



Tanta University  
Faculty of Science  
Zoology of Department

**Antifeedant and repellent properties of natural and synthetic compounds against the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) and the pulse beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae): perception, biological and behavioral contexts.**

**Thesis to faculty of science, Tanta University for the degree of  
Doctor of Philosophy in Zoology (Insects)**

**By**

**Eslam Adel Ali Negm**

**(B.Sc., Al Azhar University, 2006)**

**(M.sc., Tanta University, 2015)**

## **Supervisors**

*Prof. Dr.*

**Amal Ibrahim Seif**

**Prof. of Entomol., Zoology Dept.,  
Fac. of Sci., Tanta Univ**

*Prof. Dr.*

**Raafat Badr Abo-Arab**

**Senior Researcher of Pesticides,  
Plant Prot. Res. Inst., ARC, Egypt**

*Dr.*

**Mervat Rafick Abou Seada**

**Assistant prof., Zoology Dept.,**

**Fac. of Sci, Tanta Univ.**

**2019**

**Antifeedant and repellent properties of natural and synthetic compounds against the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) and the pulse beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae): perception, biological and behavioral contexts.**

**Abstract**

The objective of this study was to evaluate the potential of four commercially available essential oils (EOs) (cumin, mandarin, marigold and bitter orange) and three oil components (citranellol, geraniol and linalool) and one insecticide (Deltamethrin) against two of the most common stored grain pests in storage facilities in Egypt; the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and the pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). In addition, the purpose of this study was to compare the antennal morphology, types of sensilla, and their distribution of *C. maculatus* and *S. oryzae* in order to better understand the repellent and antifeeding responses of these stored grain pests to botanical insecticides. The chemical composition of EOs was determined by gas chromatography/ mass spectrometry (GC-MS). Results obtained showed that, principal compounds of bitter orange, mandarin, cumin and marigold EOs were limonene (85.47%), tricarbonyl [methyl 6-vinylidenehepta 2,4-dien-1-oate]iron (52.8%), propanal (26.07%) and  $\alpha$ -terthienyl (8.79%), respectively. Deltamethrin possessed the highest contact toxicity against both insects when compared with the other EOs. Mandarin and bitter orange EOs were the most toxic against *S. oryzae* after two and five days post exposure with LC<sub>50</sub> values of 8974 and 1752.7 ppm, respectively. Bitter orange and mandarin EOs exhibited highest contact toxicity against *C. maculatus* after two and five days post exposure with LC<sub>50</sub> values of 1138.5 and 14 ppm, respectively. Combined mixtures of EOs were more toxic to *S. oryzae* and *C. maculatus* than single EO treatments. The monoterpenoid geraniol was the most toxic compared to either linalool or citronellol five days post exposure of *C. maculatus* and *S. oryzae*. All EOs inhibited the emerged progeny and reduced the seed weight loss. Bitter orange EO and the monoterpenoid citronellol at the highest concentration showed the strongest antifeedant effect on *S. oryzae*. The oil components had the highest repellent activity against both *S. oryzae* and *C. maculatus* than that of crude EOs. Percent of repellent increased with the increasing of concentration through the period of experiment, treated grain bioassay obviously demonstrated higher repellent activity than filter paper bioassay. Three

types of sensilla were recognized on the antennae of *C. maculatus* including sensilla trichodea, Böhm bristles (BB) and sensilla chaetica (Sc). Seven types of sensilla were found on *S. oryzae* antennae including two types of sensilla trichoidea (ST1 and ST2), two types of sensilla chaetica (Sch1 and Sch2), two types of sensilla basiconica (Sb Type 1 and 2) and sensilla gemmiformia.

