



**USING UNTRADITIONAL ALTERNATIVES IN IPM PROGRAM  
TO CONTROL FRUIT FLIES AND MITES INFESTING CITRUS  
TREES IN EGYPT**

*A Thesis*

*Submitted for the requirement for Degree of philosophy doctorate in  
Science in Zoology (Entomology)*

**By**

**Sherihan Mohammed Al-Amin Ahmed Abdel-Fattah**

B.Sc. (Entomology), Faculty of Science, Assiut University, 2008

M.Sc. (Entomology), Faculty of Science, Assiut University, 2015

*Supervised by*

**Prof. Dr. Nehad A. Soliman**

Prof. of Horticultural Pests,  
Plant Protection Research Institute,  
Agricultural Research Center

**Dr. Ahmed M.A. Ibrahim**

Ass. Prof. of Entomology,  
Zoology and Entomology Dep.  
Faculty of Science,  
Assiut University

**Dr. Amira El Desouki Mesbah**

Ass. Prof. of Acarology,  
Plant Protection Research Institute,  
Agricultural Research Center

**Dr. Ali M. A. Mahmoud**

Ass. Prof. of Entomology,  
Zoology and Entomology Dep.,  
Faculty of Science,  
Assiut University

**Zoology and Entomology Department, Faculty of Science  
Assiut University, Egypt**

**2020**

## Table of Contents

Title	Page
<b>Chapter I : General Introduction</b>	<b>1</b>
<b>Chapter II : Literature Review</b>	<b>9</b>
<b>2.1. Citrus production in Egypt</b>	<b>9</b>
<b>2.2. Pests infesting citrus crops in Egypt</b>	<b>10</b>
<b>2.3. Tephritid fruit flies of economic importance</b>	<b>11</b>
<b>2.4. Mites of economic importance and citrus trees</b>	<b>18</b>
<b>2.5. Control methods of medfly, <i>C. capitata</i> and citrus brown mite</b>	<b>20</b>
<b>Chapter III: Pathogenicity of three entomopathogenic fungi against the Mediterranean fruit fly, <i>Ceratitis capitata</i> (Wiedemann) (Diptera: Tephritidae)</b>	<b>43</b>
<b>Abstract</b>	<b>43</b>
<b>3.1. Introduction</b>	<b>44</b>
<b>3.2. Materials and methods</b>	<b>45</b>
<b>3.3. Results and Discussion</b>	<b>48</b>
<b>Chapter IV: Potentiality of plant extracts against different developmental stages of <i>Ceratitis capitata</i> (Wied.) (Diptera : Tephritidae) and study its persistence</b>	<b>77</b>
<b>Abstract</b>	<b>77</b>
<b>4. 1. Introduction</b>	<b>78</b>
<b>4. 2. Materials and methods</b>	<b>81</b>
<b>4.3. Results and discussion</b>	<b>85</b>

<b>Table of Contents</b>	
<b>Chapter V: Repellent effect of leaves extracts of <i>Viola odorata</i> Linn and <i>Eucalyptus camaldeulensis</i> Dehnh against <i>Eutetranychus orientalis</i> females (Acari: Tetranychidae) under laboratory conditions</b>	<b>125</b>
<b>Abstract</b>	<b>125</b>
<b>4. 1. Introduction</b>	<b>126</b>
<b>5. 2. Materials and methods</b>	<b>129</b>
<b>5.3. Results and discussion</b>	<b>131</b>
<b>Chapter VI: Efficacy of acaricides on <i>Eutetranychus orientalis</i> (Acari: Tetranychidae) and its compatibility with predatory mite <i>Euseius scutalis</i> ( Acari: Phytoseiidae) under field conditions</b>	<b>140</b>
<b>Abstract</b>	<b>140</b>
<b>6.1. Introduction</b>	<b>141</b>
<b>6.2. Material and methods</b>	<b>143</b>
<b>6.3. Results and discussion</b>	<b>146</b>
<b>Summary</b>	<b>156</b>
<b>Conclusion</b>	<b>160</b>
<b>References</b>	<b>162</b>
<b>الملخص العربي</b>	<b>—</b>

