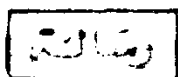


**COMPARATIVE STUDY ON THE BIOLOGY OF THE
CONFUSED AND RED FLOUR BEETLES;
TRIBOLIUM CONFUSUM (DUV.) AND *TRIBOLIUM
CASTANEUM* (HBST.) AND THEIR
SUSCEPTIBILITY TO SOME INSECTICIDES.**



A THESIS
Presented to
The Faculty of Science
Ain Shams University
For The Award of The
Ph.D. Degree

BY

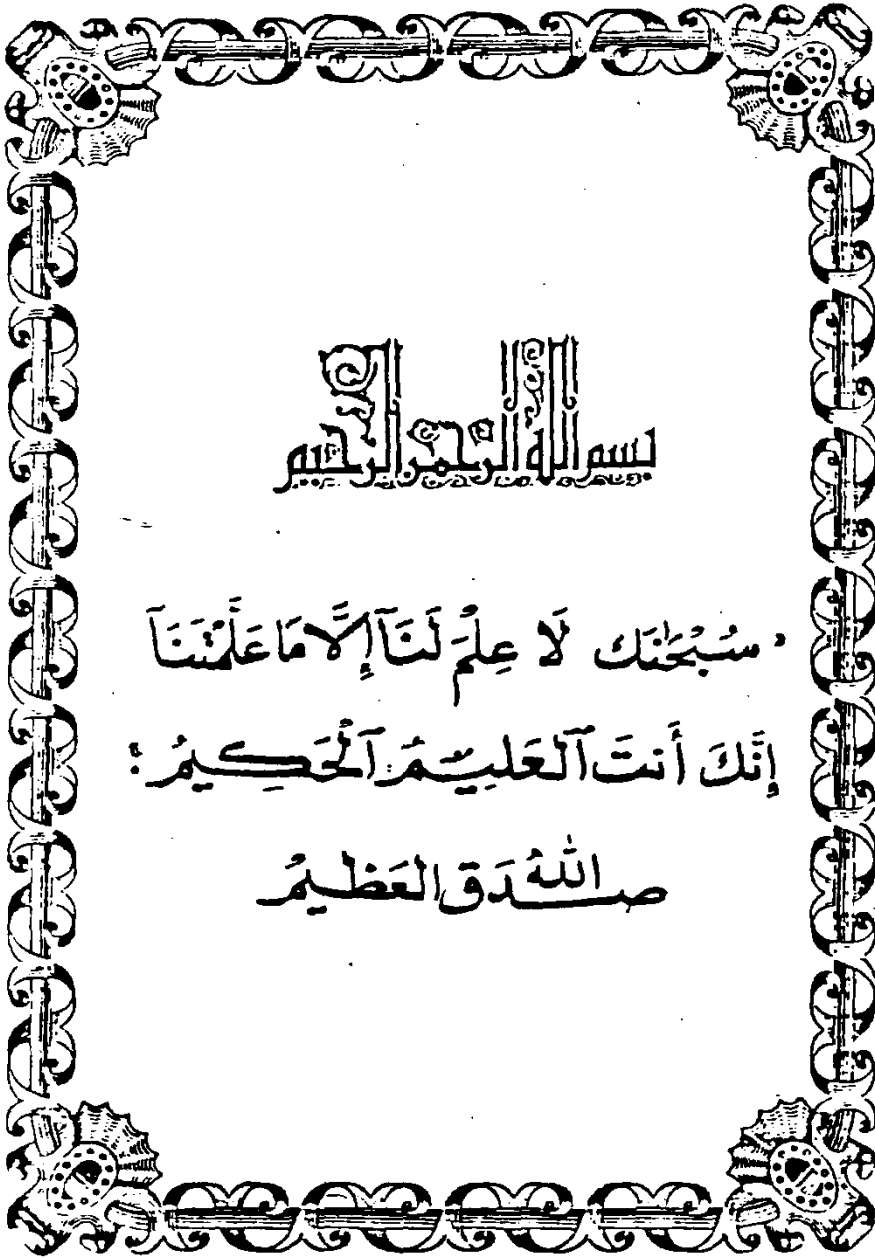
ATIF ALI EL BANNA

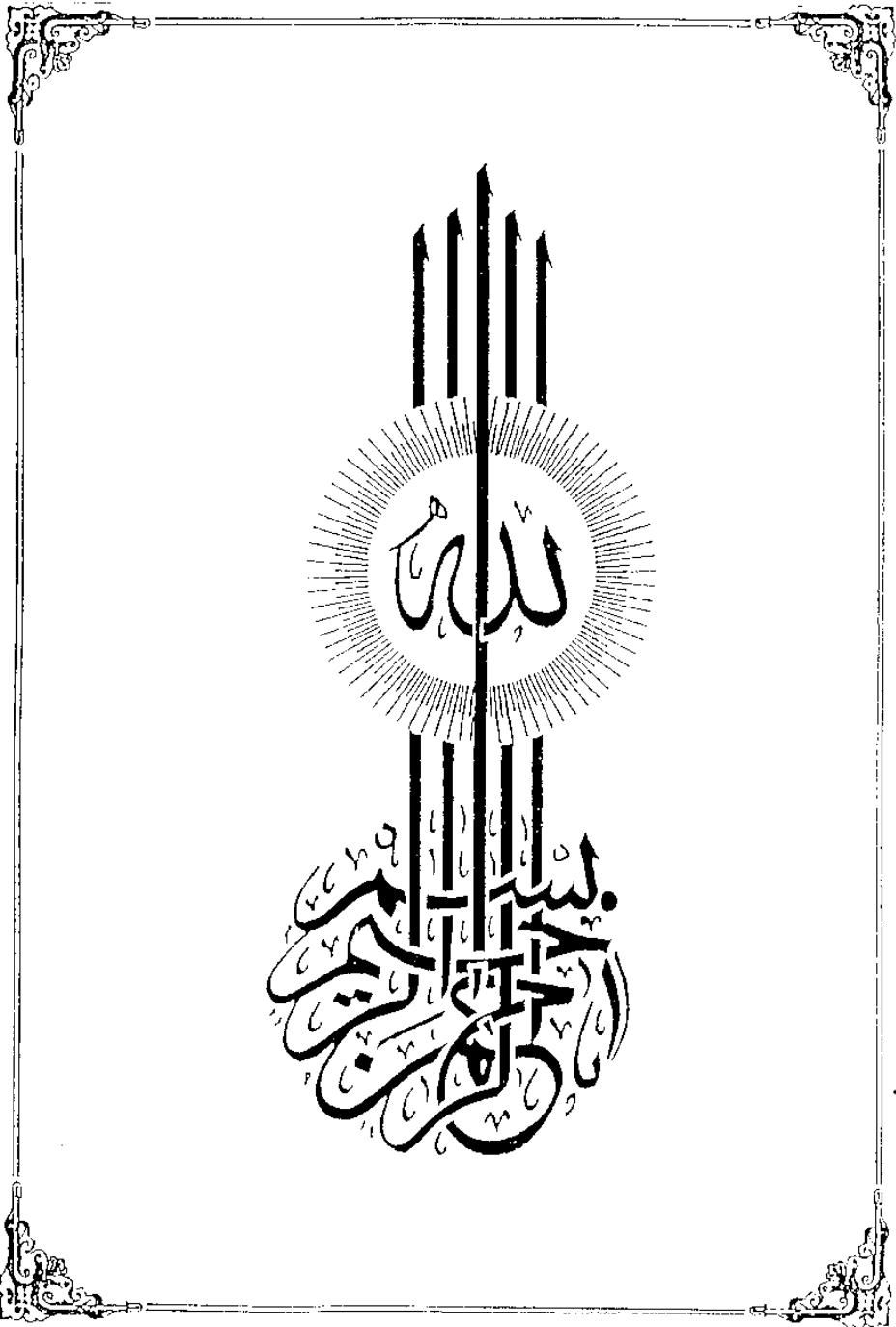
(B.Sc., M.Sc.)

