# ECOLOGICAL AND TOXICOLOGICAL STUDY ON SOME TERRESTRIAL SNAILS SPECIES

#### Submitted By

#### Walaa Mohamed Moustafa Mousa

B.Sc . of Agricultural Sciences, Faculty of Agriculture, Cairo University, 2007 Diploma in Environmental Sciences, Institute of Environmental Studies & Research, Ain Shams University, 2014

> A Thesis Submitted in Partial Fulfillment Of The Requirement for the Master Degree In Environmental Sciences

Department of Environmental Agricultural Sciences Institute of Environmental Studies and Research Ain Shams University

2019

### CONTENTS

TITLE	Pag
LIST OF TABLES	iv
LIST OF FIGERS	v
I- INTRODUCTION	1
II- REVIEW OF LITERATURE	5 5
1.1. Effect of some ecological factors on some land snail's	
Eobania vermiculata and Helicell vestalis	5
1.1.1- Effect of soil type and soil moisture	5
1.1.2- Effect of food type	6
2-Toxicological studies	15
2.1- Effects of insecticides on land snails Eobania vermiculata	
and <i>Helicell vestalis</i> under laboratory conditions	15
3- Biochemical impacts of two insecticides on immature stage and	
adult of <i>Eobania vermiculata</i>	28
3.1- Total protein content	20
3.2- Glucose content	28
3.3- Alkaline phosphatase activity	32
III-MATERIALS AND METHODS	33 36
1-Rearing and Source of snails	36
2-Ecological studies	37

#### ge

2.1. Effect of some ecological factors on some land snail's	
Eobania vermiculata and Helicell vestals under laboratory	
conditions	37
2.1.1- Effect of soil type and soil moisture	37
2.1.2- Effect of food type	37
3-Toxicological studies	38
3.1- Effects of two insecticides on two species of land	
snails Eobania vermiculata and Helicella vestalis under	
laboratory conditions	38
3.1.1-Insecticide used	38
3.1.2- Treatment for eggs	39
3.1.3-Treatment for different stages	40
4- Biochemical impacts of two insecticides on immature stage and	
adult of <i>Eobania vermiculata</i>	41
4.1- Determination of Total protein content	41
4.2-Determination of glucose	42
4.3- Estimation of Alkaline phosphatase (ALP) activity	43
IV- RESULTS AND DISCUSSION	45
1- Ecological studies	45
1.1- Effect of some ecological factors on some land snail's	
Eobania vermiculata and Helicella vestalis	45
1.1.1- Effect of soil types and soil moisture for adult land snails	
Eobania vermiculata and Helicelal vestalis under laboratory	
conditions	45
1.1.2- Effect of food types of Eobania vermiculata and Helicella	

vestalis	75
2-Toxicological studies	91
2.1- Effects of two insecticides on land snails Eobania	
vermiculata and Helicella vestalis under laboratory	
conditions	91
3-Biochemical impacts of two insecticides on immature stage	
and adult of <i>Eobania vermiculata</i>	103
3.1- Total protein content	103
3.2- Glucose content	106
3.3- Alkaline phosphatase activity	109
VI-SUMMAY	113
VII-REFRENCES	120

## LIST OF TABLES

No	Title	Page
1	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 30% soil moisture during four months.	46
2	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 60% soil moisture during four months.	48
3	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 90% soil moisture during four months.	49
4	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 30% soil moisture during four months.	51
5	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 60% soil moisture during four months.	52
6	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 90% soil moisture during four months.	54
7	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 30% soil moisture during four months.	55
8	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 60% soil moisture during four months.	57
9	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 90% soil moisture during four months.	59
10	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 30% soil moisture during four months.	61