## List of tables

No.	Title	Page
1	Brief literature survey on the Entomopathogenic fungi (EPF) used against medfly.	34
2	Effects of different botanicals (extracts and EOs) on tephritid fruit flies	39
3	Effects of different botanicals (extracts and EOs) on <i>T. urticae</i> & <i>E. orientalis</i> polyphagous mites.	43
4	Virulence of EPF; <i>Metarhizium anisopliae</i> , <i>Beauveria bassiana</i> , and <i>Paecilomyces lilacinus</i> at different concentrations against <i>Ceratitis capitata</i> full grown larvae and pupae (1, 5, and 8 day old pupae).	57
5	Toxicity values of <i>M. anisopliae</i> , <i>B. bassiana</i> and <i>P. lilacinus</i> on different stages of <i>C. capitata</i>	60
6	Mean percentage mortality( $\pm$ SD) of <i>C. capitata</i> flies at different treatment times (hours) for the different fungi.	61
7	Lethal time (hours) for <i>C. capitata</i> at different concentrations of three fungal isolates; <i>M. anisopliae</i> , <i>B. bassiana</i> and <i>P. lilacinus</i> .	63
8	GC-MS analysis of acetonic extract of <i>Viola odorata</i> Linn leaves.	87
9	GC-MS analysis of methanolic extract of <i>Eucalyptus camaldealensis</i> Dehnh leaves.	90
10	Mean percentage mortality ( $\pm$ SE) of full grown larvae and pupae (one and eight day old) of <i>C. capitata</i> treated with <i>V. odorata</i> leaves extract by spray and contact methods.	97
11	Toxicity values in ppm of <i>V. odorata</i> leaves extract on different developmental stages of <i>C. capitata</i> by spray and contact methods.	100
12	Mean percentage mortality ( $\pm$ SE) of full grown larvae and pupae (one and eight day old) of <i>C. capitata</i> treated with <i>E. camaldealensis</i> leaves extract by spray and contact methods.	101
13	Toxicity values in ppm of <i>E. camaldealensis</i> leaves extract on different developmental stages of <i>C. capitata</i> by spray and contact methods.	102
14	Mean percentage mortality ( $\pm$ SD) of adults after treatment with two plant extracts; <i>V. odorata</i> and <i>E. camaldealensis</i> in different concentrations (in ppm).	112
15	Toxicity values (in ppm) of the two treatments; <i>V. odorata</i> and <i>E. camaldealensis</i> on <i>C. capitata</i> adult flies.	113
16	Repellency rate of two extracts against citrus brown mite, <i>Eutetranychus orientalis</i> (Klein) adults on mulberry discs.	136

**List of tables**

<b>No.</b>	<b>Title</b>	<b>Page</b>
17	Number of alive females over exposure time in two treatment, <i>V. odorata</i> and <i>E. camaldealensis</i> :	137
18	Components evaluated in the assay to control <i>E. orientalis</i> and <i>E. scutalis</i>	144
19	Mean number of <i>E. orientalis</i> population before and after treatment for three days, one-week and two-week exposure.	151
20	The corrected reduction (%) of <i>E. orientalis</i> and its predatory mite, <i>E. scutalis</i> , on <i>Citrus sinensis</i> crop treated with assorted acaricides in May 2018.	152

## List of figures

No	Title	Page
1	Mean percentage mortality (% $\pm$ SD) in immature stages of <i>Ceratitis capitata</i> treated with different concentrations of <i>Metarhizium anisopliae</i> , <i>Beauveria bassiana</i> , and <i>Paecilomyces lilacinus</i> .	65
2	SEM of head and thorax of infected <i>C. capitata</i> adult with <i>M. anisopliae</i> showing dense cover of hyphae (hy) on the cuticle (x=50).	66
3	SEM of compound eyes of infected <i>C. capitata</i> adult.	67
4	SEM of infected leg of <i>C. capitata</i> adult with <i>M. anisopliae</i> at x=100.	68
5	SEM of wing of infected <i>C. capitata</i> with <i>M. anisopliae</i> (x=150) showing spores of the fungus.	69
6	SEM of infected leg densely covered with <i>M. anisopliae</i> hyphae.	69
7	SEM of abdomen of <i>C. capitata</i> densely covered with <i>M. anisopliae</i> hyphae causing great infestation.	70
8	SEM of head of infected <i>C. capitata</i> adult showing growth of <i>B. bassiana</i> fungi after three days of death (x=50).	70
9	SEM of mouthparts of infected <i>C. capitata</i> adult showing growth of <i>B. bassiana</i> on it after three days of death (x=150).	71
10	SEM of compound eyes of infected <i>C. capitata</i> adult with <i>B. bassiana</i> after three days of death (x=350).	71
11	SEM of neck of infected <i>C. capitata</i> adult with <i>B. bassiana</i> after three days of death (x=150).	72
12	SEM of thorax and legs of infected <i>C. capitata</i> with <i>B. bassiana</i> fungus after three days of death(x=50).	72
13	SEM of attached point of wing with thorax of infected <i>C. capitata</i> adult indicating growth of <i>B. bassiana</i> (x=75).	73
14	SEM of abdomen and legs of infected <i>C. capitata</i> adult with <i>B. bassiana</i> (x=50).	73
15	Scanning Electron Microscopy (SEM) of head of infected <i>C. capitata</i> adult showing dense cover of <i>P. lilacinus</i> mycelium growing on the cuticle (hy: hyphae) after three days from death (x=50).	74