Department of Entomology
Faculty of Science
Ain Shams University
CAIRO

1994







THESIS EXAMINATION COMMITTEE

Name	Title	Signature
------	-------	-----------

-----	-----	-----
-----	-----	-----
-----	-----	-----

SUPERVISORS:

Prof. Dr. Abdel-Aziz Abdel-Hafez Soliman. *A. A. Soliman*

Prof. Dr. Soad Abu El-Seoud El-Sayed. *Soad*

Prof. Dr. Akila Mohammed El Shafei. *Akila El Shafei*

Dr. Adel Mohammed Sobhi El Akad. *Adel El-Akad*

Head of Entomology Department

Bahira El Sawaf

Prof. Dr. Bahira El Sawaf

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Professor Dr. Abdel-Aziz A. Soliman Professor of Entomology, for suggesting the subject of work, for his valuable advice and keen guidance during the course of study.

I owe a great debt to Professor Dr. Soad Abu El-Seoud Professor of Entomology, for her valuable assistance, kind support and encouraging attitude which contributed to achievement of progress in the present research.

Deep appreciation and thanks are due to Professor Dr. Akila M. El-Shafei Professor of Entomology, for her active supervision, sound suggestions and comments throughout the period of study.

I also wish to thank Dr. Adel M. S. El Akad assistant Professor of Entomology, for his various remarks and help.

I am grateful to Dr. Jan Humphreys Professor of Entomology, Biology Department, IUP (Indiana University of Pennsylvania), USA, for supplying me with the equipments, materials and different facilities for the laboratory work during the period of study in the USA.

I would also like to thank Dr. Gould F. Shrock Professor of Mycology, Biology Department, IUP, for his help in the field of Mycology.

Thanks are also due to the ATCC (American Type Culture Collection), Rockville, Maryland, USA, for supplying the fungal strains, and to the Carolina Biological supply Company, Burlington, South Carolina, USA, for supplying the initial insect cultures used during the experiments carried out in the USA.