11	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 60% soil moisture during four months.	63
12	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 90% soil moisture during four months.	64
13	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 30% soil moisture during four months.	66
14	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 60% soil moisture during four months.	67
15	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 90% soil moisture during four months.	69
16	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 30% soil moisture during four months.	70
17	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 60% soil moisture during four months.	72
18	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 90% soil moisture during four months.	74
19	Daily food consumption gm day <sup>-1</sup> of <i>Eobania vermiculata</i> snail in non- choice test of food types (vegetable, ornamental and field crops) under laboratory conditions.	76
20	Daily food consumption gm day <sup>-1</sup> of <i>Helicella vestalis</i> snail in non-choice test of food types (vegetable, ornamental and field crops) under laboratory conditions.	80
21	Daily food consumption gm day <sup>-1</sup> of <i>Eobania vermiculata</i> snail in free- choice test of food types (vegetable, ornamental and field crops) under laboratory conditions.	84

22	Daily food consumption gm day <sup>-1</sup> and number of <i>Helicella vestalis</i> snail in free-choice test of food types (vegetable, ornamental and field crops) under laboratory conditions.	89
23	Toxicity of Propinb 70% W.P on different stages of <i>Eobania vermiculata</i> under laboratory conditions.	93
24	Toxicity of Propinb 70% W.P on different stages of <i>Helicella vestalis</i> under laboratory conditions.	95
25	Toxicity of Protecto 9.4% W.P on different stages of <i>Eobania vermiculata</i> under laboratory conditions.	99
26	Toxicity of Protecto 9.4 % W.P on different stages of <i>Helicella vestalis</i> under laboratory conditions.	101
27	Average total protein content of immature stage of <i>Eobania vermiculata</i> as affected by fungicide and bactericidal after one and 72 h under laboratory conditions.	103
28	Average total protein content of adult stage of <i>Eobania vermiculata</i> as affected by fungicide and bactericidal after one and 72 h under laboratory conditions.	105
29	Average glucose content of immature stage of <i>Eobania</i> <i>vermiculata</i> as affected by fungicide and bactericidal after one and 72 h under laboratory conditions	107
30	Average glucose content of adult stage of <i>Eobania vermiculata</i> as affected by fungicide and bactericidal after one and 72h under laboratory conditions	108
31	Average alkaline phosphatase activity of immature stage of <i>Eobania vermiculata</i> as affected by fungicide and bactericidal after one and 72 h under laboratory conditions	110
32	Average alkaline phosphatase activity of adult stage of <i>Eobania vermiculata</i> as affected by fungicide and bactericidal after one and 72 h under laboratory conditions	111

## LIST OF FIGURES

No	Title	Page
1	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 30% soil moisture during four months.	47
2	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 60% soil moisture during four months.	48
3	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 90% soil moisture during four months.	50
4	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 30% soil moisture during four months.	51
5	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 60% soil moisture during four months.	53
6	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 90% soil moisture during four months.	54
7	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 30% soil moisture during four months.	56
8	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 60% soil moisture during four months.	57
9	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Eobania vermiculata</i> as affected by three types of soil under 90% soil moisture during four months.	59

10	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 30% soil moisture during four months.	61
11	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 60% soil moisture during four months.	63
12	Average food consumption gm month <sup>-1</sup> of lettuce leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 90% soil moisture during four months.	65
13	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 30% soil moisture during four months.	66
14	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 60% soil moisture during four months.	68
15	Average food consumption gm month <sup>-1</sup> of mint leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 90% soil moisture during four months.	69
16	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 30% soil moisture during four months.	71
17	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 60% soil moisture during four months.	72
18	Average food consumption gm month <sup>-1</sup> of wheat leaves for adult of <i>Hellicela vestalis</i> as affected by three types of soil under 90% soil moisture during four months.	74
19	Daily food consumption gm day <sup>-1</sup> of <i>Eobania vermiculata</i> snail in non-choice test of vegetable crops under laboratory conditions.	77
20	Daily food consumption gm day <sup>-1</sup> of <i>Eobania vermiculata</i> snail in non-choice test of ornamental plants under laboratory conditions.	77

