



UNIVERSITI PUTRA MALAYSIA

***TOXICITY OF METHANOLIC CRUDE EXTRACTS OF
AZADIRACHTIN AGAINST *Plutella xylostella* (L.)
(Lepidoptera: Plutellidae)***

NOR SHUHAIDAH NORIZAN

FP 2013 76

**TOXICITY OF METHANOLIC CRUDE EXTRACTS OF
AZADIRACHTIN AGAINST *Plutella xylostella* (L.)**

(Lepidoptera: Plutellidae)

BY

NOR SHUHAIDAH NORIZAN

A project report submitted to Faculty of Agriculture, Universiti Putra
Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year
Project) for the award of the degree of Bachelor of Agricultural Science

FACULTY OF AGRICULTURE

UNIVERSITY PUTRA MALAYSIA

SERDANG, SELANGOR DARUL EHSAN

2012/2013

This project report entitled Toxicity of Methanolic Crude Extracts of Azadirachtin against *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is prepared by Nor Shuhaidah bt Norizan and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

Student's name:

Nor Shuhaidah bt Norizan

Student's signature:

Certified by:

Professor Dr. Dzolkifhli Omar

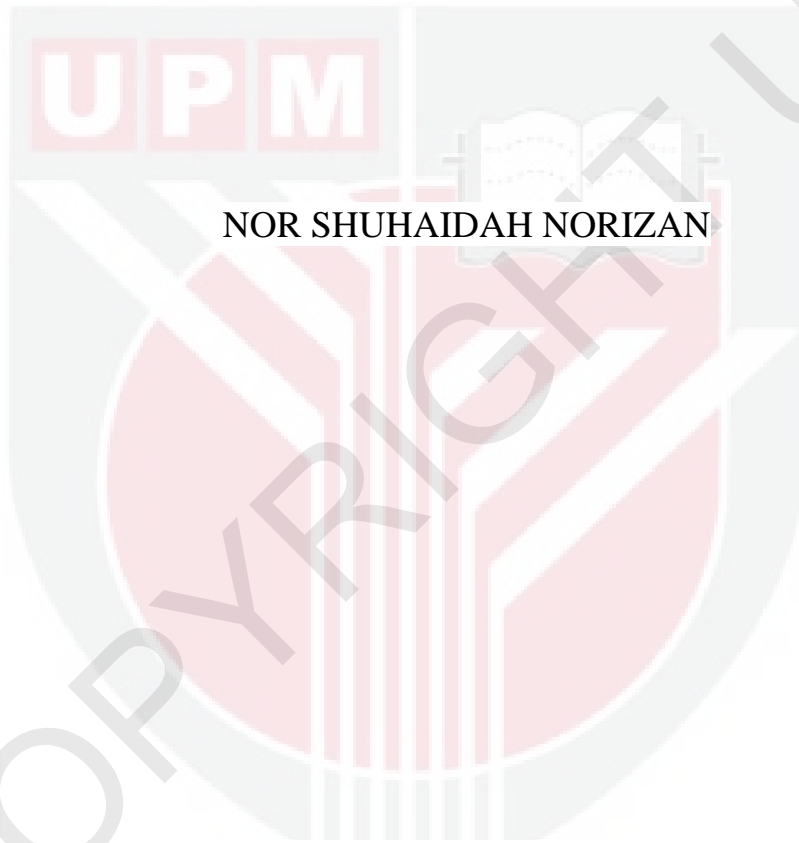
Department of Plant Protection

Date:



**TOXICITY OF METHANOLIC CRUDE EXTRACTS OF
AZADIRACHTIN AGAINST *Plutella xylostella* (L.)**

(Lepidoptera: Plutellidae)



