



Kafr El-Sheikh University
Faculty of Agriculture
Pesticides Chemistry and Toxicology Dept.

**NEW APPROACHES FOR STORED GRAIN
INSECTS CONTROL**

By

Ahmed Fayez Eid Omar

B. Sc. Agric. (Pesticides), 2006

Faculty of Agriculture, Kafr El-Sheikh University

M. Sc. (pesticides). Fac. of Agric. Menufiya Univ., 2012

THESIS

Submitted in partial fulfillment of the requirement
for the degree of

DOCTOR OF PHILOSOPHY

In

**AGRICULTURAL SCIENCE
(PESTICIDES)**

Pesticides Chemistry and Toxicology Department
Faculty of Agriculture
Kafr El-Sheikh University

2017



Kafr El-Sheikh University
Faculty of Agriculture
Pesticides Chemistry and Toxicology Dept.

NEW APPROACHES FOR CONTROLLING STORED GRAIN INSECTS.

By

Ahmed Fayez Eid Omar

B. Sc. Agric. (Pesticides), 2006

M. Sc. (pesticides). Fac. of Agric. Menufiya Univ., 2012

Supervision Committee:

Prof. Dr. Ahmed EL-Sayed Ahmed Salama

Emeritus Professor of Pesticides Chemistry and Toxicology
Faculty of Agriculture, Kafr El-Sheikh University

Prof. Dr. Attiah Youssef Keratum

Emeritus Professor of Pesticides Chemistry and Toxicology
Faculty of Agriculture, Kafr El-Sheikh University

Dr. Hesham Moustafa M. El-Zun

Senior Researcher, Plant Protection Research Institute,
Agricultural Research Center, Dokki

2017

CONTENTS

Subject	Page
INTRODUCTION	1
REVIEW OF LITERATURE	4
1. Background.	4
2. Bioactivity of the essential oils and their nano-emulsions against grain storage insects..	5
3. Nanotechnology in agriculture and management of insect pests.	13
4. Role of Natural products in protection of grain storage insects..	16
5. Role of pesticides in stored product protection.	19
6. Toxicological Study.	22
MATERIALS AND METHODS	27
1. Tested insect	27
2. Rearing of test insects	27
3. Tested Animals	27
4. Materials used	27
4.1 Essential oils	27
4.2 Essential oils nanoparticales (EO-NPs)	27
4.3 Silica nanoparticles	28
4.4 Essential oil nanoemulsion	30
4.5 Nano SiO₂ water suspensions.	30
4.6 Natural products	30
4.6.1. Garlic powder	30
4.6.2. Lemongrass powder	30
4.7 insecticides	31
4.7.1. Chlorpyrifos 2%	31
4.7.2. Deltadust® insecticide (Finito)	31
5. Biological activity of essential oils (EOs) against adults of <i>Rhyzopertha dominica</i> and <i>Tribolium castaneum</i>	32
6. Biological activity of EOs-NPs against adults of <i>Rhyzopertha dominica</i> and <i>Tribolium castaneum</i>.	32
7. Toxicity assessment of silica nanoparticles dust against adults of <i>Rhyzopertha dominica</i> and <i>Tribolium castaneum</i>	33
8 Insecticidal activity of essential oil nanoemulsions against <i>Rhyzopertha dominica</i> and <i>Tribolium castaneum</i>	33
9. Insecticidal activity of sio₂ nanoparticles water suspensions against adults of <i>Rhyzopertha dominica</i> and <i>Tribolium castaneum</i>	33
10. Insecticidal effect of plant derived powders on <i>Rhyzopertha dominica</i> and <i>Tribolium castaneum</i>	34
11. Toxicity bioassay of Chlorpyrifos 2 % and Deltamethrin 0.5 % powder insecticide on <i>Rhyzopertha dominica</i> and <i>Tribolium castaneum</i>	34

