



# ECOLOGICAL AND BIOLOGICAL CONTROL STUDIES ON CERTAIN SUBTERRANEAN TERMITE SPECIES AT EL-FAYOUM GOVERNORATE, EGYPT

By

### Ahmed Mohammed Abd El-Qawi

B. Sc. Agric. Sci. (Plant protection), Fac. Agric., Fayoum Univ., 2011

Thesis

Submitted in partial fulfillment

Of

The requirements for the degree of

Master of science

In

**Agriculture science** 

### (Economic Entomology)

Department of Plant Protection

Faculty of Agriculture

# FAYOUM UNIVERSITY

# 2022

#### Abstract

#### Ecological and biological control studies on certain subterranean termite species at El-Fayoum governorate, Egypt.

The present study involves some ecological and biological control studies on *Anacanthotermes ochraceus* and *Psammotermes hybostoma*. The ecological studies represented by Surface and subsurface foraging activity, which were carried out by two trap designs for one year extended from the 1<sup>st</sup> of Oct. 2016 - 30<sup>th</sup> of Sep. 2017 at Sanhour village, Sennoris district for *A. ochraceus*. and from the 1<sup>st</sup> of Oct. 2018 - 30<sup>th</sup> of Sep. 2019 for *P. hybostoma* at El-Lahoun village, El-Fayoum district, El-Fayoum governorate. The biological control studies were carried out to evaluate the efficacy of some entomopathogenic fungi (*Metarhizium pemphigi, Metarhizium brunneum* and *Beauveria bassiana*) against both *A. ochraceus* and *P. hybostoma*.

#### The obtained results included the following.

#### 1- Ecological studies

#### 1.1- Surface and subsurface foraging activity of A. ochraceus.

**a- Surface activity:** The rate of food consumption was low during both winter and spring, moderate during summer and relatively high in autumn with rates, (6.44, 6.34, 10.27 and 19.58 g./trap/m<sup>2</sup>), for winter, spring, summer and autumn, respectively. The seasonal percentages of soil translocation represented (11.82, 17.74, 35.0 and 35.45 %) for winter, spring, summer and autumn, respectively. The seasonal means of number of captured workers / 30 traps were 740.3, 500.33, 96.83 and 402.66 for winter, spring, summer and autumn, respectively. The percentage of infested traps was relatively high during autumn (18.89%) and relatively low during both spring (8.89%) and summer (8.34%) while it was moderate in winter (13%).

**b- subsurface activity** could not be studied because of the high level of ground-water level.

#### **1.2-** Surface and subsurface foraging activity of *P. hybostoma*:

**a- Surface activity:** Food consumption, was relatively low during winter (13.23 g./trap/m<sup>2</sup>). While, it was relatively higher during spring (76.1 g./trap/m<sup>2</sup>) and more or less moderate during both summer (48.1 g./trap/m<sup>2</sup>) and autumn (58.7 g./trap/m<sup>2</sup>). The highest seasonal means of soil translocation took place during spring (155 g./trap/m<sup>2</sup>) and the lowest occurred during winter (about 25 g./trap/m<sup>2</sup>). Number of captured workers was relatively low during winter (38916/35 traps), more or less moderated during both spring (167409/35 traps) and summer (196168/35 traps), while, the highest number occurred during autumn (337325/35 traps). The lowest percentage of infested traps occurred during winter (17-40 %), while it reached the top peak in autumn (89%).

**b-** Subsurface activity: The highest seasonal means of surface food consumption (0-30 cm vertical distance) occurred in autumn (210.88 g./5 column traps), followed spring (177.29 g./5 column traps), while the lowest occurred during summer (25.88 g./5 column traps). The maximum seasonal means of subsurface food consumption recorded 1806.92 g./5 column traps during autumn throughout four levels, (from the 2<sup>nd</sup> to the 5<sup>th</sup> level), while the minimum seasonal