NOR SHUHAIDAH NORIZAN

FACULTY OF AGRICULTURE

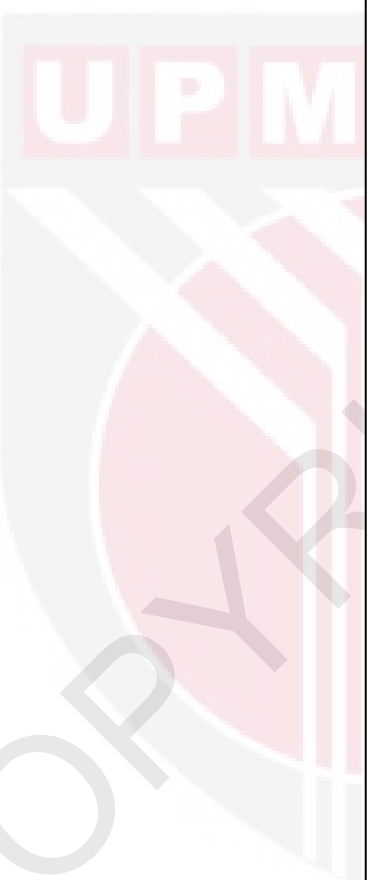
UNIVERSITY PUTRA MALAYSIA

SERDANG, SELANGOR DARUL EHSAN

2012/2013

NOR SHUHDAH BT NORIZAN

BACHELOR OF AGRICULTURAL SCIENCE



UPM

©

ACKNOWLEDGEMENT

First of all, I wish thankful to Allah the Almighty for His blessings on the completion of this final year project.

Secondly, I wish to express my sincerest gratitude and appreciation to Professor Dr. Dzolkifhli Omar, who played to the role of a mentor and supervisor for the support, guidance, constructive ideas and comments, and invaluable advice throughout the duration of this project.

I would like to thanks Mr. Zaki, Mr. Jarkasi and Mr. Tamsil, the lab assistance of Department of Plant Protection, Faculty of Agriculture, for their assistance and guidance in laboratory practical, plant cultivation and preparation of the tools needed on my experiment.

I would like to acknowledge with thanks to Ms. Noorhazawani Kamaruddin and Ms. Nurhayu Asib, the research assistants and my fellow friends for their cooperation and assistance throughout field and laboratory works.

Finally, appreciation is also expressed to my loving parents and family for their support and encouragement and kind help during the construction of this project.

LIST OF CONTENTS

CONTENT	PAGE
ACKNOWLEDGEMENT	I
LIST OF CONTENTS	II
LIST OF FIGURE	V
LIST OF PLATES	VI
LIST OF TABLE	VII
LIST OF APPENDIXES	VIII
ABTRACT	IX
ABSTRAK	X
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	4
2.1 Taxonomy of <i>P. xylostella</i>	4
2.2 Origin and distribution	4
2.3 Occurrence and importance in Malaysia	5
2.4 Hosts	5
2.5 Biology	6
2.5.1 Adult	6
2.5.2 Egg	7
2.5.3 Larvae	8
2.5.4 Pupae	9

2.6 Damages	10
2.7 Management of the <i>P. xylostella</i>	11
2.7.1 Cultural method	11
2.7.2 Biological method	12
2.7.3 Chemical method	13
2.8 <i>A. indica</i> , Neem	
2.8.1 Taxonomy	14
2.8.2 Habitat and distribution	14
2.8.3 Botanical description	15
2.8.4 Phytochemistry of <i>A.indica</i> ingredients	15
2.8.5 Insecticidal properties	17
2.8.6 Use of <i>A. indica</i>	18
2.9 <i>M. azedarach</i> , China berry	
2.9.1 Taxonomy	19
2.9.2 Habitat and distribution	19
2.9.3 Botanical description	20
2.9.4 Phytochemistry of <i>M. azedarach</i> ingredients	20
2.9.5 Insecticidal properties	21
2.9.6 Use of <i>M. azedarach</i>	21
2.10 <i>A. excelsa</i> , Sentang	
2.10.1 Taxonomy	22
2.10.2 Habitat and distribution	22
2.10.3 Botanical description	23
2.10.4 Phytochemistry of <i>A. excelsa</i> ingredients	23
2.10.5 Insecticidal properties	24
2.10.6 Use of <i>A. excelsa</i>	24

3. MATERIALS AND METHODS	
3.1 Plants and planting materials	25
3.2 Sampling and rearing of the <i>P. xylostella</i>	26
3.3 Preparations of dilutions of <i>A. indica</i> , <i>M. azedarach</i> and <i>A. excelsa</i> seed kernel extract.	27
3.4 Bioassays	
3.4.1 Mortality	28
3.4.2 Leave area consumption	29
3.5 Statistical analysis	30
4. RESULT AND DISSCUSION	
4.1 RESULTS	
4.1.2 Mortality of <i>P. xylostella</i> after 24, 48 and 72 hour exposed to <i>A. indica</i> , <i>A. excelsa</i> And <i>M. azedarach</i>	31
4.1.2 Leaf consumed by larvae <i>P. xylostella</i>	36
4.2 DISCUSSION	38
5. CONCLUSION	40
BIBILIOGRAPHY	41
APPENDIXES	45