21	Daily food consumption gm day <sup>-1</sup> of <i>Eobania vermiculata</i> snail in non-choice test of field crops under laboratory conditions.	78
22	Daily food consumption gm day <sup>-1</sup> of <i>Helicella vestalis</i> snail in non-choice test of vegetable crops under laboratory conditions.	80
23	Daily food consumption gm day <sup>-1</sup> of <i>Helicella vestalis</i> snail in non-choice test of ornamental plants under laboratory conditions.	81
24	Daily food consumption gm day <sup>-1</sup> of <i>Helicella vestalis</i> snail in non-choice test of field crops under laboratory conditions.	81
25	Daily food consumption gm day <sup>-1</sup> of <i>Eobania vermiculata</i> snail in free- choice test of vegetable crops under laboratory conditions.	85
26	Daily food consumption gm day <sup>-1</sup> of <i>Eobania vermiculata</i> snail in free- choice test of ornamental plants under laboratory conditions.	85
27	Daily food consumption gm day <sup>-1</sup> of <i>Eobania vermiculata</i> snail in free- choice test of field crops under laboratory conditions.	86
28	Daily food consumption gm day <sup>-1</sup> and number of <i>Helicella vestalis</i> snail in free choice test of vegetable crops under laboratory conditions.	90
29	Daily food consumption gm day <sup>-1</sup> and number of <i>Helicella vestalis</i> snail in free choice test of ornamental plants under laboratory conditions.	90
30	Daily food consumption gm day <sup>-1</sup> and number of <i>Helicella vestalis</i> snail in free choice test of field crops under laboratory conditions.	91
31	Toxicity of Propinb 70% W.P on different stages of <i>Eobania vermiculata</i> under laboratory conditions.	94
32	Toxicity of Propinb 70% W.P on different stages of <i>Helicella vestalis</i> under laboratory conditions.	96
33	Toxicity of Protecto 9.4 % W.P on different stages of <i>Eobania vermiculata</i> under laboratory conditions.	100

34	Toxicity of Protecto 9.4 % W.P on different stages of <i>Helicella vestalis</i> under laboratory conditions.	102
35	Average total protein content of immature stage of <i>Eobania vermiculata</i> as affected by fungicide and bactericidal after one and 72 h under laboratory conditions.	104
36	Average total protein content of adult stage of <i>Eobania vermiculata</i> as affected by fungicide and bactericidal after one and 72 h under laboratory conditions.	106
37	Average glucose content of immature stage of <i>Eobania vermiculata</i> as affected by fungicide and bactericidal after one and 72 h under laboratory conditions.	108
38	Average glucose content of adult stage of <i>Eobania vermiculata</i> as affected by fungicide and bactericidal after one and 72 h under laboratory conditions.	109
39	Average alkaline phosphatase activity of immature stage of <i>Eobania vermiculata</i> as affected by fungicide and bactericidal after one and 72 h under laboratory conditions	111
40	Average alkaline phosphatase activity of adult stage of <i>Eobania vermiculata</i> as affected by fungicide and bactericidal after one and 72 h under laboratory conditions.	112

#### ABSTRACT

Walaa Mohamed Moustafa Mousa: Ecological and Toxicological Study On Some Terrestrial Snails Species. Unpublished M.Sc. Thesis, Institute of Environmental Studies & Research, Ain Shams University, 2019.

Ecological studies indicated that, type of soil and soil moisture playing an important role of increase or decrease the food consumption, whereas, lettuce leaves for adult of E. vermiculata at 60% soil moisture in clay soil was recorded highly food consumption at December with an average 135.6±4.35 gm month<sup>-1</sup> followed by November, January and February 133.64±4.06, 123.78±3.68 and  $115.32\pm3.17$  gm month<sup>-1</sup> respectively, while at 30% soil moisture in sand soil was recorded the lowest food consumption 98.64±1.64 gm month<sup>-1</sup>. While used 90% soil moisture, Food consumption were slightly high in clay soil in November and December with an average 125.38 $\pm$ 3.26, 125.36 $\pm$ 5.55 gm month<sup>-1</sup> For mint leaves at 30% soil moisture, the highest food consumption was recorded at November and December in clay soil  $43.06\pm3.75$  and  $40.88\pm2.58$  gm month<sup>-1</sup>, respectively, then peat soil the food consumption was recorded  $33.58\pm2.66$  and  $35.44\pm2.75$  gm month<sup>-1</sup>, respectively. While in 60% was recorded the highest food consumption in clay soil  $46.84 \pm 4.06$ gm month<sup>-1</sup>, following at November, January and February were recorded 45.04 $\pm$  7.14, 41.66 $\pm$  4.07 and 32.84 $\pm$  4.47 gm month<sup>-1</sup>, respectively. In 90% soil moisture was recorded the lowest food consumption in sand soil  $26.72 \pm 4.59$  gm month<sup>-1</sup> For wheat leaves, results, were recorded no significant differences between three type of soil during different months, in 30% soil moisture was recorded highest food consumption at February in clay soil 16.12± 1.23 gm month<sup>-1</sup> following peat and sand soil with an average  $14.60 \pm 1.28$  and 14.10± 1.57 gm. / month. In 60% soil moisture at November, the highest food consumption was in clay soil 18±0.5 gm month<sup>-1</sup> then