## CONTENTS

1. INTRODUCTION .....	1
2. LITERATURE REVIEW .....	6
2. 1. Primary coleopteran pests of stored cowpea.....	6
2.2. Primary coleopteran pests of stored rice .....	8
2. 3. Control of stored grain pests.....	10
2.4. Alternative strategies for stored products pest management ....	11
2. 5. Plant essential oils.....	12
2.5.1. Chemical composition of essential oils.....	15
2.5.2. Co-toxicity of essential oils binary mixtures .....	16
2.5.3. Toxicity of essential oils component .....	18
2.5.4. Effect of essential oils on the biology of stored grain pests.	19
2.5.5. Antifeedant properties of essential oils.....	22
2.5.6. Antifeedant activity of essential oil components on stored grain pests .....	24
2.5.7. Repellent activity of essential oils .....	25
2.5.7.1. Repellent activity of essential oils to stored grain.....	26
2.5.7.2. Repellent effect of essential oil components.....	28
2.5.8.The morphology and distribution of antennal sensilla.....	30
3. MATERIALS AND METHODS .....	34
3. 1. Mass culturing of <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> .....	34
3. 2. Tested essential oils .....	34
3. 3. Tested monoterpenoids .....	35
3.4. Tested chemical insecticide .....	35
3. 5. Concentration preparation.....	35
3. 6. Chemical analysis and identification of essential oils: .....	35
3. 7. Grain treatment and contact toxicity bioassays .....	36
3. 8. Co-toxicity of tested oils.....	37
3.9.Effect of essential oils, monoterpenoids and Deltamethrin on the biology of <i>S. oryzae</i> and <i>C. maculatus</i> .....	37
3. 10. Feeding deterrent activity bioassays.....	38
3.11. Repellency bioassays.....	40
3. 12. Scanning electron microscopy.....	42
3. 13. Data analysis.....	42
4. RESULTS.....	43
4. 1. Chemical composition of essential oils.....	43
4.1.1. Chemical composition of essential oil of bitter orange, <i>Citrus aurantium</i> peel.....	43
4.1.2. Chemical composition of essential oil of mandarin, <i>Citrus</i>	

<i>reticulata</i> peel.....	46
4.1.3. Chemical composition of essential oil of cumin, <i>Cuminum</i> <i>cuminum</i> seeds.....	49
4.1.4. Chemical composition of essential oil of, marigold, <i>Tagetes</i> <i>erecta</i> flowers.....	52
4.2. Toxicity of essential oils alone and in mixtures, monoterpenoids and Deltamethrin against <i>Sitophilus oryzae</i> and <i>Callosobruchus</i> <i>maculatus</i> adults.....	56
4.2.1. Toxicity of essential oils alone and Deltamethrin against <i>Sitophilus oryzae</i> adults.....	56
4.2.2. Contact toxicity of essential oils alone and Deltamethrin against <i>Callosobruchus maculatus</i> adults.....	59
4.2.3. Co-toxicity of essential oil mixtures against <i>Sitophilus</i> <i>oryzae</i> and <i>Callosobruchus maculatus</i> adults.....	62
4.2.4. Toxicity of the monoterpenoids citrenallol, geraniol and linalool against <i>Sitophilus oryzae</i> and <i>Callosobruchus</i> <i>maculatus</i> adults.....	64
4.3. Effect of essential oils, monoterpenoids and Deltamethrin on the biology of <i>Sitophilus oryzae</i> and <i>Callosobruchus</i> <i>maculatus</i> .....	67
4.3.1. Effect of essential oils on <i>Sitophilus oryzae</i> adult emergence.....	67
4.3.2. Effect of monoterpenoids on <i>Sitophilus oryzae</i> adult emergence.....	69
4.3.3. Effect of Deltamethrin on <i>Sitophilus oryzae</i> adult emergence.....	70
4.3.4. Effect of essential oils on <i>Callosobruchus maculatus</i> adult emergence and reproductive potential and cowpea seed weight loss.....	70
4.3.5. The effect of monoterpenoids on <i>Callosobruchus maculatus</i> adult emergence and reproductive potential and cowpea seed weight loss.....	73
4.3.6. Effect of Deltamethrin on <i>Callosobruchus maculatus</i> adult emergence and reproductive potential and cowpea seed weight loss.....	75
4.4. Antifeedant activity of essential oils, monoterpenoids and Deltamethrin to <i>Sitophilus oryzae</i> adults.....	76
4.4.1. Antifeedant activity of essential oils to <i>Sitophilus oryzae</i> adults.....	76
4.4.2. Antifeedant activity of monoterpenoids to <i>Sitophilus oryzae</i> adults.....	78
4.4.3. Antifeedant activity of Deltamethrin to <i>Sitophilus oryzae</i> adults.....	80

