



Ain Shams University
Faculty of science
Entomology Department

Effect of Some Natural Products on The Cowpea Seed Beetle, *Callosobruchus maculatus* (F.) (Coleoptera : Bruchidae)

THESIS

Presented to the Faculty of Science – Ain Shams University

**In Partial Fulfillment for the award of the M.Sc. Degree
(Entomology)**

By

Rasha Farid Emam Ahmed Sokker

B.Sc. Science (Entomology), Ain Shams University, 2004.

Supervisors:

Prof. Dr. Mohamed Adel Hussein: Professor of Entomology, Entomology Department, Faculty of Science, Ain Shams University.

Prof. Dr. Ragaa Kotb Abdel Gaber Hamed: Professor of Entomology, Entomology Department, Faculty of science, Ain Shams University.

Prof. Dr. Salwa Mostafa Saied Ahmed: Professor of Agricultural Stored Grain Pests Research Department, Plant Protection Research Institute, Agricultural Research Center.

Entomology Department

Faculty of Science

Ain Shams University

2013

APPROVAL SHEET

NAME: Rasha Farid Emam Ahmed Sokker

**TITLE: Effect of Some Natural Products on The
Cowpea Seed Beetle, *Callosobruchus
maculatus* (F.) (Coleoptera : Bruchidae)**

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree
of Master

In

ENTOMOLOGY

Department of Entomology

Faculty of Science

Ain-Shams University

2013

Approved By:

Prof. Dr. Zenab Hassan El Besheir

Department of Entomology, Faculty of Science, Zagazig University

Prof. Dr. Aly Ahmad Younes

Department of Entomology, Faculty of Science, Cairo University

Prof. Dr. Mohamed Adel Hussein

Department of Entomology, Faculty of Science, Ain Shams University.

Prof. Dr. Ragaa Kotb Abdel Gaber Hamed

Department of Entomology, Faculty of Science, Ain Shams University.

Date: / / 2013

**TITLE:Effect of Some Natural Products on The
Cowpea Seed Beetle, *Callosobruchus maculatus*
(F.) (Coleoptera : Bruchidae)**

NAME: Rasha Farid Emam Ahmed Sokker

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree
of Master

In

ENTOMOLOGY

Department of Entomology

Faculty of Science

Ain-Shams University

2013

Supervision Committee:

Prof. Dr. Mohamed Adel Hussein: Professor of Entomology,
Entomology Department, Faculty of Science, Ain Shams University.

Prof. Dr. Ragaa Kotb Abdel Gaber Hamed: Professor of
Entomology, Entomology Department, Faculty of science, Ain
Sgams University.

Prof. Dr. Salwa Mostafa Saied Ahmed: Professor of Agricultural
Stored Grain Pests Research Department, Plant Protection Research
Institute, Agricultural Research Center.

ABSTRACT

The present work was carried out to evaluate the toxicity of katel-sous dust, clove, cinnamon and dill seed powders and the combinations of the three powders with katel-sous dust. Evaluation has been conducted on *Callosobruchus maculatus* adults to determine the toxicological effect, residual effect of these products on the cowpea seeds, the changes in insect biology caused by these products, some biochemical studies and seed viability after treatment with the tested products. The results referred to the availability to use these products to control cowpea seed beetle without affect the seeds viability and consider clove powder as spiracle-blocking insecticides. The tested products and its combinations made a sharp reduction in the number of eggs laid and the number of adults emerged. Mixing clove powder with katel-sous dust was helping in using katel-sous dust in pest management without any problems such as the large amount of material (high concentration) needed to obtain commercial kill of insect pest which make us suffered from the expensive costs and hardly remove the dust.

Key words: Katel-sous dust, Clove powder, Cinnamon powder, Dill seed powder, Control, Bruchids, Coleoptera, *Callosobruchus maculatus*, Cowpea seeds beetle, Biology and Scanning.