LIST OF FIGURES	PAGE
Figure 1: Life cycle of <i>P. xylostella</i>	6
Figure 2: Adult of <i>P. xylostella</i>	7
Figure 3: Eggs of <i>P. xylostella</i>	8
Figure 4: Larva of <i>P. xylostella</i>	9
Figure 5: Pupa of <i>P. xylostella</i>	9
Figure 6: Cabbage damage by	10
Figure 7: Damage by young caterpillars	10
Figure 8: Chemical structure of azadirachtin	16
Figure 9: Total number of <i>P. xylostella</i> larvae at 24, 48 and 72 hour exposed to crude extracts <i>A. excelsa</i> , <i>M. azedarach</i> and Neem oil	33
Figure 10: The mortality response at 24 hours for each treatment	34
Figure 11: The mortality response at 48 hours for each treatment	34
Figure 12: The mortality response at 72 hours for each treatment	34

LIST OF PLATES	PAGE
Plate 1: Sampling of <i>P. xylostella</i>	25
Plate 2: <i>B. rapa</i> and <i>B. oleracea</i>	25
Plate 3: Sampling location of <i>P. xylostella</i>	26
Plate 4: Rearing the <i>P. xylostella</i>	26
Plate 5: Neem oil 3% A.i (Neemix)	27
Plate 6: Crude extracts (3ppm) of <i>A. excelsa</i>	27
Plate 7: Crude extracts (3ppm) of <i>M. azedarach</i>	27
Plate 8: Mortality parameter	28
Plate 9: Automatic Leaf Area Meter(LICOR Model 3100, LI-COR Inc, Lincoln, NE, USA).	29

LIST OF TABLES

PAGE

Table 1: Linear relationship and R-square (R^2) value of mortality at 24, 48 and 72 hours exposed to crude extracts <i>M. azedarach</i> , <i>A. excelsa</i> and Neem oil	33
Table 2: Probit dose-response of data for early 3 rd instars <i>P. xylostella</i> for Neem oil, <i>M. azedarach</i> and <i>A. excelsa</i> treatment at 24, 48 and 72 hours.	35
Table 3: Size of leaf area (antifeedant activities) of leave treated by Neem oil, <i>M. azedarach</i> and <i>A. excelsa</i> extracts after 72 hour exposed to larvae <i>P. xylostella</i> following the Tukey Test.	37
Table 4: Size of leaf area (antifeedant activities) of leave treated by different concentrations by crude extracts of <i>M. azedarach</i> , <i>A. excelsa</i> and Neem oil exposed to larvae <i>P. xylostella</i> following the Tukey Test.	37

LIST OF APPENDIXES	PAGE
Appendixes 1: Number of larvae mortality <i>P. xylostella</i> at 24, 48 and 72 hour for each three treatments.	45
Appendix 2: SAS Mortality of <i>M. azedarach</i>	46
Appendix 2.1: Tukey's studentized Range (HSD) Test for mortality (treatment & time)	47
Appendix 3 : SAS Mortality of <i>A. indica</i>	48
Appendix 3.1: Tukey's studentized Range (HSD) Test for mortality (treatment & time)	49
Appendix 4 : SAS Mortality of <i>A. excelsa</i>	50
Appendix 4.1: Tukey's studentized Range (HSD) Test for mortality (treatment & time)	51
Appendixes 5 : Epa probit analysis program for Melia 24, 48 and 72 hour	52
Appendixes 6 : Epa probit analysis program for Neem 24, 48 and 72 hours	55
Appendixes 7: Epa probit analysis program for Sentang 24, 48 and 72 hour	58