4.5. Repellent activity of essential oils, monoterpenoids and Deltamethrin to <i>Sitophilus oryzae</i> and <i>Callosobraccus maculatus</i> adults.....	81
4.5.1. Repellent activity of cumin essential oil against <i>Sitophilus oryzae</i> and <i>Callosobraccus maculatus</i> adults.....	81
4.5.2. Repellent activity of marigold essential oil against <i>Sitophilus oryzae</i> and <i>Callosobraccus maculatus</i> adults.....	84
4.5.3. Repellent activity of bitter orange essential oil against <i>Sitophilus oryzae</i> and <i>Callosobraccus maculatus</i> adults.....	87
4.5.4. Repellent activity of mandarin essential oil against <i>Sitophilus oryzae</i> and <i>Callosobraccus maculatus</i> adults.....	90
4.5.5. Repellent activity of monoterpenoid citronellol against <i>Sitophilus oryzae</i> and <i>Callosobraccus maculatus</i> adults.....	93
4.5.6. Repellent activity of monoterpenoid geraniol against <i>Sitophilus oryzae</i> and <i>Callosobraccus maculatus</i> adults.....	96
4.5.7. Repellent activity of monoterpenoid linalool against <i>Sitophilus oryzae</i> and <i>Callosobraccus maculatus</i> adults.....	99
4.5.8. Repellent activity of Deltamethrin against <i>Sitophilus oryzae</i> and <i>Callosobraccus maculatus</i> adults.....	102
4.6. Ultrastructure of sensilla on the antennae of <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults.....	105
4.6.1. General morphology of <i>Callosobruchus maculatus</i> adult antennae.....	105
4.6.2. General morphology of <i>Sitophilus oryzae</i> antennal sensilla.....	109
<b>5. DISCUSSION.....</b>	<b>114</b>
<b>6. SUMMARY.....</b>	<b>146</b>
<b>7. LITERATURE CITED.....</b>	<b>154</b>

**ARABIC SUMMARY**

## LIST OF TABLES

Table 1.	Chemical composition of essential oil of bitter orange, <i>Citrus aurantium</i> peel analyzed by gas chromatography-mass spectrometry (GC-MS).....	44
Table 2.	Chemical composition of essential oil of mandarin, <i>Citrus reticulata</i> peel analyzed by gas chromatography-mass spectrometry (GC-MS).....	47
Table 3.	Chemical composition of essential oil of cumin, <i>Cuminum cyminum</i> seeds analyzed by gas chromatography-mass spectrometry (GC-MS).....	50
Table 4.	Chemical composition of essential oil of, marigold, <i>Tagetes erecta</i> flowers analyzed by gas chromatography-mass spectrometry (GC-MS).....	53
Table 5.	Calculated LC <sub>50</sub> values (ppm) of certain essential oils against <i>Sitophilus oryzae</i> adults two days post exposure.....	57
Table 6.	Calculated LC <sub>50</sub> (ppm) values of certain essential oils against <i>Sitophilus oryzae</i> adults five days post exposure.....	58
Table 7.	Calculated LC <sub>50</sub> (ppm) values of certain essential oils against <i>Callosobruchus maculatus</i> adults two days post exposure.....	60
Table 8.	Calculated LC <sub>50</sub> values (ppm) of certain essential oils against <i>Callosobruchus maculatus</i> adults five days post exposure.....	61
Table 9.	Calculated LC <sub>50</sub> values (ppm) and co-toxicity coefficient of certain essential oil mixtures against <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults two days post exposure. ....	63
Table 10.	Calculated LC <sub>50</sub> values (ppm) and toxicity indices of the monoterpenoids citronellol, geraniol and linalool against <i>Sitophilus oryzae</i> adults two and five days post exposure. ....	65
Table 11.	Calculated LC <sub>50</sub> values (ppm) and toxicity indices of the monoterpenoids citronellol, geraniol and linalool against <i>Callosobruchus maculatus</i> adults two and five days post exposure. ....	66
Table 12.	Percentage reduction of F <sub>1</sub> offspring of <i>Sitophilus oryzae</i> reared on rice grain treated with different concentrations of cumin, mandarin, marigold and bitter orange essential oils .....	68
Table 13.	Percentage reduction of F <sub>1</sub> offspring of <i>Sitophilus oryzae</i> reared on rice grain treated with different concentrations of the monoterpenoids citronellol, geraniol and linalool.....	69
Table 14.	Percentage reduction of F <sub>1</sub> offspring of <i>Sitophilus oryzae</i> reared on rice grains treated with different concentrations of Deltamethrin.....	70
Table 15.	Effect of cumin, mandarin, marigold and bitter orange	