peat and soil were  $16.80\pm0.88$  and  $15.80\pm0.73$  gm month<sup>-1</sup>, respectively. In 90% was recorded the lowest food consumption in sand soil at February  $13.98\pm1.45$  gm month<sup>-1</sup>.

For adult of snail H. vestalis, lettuce leaves in 30% soil moisture at December there was highly significant differences between sand soil comparing with peat and clay soil with an average  $124\pm3.12$ , 118.02±6.60 and 113.64±4.46 gm month<sup>-1</sup> respectively. In 60 % at December no significant differences between sand and peat soil comparing with clay soil with an average 139.38±5.47 and 136.02±79 gm month<sup>-1</sup> respectively, but in clay soil the lowest food consumption 123.10±6.84 gm month<sup>-1</sup>. While the lowest food consumption was recorded in clay soil at January and February with an average  $99.36\pm6.64$  and  $93.72\pm5.48$  gm month<sup>-1</sup> in 90% soil moisture. For mint leaves in 30% soil moisture was recorded the lowest food consumption at February in clay soil with an average  $20.38 \pm 1.06$  gm month<sup>-1</sup>. In 60% soil moisture at November, sand soil was recorded the highest food consumption  $35.78\pm3.23$  gm month<sup>-1</sup> comparing with peat and clay soil with an average, 29.44±1.57 and  $28.40\pm1.17$  gm month<sup>-1</sup> respectively. while at February no significant differences between three types of soil but in sand is considered slightly high of food consumption 27.22±2.86 gm month<sup>-1</sup> comparing with peat and clay  $24.34\pm3.87$  and  $22.56\pm2.06$  gm month<sup>-1</sup> respectively, in 90% soil moisture. For wheat leaves the lowest food consumption was recorded in clay soil  $10.94\pm0.81$  gm month<sup>-1</sup> at February in 30% soil moisture while in 60% at December the highest food consumption was recorded in sand soil  $17.66 \pm 1.75$  gm month<sup>-1</sup>, but in 90% the lowest food consumption was recorded in clay soil at February 10.86 $\pm$ 0.48 gm month<sup>-1</sup>.

On the other hand, daily food consumption during 10 days for *E. vermiculata* and *H. vestalis* in non-choice test under laboratory conditions. In *E. vermiculata* snails showed that, the highest values of daily food consumption on lettuce and cabbage leaves,  $4.37\pm0.38$  and  $3.22\pm0.40$  gm day<sup>-1</sup> respectively. The lowest values of food consumption of vegetable crops were  $2.28\pm0.24$  gm day<sup>-1</sup> when snails fed on watercress leaves, while in ornamental plants was recorded the lowest values  $0.84\pm0.14$  gm day<sup>-1</sup> on mint leaves, but in field crops barley leaves was recorded the lowest food consumption with an average  $0.91\pm0.2$  gm day<sup>-1</sup> but regarding *H. vestalis* snail, also showed that, the highest values of daily food consumption on lettuce and cabbage leaves  $3.81\pm0.60$  and  $3.42\pm0.07$  gm day<sup>-1</sup> respectively. Also, in ornamental plants, mint was recorded the lowest values of daily food consumption  $0.94\pm0.27$  gm day<sup>-1</sup> and in field crops barley was recorded the lowest daily food consumption  $0.91\pm0.34$  gm day<sup>-1</sup>.