ABSTRACT

Leaf mustard is the most popular leaf vegetables in Malaysia, occupying more than 3,000 ha and heavily sprayed with synthetic insecticides against its insect pest *Plutella xylostella*. The use synthetic insecticides can result resistance problem, residues exceeding the MRL, health hazard to consumers, crops and ecosystem. The use of the botanical insecticides could solve these problems due to their bioactive compounds are fairly complex, making it more difficult for the pest to develop resistance and easily degraded. The toxicity of the methanolic crude extracts *Azadirachta excelsa*, *Melia azedarach* and Neem oil against *P. xylostella* was evaluated by leaf dip bioassay using early 3rd instars larvae in the laboratory. A minimum of 6 concentrations (0.75, 1.00, 1.50, 2.00 and 2.50ppm) for each treatment and 6 replications arranged in Randomized Complete Block Design are utilized to obtain the LC₅₀ values for each extracts. The mortality and leaf consumed was recorded at 24, 48 and 72 hours after treatment and the data were subjected to probit analysis. The amount of leaf consumed by the larvae was also measured and the data were subjected to analysis of variance (ANOVA). The methanolic extract of *A. excelsa* showed the higher toxicity with lower LC₅₀ value of 0.590 ppm and the lowest leave consumed by the larvae compared with *M. azedarach* (0.794 ppm) and Neem oil (1.716 ppm). *Azadirachta excelsa* contained an active compound known as Marrangin (azadirachtin L) that are more toxic than azadirachtin A and B in Neem oil and *M. azedarach*.

ABSTRAK

Sawi adalah sayuran daun yang paling popular di Malaysia, menduduki lebih daripada 3,000 ha dan banyak disemur dengan racun serangga terhadap serangga perosak *Plutella xylostella* itu. Penggunaan racun serangga boleh menyebabkan masalah rintangan, sisa melebihi MRL, bahaya kepada kesihatan pengguna, tanaman dan ekosistem. Penggunaan racun serangga botani boleh menyelesaikan masalah-masalah ini disebabkan oleh sebatian bioaktif mereka adalah agak kompleks, menjadikannya lebih sukar untuk perosak untuk membangunkan rintangan dan mudah dihina. Ketoksikan mentah metanol ekstrak *Azadirachta excelsa*, *Melia azedarach* dan minyak Neem terhadap *P. xyloxtella* telah dinilai oleh bioesei rendaman daun menggunakan larva awal instar ketiga di dalam makmal. Sekurang-kurangnya 6 kepekatan (0.75, 1.00, 1.50, 2.00 dan 2.50ppm) bagi setiap rawatan dan 6 replikasi disusun dalam Reka Bentuk Rawak Lengkap digunakan untuk mendapatkan nilai LC50 bagi setiap ekstrak. Kadar kematian dan daun yang digunakan dicatatkan pada 24, 48 dan 72 jam selepas rawatan dan data tertakluk kepada analisis probit. Jumlah daun dimakan oleh larva juga diukur dan data tertakluk kepada analisis varians (ANOVA). Ekstrak metanol *A. excelsa* menunjukkan tahap ketoksikan yang lebih tinggi dengan nilai LC50 rendah 0.590 ppm dan yang paling rendah meninggalkan kesan dimakan oleh larva berbanding dengan *M. azedarach* (0.794 ppm) dan minyak Neem (1.716 ppm). *Azadirachta excelsa* terkandung sejenis bahan aktif yang dikenali sebagai Marrangin (Azadirachtin L) yang lebih toksik daripada Azadirachtin A dan B dalam minyak Neem dan *M. azedarach*.

CHAPTER 1

INTRODUCTION

Plutella xylostella (L.) (Lepidoptera:Plutellidae) or commonly known as diamondback moth (DBM) is a cosmopolitan and oligophagous pest of cruciferous crops (Thorsteinson, 1953; Ahmad et al., 2009). It is the most important pest of cruciferous crops throughout the world, causing direct damage to cabbage with losses as much as 100% (Castelo-Branco & Gatehouse, 2001). The insect feeds on numerous cruciferous plants that contain mustard oils and glucosides and attacks the crop from the nursery stage and can cause up to 52% loss in marketable yield in cabbage. Srinivasan (1984) reported that 90–92% loss could occur if cabbage plants were left unprotected and Lingappa et al., (2000) reported that losses vary from 30 to 100%.