Finally, I would like to thank Dr. Mona Shoukry assistant Professor, Dr. Nadia Lotfi assistant Professor, staff members and colleagues of the Entomology Department, Faculty of Science, Ain Shams University, for their various help.

~~~~~

## ***ABSTRACT***

This comparative study investigates the biology of the two flour beetles *Tribolium confusum* and *T. castaneum* and their susceptibility to microbial insecticides using entomopathogenic fungi, and to chemical insecticides under two temperatures. Temperature was found to be a limiting factor influencing the efficiency of controlling these pests.

## ***KEY WORDS***

- \* Flour beetles.
- \* *Tribolium confusum*.
- \* *Tribolium castaneum*.
- \* Coleoptera.
- \* Tenebrionidae.
- \* Biology.
- \* Microbial insecticides.
- \* Microbial control.
- \* Entomopathogenic fungi.
- \* Insect-fungus interaction.
- \* Chemical insecticides.
- \* Temperature.

## **CONTENTS**

|                                                                                                                | Page |
|----------------------------------------------------------------------------------------------------------------|------|
| I- INTRODUCTION.....                                                                                           | 1    |
| II- LITERATURE REVIEW.....                                                                                     | 4    |
| [A] Biological studies.....                                                                                    | 4    |
| [B] Susceptibility of <i>Tribolium confusum</i> and<br><i>T. castaneum</i> to some entomopathogenic fungi..... | 8    |
| [C] Susceptibility of <i>T. confusum</i> and<br><i>T. castaneum</i> to some insecticides.....                  | 13   |
| III- MATERIALS AND METHODS.....                                                                                | 17   |
| [A] Origin and maintenance of cultures.....                                                                    | 17   |
| 1) Origin of cultures.....                                                                                     | 17   |
| 2) Laboratory rearing and maintenance of<br>stock cultures.....                                                | 17   |
| [B] Biology and life table studies.....                                                                        | 18   |
| [C] Susceptibility of <i>T. confusum</i> and <i>T.</i><br><i>castaneum</i> to some entomopathogenic fungi..... | 20   |
| a- The fungi used in the present study.....                                                                    | 20   |
| 1) Source of cultures.....                                                                                     | 20   |
| 2) Taxonomic status and classification.....                                                                    | 20   |
| 3) Identification and diagnostic symptoms<br>of infection with the fungi.....                                  | 22   |
| 1. <i>Beauveria bassiana</i> .....                                                                             | 22   |
| 2. <i>Metarhizium anisopliae</i> .....                                                                         | 22   |
| 3. <i>Paecilomyces farinosus</i> .....                                                                         | 23   |
| 4. <i>Hirsutella thompsonii</i> .....                                                                          | 23   |
| 5. <i>Nomuraea rileyi</i> .....                                                                                | 23   |



|                                                                                                           |    |
|-----------------------------------------------------------------------------------------------------------|----|
| 6. <i>Verticillium lecanii</i> .....                                                                      | 23 |
| 7. <i>Aspergillus flavus</i> .....                                                                        | 24 |
| 8. <i>Cordyceps millitaris</i> .....                                                                      | 24 |
| b- Laboratory preparation and growth of the<br>fungal cultures.....                                       | 36 |
| 1) Preparation of the nutrient media.....                                                                 | 36 |
| i. PDA.....                                                                                               | 36 |
| ii. Rabbit food agar.....                                                                                 | 36 |
| 2) Reviving of the cultures received from the<br>ATCC.....                                                | 37 |
| 3) Inoculation of the nutrient media by the<br>streak- plate technique.....                               | 38 |
| c- Preliminary experiments.....                                                                           | 39 |
| 1) Infection of the beetles.....                                                                          | 39 |
| 2) Scanning electron microscopy.....                                                                      | 40 |
| i. Preparation of specimens.....                                                                          | 40 |
| ii. Critical- point drying.....                                                                           | 40 |
| iii. Specimen coating.....                                                                                | 41 |
| d- Susceptibility tests.....                                                                              | 41 |
| 1) Harvesting and suspension of the conidia.....                                                          | 41 |
| 2) Concentration of the conidia<br>by centrifugation.....                                                 | 42 |
| 3) Enumeration of the conidia in-suspension.....                                                          | 42 |
| 4) Determination of the activity (the<br>true titer) of the fungal preparations.....                      | 43 |
| 5) Inoculation of <i>T. confusum</i> and <i>T.</i><br><i>castaneum</i> with the conidial suspensions..... | 44 |
| 6) Post-treatment conditions and mortality<br>counts.....                                                 | 44 |
| 7) Assessment of results and statistical<br>analysis of data.....                                         | 45 |

|                                                                                            |    |
|--------------------------------------------------------------------------------------------|----|
| [D] Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to some insecticides..... | 47 |