Contents

INTRODUCTION.....	1
REVIEW OF LITERATURES.....	5
1. Toxicological evaluation of natural products efficacy against some coleopteran insects	5
2. Residual activity of the natural products on the seeds and grains after different periods.....	11
3. The effect of natural products on the biological aspects of some stored grain pests.....	14
4. Biochemical studies on insects after treatment with natural products.....	28
5. Scanning electron microscope studies.....	33
6. Effect of the natural products on the seeds viability.....	34
7. The combination between the natural grain protectants.....	37
MATERIALS AND METHODS.....	42
1. Insect Culture.....	42
2. Types and sources of plant materials used for controlling the cowpea seed beetle, <i>Callosobruchus maculatus</i>	44
3. Toxicological evaluation of the tested treatments and mixtures on <i>Callosobruchus maculatus</i> adults.....	46
4. Experimental design and analysis of data.....	47
5. Residual activity of the tested treatments and mixtures on the cowpea seeds after different periods.....	50
6. Effect of katel-sous dust, clove, cinnamon, dill seeds powder and mixtures on some biological aspects of <i>Callosobruchus maculatus</i>	51
6.1. Number of eggs, hatchability and oviposition.....	51
6.2. Percentage adult emergence.....	53

7. Biochemical studies on the <i>Callosobruchus maculatus</i> adults after treatment with the four tested natural products	54
7.1. Determination of the main metabolites in the adults <i>Callosobruchus maculatus</i> after treatment with the four tested natural products.....	55
7.1.1. Total protein.....	55
7.1.2. Total carbohydrates.....	56
7.1.3. Total lipids.....	57
7.2. Determination of the phosphatases activity in the <i>Callosobruchus maculatus</i> adults after treatment with the four tested natural products.....	59
7.2.1. Acid phosphatase activity.....	59
7.2.2. Alkaline phosphatase activity.....	60
7.3. Determination of the acetylcholinesterase activity (AChE) in the adults <i>Callosobruchus maculatus</i> after treatment with the four tested natural products.....	60
8. Scanning electron microscope study on spiracles of <i>Callosobruchus maculatus</i> adults after clove powder treatment.....	61
9. Effect of the katel-sous dust, clove, cinnamon, dill seeds powders and their mixtures on cowpea seeds viability.....	65
9.1. Seeds germination.....	65
9.2. Water absorption.....	68
10. Statistical analysis.....	69
RESULTS	71
1. Toxicological evaluation of the tested treatments and mixtures on <i>Callosobruchus maculatus</i> adults	71
2. Residual activity of the tested treatments and mixtures on the cowpea seeds after different periods	79
2.1. Residual activity of katel-sous dust.....	79

2.2. Residual activity of clove powder.....	79
2.3. Residual activity of cinnamon powder.....	79
2.4. Residual activity of dill seeds powder.....	80
2.5. Residual activity of the mixtures of katel-sous dust and clove powder (Substance no. 1)	86
2.6. Residual activity of the mixtures of katel-sous dust and cinnamon powder (Substance no. 2)	91
2.7. Residual activity of the mixtures of katel-sous dust and dill seeds powder (Substance no. 3)	96
3. Effect of the tested treatments and mixtures on some biological aspects of <i>Callosobruchus maculatus</i>	103
3.1. Number of eggs, hatchability and oviposition.....	103
3.2. Percentage adult emergence.....	104
4. Biochemical studies.....	111
4.1. Determination of the main metabolites in the adults <i>Callosobruchus maculatus</i> after treatment with the four tested natural products.....	111
4.2. Determination of the phosphatases activity in <i>Callosobruchus maculatus</i> adults after treatment with the four tested natural products.....	114
4.2.1. Acid phosphatase activity.....	114
4.2.2. Alkaline phosphatase activity.....	115
4.3. Determination of the acetyl cholinesterase activity (AChE) in <i>Callosobruchus maculatus</i> adults after treatment with the four tested natural products.....	116
5. Scanning electron microscope study on spiracles of <i>Callosobruchus maculatus</i> adults after clove powder treatment.....	118
6. Effect of the tested treatments and mixtures on cowpea seeds viability	123
6.1. Seeds germination.....	123