12. Effect of the tested materials on F1 progeny of <i>Rhyzopertha dominica</i> and <i>Tribolium castaneum</i>	34
13. Residual activity of the tested materials against <i>Rhyzopertha dominica</i> and <i>Tribolium castaneum</i>	35
14. Chemical analysis and identification of essential oils	35
15. Toxicity assessment of tested materials	35
15.1. Repeated dose 30-day dietary toxicity test followed by 10-day recovery Period.	35
15.2. Clinical observation and body weight	36
15.3. Biochemistry determinations	36
15.4. Serum uric acid assay.	37
15.5. Serum creatinine assay	38
15.6. Estimation of total protein	38
15.7. Estimation of albumin	39
15.8. Assessment of acetyl cholinesterase activity.	40
15.9. Assessment of aspartate aminotransferase (AST) activity	41
15.10. Estimation of alanine aminotransferase (ALT)	42
15.11. Estimation of alkaline phosphatase (ALP)	43
16. Histopathological assay	44
17. Statistical analysis.	44
RESULTS AND DISCUSSION	45
1. Insecticidal activity of essential oils .	45
2. Biological activity of EOs-NPs of geranium, basil oil and silica nanoparticles	48
3. Insecticidal activity of geranium, basil oil nanoemulsions and SiO₂ nanosuspension.	51
4. Insecticidal effect of plant derived powders.	56
5. Insecticidal effect of tested pesticides	59
6. Chemical constituents of the essential oils	62
7. Effect on F1 progeny	63
7.1. Activity of essential oils	63
7.2. Activity of nanoformulation	64
7.3. Activity of plant powder on F1 progeny.	67
7.4. Activity of tested insecticides	69
8. Residual activity of the tested materials	70
8.1. Residual activity of insecticides	70
8.2. Residual activity of geranium, basil oil and its nanoformulation	71
8.3. Residual activity of silica nanoparticles and sio₂ suspensions	73
8.4. Residual activity of plant powders	74

9. Toxicological study	75
9.1. Effect of 30 days dietary treatment followed by 10 days feeding on untreated diet on weight of rats and weight of some internal organs	75
9.2. Studies on serum biochemical component	79
9.2.1. Uric acid and creatinine	79
9.2.2 Total protein, albumin and acetylcholine esterase	82
9.2.3. Enzymatic activity of serum ALT, AST and ALP	87
10. Histopathological changes in liver and kidney of albino rats after 30 days subacute dietary	99
10.1. Liver histopathology.	99
10.2. Kidney histopathology	102
11. Histopathological changes in liver and kidney of albino rats after 10 days post exposure	108
11.1. Liver histopathology	108
11.2. Kidney histopathology	119
SUMMARY.	122
REFERENCES CITED.	129
ARABIC SUMMARY.	

List of Tables

<i>No</i>	<i>Title</i>	<i>Page</i>
1.	Insecticidal effects of geranium oil, <i>Pelargonium graveolens</i> and basil oil <i>Ocimum basilicum</i> on <i>Tribolium castaneum</i> adults after 3, 5 and 7 days of treatment	46
2.	Insecticidal effects of geranium oil, <i>Pelargonium graveolens</i> and basil oil, <i>Ocimum basilicum</i> , on <i>Rhyzopertha dominica</i> adults after 1, 3 and 5 days of treatment.	47
3.	Insecticidal effects of silica nanoparticles, EO-NPs of geranium and basil oils on <i>Tribolium castaneum</i> 3,5and 7days after treatment.	50
4.	Insecticidal effects of silica nanoparticles, EO-NPs of geranium and basil oils on <i>Rhyzopertha dominica</i> adults 1, 3and 5 after treatment.	51
5.	Insecticidal effects of SiO ₂ water suspension, geranium and basil oil nanoemulsion on <i>Tribolium castaneum</i> adults 3, 5 and 7 days after treatment	54
6.	Insec Insecticidal effects of SiO ₂ water suspension, geranium and basil oil nanoemulsions on <i>Rhyzopertha dominica</i> adults 1, 3 and 5 days after treatment	55
7.	In insecticidal effects of lemon grass and garlic powders on <i>Tribolium castaneum</i> adults after 3, 5and 7days of treatment.	57
.8	Insecticidal effects of lemon grass and garlic powders on <i>Rhyzopertha dominica</i> adults 1, 3and 5 of treatment.	58
9.	Insecticidal effects of chlorpyrifos 2% and deltamethrin0.5% powders on <i>Tribolium castaneum</i> adults one, two and three days after treatment	60
10.	Insecticidal effects of chlorpyrifos 2% and deltamethrin 0.5% powders on <i>Rhyzopertha dominica</i> adults one and two days' post- treatment.	61
11.	Chemical composition of geranium, <i>Pelargonium graveolens</i> (Herb), and basil, <i>Ocimum basilicum</i> (Herb) essential oils.	62
12.	Efficacy of geranium oil on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adult	64
13.	Efficacy of basil oil on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adult	64
14.	Efficacy of geranium oil nanoemulsion on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adult	65
15.	Efficacy of basil oil nanoemulsion on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adult	65
16.	Efficacy of nano SiO ₂ water suspension on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adults	66
17.	Efficacy of EO-NPs of geranium oil on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adults	66
18.	Efficacy of EO-NPs of basil oil on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adults.	66
19.	Efficacy of silica nanoparticles on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adults	67

