate, hydrochlorin and carbamate are considerably toxic and can cause hazard to both environment and human health (Rai and Yadav, 2005). Many studies proves that the application of pesticides will cause long term health problems including respiratory problem, memory disorder, cancer, depression, neurological deficit, miscarriage and birth defects (Shamsudin et al., 2010).

Thus, the application of biopesticides are highly recommended to replace the chemical pesticides as it safe to human health (Rai and Yadav, 2005). The plant such as *chrysanthemum sp.* that contain pyrethrin extract (Matsuda et al., 2005) is among the best option to control this type of pest from crucifers family. Nevertheless, the application of biopesticides extract from flower could lead to the environmental friendly approaches that fulfil the standard of IPM (Rai et al., 2005).
1.2 Justification

This study has been designed to investigate the potential of *Chrysanthemum sp.* extract to control the infestation of Diamondback moth (*P. xylostella*) on mustard crop. This is an alternative to replace the use of synthetic insecticides that are harmful to both human and nature.

1.3 Objectives

The objectives of this study are;

a) to evaluate the active constituents present in *Chrysanthemum sp.* extracts toward the control of *P. xylostella*.

b) to investigate the effectiveness of *Chrysanthemum* flower and leaves extracts on larvae population of diamondback moth in mustard crop.
CHAPTER 2

LITERATURE REVIEW

2.1 Chrysanthemum sp.

2.1.1 Family and Related Taxa

Chrysanthemum sp. has been classified under family Compositae Giseke. Wodehouse (1935) reported that the genus Chrysanthemum comprising of about 100 species is widely distributed in the Northern Hemisphere. All are insect pollinated (Meo and Khan, 2006). Compositae is a huge family with many important genera including Aster, Calendula, Callistephus, Centaurea, Cosmos, Dahlia, Echinacea, Gerbera, Helianthus, Liatris, Pericallis, Solidago, Tagetes and Zinnia (Dole, 2005). All members of the genus chrysanthemum L. are annuals, of which C. Coronarium L., the rainbow daisy is of horticultural interest. Several other related species, which were formerly in the genus chrysanthemum which were commonly grown include Tanecetum parthenium (L.) Schultz-Bip; pyrethrum, Tanecetum cinerifolium (L.) Schultz-Bip; and shasa daisy, feverfew, Leucanthemum superbum (J. Ingram) Bergams ex Ker (Figure 2.1). A brief listing of additional horticultural species and their scientific name are included in Appendix A.

2.1.2 Origin

Chrysanthemums were first cultivated in China as a flowering herb as far back as the 15th century BC. The Chrysanthemums garden were grown in Europe in 18th century.
2.1.3 Physical Characteristics

*Chrysanthemum* *sp.* are herbaceous perennial plant that can grow up to 50-150 cm tall, with deeply leaves and large flower heads, white, yellow or pink in the wild species (Kiriamti *et al.*, 2002). From the previous research, the active constituents of *Chrysanthemum* which is phyrethrin are ranges from 0.5 to 2% of the dry flower mass. The seed of the *chrysanthemum* *sp.* contain more phyrethrin than the flower part (Shamsudin *et al.*, 2010).

2.1.4 Economic Uses and Latest Status

a. As Potted Plant and Cut Flower

*Chrysanthemums* also grown as cut flower, potted flowering plant or garden plant for fall colour. Potted and cut *Chrysanthemums* are available as lateral disbuds or standard, respectively with one colour or shoot or as center disbudded or sprays respectively with several flower or shoot. Center disbuddded pots and cuts sprays are most commonly grown. Potted flowering *Chrysanthemums* rank as one of most popular
plants for home and widely grown in the Northern Hemisphere. Garden Chrysanthemums are either sold in the spring in flower or cut back in the home garden after the flowers have faded to allow regrowth and fall flowering, or sold as large flowering specimens in the fall for autumn colour (Dole, 2005).