## List of figures

No.	Title	Page
16	SEM in the head cavity of infected <i>C. capitata</i> adult after three days of death showing network of <i>P. lilacinus</i> mycelium; hy: hypha, Co: Conidiophore (x=100).	74
17	SEM of <i>P. lilacinus</i> showing spores proliferation (S), hyphae (hy) and conidiophores' shape (Co) inside head cavity of infected <i>C. capitata</i> adult. (x=1000).	75
18	SEM of attached point of leg with thorax of infected <i>C. capitata</i> adult covered showing hyphal dense growth of <i>P. lilacinus</i> (x=150).	75
19	SEM of abdomen and part of leg (L) of infected <i>C. capitata</i> adult showing cuticle proliferation (cu-pro) of <i>P. lilacinus</i> in all the cuticle (x=100).	76
20	SEM of abdomen of infected <i>C. capitata</i> adult showing cuticle proliferation (cu-pro) and spores (s) of <i>P. lilacinus</i> in all the cuticle (x=750).	76
21	GC-MS spectra of acetonic extract of <i>V. odorata</i> L leaves extract	89
22	GC-MS spectra of methanolic extract of <i>E. camaldealensis</i> leaves.	92
23	different forms of pupal abnormalities resulting from exposing <i>Ceratitis capitata</i> pupae to different concentrations of violet dough extracts, <i>Viola odorata</i> .	104
24	Deformation of pupal and adult stages of <i>C. capitata</i> (Weid.) after treatment with ethanolic extract of <i>E. camaldealensis</i>	105
25	Comparison between mean percentages of mortality at the different treatments using different concentrations of <i>V. odorata</i>	107
26	Comparison between mean percentages of mortality at the different treatments using different concentration of <i>E. camaldealensis</i>	108
27	Dissipation curve of two plant extracts.	109
28	lateral view of antennae of <i>C. capitata</i> adult on control treatment indicating normal arista (Na) and normal funiculus(Nf) (x=200).	114

### List of figures

No.	Title	Page
29	SEM of lateral view of antenna of <i>C. capitata</i> adult treated with <i>V. odorata</i> leave extract indicating coated aristae (Ca) and coated funiculus (Cf) with treatment (x=150).	114
30	SEM of part of sensillae on control antenna indicating normal funicular sensillae (x=3500).	115
31	SEM of part of sensillae on treated <i>C. capitata</i> adult with <i>V. odorata</i> leave extract indicating its change to curvy funicular sensillae (x=3500).	115
32	SEM of normal compound eye of <i>C. capitata</i> indicating normal shape of ommatidia (NO) and ommatidial sensillae (NOs) during control treatment (x=500).	116
33	SEM of abnormal compound eye of <i>C. capitata</i> indicating shrunk shape of ommatidia (ShOs) and curvy sensillae (cs) during treatment of <i>V. odorata</i> leave extract (x=1500).	116
34	SEM of abnormal compound eye of <i>C. capitata</i> indicating shrinkage of ommatidium (Sh o) and curvy sensillae (CS) after treatment of <i>V. odorata</i> leave extract(x=3500).	117
35	Mean number of <i>E. orientalis</i> individuals /10 leaf: pre and post spray on citrus crop in Assiut Governorate.	153
36	Mean number of <i>Eusius scutalis</i> individual/10leaf on citrus crop in Assiut Governorate.	154