means occurred during spring (1019.15 g./5 column traps). The seasonal means in winter and summer were more or less moderate (1265.73 and 1150.21 g./5 column traps, respectively). The highest seasonal means of surface soil translocation took place during autumn (496.23 g./5 column traps) and the lowest occurred during summer (35.01 g./5 column traps). The maximum seasonal means subsurface soil translocation took place during autumn (4841.09 g./5 column traps) and the minimum occurred during spring (1384.41 g./5 column traps). Seasonal means of surface foraging activity (number of captured workers) was the lower activity in winter (1167.67 workers/5 column traps) while its peak occurred in spring (7586 workers/5 column traps). Seasonal means of subsurface foraging activity was relatively low in summer (15370 workers/5 column traps) while the maximum took place during spring (36090.67 workers/5 column traps).

#### **1.3-** Nest structure of *A. ochraceus* and *P. hybostoma*.

**a- Nest of** *A. ochraceus* was studied under high level condition of ground water, at depth of 40 cm of soil surface. The nest starts with the tunnel openings on the soil surface. Storage chambers were found at different depths of the soil surface starting from 2 cm to 16 cm, takes a pyramidal shape with the upper surface. The dwelling chambers and center of colony were found at a depth of 25 cm from the soil surface, containing a number of replacement individuals, about 10 individuals, and groups of workers, small larvae, and newly hatched larvae.

**b-** Nest of *P. hybostoma* was found to be similar in structure to a building, as it consists of several floors connected to each other by longitudinal tunnels. Each floor consists of several rooms connected to each other by transverse tunnels, and may unite with each other to form a common vestibule. the center of colony including all castes and replacement individuals were found at a depth of 60-80 cm from the surface of the soil.

#### 2- Biological control studies:

#### 2.1- Molecular identification of M. pemphigi Ph4 isolate using nuclear rDNA.

*M. pemphigi* Ph4 isolate was identified and submitted in gene bank under accession number (OL305760.1). The alignment showed that the retrieved sequence of the tested fungal strain was closely related to all of *M. pemphigi* with accession numbers, KY087809.1, MH143795.1 and AB524444.1.

# 2.2- Efficacy of *M. pemphigi* Ph4, *M. brunneum* V275 and *B. bassiana* fungi against *A. ochraceus* workers under laboratory conditions.

All concentrations caused 100% mortality rate on the 9<sup>th</sup> day post inoculation except the concentration  $1 \times 10^5$  conidia/ml suspension caused 95, 85 and 93.3 % mortality rates on the 10<sup>th</sup> day post – treatment for *M. pemphigi* Ph4, *M. brunneum* V275 and *B. bassiana* treatments, respectively. Statistical analysis results showed that on the 10<sup>th</sup> day post treatment, no significant differences between all four tested concentrations for three tested fungi except concentration of 1  $\times$  10<sup>5</sup> conidia/ml for *M. brunneum* which recorded the least effective (85.0 % mortality) against *A. ochraceus* workers. Results demonstrated that LT<sub>50</sub> and LT<sub>90</sub> values were shorter for *M. pemphigi* (Ph4) treatment and estimated by 3.04 & 4.52 days at concentration of 1  $\times$  10<sup>8</sup>.

# 2.3- Efficacy of *M. pemphigi* Ph4, *M. brunneum* V275 and *B. bassiana* fungi against *P. hybostoma* workers under laboratory conditions.

On the 10<sup>th</sup> day all treatments caused 100% mortality with all concentrations, except the lowest concentration of  $1 \times 10^5$  conidia/ml with *M. brunneum*, where its mortality was 72.7%. Statistical analysis showed that on the 10<sup>th</sup> day post treatment, no significant differences between all concentrations for three tested fungi except the concentration of  $1 \times 10^5$  for *M. brunneum* treatment was the least pathogenic where mortality rate was 72.7%. Obtained results demonstrated that at high concentration of  $1 \times 10^8$ , LT<sub>50</sub> and LT<sub>90</sub> values were shorter for *M. brunneum*. Where estimated by 3.12 & 4.70 days

# 2.4- Laboratory evaluation of treatment techniques effect on the pathogenicity of M. *pemphigi*, M. *brunneum* and B. *bassiana* fungi against A. *ochraceus* workers at a concentration of $1 \times 10^8$ conidia/ml.