b. As a herbal drink and traditional medicine

Yellow or white chrysanthemum flowers are boiled to make sweet drinks in some parts of Asia. The resulting beverage is known simply as 'Chrysanthemum tea'. Drunk with meals it helps to aid digestion, especially of greasy foods. It is also commonly taken to help strengthen the lungs and relieve head congestion. When made from fresh flowers, the flowers can be applied to the eyes to relieve dryness and itching. Chrysanthemum flowers (Chrysanthemum morifolii), known as Ju Hua, have been used in traditional Chinese medicine for centuries. They are found in many ancient formulas. But simple chrysanthemum flower tea is also a very common beverage in China, as the Chinese take into account the health benefits of the food and drink they consume. Ju Hua is classified as a cool, acrid herb which is good for relieving heat of the upper body—i.e. head and chest, being especially helpful for red, itchy eyes (Anonymous, 2007).

c. As source of Biopesticides

Chrysanthemum sp. can be extracted and be used as active components of insecticides. The flowers are pulverized and are harvested shortly after blooming and the active components called pyrethrins which contained in the seed cases. The flower are either dried or powdered or extracted with solvents and sold in the form of an oleoresin. The extract of Chrysanthemum sp. contains active insecticides components called pyrethrins. There are two types of pyrethrins that is pyrethrin-I and pyrethrin-II. The pyrethrins consist of another four different ingredients that is Cinerin-I and II and Jasmolin I and II (Kiriamti et al., 2002). Despite of it potential as insecticides, the pyrethrins is principally low toxicity to mammals, non-persistence and high toxicity to insect coupled with its remarkable in causing insect knockdown (Perry et al., 1998). However, the rapid paralytic effect of pyrethrins on insect “quick knockdown” is not always lethal (Waxman, 1998). Phrethrins is non-toxic to most mammals, making it among the safest insecticides in use and it being biodegradable and also breaking
down easily on exposure to light. The environmental protection agency has approved it for more uses than any other insecticides (Rai and Yadav, 2005).

2.2 *Brassica chinensis* var. *Parachinensis* (Mustard)

*Brassica sp.* is one of the leafy vegetables or commodity known as mustards and are well grown in Malaysia. *Brassica chinensis* var. *Parachinensis* is also known as flowering white mustard. This vegetable being planted in a period of season which has straight leaves dark green in colour at about 20-25 cm long and also the flowers are yellow in colour. Mustard is suitable to be planted in high humidity area which of about 23-25 °C. Mustard need high volume of water in their growth (Shamsudin *et al.*, 2010).

The whole part of mustard are green in colour (Figure 2.2). Mustard can be planted by seeds and it can be harvested after 8-10 weeks afterwards. It will produce small flower which is yellow in colour and at the time of harvest, the flower of the plant will open (Halimathul Saadiah, 2008).

In 2007, mustard has been planted on five hundred and thirty two (532.8) hectare land in Sabah with over eight thousands and seventy four (8,074.5) tonne production in a year. This plant has cover over 20% of land use for vegetables planting from total two thousand and sixty six (2066) hectare land in Sabah (Sabah Department of Agriculture, 2007).

![Figure 2.2 Mustard are crop that commonly grown at Kundasang](image)
2.3 The Diamondback Moth (*Plutella xylostella*)

*Plutella xylostella* or mostly known as Diamondback moth or cabbage moth is a vegetable pest in Cruciferae family. *P. xylostella* is included in *Yponomeutidae* family and order *Lepidoptera* (Wakisaka et al., 1992; Muckenfuss et al., 1990). The *P. xylostella* is the most important pest of crucifers in Malaysia and indeed in most tropical countries, probably this pest came into this country together with crucifers plants. This pest also is the first agricultural pest which developed resistance and cross-resistance to virtually all insecticides and has almost impossible to grow cruciferous crops economically (Shamsudin et al., 2010).

2.3.1 Life Cycle and Description

Total developments of *P. xylostella* from egg to pupal stage averages in about 25-30 days. In Malaysia, the life cycle of *P. xylostella* is completed in about 27 days in the highlands, while in the warmer lowlands the life cycle of this pest completed in half time of that time (Chong et al., 1991; Wakisaka et al., 1992).

Diamondback moth eggs (Figure 2.3a) are oval and flattened, and measure 0.44 mm long and 0.26 mm wide. They are yellow pale green, and are deposited singly or in small groups of 2-8 eggs in depressions on the surface of foliage, or sometime on other plant parts. Females may deposit 250-300 eggs early in the year, but the number decrease in later generations by 90%; average total production is probably 150 eggs. Development time averages 5.6 days (range 4-8 days) (Capinera, 2001; Talekar, 1992).