20.	Efficacy of lemon grass powder on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adults	68
21.	Efficacy of garlic powder on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adults	68
22.	Efficacy of chlorpyrifos 2% powder on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adults	69
23.	Efficacy of deltamethrin 0.5% powder on the F1 emergence of <i>Rhizopertha dominica</i> and <i>Tribolium castaneum</i> adults	70
24.	Residual activity of chlorpyrifos 2% powder and deltamethrin 0.5% powder against <i>Tribolium castaneum</i> and <i>Rhizopertha dominica</i> adults after different periods of storage	71
25.	Residual activity of geranium oil, EO-NPs of geranium and geranium oil nanoemulsion against <i>Tribolium castaneum</i> and <i>Rhizopertha dominica</i> adults after different periods of storage	71
26.	Residual activity of basil oil, EO-NPs of basil and basil oil nanoemulsion against <i>Tribolium castaneum</i> and <i>Rhizopertha dominica</i> adults after different periods of storage	72
27.	Residual activity of silica nanoparticles and nano silica suspension against <i>Tribolium castaneum</i> and <i>Rhizopertha dominica</i> adults after different periods of storage	73
28.	Residual activity of lemon grass powder and garlic powder against <i>Tribolium castaneum</i> and <i>Rhizopertha dominica</i> adults after different periods of storage.	75
29.	Mean values of body weights of male albino rats fed for 30 days on diet treated with LC ₉₀ of chlorpyrifos 2%, deltamethrin 0.5% powder, EO-NPs of geranium and Nano-silica powder followed by 10 days post exposure period.	75
30.	Mean weights of liver and kidney from albino rat males fed for 30 days on diet treated with LC ₉₀ of chlorpyrifos 2% powder, deltamethrin 0.5% powder, EO-NPs of geranium and nano silica Powder followed by 10 days post exposure period.	77
31.	Uric acid and creatinine levels in serum from albino rats male fed for 30 days on diet treated with LC ₉₀ of chlorpyrifos powder 2%, deltamethrin 0.5% powder, EO-NPs of geranium and nano-silica powder followed by 10 days post exposure period.	80
32.	Total protein, albumin and acetylcholinesterase (AChE) levels in serum from albino rats male fed for 30 day on diet treated with LC ₉₀ of Chlorpyrifos powder, deltamethrin 0.5% powder, EO-NPs of geranium and nano- silica powder followed by 10 days post exposure period.	84
33.	Serum biochemical parameters in male albino rats fed for 30 days on diet treated with LC ₉₀ of chlorpyrifos 2% powder, deltamethrin 0.5% powder, EO-NPs of geranium and nano silica powder followed by 10 days post exposure period.	89