Statistical analysis revealed that on the 7<sup>th</sup> day of treatment, no significant differences were observed among three tested treatment techniques (food, soil and insect) with three tested fungi (*M. pemphigi*, *M. brunneum* and *B. bassiana*) except, *M. pemphigi* and *B. bassiana* fungi with soil treatment technique differed significantly whereas mortality rates reached 77.6 and 71.6 %, respectively. Results showed that the shortest values of LT<sub>50</sub> and LT<sub>90</sub> were 3.38 & 4.36 days with soil treatment technique for *M. brunneum*.

# 2.5- Laboratory evaluation of treatment techniques effect on the pathogenicity of M. *pemphigi*, M. *brunneum* and B. *bassiana* fungi against P. *hybostoma* workers at a concentration of $1 \times 10^8$ conidia/ml.

Statistical analysis results showed that on the 7<sup>th</sup> day of treatment there were significant differences between *M. brunneum* (100% mortality rate) and other fungi *M. pemphigi* and *B. bassiana* (98.6% mortality) for soil treatment technique. Results showed that the shortest values of LT<sub>50</sub> and LT<sub>90</sub> were 2.18 & 2.97 days for *M. brunneum* with insect treatment technique.

# 2.6- Efficacy of termite' workers of *P. hybostoma* as carriers for *M. pemphigi* fungus at a concentration of $1 \times 10^8$ conidia/ml under laboratory conditions.

Results showed that on the 7<sup>th</sup> day after treatment, the mortality percentages reached 32.3, 53 and 60 % for 1, 2 and 4 contaminated workers compared with 0.7 % mortality in the check treatment. On both 5<sup>th</sup> and 7<sup>th</sup> day post treatment no significant differences were observed between 2 and 4 carrier workers / 100 healthy workers but there were significant differences between those previously mentioned two treatments (2 and 4 carrier / 100 healthy workers) and 1 carrier worker / 100 healthy workers. Results demonstrated that LT<sub>50</sub> and LT<sub>90</sub> values were 8.39 and 17.01 days with one carrier worker / 100 healthy workers, 6.28 and 12.35 days with 2 carrier workers / 100 healthy workers and lastly 5.74 and 10.23 days with 4 carrier workers / 100 healthy workers, respectively.

## CONTENTS

		Title	Page	
I-	IN	TRODUCTIONS	1	
II-	RF	<b>CVIEW OF LITERATURE</b>	4	
1.	Ecol	ogical studies	4	
	1.1-	Globally geographical distribution of	4	
		Egyptian subterranean termite	4	
	1.2-	Economic importance and damage of	5	
		subterranean termite	5	
	1.3-	Seasonal forging activity of subterranean	6	
		termite	0	
	1.3	.1- Surface activity	6	
	1.3	.2- Subsurface activity	10	
	1.4-	Colony size and foraging territories	11	
	1.5-	Cate composition	11	
	1.6-	Baiting materials and subterranean termite	12	
		traps	12	
	1.6	.1- Surface foraging activity	12	
	1.6	.2- Sub-surface foraging activity	14	
	1.7-	Swarming behavior	14	
	1.8-	Nesting system of subterranean termites	15	
2.	Mi	crobial control studies	18	
	2.1-	Entomopathogenic fungi EPFs against	10	
		termite	18	
	2.2-	Efficacy of the entomopathogenic fungi,		
		Beauveria spp. And Metarhizium spp. As	20	
		biological control agents against subterranean		
		termites		