6.2. Water absorption.....	129
DISCUSSION	136
REFERENCES	158
SUMMARY	187
ARABIC SUMMARY	

List of Tables

1. Plants used in the present work.....	44
2. Mixture concentrations between katel- sous and the other three tested powders.....	49
3. Percentage mortality of <i>Callosobruchus maculatus</i> adults treated with different natural products treatments	72
4. Toxicological evaluation of tested natural products after two days of exposure against <i>Callosobruchus maculatus</i> adults...	74
5. Percentages corrected mortality of <i>Callosobruchus maculatus</i> adults treated with different mixtures of natural powders with katel-sous at different concentrations.....	77
6. Percentage mortality of <i>Callosobruchus maculatus</i> adults exposed to cowpea seeds treated with LC ₉₅ of clove powder after different periods.....	81
7. Percentage mortality of <i>Callosobruchus maculatus</i> adults exposed to cowpea seeds treated with LC ₉₅ of cinnamon Powder after different periods.....	82
8. Percentage mortality of <i>Callosobruchus maculatus</i> adults exposed to cowpea seeds treated with LC ₉₅ of dill seeds powder after different periods.....	83
9. Percentage mortality of <i>Callosobruchus maculatus</i> adults exposed to cowpea seeds treated with mixture no. (1) [LC ₂₅ katel-sous+ LC ₇₅ clove] after different periods.....	87
10. Percentage mortality of <i>Callosobruchus maculatus</i> adults exposed to cowpea seeds treated with mixture no. (2) [LC ₅₀ katel-sous+ LC ₅₀ clove] after different periods.....	88

11. Corrected percentage mortality of <i>Callosobruchus maculatus</i> adults exposed to cowpea seeds treated with mixture no. (4) [LC ₂₅ katel-sous+ LC ₇₅ cinnamon] after different periods.....	92
12. Percentage mortality of <i>Callosobruchus maculatus</i> adults exposed to cowpea seeds treated with mixture no. (5) [LC ₅₀ katel-sous+ LC ₅₀ cinnamon] after different periods.....	93
13. Percentage mortality of <i>Callosobruchus maculatus</i> adults exposed to cowpea seeds treated with mixture no. (7) [LC ₂₅ katel-sous+ LC ₇₅ dill seeds powder] after different periods.....	97
14. Percentage mortality of <i>Callosobruchus maculatus</i> adults exposed to cowpea seeds treated with mixture no. (8) [LC ₅₀ katel-sous + LC ₅₀ dill seeds powder] after different periods...	98
15. Percentage mortality of <i>Callosobruchus maculatus</i> adults exposed to cowpea seeds treated with all tested treatments after different storage periods: (Residual activity).....	102
16. Efficacy of the tested plant powders on the oviposition and hatchability of eggs of <i>Callosobruchus maculatus</i> adults.....	105
17. Efficacy of the tested mixtures on percentage oviposition and hatchability of <i>Callosobruchus maculatus</i> adults.....	106
18. Efficacy of the tested plant powders on percentage adult emergence of <i>Callosobruchus maculatus</i>	108
19. Efficacy of the tested mixtures on the percentage adult emergence of <i>Callosobruchus maculatus</i> adults.....	109
20. Determination of the main metabolites in the insect's body after treatment with the tested natural products.....	112
21. Determination of phosphatases in <i>Callosobruchus maculatus</i> adults after the tested natural products treatment.....	115

22. Determination of Acetyl cholinesterase activity in <i>Callosobruchus maculatus</i> adults after treating with the tested natural products.....	117
23. Effect of the four tested natural products on cowpea seeds germination at initial treatment.....	124
24. Effect of the four tested natural products on cowpea seeds germination after storage period (90 days).....	125
25. Germination efficacy of cowpea seeds treated with the tested mixtures (at initial time & after storage period).....	127
26. Water absorption of cowpea seeds treated with the tested natural products (at initial time of treatment).....	130
27. Water absorption of cowpea seeds treated with the tested natural products (after storage period).....	131
28. Efficacy of water absorption of cowpea seeds treated with the tested mixtures at initial treatment.....	133
29. Efficacy of water absorption of cowpea seeds treated with the tested mixtures (after storage period).....	134

List of Figures

1 a. Male of <i>Callosobruchus maculatus</i> adult.....	43
1 b. Female of <i>Callosobruchus maculatus</i> adult.....	43
2. Insect Culture.....	43
3. Clove.....	45
4. Dill Seeds.....	45
5. Cinnamon.....	46
6. Hatched and unhatched eggs.....	52
7. Adult emergence and emergence holes.....	54
8. A typical SEM instrument, showing the electron column, sample chamber, EDS detector, electronics console, and visual display monitors.....	64
9. A SEM samples are coated with a very thin layer of gold by a machine called a sputter coater.....	64
10. The sample is placed inside the microscope's vacuum column through an air-tight door.....	65
11. Preparation of seeds germination.....	67
12. Water absorption of seeds.....	69
13. Percentage mortality of <i>C. maculatus</i> at different concentrations of the four tested products.....	73
14. Concentration /mortality regression lines for adults of <i>C.</i> <i>maculatus</i> treatd with tested products.....	75
15. LC ₅₀ values of the tested products.....	75
16. Percentage mortality of <i>Callosobruchus maculatus</i> adults (after two days of exposure) caused by the tested mixtures.....	78