Concerning with food consumption in free- choice test of *E. vermiculata* was illustrated that, in vegetable crops lettuce leaves followed by cabbage leaves were recorded highly food consumption  $3.73\pm1.13$  and  $2.61\pm0.19$  gm day<sup>-1</sup> respectively, while in ornamental plants, thyme leaves was recorded the lowest food consumption  $0.71\pm0.09$  gm day<sup>-1</sup> but, in field crops also, barley leaves was recorded the lowest food consumption  $0.21\pm0.05$  gm day<sup>-1</sup>, in freechoice test of *H.vestalis* was conducted that, lettuce and cabbage leaves were recorded the highest daily food consumption  $4.20\pm0.65$ and  $3.40\pm0.32$  gm day<sup>-1</sup>, but in ornamental plant and field crops, thyme and barley leaves were recorded the lowest food consumption  $0.76\pm0.12$  and  $0.20\pm0.12$  gm day<sup>-1</sup> respectively.

The Effects of bio insecticides on land snails of fungicide (propineb70% W.P) and bio insecticides (Protecto 9.4% W.P) under laboratory conditions at different concentrations against eggs and immature stages and adult stage of *Eobania vermiculata* and *Helicella vestalis* by using leaf dipping technique under laboratory conditions revealed that, eggs was more sensitive to propineb 70%, since *H. vestalis* recorded higher mortality percentage compared to *E*.

*vermiculata*. At the concentrations 0.1, 0.2, 0.3 and 0.4 %, with LC<sub>50</sub> values 0.17 and 0.19%, respectively. Regarding immature stages (one week) also, *H. vestalis* recorded higher mortality percentage than *E.vermiculata* 23, 40, 73 and 97% and 20, 33, 70 and 93% respectively at concentrations 0.2, 0.4 0.6 and 0.8%. But for four weeks' age was more sensitive than eight weeks and adult. Protecto 9.4% W.P also, eggs were more susceptible than immature stages and adult. A high mortality percentage was recorded to *H. vestalis* compared to *E. vermiculata* (20, 33.30, 70 and 76.70%) and (10, 30, 60 and 70%) respectively while for immature stages (one week) also, it was more sensitive than four, eight weeks and adult for *H. vestalis* compared to *E. vermiculata*.

Biochemical effects of fungicide (propineb70% W.P) and bactericide (Protecto 9.4% W.P) on immature stage and adult Eobania vermiculata, on total protein content, effect of sub-lethal concentration  $(1/4 \text{ Lc}_{50})$  of the two tested compounds on the total protein in immature and adults stage of E. vermiculata land snail showed that, Fungicide (Propineb 70% W.P) treatment increased total protein with 333.33 and 327.77 % after one and 72 hours in comparison with control. On the other side bio insecticides (Protecto) increased total protein by 644.44 and 727.77 % than control after one and 72 hours from treatment in immature stage respectively, Fungicide treatment high significant decreased total protein with -39.28 % after 1 hour while non-significant increase with 82.14 % 72 hours after treatment, respectively. On the other side bio insecticides compound non-significant increased total protein by -3.32 and -6.06 % compared with the control after 1 and 72 hours of treatment. While on glucose, results cleared that, glucose level significantly increased by fungicide (Propineb 70% W.P) and bio insecticides pesticides (Protecto) treatments with difference percentage 222.22% and 277.77 % after one and 72 hours after treatment by bio insecticides (Protecto). Whereas on alkaline phosphatase activity results indicated that, the maximum reduction of acid and alkaline phosphatase enzyme (ALP) activity occurred after fungicide (Propineb 70% W.P) and bio insecticides pesticides (Protecto) treatments immature stage *E. vermiculata* with the different percentage -30.73 % and 4.76 % after one hours post treatment for each compound respectively, but non-significant change after 72 hours after treatment.

Key words: Terrestrial snails, *Eobania vermiculata*, *Helicella vestalis*, type of soil, soil moisture, Vegetable crops, Ornamental plants, Field crops, fungicide, bio insecticides, total protein, glucose, alkaline phosphatase.