List of Figures

No.	Title	Page
1.	X-ray diffraction pattern of Silica nanoparticles	29
2.	Average initial and final body weight of rats treated with LC ₉₀ of the tested materials for 30 days treatment followed by 10 days post exposure period.	76
3.	Average Liver weight of rats treated with LC ₉₀ of the tested materials for 30 days treatment followed by 10 days post exposure period.	77
4.	Average Kidney weight of rats treated with LC ₉₀ of the tested materials for 30 days of treatment followed by 10 days post exposure period.	78
5.	Uric acid levels in serum of rats from groups treated with LC ₉₀ of the tested materials for 30 of day's treatment followed by 10 days post exposure period.	81
6.	Creatinine level in serum of rats from groups treated with LC ₉₀ of the tested materials for 30 days treatment followed by 10 days post exposure period.	81
7.	Total protein level in serum of rats group treated with LC ₉₀ of the tested materials for 30 days treatment followed by 10 days post exposure period.	84
8.	Albumin level in serum of rats from group treated with LC ₉₀ of the tested materials for 30 days treatment followed by 10 days post exposure period.	85
9.	Acetyl cholinesterase level in serum of rats from group treated with LC ₉₀ of the tested materials for 30 days treatment followed by 10 days post exposure period.	85
10.	ALT (alanine aminotransferase) level in serum of rats from group treated with LC ₉₀ of the tested materials for 30 days treatment followed by 10 days post exposure period.	89
11.	AST (aspartate aminotransferase) level in serum of rats from groups treated with LC ₉₀ of the tested materials for 30 days treatment followed by 10 days post exposure period.	90
12.	Alkaline phosphatase(ALP) level in serum of rats from groups treated with LC ₉₀ of the tested materials for 30 days treatment followed by 10 days post exposure period.	90
13.	Liver of rat from control group (H & E X 400).	94
14.	Liver of rat from control group (H & E X 400).	95
15.	Liver of rats exposed to LC ₉₀ of chlorpyrifos 2% Powder for 30 day dietary. (H & E X 400).	96
16.	Liver of rats exposed to LC ₉₀ of chlorpyrifos 2% powder for 30 day dietary. (H & E X 400).	96
17.	Liver of rats exposed to LC ₉₀ of chlorpyrifos 2% powder for 30 day dietary. (H & E X 400).	97
18.	Liver of rats exposed to LC ₉₀ of deltamethrin 0.5% powder for 30 day dietary (H & E X 400).	98
19.	Liver of rats exposed to LC ₉₀ of deltamethrin 0.5% powder for 30 day dietary (H & E X 400).	98
20.	Liver of rats exposed to LC ₉₀ of deltamethrin 0.5% powder for 30 day dietary (H & E X 400).	98
21.	Liver of rats exposed to LC ₉₀ of deltamethrin 0.5% powder for 30 day dietary (H & E X 400).	98
22.	Liver of rats exposed to LC ₉₀ of EO-NPs of geranium for 30 days dietary.(H & E X 400)	99

No.	Title	Page
23.	Liver of rats exposed to LC ₉₀ of EO-NPs of geranium for 30 days dietary.((H & E X 400)	100
24	Liver of rats exposed to LC ₉₀ of EO-NPs of geranium for 30 days dietary (H & E X 400).	100
25.	Liver of rats exposed to LC ₉₀ of silica nanoparticles for 30 days dietary (H & E X 400)	101
26.	Liver of rats exposed to LC ₉₀ of silica nanoparticles for 30 days dietary (H & E X 400).	101
27.	7 Liver of rats exposed to LC ₉₀ of silica nanoparticles for 30 days dietary (H & E X 400).	101
28.	Kidney rats from control group showing the normal histological structure of renal parenchyma (H & E X 400).	103
29.	Kidney of rats from group control showing the normal histological structure of renal parenchyma (glomerulus of Bowman capsules and renal tubules) ((H & E X 400).	103
30.	Kidney of rats exposed to LC ₉₀ of chlorpyrifos 2% powder for 30 days dietary (H & E X 400).	104
31.	Kidney of rats exposed to LC ₉₀ of chlorpyrifos 2% powder for 30 days dietary (H & E X 400).	104
32.	Kidney of rats exposed to LC ₉₀ of chlorpyrifos 2% powder for 30 days dietary (H & E X 400).	104
33.	Kidney of rats exposed to LC ₉₀ of deltamethrin 0.5% powder for 30 days dietary (H & E X 400).	105
34.	Kidney of rats exposed to LC ₉₀ of deltamethrin 0.5% powder for 30 days dietary (H & E X 400).	105
35.	Kidney of rats exposed to LC ₉₀ of EO-NPS of geranium for 30 days dietary (H & E X 400).	106
36.	Kidney of rats exposed to LC ₉₀ of EO-NPS of geranium for 30 days dietary (H & E X 400).	106
37.	Kidney of rats exposed to LC ₉₀ of EO-NPS of geranium for 30 days dietary (H & EX 400).	107
38.	Kidney of rats exposed to LC ₉₀ of silica-nanoparticles for 30 days dietary (H & E X 400).	107
39.	Kidney of rats exposed to LC ₉₀ of silica-nanoparticles for 30 days dietary (H & E X 400).	107
40.	Kidney of rats exposed to LC ₉₀ of silica-nanoparticles for 30 days dietary (H & E X 400).	108
41.	Liver of rats from Chlorpyrifos 2% group feeding untreated diet for 10 days post exposure (H & E X 400).	109
42.	Liver of rats from Chlorpyrifos 2% group feeding untreated diet for 10 days post exposure (H & E X 400).	109
43.	Liver of rats from Chlorpyrifos 2% group feeding untreated diet for 10 days post exposure H & E X 400).	109
44.	Liver of rats from deltamethrin0.5% group feeding untreated diet for 10 days post exposure (H & E X 400).	110
45.	Liver of rats from deltamethrin0.5% group feeding untreated diet for 10 days post exposure (H & E X 400).	110