In Malaysia, *P. xylostella* has become resistant to all groups of conventional insecticides in some major Cruciferae family production areas including in India, Cameron Highlands and Thailand. Resistance is “a genetic change in response to selection by toxicants (i.e., insecticides) used to control *P. xylostella* in the field.” (Sawicki,1987). By 1981, *P. xylostella* had become resistant to more than 36 insecticides across multiple chemical classes including chlorinated hydrocarbons, carbamates, organophosphates and pyrethroids (Miyata et al., 1986). By 1990, *P. xylostella* was resistance to abamectin, benzophenylureas, and various strains of *Bacillus thuringiensis* (Sun, 1990).

The damages become serious from the abuse of chemicals by spraying excessively, mixtures of chemicals, increased dosage and frequency of spraying. It has led to the resistance and absence of effective natural enemies by the use of broad-spectrum insecticides such as Ambush (permethrin) and Dibrom (naled) (Chua & Ooi, 1986). This is primarily because of its genetic elasticity, and as a consequence the *P. xylostella* has become one of the most difficult pests to control in the past 50 years worldwide (Attique et al., 2006; Sarita et al., 2010b & Gong et al., 2010).

The rapid widespread and frequent use of newer insecticides is usually followed by the rapid evolution of resistance (Amit et al., 2004). In South Asia, substantial resistance to new insecticides has appeared within as little as two years of first use (Wright, 2004) and in Hawaii after only 30 months (Mau & Gusukuma-Minuto, 2004). There is frequent “burn out” of new products such as spinosad, avermectin and indoxacarb in the intensive cruciferous crop production areas in Cameron Highland and Thailand (Wright, 2004).

Plant-derived extracts and phytochemicals have long had been a subject of research in an effort to develop alternatives to conventional insecticides but with reduced health and environmental impacts. In this review we compare bioactivities of some plants extracts against the cruciferous crops pest, *P. xylostella*. There is a great variety of families of plants that possess potent anti-insect compounds.

From the Meliaceae family, strong insecticide molecules have been isolated with the limonoid azadirachtin obtained from *A. indica* being the most potent and studied (Govindachari, 1992; Schmutterer, 1995). *Melia azedarach*, commonly named is china berry is known to be toxic to higher animals. However, its application in pest control is still limited. A limonoid has been isolated from this tree as the most potent substance for controlling insects. The others Meliaceae family is *A. excelsa* and limonoids are known to be excellent antifeedants for many pest species with no deleterious effects on humans, animals or beneficial insects (Mordue & Blackwell, 1993). A methanolic extract of *A. excelsa* wood inhibited growth, feeding and was toxic to the larvae of *Crocidolomia binotalis* (Ng et al., 2003).

The objective of this study was to evaluate of *P. xylostella* larvae against Neem oil and methanolic crude extracts of *A. excelsa* and *M. azedarach*.

BIBLIOGRAPHY

Insecticide resistance management for Diamondback moth in Cole crops C 889.

Diamondback moth (DBM) resistance. Retrieved September 16, 2012 from http://www.caes.uga.edu/publications/pubDetail.cfm?pk_id=7458

Insecticide resistance in *Plutella xylostella* (L.) (Lepidoptera : Yponomeutidae) in the

federal district, Brazil. Material and methods. Retrieved September 22, 2012 from http://www.scielo.br/scielo.php?pid=S030180591997000100010&script=sci_arttext.

Government of Saskatchewan. Diamondback moth damage. Retrieved September 22,

2012 from <http://www.agriculture.gov.sk.ca/Default.aspx?DN=688b2f99-ad99423d-900c-c01a1c45d8a1>

Diamondback moth, *Plutella xylostella* (L.), resistance management in Hawaii

Introduction. Retrieved September 24, 2012 from <http://regional.org.au/au/esa/ws/contributed/8/Mau%2023Nov02.htm>.

Dept. of Horticulture, University of Kentucky. How to rear diamondback moth (DBM)

larvae to look for parasitoids. Retrieved September 24, 2012 from http://thailand.ipm info.org/natural_enemies/parasitoids/dbm/Rearing_DBM.htm.

A.S. Atwal & G.S Dhaliwal. Agricultural pests of south Asia and their management.