Larvae of diamondback moth (Figure 2.3b) has four instars. Average and range of development time is about 4.5 (3-7 days), 4 (2-7 days), 4 (2-8 days), 5 (2-10 days), respectively. Throughout their development, larvae remain quite small and active. If disturbed, they often wriggle violently, move backward, and spin down from the plant on a strand of silk. Overall length of each instar rarely exceeds 1.7, 3.5, 7.0 and 11.2 mm, respectively, for instars 1-4. The larvae are colorless in the first instar, but thereafter they become green. The body bears relatively few hairs, which are short in length, and most are marked by the presence of small white patches. They are five pairs of prolegs. Initially, the feeding habit of first instar larvae is leaf mining, though they are so small that the mines are difficult to notice. The larvae emerge from their mines at the conclusion of the first instar, molt beneath the leaf. Their chewing results
in irregular patches of damage, and the upper leaf epidermis is often left intact (Capinera, 2001; Talekar, 1992).

*P. xylostella* pupa (Figure 2.3c) is green in colour and change to yellowish or brownish in 24 hours. Pupation occurs in a loose silk cocoon, usually formed on the lower or outer leaves. The yellowish pupa is 7-9 mm long. The duration of the cocoon averages about 8.5 days (range 5-15 days), but during this time the insect is in the pre-pupal rather than the pupal stage (Capinera, 2001; Talekar, 1992).

The adult of diamondback moth (Figure 2.3d) is a small, slender, grayish brown moth with pronounced antennae. It is about 6 mm long, and marked with a broad cream or light brown band along the back. The band is sometimes constricted to form one or more light-colored diamonds on the back, which is the basis for the common name of this insect. When viewed from the side, the tips wings can be seen to turn upward slightly. Moth usually mate at dusk, immediately after emergence from the cocoon. Flight and oviposition take place from dusk to midnight, and moths can be found feeding at blossoms on nectar. Adult males and females live about 12-16 days, respectively, and females deposit eggs for about 10 days. The moths are weak fliers, usually flying within 2 m of the ground, and not flying long distances. However, they are readily carried by the winds. The adult is the overwintering stage in temperate areas but moths do not survive cold winters (Capinera, 2010; Talekar, 1992).
2.3.2 Damage

Damage is caused by larval feeding (Figure 2.4). Feeding habit start from first instar larvae that caused leaf mining. The chewing habit will last until the larvae become adult (Capinera, 2010; Talekar, 1992). When the large numbers of this feeding occur, the leaves will be skeletonized. Severe damage will kill the young plant (Shamsudin et al., 2010). Although the larvae are very small, they can be quite numerous, resulting in complete removal of foliar tissue except for the leaf veins. They are particularly damaging to seedlings, and may disrupt head formation in cabbage, broccoli and cauliflower. The presence of larvae in florets can result in complete rejection of produce, even if the level of plant tissue removal is insignificant (Capinera, 2001).

Diamondback moth was long considered a relatively insignificant pest. Its impact was overshadowed by such serious defoliators as imported cabbageworm, *Pieris rapae* (Linnaeus), and cabbage looper, *Trichoplusia ni* (Hübner). However, in the
1950s the general level of abundance began to increase and by the 1970s it became troublesome to crucifers in some areas (Capinera, 2010).

![Damage caused by larval feeding of P. xylostella on mustard crop](image)

**Figure 2.4** Damages caused by larval feeding of *P. xylostella* on mustard crop

Bar = 1 cm

### 2.3.3 Control management

According to integrated Pest management (IPM) practices, control will be taken when the larvae population are beyond the Economic Injury Level which is seven larvae per crop plant and parasitism percentage are below 40% (Chong *et al*., 1991; Shamsudin *et al*., 2010).

Protection of crucifers crops from damage often requires application of insecticide to plant foliage (Capinera, 2010). However, the application of chemical insecticides must be minimized and used when needed only. The example of effective insecticides are avermectin, fipronil, diflubenzuron, profenofos, prothiophos, and deltamethrin (Shamsudin *et al*., 2010). At present the main problem encountered in
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