2.3- Efficacy of fungal commercia against termites	al formulation 26
2.4- Fungal application methods i	n the field as 26
biological control agent	20
2.5- Efficacy of grooming behavio	r in spreading
diseases and transferring infect	ion
III- MATERIALS AND METHODS	30
1. Ecological studies	30
1.1- Time and location of experime	nts performing 30
1.2- Trap designs	30
1.3- Surface foraging activity	33
1.4- Subsurface foraging activity	35
1.5- Statistical analysis	36
2. Microbial control studies	36
2.1- source of fungal isolates	36
2.2- preparation of fungal growth m	nedia 38
2.2.1- Potato-dextrose agar (PDA	38
2.2.2- Sabouraud dextrose agar + (SDAY)	yeast extract 38
2.3- Isolation and purification of <i>M</i> .	. pemphigi 39
2.4- Preparation of conidial suspens	sion 40
2.5- Termite's collection	41
2.6- Molecular identification of	M. pemphigi 41
isolate Ph4	
2.6.1- Extraction of DNA	41
2.6.2- Polymerase chain read amplification of its region	ction (PCR) 42
2.6.3- Agarose gel electrophoresis	42
2.6.4- Elution (Gel extraction)	43

2.6.5- Sequencing	43
2.7- Bioassay	43
2.7.1- Comparing the virulence and pathogenicity of <i>M. pemphigi</i> , <i>M.</i> <i>brunneum</i> and <i>B. bassiana</i> against <i>A.</i> <i>ochraceus</i> and <i>P. hybostoma</i>	43
2.7.2- Effect of treatment techniques on the efficacy of fungal isolates and infection occurrence	44
2.7.3- Effect of grooming behavior on spread the infection between the healthy individuals	45
IV- RESULTS AND DISCUSSIONS	47
1. Ecological studies	47
1.1- Foraging activity parameters	47
1.1.1- Surface foraging activity of A. ochraceus	47
1.1.1.1- Food consumption	47
1.1.1.2- Soil translocation	51
1.1.1.3- Number of captured workers	53
1.1.1.4- Percentage of infested traps	54
1.1.1.5- Caste composition	54
1.1.1.6- Statistical analysis	57
1.1.2- Subsurface foraging activity of <i>A</i> . <i>ochraceus</i>	58
1.1.3- Surface foraging activity of <i>P. hybostoma</i>	59
1.1.3.1- Food consumption	59
1.1.3.2- Soil translocation	63
1.1.3.3- Number of captured workers	64
1.1.3.4- Percentage of infested traps	65
1.1.3.5- Caste composition	66

1.1.3.6- Statistical analysis	69
1.1.4- Subsurface foraging activity of <i>P. hybostoma</i>	70
1.1.4.1- Food consumption	70
1.1.4.2- Soil translocation (construction activity)	74
1.1.4.3- Number of captured workers	78
1.1.4.4- Number of captured soldiers	81
1.2- Nest structure	85
1.2.1- Nest structure of A. ochraceus	85
1.2.2- Nest structure of <i>P. hybostoma</i>	90
1.3- Swarming season of <i>P. hybostoma</i>	97
1.4- Predators of A. ochraceus and P. hybostoma	99
2. Microbial control studies	101
2.1- Molecular identification of <i>M. pemphigi</i> ph4 isolate using nuclear rDNA	101
2.2- Bioassay	106
2.2.1- Efficacy of <i>M. pemphigi</i> ph4, <i>M. brunneum</i> v275 and <i>B. bassiana</i> against <i>A. ochraceus</i> under laboratory conditions	106
2.2.2- Efficacy of <i>M. pemphigi</i> ph4, <i>M. brunneum</i> v275 and <i>B. bassiana</i> against <i>P. hybostoma</i> under laboratory conditions	112
2.2.3- Laboratory evaluation of treatment techniques effect on the pathogenicity of <i>M. pemphigi, M. brunneum</i> and <i>B.</i> <i>bassiana</i> fungi against <i>A. ochraceus</i> at a concentration of 1 ×10 <sup>8</sup> conidia/ml	119

	2.2.4- Laboratory evaluation of treatment	
	techniques effect on the pathogenicity of	
	M. pemphigi, M. brunneum and B.	124
	bassiana fungi against P. hybostoma at a	
	concentration of $1 \times 10^8$ conidia/ml	
	2.2.5- Efficacy of termite' workers of P.	
	hybostoma as carriers for M. pemphigi	120
	fungi at a concentration of $1 \times 10^8$	130
	conidia/ml under laboratory condition	
V-	SUMMARY	134
VI-	REFERENCES	148