17. Percentage mortality of <i>C. maculatus</i> adults treated with clove powder after different periods of storage.....	84
18. Percentage mortality of <i>C. maculatus</i> adults treated with cinnamon powder after different periods of storage	84
19. Percentage mortality of <i>C. maculatus</i> adults treated with dill seeds powder after different periods of storage	85
20. Comparison between the percentage mortality of <i>C. maculatus</i> adults induced after 14 days of treatment by the four tested products.....	85
21. Percentage mortality of <i>C. maculatus</i> adults treated with mixture number one after different periods of storage.....	89
22. Percentage mortality of <i>C. maculatus</i> adults treated with mixture number two after different periods of storage.....	89
23. Comparison between the percentage mortality of <i>C. maculatus</i> adults after 22 days of storage period after treatment with the three mixtures of katel-sous with clove (Substance no. 1).....	90
24. Percentage mortality of <i>C. maculatus</i> adults treated with mixture number four after different periods of storage.....	94
25. Percentage mortality of <i>C. maculatus</i> adults treated with mixture number five after different periods of storage.....	94
26. Comparison between the percentage mortality of <i>C. maculatus</i> adults after 22 days of storage period after treatment with the three mixtures of katel-sous percentage with cinnamon (Substance no. 2)	95
27. Percentage mortality of <i>C. maculatus</i> adults treated with mixture number seven after different periods of storage.....	99
28. Percentage mortality of <i>C. maculatus</i> adults treated with mixture number eight after different periods of storage.....	99

29. Comparison between the percentage mortality of <i>C. maculatus</i> adults after 16 days of percentage treatment with the three mixtures of katel-sous dust with dill seeds powder (sub. 3).....	100
30. Comparison between the persistence periods of the three groups of mixtures (Sub. 1, 2 and 3).....	101
31. Comparison between the percentage mortality of <i>C. maculatus</i> adults treated with the all tested mixtures after 16 days of storage.....	101
32. Values of total number of eggs laid by one female of <i>C. maculatus</i> adults treated with LC ₅₀ & LC ₉₅ of the tested products after 10 days of mating.....	107
33. Values of total number of eggs laid by one female of <i>C. maculatus</i> adults treated with tested mixtures after 10 days of mating.....	107
34. Total number of emerged adults of <i>Callosobruchus maculatus</i> treated with LC ₅₀ & LC ₉₅ of the four tested products after 10 days of mating.....	110
35. Total number of emerged adults of <i>Callosobruchus maculatus</i> treated with the nine tested mixtures after 10 days of mating ...	110
36. Total protein content in <i>Callosobruchus maculatus</i> adults treated with the tested natural products.....	113
37. Total carbohydrates content in <i>Callosobruchus maculatus</i> adults after treatment with the tested natural products.....	113
38. Total lipids content in the body of the adults of the tested insect <i>C. maculatus</i> treated with the tested natural products...	114
39. Phosphatase activity in the body of <i>C. maculatus</i> adults after treatment with the tested natural products.....	116
40. Acetyl cholinesterase activity in the adults of <i>Callosobruchus maculatus</i> treated with the tested products.....	118

41. The scanning electron microscope (SEM) showing detailed 3-dimensional images showing the normal spiracles on the abdomen of the untreated <i>Callosobruchus maculatus</i> adults...	120
42. The scanning electron microscope (SEM) showing detailed 3-dimensional images showing the spiracles, spiracle openings on the abdomen and the cilia which surround the spiracle openings of untreated <i>Callosobruchus maculatus</i> adults.....	121
43. The scanning electron microscope (SEM) showing detailed 3-dimensional images showing the closed spiracles, spiracle openings on the abdomen and the cilia which surround the spiracle openings of <i>Callosobruchus maculatus</i> adults after treatment with clove powder.....	122
44. Effect of the four tested natural products on cowpea seeds germination at initial treatment and after storage period (90 days).....	126
45. Germination efficacy of cowpea seeds after treated with the tested mixtures at initial treatment and after storage period.....	128
46. The effect of all treatments on germination percentages of cowpea seeds at initial time and after treatment.....	128
47. Comparison between percentage water absorption of cowpea seeds after treatment with the tested natural products at initial and after storage time.....	132
48. Comparison between percentage water absorption of cowpea seeds after treatment with the tested mixtures at initial and after storage time.....	135