| a- Insecticides tested and properties.....                                                 | 47 |
| 1) Malathion.....                                                                          | 47 |
| 2) Diazinon.....                                                                           | 48 |
| 3) Dursban.....                                                                            | 49 |
| 4) Pyrethrum.....                                                                          | 49 |
| b- Susceptibility tests.....                                                               | 50 |
| 1) Preparation of discs impregnated with insecticidal solutions.....                       | 50 |
| 2) Application techniques.....                                                             | 50 |
| c- Assessment of results and statistical analysis of data.....                             | 51 |
| IV- PRESENT INVESTIGATIONS AND EXPERIMENTAL RESULTS.....                                   | 52 |
| [A] Biology and life-table studies.....                                                    | 52 |
| 1) The egg stage.....                                                                      | 52 |
| Incubation period and hatchability.....                                                    | 52 |
| 2) The larval stage.....                                                                   | 55 |
| i. Duration.....                                                                           | 55 |
| ii. Percentage of mortalities.....                                                         | 58 |
| 3) The pupal stage.....                                                                    | 62 |
| i. Duration.....                                                                           | 62 |
| ii. Percentage of mortalities.....                                                         | 64 |
| iii. Sex ratio.....                                                                        | 66 |
| 4) The adult stage.....                                                                    | 68 |
| i. Sex ratio.....                                                                          | 68 |
| ii. Pre-oviposition period.....                                                            | 70 |

|                                                                                                      |     |
|------------------------------------------------------------------------------------------------------|-----|
| [B] Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to some entomopathogenic fungi..... | 73  |
| (1) <i>Beauveria bassiana</i> .....                                                                  | 73  |
| (2) <i>Metarhizium anisopliae</i> .....                                                              | 79  |
| (3) <i>Paecilomyces farinosus</i> .....                                                              | 85  |
| (4) <i>Hirsutella thompsonii</i> .....                                                               | 90  |
| (5) <i>Nomuraea rileyi</i> .....                                                                     | 95  |
| (6) <i>Verticillium lecanii</i> .....                                                                | 101 |
| (7) <i>Aspergillus flavus</i> .....                                                                  | 106 |
| (8) <i>Cordyceps militaris</i> .....                                                                 | 112 |
| [C] Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to some insecticides.....           | 114 |
| (1) Malathion.....                                                                                   | 114 |
| (2) Diazinon.....                                                                                    | 120 |
| (3) Dursban.....                                                                                     | 125 |
| (4) Pyrethrum.....                                                                                   | 130 |
| V- DISCUSSION OF RESULTS AND CONCLUSIONS.....                                                        | 137 |
| [A] Biological studies.....                                                                          | 138 |
| [B] Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to some entomopathogenic fungi..... | 139 |
| [C] Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to some insecticides.....           | 144 |
| VI- SUMMARY.....                                                                                     | 148 |
| VII- LITERATURE CITED.....                                                                           | 154 |
| VIII- ARABIC SUMMARY.                                                                                |     |

## LIST OF TABLES

| Table                                                                                                                                                                           | page |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Table (1) Incubation Period and hatchability of eggs of <i>Tribolium confusum</i> and <i>T. castaneum</i> at temperatures of 24 and $30 \pm 1^{\circ}\text{C}$ , and 60-75% RH. | 53   |
| Table (2) Duration of larval instars of <i>Tribolium confusum</i> and <i>T. castaneum</i> at temperatures 24 and $30 \pm 1^{\circ}\text{C}$ , and 60-75% RH.                    | 56   |
| Table (3) Percent mortality in the larval instars of <i>Tribolium confusum</i> and <i>T. castaneum</i> at 24 and $30 \pm 1^{\circ}\text{C}$ , and 60-75% RH.                    | 59   |
| Table (4) Duration of pupal stage of <i>T. confusum</i> and <i>T. castaneum</i> at temperatures 24 and $30 \pm 1^{\circ}\text{C}$ , and 60-75% RH.                              | 62   |
| Table (5) Mortality percentage for <i>Tribolium confusum</i> and <i>T. castaneum</i> pupae at temperatures of 24 and $30 \pm 1^{\circ}\text{C}$ , and 60-75% RH.                | 65   |
| Table (6) Sex ratio for pupae of <i>Tribolium confusum</i> and <i>T. castaneum</i> at 24 and $30 \pm 1^{\circ}\text{C}$ , and 60-75% RH.                                        | 67   |
| Table (7) Sex ratio for adults of <i>Tribolium confusum</i> and <i>T. castaneum</i> at 24 and $30 \pm 1^{\circ}\text{C}$ , and 60-75% RH.                                       | 69   |
| Table (8) Pre-oviposition period for females of <i>Tribolium confusum</i> and <i>T. castaneum</i> at temperatures of 24 and $30 \pm 1^{\circ}\text{C}$ , and 60-75% RH.         | 71   |
| Table (9) Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Beauveria bassiana</i> at 24 and $30 \pm 1^{\circ}\text{C}$ , and 92-97% RH.                       | 74   |
| Table (10) Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Metarhizium anisopliae</i> at 24 and $30 \pm 1^{\circ}\text{C}$ , and 92-97% RH.                  | 79   |