essential oils on fecundity, egg hatchability, adult emergence of F <sub>1</sub> offspring of <i>Callosobruchus maculatus</i> and cowpea seeds weight loss. ....	71
Table 16. Effect of the monoterpenoids citronellol, geraniol and linalool on fecundity, egg hatchability, adult emergence of F <sub>1</sub> offspring of <i>Callosobruchus maculatus</i> and cowpea seeds weight loss.....	74
Table 17. Effect of Deltamethrin on fecundity, egg hatchability, adult emergence of F <sub>1</sub> offspring of <i>Callosobruchus maculatus</i> and cowpea seeds weight loss.....	75
Table 18. Nutritional and feeding deterrence indices of <i>Sitophilus oryzae</i> adults fed on wheat flour disks treated with different concentrations of mandarin, cumin, marigold and bitter orange essential oils 3 days post treatment. ....	77
Table 19. Nutritional and feeding deterrence indices of <i>Sitophilus oryzae</i> adults fed on wheat flour disks treated with different concentrations of the monoterpenoids citronellol, geraniol and linalool 3 days post treatment. ....	79
Table 20. Nutritional and feeding deterrence indices of <i>Sitophilus oryzae</i> adults fed on wheat flour disks treated with different concentrations of Deltamethrin 3 days post treatment.....	80
Table 21. Repellency of cumin essential oil at different concentrations and exposure periods to <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using filter paper assay.....	82
Table 22. Repellency of cumin essential oil at different concentrations against adults of <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using whole grain test.....	83
Table 23. Repellency of marigold essential oil at different concentrations and exposure periods to <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using filter paper assay. ....	85
Table 24. Repellency of marigold essential oil at different concentrations and exposure periods to <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using whole grain assay.....	86
Table 25. Repellency of bitter orange essential oil at different concentrations against adults of <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using filter paper test.....	88
Table 26. Repellency of bitter orange essential oil at different concentrations against adults of <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using whole grain test.....	89
Table 27. Repellency of mandarin essential oil at different concentrations against adults of <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using filter paper test.....	91
Table 28. Repellency of mandarin essential oil at different	



concentrations against adults of <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using whole grain test .....	92
Table 29. Repellency of the monoterpenoid citronellol at different concentrations and exposure periods to <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using filter paper assay. ....	94
Table 30. Repellency of the monoterpenoid citronellol at different concentrations and exposure periods to <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using whole grain assay. ....	95
Table 31. Repellency of the monoterpenoid geraniol at different concentrations and exposure periods to <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using filter paper assay .....	97
Table 32: Repellency of the monoterpenoid geraniol at different concentrations and exposure periods to <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using whole grain assay .....	98
Table 33: Repellency of the monoterpenoid linalool at different concentrations and exposure periods to <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using filter paper assay .....	100
Table 34: Repellency of the monoterpenoid linalool at different concentrations and exposure periods to <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using whole grain assay .....	101
Table 35: Repellency of Deltamethrin at different concentrations and exposure periods to <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using filter paper assay .....	103
Table 36: Repellency of Deltamethrin at different concentrations and exposure periods to <i>Sitophilus oryzae</i> and <i>Callosobruchus maculatus</i> adults using grain treated assay .....	104
Table 37: Lengths ( $\mu\text{m}$ ) of antennal segments of <i>Callosobruchus maculatus</i> and <i>Sitophilus oryzae</i> .....	113

## LIST OF FIGURES

Figure 1: First device choice bioassay system.....	40
Figure 2: Second device choice bioassay system.....	41
Figure 3: Scanning electron micrograph of antenna of <i>Callosobruchus maculatus</i> adult showing general morphology of antenna and parts of the antenna. Scape (Sc), Pedicel (Pe) and nine flagellomeres (F1- F9). Bar= 0.0298 $\mu\text{m}$ .....	105
Figure 4: Scanning electron micrograph of antenna of <i>Callosobruchus maculatus</i> adult showing sensilla trichodea types recorded on the antennae. Figure shows type I sensilla trichodea (ST1) and type II (ST2). Bar= 0.418 $\mu\text{m}$ .....	107
Figure 5: Scanning electron micrograph of antenna of <i>Callosobruchus maculatus</i> adult showing scape (Sc) and pedicel (Pe) regions and clusters of Böhm bristles (BB) on the base of pedicel and between the scape and the head. Bar= 0.185 $\mu\text{m}$ .....	108
Figure 6: Scanning electron micrograph of antenna of <i>Callosobruchus maculatus</i> adult showing chaetie sensilla, Bar= 0.697 $\mu\text{m}$ .....	109
Figure 7: Scanning electron photomicrograph of <i>Sitophilus oryzae</i> showing clubbed antennae. Scape (Sc), pedicel (Pe) and flagellomes (F 1-6). The apical region of the club shaped flagellomere 6 carries numerous sensilla, while other antennal segments reveal few sensilla. Cuticular grooves (Cg) covering the whole antennal segments, Scale bar= 0. 470 $\mu\text{m}$ .....	110
Figure 8: Scanning electron photomicrograph of antennal club segment of <i>Sitophilus oryzae</i> adult showing trichodea sensilla (ST1 and ST2), chaetica sensillae Type I and II and sensilla gemmiformia (Sg) at the proximal end of the antennal club in the joint between club and flagellomere 5. Cuticular grooves (Cg) covering the whole club segment, Scale bar= 3.36 $\mu\text{m}$ .....	111
Figure 9: Scanning electron photomicrograph of antennal club segment of <i>Sitophilus oryzae</i> adult showing Sensilla basiconica Type I and Type II, Scale bar= 22.3 $\mu\text{m}$ .....	112