<i>No.</i>	<i>Title</i>	<i>Page</i>
46.	Liver of rats from EO-NPs of geranium group feeding untreated diet for 10 days post exposure (H & E X 400).	111
47.	Liver of rats from EO-NPs of geranium group feeding untreated diet for 10 days post exposure (H & E X 400).	111
48.	Liver of rats from EO-NPs of geranium group feeding untreated diet for 10 days post exposure (H & E X 400).	111
49.	Liver of rats from nano silica powder group feeding untreated diet for 10 days post exposure (H & E X 400).	113
50.	Liver of rats from nano silica powder group feeding untreated diet for 10 days post exposure (H & E X 400).	113
51.	Liver of rats from nano silica powder group feeding untreated diet for 10 days post exposure (H & E X 400).	113
52.	Liver of rats from nano silica powder group feeding untreated diet for 10 days post exposure (H & E X 400).	114
53.	Kidney rats from chlorpyrifos 2% group feeding untreated diet for 10 days post exposure (H & E X400).	115
54.	Kidney rats from chlorpyrifos 2% group feeding untreated diet for 10 days post exposure (H & E X400).	115
55.	Kidney rats from chlorpyrifos 2% group feeding untreated diet for 10 days post exposure (H & E X400).	115
56.	Kidney rats from chlorpyrifos 2% group feeding untreated diet for 10 days post exposure (H & E X400).	116
57.	Kidney rats from deltamethrin 0.5% group feeding on untreated diet for 10 days post exposure (H & E X400)	116
58.	Kidney rats from deltamethrin 0.5% group feeding untreated diet for 10 days post exposure (H & E X400).	116
59.	Kidney rats from deltamethrin 0.5% group feeding on untreated diet for 10 days post exposure (H & E X400).	117
60.	Kidney rats from deltamethrin 0.5% group feeding on untreated diet for 10 days post exposure (H & E X400).	117
61.	Kidney rats from EO-NPs of geranium group feeding on untreated diet for 10 days post exposure (H & E X400).	117
62.	Kidney rats from EO-NPs of geranium group feeding on untreated diet for 10 days post exposure (H & E X400)	118
63.	Kidney rats from EO-NPs of geranium group feeding on untreated diet for 10 days post exposure (H & E X400).	118
64.	Kidney rats from silica nanoparticles group feeding on untreated diet for 10 days post exposure (H & E X400).	118
65.	Kidney rats from silica nanoparticles group feeding untreated diet for 10 days post exposure (H & E X400).	119

ABSTRACT

In the present study, twelve material (two essential oils geranium and basil , their nanoemulsions , EO-NPs of the two oils loaded in poly ethylen glycol 6000 PEG, silica nanoparticles(SNP), nano SiO_2 water suspension, lemon grass powder, garlic powder finally two of pesticides chloropyrifos 2% and deltamethrin 0.5%) were investigated to examine their insecticidal activity against two of the stored product insects *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae).Results indicated that the tested pesticides were the most effective materials followed by silica nanoparticles, EO-NPs and nanoemulsion of the selected essential oils, and nano SiO_2 water suspension while natural powder were the least effective compounds for both tested insects against all stages of the tested insects. Moreover their long residual effect which lasts for 90 days of storage. For test the risk of exposure of both pesticides and nanomaterials, health hazards and safety considerations for such materials should be examined. Male albino rats were dietary exposed to LC_{90} value of the two pesticides, nanosilica and EO-NPs of the geranium oil for 30 days followed by 10 days as recovery period to examine the changes in both liver and kidney function which consider markers in changes of serum, ALT, AST, ALP, T.P, Albumin, Uric Acid, Creatinin and Acetylcholine-esterase. Also Histological analysis revealed the effects of tested materials on liver and kidney functions after 30 days of exposure while the histological analysis after 10 days feeding on normal diet post exposure period revealed that, the serum enzymatic level showed decreasing than the treated period also histological changes showed activated kupffer cells (KCs) phagocytic cells in liver which have the ability of elimination of foreign materials from the body.