## LIST OF TABLES

No.	Title	Page
1	Half-monthly and seasonal means of food	
	consumption and soil translocation by A. ochraceus	10
	at El-Fayoum governorate for one-year extended	40
	October 2016 to September 2017.	
	Half-monthly and seasonal numbers of captured	
2	termite castes of A. ochraceus at El-Fayoum	55
2	governorate for one year extended from October	55
	2016 to September 2017.	
	Simple correlation (r) and simple regression (b)	
	coefficients for the relationship between some	
3	weather factors and the foraging activity parameters	59
5	of A. ochraceus at El-Fayoum governorate	50
	throughout one year extended from October 2016 to	
	September 2017.	
	Half-monthly and seasonal means of food	
Δ	consumption and soil translocation by P. hybostoma	60
-	at El-Fayoum governorate for one-year extended	00
	from October 2018 to September 2019.	
	Half-monthly and seasonal numbers of captured	
5	termite castes of P. hybostoma at El-Fayoum	67
5	governorate for one-year extended from October	
	2018 to September 2019.	
	Simple correlation (r) and simple regression (b)	
6	coefficients for the relationship between some	69
0	weather factors and the foraging activity parameters	07
	of <i>P. hybostoma</i> at Fayoum governorate throughout	

	one year extended from October 2018 to September	
	2019	
7	Half-monthly and seasonal means of food	
	consumption of P. hybostoma at different vertical	72
	distances of soil surface at El Fayoum governorate,	12
	Egypt in 2018/2019.	
	Half-monthly and seasonal means of soil	
Q	translocation rates of P. hybostoma at different	76
0	vertical depth of soil surface at El Fayoum	70
	governorate in 2018/2019	
	Half-monthly and seasonal numbers of captured	
0	workers of P. hybostoma at different vertical	70
7	distance of soil surface at El Fayoum governorate in	13
	2018/2019.	
	Half-monthly seasonal numbers of captured	
10	soldiers of P. hybostoma at different vertical depth	82
10	of soil surface at El Fayoum governorate in	05
	2018/2019	
	Total numbers of captured alates of P. hybostoma at	
11	El-Fayoum governorate throughout the study year	98
	of 2018-2019.	
	Cumulative mortality percentages of A. ochraceus	
12	workers treated with M. pemphigi, M. brunneum	108
	and B. bassiana under laboratory condition	
	Comparison of $LT_{50}$ and $LT_{90}$ values for three tested	
13	fungi M. pemphigi, M. brunneum and B. bassiana at	111
	four different concentrations against A. ochraceus	111
	workers	
1/	Cumulative mortality percentages of P. hybostoma	112
14	workers treated with three entomopathogenic fungi,	115

	M. pemphigi, M. brunneum and B. bassiana under	
	laboratory conditions	
	Comparison of $LT_{50}$ and $LT_{90}$ values for three tested	
15	fungi M. pemphigi, M. brunneum and B bassiana at	118
	four different concentrations against P. hybostoma	110
	workers	
	Effect of treatment technique on mortality	
	percentages of the subterranean termites A.	
16	ochraceus workers treated with M. pemphigi, M.	120
	brunneum and B. bassiana fungi at a concentration	
	of $1 \times 10^8$ conidia/ml	
	Comparison of LT <sub>50</sub> and LT <sub>90</sub> values among three	
	treatment techniques (food, soil and insect) for three	
17	tested fungi (M. pemphigi, M. brunneum and B.	123
	<i>bassiana</i> ) at a concentration of $1 \times 10^8$ conidia/ml.	
	against A. ochraceus workers	
	Effect of treatment techniques on mortality	
18	percentages of the subterranean termites P.	125
10	hybostoma workers treated with M. pemphigi, M.	123
	brunneum and B. bassiana fungi	
	Comparison of LT <sub>50</sub> and LT <sub>90</sub> values among three	
	treatment techniques (food, soil and insect) for three	
19	tested fungi (M. pemphigi, M. brunneum and B.	129
	<i>bassiana</i> ) at a concentration of $1 \times 10^8$ conidia/ml.	
	against P. hybostoma workers	
20	Cumulative mortality percentages of P. hybostoma	
	workers mixed with 1, 2, and 4 workers treated with	132
	<i>M. pemphigi</i> $(1 \times 10^8 \text{ conidia/ml})$ as carrier termite	132
	for one week post treatment.	