| Table (11) Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Paecilomyces farinosus</i> at 24 and $30 \pm 1^{\circ}\text{C}$ , and 92-97% RH.                  | 85   |
| Table (12) Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Hirsutella thompsonii</i> at 24 and $30 \pm 1^{\circ}\text{C}$ , and 92-97% RH.                   | 90   |

| Table                                                                                                                                         | Page |
|-----------------------------------------------------------------------------------------------------------------------------------------------|------|
| Table (13) Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Nomuraea rileyi</i> at 24 and 30 $\pm$ 1°C, and 92-97% RH.      | 96   |
| Table (14) Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Verticillium lecanii</i> at 24 and 30 $\pm$ 1°C, and 92-97% RH. | 101  |
| Table (15) Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Aspergillus flavus</i> at 24 and 30 $\pm$ 1°C, and 92-97% RH.   | 107  |
| Table (16) Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to malathion at 24 and 30 $\pm$ 1°C, and 60-75% RH.                   | 115  |
| Table (17) Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to diazinon at 24 and 30 $\pm$ 1°C, and 60-75% RH.                    | 120  |
| Table (18) Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to dursban at 24 and 30 $\pm$ 1°C, and 60-75% RH.                     | 125  |
| Table (19) Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to pyrethrum at 24 and 30 $\pm$ 1°C, and 60-75% RH.                   | 131  |

## **LIST OF FIGURES**

| Figure                                                                                                                                                            | page |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| (1): <i>Beauveria bassiana</i> mycosis in <i>T. confusum</i> showing primary infection on softer areas of the body (dorsal view).                                 | 26   |
| (2): <i>B. bassiana</i> mycosis in <i>T. confusum</i> (ventral view).                                                                                             | 26   |
| (3): Advanced <i>B. bassiana</i> mycosis in <i>T. confusum</i> (ventral view).                                                                                    | 26   |
| (4): Characteristic mycosis pattern of <i>Metarhizium anisopliae</i> on softer areas of body of <i>T. castaneum</i> .                                             | 28   |
| (5): <i>M. anisopliae</i> Mycosis on softer areas of <i>T. confusum</i> .                                                                                         | 28   |
| (6): Mummification of bodies of <i>Tribolium</i> beetles in advanced stages of <i>M. anisopliae</i> mycosis.                                                      | 28   |
| (7): <i>Paecilomyces farinosus</i> infection in <i>Tribolium</i> spp.                                                                                             | 30   |
| (8): Advanced stage of <i>P. farinosus</i> mycosis.                                                                                                               | 30   |
| (9): Beetles infected with <i>Hirsutella thompsonii</i> showing mycelia and primary sporulation.                                                                  | 30   |
| (10): <i>Nomuraea rileyi</i> mycosis on ventral side of <i>T. confusum</i> .                                                                                      | 32   |
| (11): <i>Tribolium confusum</i> entangled in network of mycelial strands of <i>Verticillium lecanii</i> . (dorsal view)                                           | 32   |
| (12): Advanced stage of <i>Aspergillus flavus</i> mycosis in <i>Tribolium castaneum</i> showing dense sporulation around the beetle cadaver. (dorso-lateral view) | 32   |

| Figure                                                                                                                                                                                                       | page    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| (13-17): Gradual magnifications (using Scanning electron microscope) showing fruiting conidia and sprouting mycelia of <i>Aspergillus flavus</i> , penetrating the intact integument of <i>T. confusum</i> . | 34&35   |
| (18a&b): Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Beauveria bassiana</i> at temperatures of 24 and 30 $\pm$ 1°C.                                                                   | 76&78   |
| (19a&b): Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Metarhizium anisopliae</i> at 24 and 30 $\pm$ 1°C.                                                                               | 81&83   |
| (20a&b): Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Paecilomyces farinosus</i> at 24 and 30 $\pm$ 1°C.                                                                               | 87&89   |
| (21a&b): Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Hirsutella thompsonii</i> at 24 and 30 $\pm$ 1°C.                                                                                | 92&94   |
| (22a&b): Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Nomuraea rileyi</i> at 24 and 30 $\pm$ 1°C.                                                                                      | 98&100  |
| (23a&b): Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Verticillium lecanii</i> at 24 and 30 $\pm$ 1°C.                                                                                 | 103&105 |
| (24a&b): Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to <i>Aspergillus flavus</i> at 24 and 30 $\pm$ 1°C.                                                                                   | 109&111 |
| (25a&b): Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to malathion at 24 and 30 $\pm$ 1°C.                                                                                                   | 117&119 |
| (26a&b): Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to diazinon at 24 and 30 $\pm$ 1°C.                                                                                                    | 122&124 |
| (27a&b): Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to dursban at 24 and 30 $\pm$ 1°C.                                                                                                     | 127&129 |
| (28a&b): Susceptibility of <i>T. confusum</i> and <i>T. castaneum</i> to pyrethrum at 24 and 30 $\pm$ 1°C.                                                                                                   | 133&135 |