Kalyani publisher, New Delhi, India. pp. 264-265, pp 156-158

A.Regupathy. Insecticide resistance in diamondback moth (DBM), *Plutella xylostella*

(L.): status and prospects for its management in India. pp. 233-234

Ahmad Ansari, M. S., & Hasan, F. (2012). Effects of neem based insecticides on *Plutella xylostella* (linn.). *Crop Protection*, 34(0), 18-24. doi : 10.1016/j.cropro.2011.12.010

Andreu Juan, Albert Sans and MagfRiba (2000). Antifeedant Activity of Fruit and Seed Extracts of *Melia azedarach* and *Azadirachta indica* on Larvae of *Sesamia nonagrioides*. Spanish Agency CICYT-CIRIT, project. *Phytoparasitica* 28(4):311-319

Branco M.C^I & Gatehouse A.G^{II}. Insecticide resistance in *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) in the Federal District, Brazil. 3:11 Capinera, J. L. (2008). Diamondback moth, *plutella xylostella* (L.) (lepidoptera: Plutellidae). In J. L. Capinera (Ed.), *Encyclopedia of entomology* (pp. 1202-1206) Springer Netherlands. doi: 10.1007/978-1-4020-6359-6_901

Charleston D.S, Kfir R., Vet L.E.M, Dicke M. (2005). Behavioural responses of Diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) to extracts derived from *Melia azedarach* and *Azadirachta indica*. 95, 457-465. DOI: 10.1079/BER 2005377

D.g. voice & R.b. chapman. Imported insecticide resistance in Diamondback moth. Lincoln University, Canterbury 4: 83-84

H. Yeo, J.K Pell, M. Walter, K.S.H. Boyd-Wilson, C. Snelling & D.M Suckling. (2001).

Suceptibility of Diamondback moth (*Plutella xylostella* (L.) larvae to the entomopahtogenic fungus, *Zoophthora radicans* (brefeld)batko.

K. Umeda, D. MacNeil, D. Roberts. New Insecticides for Diamondback Moth Control in Cabbage. 4(10): 1

Lean T.N, Pak M.Y, Wai H.L, Azizol. (2003). Effects of *Azadirachta excelsa* on feeding behavior, body weight and mortality of *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae). Journal of the Science of Food and Agriculture. 83: 1327-1330. DOI:10.1002/jsfu.1542.

Liang, G., Chen, W., & Liu, T. (2003). Effects of three Neem-based insecticides on Diamondback moth (lepidoptera: Plutellidae). *Crop Protection*, 22(2), 333-340. DOI: 10.1016/S0261-2194(02)00175-8

Lloyd M. Dosedall, Julie J. Soroka & Owen Olfert. (2011). The Diamondback Moth in Canola and Mustard: Current Pest Status and Future Prospects. *Prairie Soils & Crops Journal* Vol 4: 66-68

M. S. Mosiane. Diamondback Moth, *Plutella xylostella* (L.), (Lepidoptera: Plutellidae), and Other Insects of Canola, *Brassica napus* L., in Gauteng Province, South Africa. pp. 11-13

Mohamed F. R. Khan, Randy P. Griffin, Gerald R. Carner, Clyde S. Gorsuch. Susceptibility of Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera:

Plutellidae), from Collard Fields in South Carolina to *Bacillus thuringiensis*. pp 18-19

Nor aini, K.S Loo. (2006). Azadirachtin variation of six provenances of *Azadirachta excelsa* (Jack) Jacob. Pakistan Journal of Biological Science, 9 (5): 833-836.

N.S Talekar, A.M Shelton. (1993). Biology, Ecology, and Management of the Diamondback Moth. Annual Review Entomology, Vol 38:275-301

Peter A. C. Ooi. (1997). Role of Parasitoids in Managing Diamondback Moth in the Cameron Highlands, Malaysia. International Journal of Pest Management. Vol 43: 255-260

Schumutterer H. (1990). Properties and potential of natural pesticides from the Neem tree, *Azadirachta indica*. Annual review entomology. Vol 35:27 1-97.

Tang, J. D., Shelton, A. M., Van Rie, J., De Roeck, S., Moar, W. J., Roush, R. T., & Peferoen, M. (1996). Toxicity of *Bacillus thuringiensis* spore and crystal protein to resistant Diamondback moth (*Plutella xylostella*). Applied and Environmental Microbiology, 62(2),564-569.

Wheeler, D. E., Tuchinskaya, I., Buck, N. A., & Tabashnik, B. E. (2000). Hexameric storage proteins during metamorphosis and egg production in the Diamondback