	LT <sub>50</sub> and LT <sub>90</sub> values for <i>P. hybostoma</i> workers	
01	mixed with 1, 2 and 4 carrier workers treated with	122
21	<i>M. pemphigi</i> at a concentration of $1 \times 10^8$ conidia/ml	132
	for one week post - treatment.	

## LIST OF FIGURES

No.	Figure	Page
1	Plastic box trap provided with corrugated cardboard	31
	paper as food material as bait	51
2	The parts of cylindrical column traps	32
3	Distribution of both column and plastic box traps in	22
5	the experimental area	55
4	Contents of infested trap with P. hybostoma	34
5	The position of cylindrical column traps in the soil	35
6	Galleria mellonella infested with M. brunneum	27
0	(V275), M. pemphigi (Ph4) and B. bassiana	57
	Pure colony of <i>M. brunneum</i> (V275) on SDAY	
7	media, M. pemphigi (Ph4) and B. bassiana on PDA	38
	media	
0	Incubated box trap contains infected workers of P.	20
0	hybostoma by M. pemphigi	39
0	Isolation and purification of <i>M. pemphigi</i> on PDA	40
9	media	40
	Half-monthly means of food consumption and soil	
10	translocation by A. ochraceus at El-Fayoum	40
10	governorate for one-year extended from October	49
	2016 to September 2017	
	Seasonal percentages of food consumption and soil	
11	translocation by A. ochraceus at El-Fayoum	50
	governorate for one-year extended from October	50
	2016 to September 2017	
	Half-monthly numbers of captured workers of A.	
10	ochraceus and percentage of infested traps at El-	56
12	Fayoum governorate throughout one-year extended	
	from October 2016 to September 2017	

	Half-monthly means of food consumption and soil translocation by $P$ by bostoma at El-Eavour	
13	governorate for one-year extended form October	61
	2018 to September 2019	
	Seasonal percentage means of food consumption	
14	and soil translocation by <i>P. hybostoma</i> at El-	$\sim$
	Fayoum governorate for one-year extended from	62
	October 2018 to September 2019	
	Half-monthly numbers of captured workers of P.	
15	hybostoma and percentage of infested traps at El-	68
15	Fayoum governorate throughout one-year extended	00
	from October 2018 - September 2019	
	Seasonal means of food consumption in (g. / level /	
16	5 traps) of P. hybostoma throughout five vertical	73
	levels of termites foraging	
	Seasonal means of soil translocation in (g./level/5	
17	traps) of P. hybostoma throughout five vertical	77
	levels of termites foraging	
	Seasonal means of captured workers' number / level	
18	/ 5 traps of <i>P. hybostoma</i> throughout five vertical	80
	levels of termites foraging	
	Seasonal means of captured soldiers' number / level	
19	/ 5 traps of <i>P. hybostoma</i> throughout five vertical	84
	levels of termites foraging	
	a- Food storage chambers. b- Dwelling chamber of	
20	Anacanthotermes ochraceus with many individuals	87
20	of workers and larvae inside it. c- Vestibule of two	01
	chambers	
21	Many floors of Anacanthotermes ochraceus nest at	88
	different vertical depth of soil surface	00

determine the optimum foraging season and foraging level for applying control.

b- Evaluating the virulence and pathogenicity of some entomopathogenic fungi (*Metarhizium pemphigi, Metarhizium brunneum* and *Beauveria bassiana*) against *A. ocraceus* and *P. hybostoma*. Also, testing different treatment techniques to select the best one in the field treatment.