compounds to protect crops from insect invasion, because of their low cost and because they are bio- degradable and thus are ecofriendly. In this study, violet dough extract, *V.odorata* and *Eucalyptus* leaves extract *E. camaldealensis* were applied on immature stages (full-grown larvae and pupae) and adults of *Ceratitis capitata*. With different concentrations;  $0.25 \times 10^3$ ,  $0.5 \times 10^3$ ,  $1 \times 10^3$ ,  $2 \times 10^3$  and  $3 \times 10^3$  ppm in immature stages by two methods; spray and contact treatment. The larval and pupal response to the two leave extract concentrations varied according to the method of treatment. The Mediterranean fruit fly, *C.capitata* one-day and eight-days old pupae were more sensitive to the Violet dough different concentrations treatments by using the spray and contact treatment methods principally at the high concentrations than full- grown larvae. In *Eucalyptus* leave, there were fluctuation of mortality percentages among different concentrations. According to  $LC_{50}$  and  $LC_{90}$  calculated values, the mortality percentage of *C. capitata* full-grown larvae, one-day and eight-day pupae was higher by using the contact treatment method than the spray method. The adult individuals treated with *V. odorata* were more sensitive and recorded higher mortality rates than that treated with *E. camaldealensis*. Consequently, deformed sensillae on antennae and ommatedia were observed by scanning electron microscopy on individuals treated with *V. odorata*.

### **3. Repellent effect of leaves extracts of *Viola odorata* Linn and *Eucalyptus camaldealensis* Dehnh against *Eutetranychus orientalis* Females (Acari:Tetranychidae) under laboratory conditions:**

Brown spider mites can rapidly develop resistance to pesticides. Organic control may be considered as an alternative and eco-friendly approach. Two plant extracts were tested for their repellence against adult females of

*Eutetranychus orientalis* (Klein). In this study, the violet leaves extract, *Viola odorata* L. (Violaceae) and Camphor leaves extract, *Eucalyptus camaldealensis* Dehnh (Myrtaceae) have been tested as repellent agents via assorted exposure periods ( 6, 12, 24, 48, 96, and 72hr) at five assorted doses ( $0.25 \times 10^3$ ,  $0.5 \times 10^3$ ,  $1 \times 10^3$ ,  $2 \times 10^3$  and  $3 \times 10^3$  ppm/L). We observed repellency effect in all doses and for the two extracts, whereas *E. camaldealensis* was proven to be a slight stronger repellent compared to *V. odorata* at  $2 \times 10^3$  and  $3 \times 10^3$  ppm/L along all exposure time. This repellence effect is due to terpenoid constituents presented in the two extracts. There were significant differences between two extracts whereas the high diversity of terpenoid groups in *E. camaldealensis* than in *V. odorata*. The present work was carried out to study the behavioral effect represented in the repellence of two plant extracts on the mite, *E. orientalis*, a pest of citrus and could be a potential alternative for the development of eco-friendly products used to control pests which is very harmful to agriculture.

#### **4. Efficacy of Acaricides on *Eutetranychus orientalis* (Acari: Tetranychidae) and Its Compatibility with Predatory Mite *Euseius scutalis* (Acarei: Phytoseiidae) under Field Conditions:**

Efficacy evaluation of seven acaricides, i.e. Acarine (Abamectin 5% EC), Gat Fast (2% Abamectin +10% Thiamthoxam (12% SC)), OrtisSuper (Fenpyroximate 5% EC), Concord (Chlorfenapyr 24% EC), Perfect (2% Abamectin+10% Chlorfenapyr (12% EW)), Micronet S (Sulfur 80% WP) and Acarots (Fenpyroximate 5% SC) at recommended dose (RD), against the brown spider mite, *Eutetranychus orientalis* (Tetranychidae) and its predatory mite, *Euseius scutalis* (Phytoseiidae), was applied on citrus crop in Assiut Governorate under field conditions. Three assorted exposure eras: three days, one week and two weeks, were achieved in May 2018. It was

found that a total reduction rate of these 7 acaricides against *E. orientalis* was 88.26, 90.40, 87.99, 88.91, 88.78, 88.41 and 87.82% and against *E. scutalis* was 23.69, 19.61, 14.33, 12.7, 15.52, 16.51 and 15.33%, respectively. Abamactin 5% was significantly higher than other acaricides ( $p < 0.05$ ) followed by Fenpyroximate 5%EC and Fenpyroximate 5%SC. On the other hand, the rest of acaricides appeared to be insignificant ( $p > 0.05$ ). Acaricides can be used against *E. orientalis* without affecting *E. scutalis* where the results showed compatibility between acaricide and predatory mites in the field. For mode of action, Fenpyroximate is safer for human and animal than others because it acts as mitochondrial electron transport inhibitor with contact action. Application of serial concentrations from these compounds is recommended to reduce its toxicity in the environment.