BIOASSAY OF SOME ENTOMOPATHOGENES ON THE RED PALM WEEVIL

By

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2020

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Abstract

The red palm weevil *Rhynchophorus ferrugineus* (RPW) became the most dangerous insect pests to date palm trees and causes heavy losses to farmers, and due to the negative impact of synthetic pesticides on human health and beneficial organisms…etc in the environment, the use of biological elements was used to treat this insect. Laboratory and field studies were conducted to investigate the potential of two types of fungi *Beauveria bassiana* and *Metarhizium anisopliae* on RPW larvae and adults. The biology of red palm weevil were studied on two types of diet, Diet A (sugarcane stems) and Diet B (ground sugarcane + additives). By rearing on Diet A at 27 ± 2 °C and 70 ± 5 % R.H. incubation period of eggs was 3.53 days, led to subsequently, to 80% hatching, opposed to 3.62 days with 84% hatchability when rearing on Diet B. the total larval duration lated 103.73 and 98.30 days, respectively, being longer on diet (A) than diet (B). The pupal duration was 17.10 days after larvae rearing on diet (A) and 20.10 days on diet (B). Female and male longevities by rearing on diet (A) was 51.9 and 47.4 days, respectively, opposed to 43.6 and 39.5 days, respectively by rearing on diet (B). That indicated diet (B) proved, generally better for successful rearing of RPW. After treatment of RPW larvae of the 1^{st}, 5^{th} and 10^{th} instars with conidial suspension of Newvar (*B. bassiana*) and Metmite (*M. anisopliae*), the recorded mortalities reached 95% in the 1^{st}, 85% in the 5^{th} and 65% in the 10^{th} instar when treated with $1x10^8$CFU's/100ml from Newvar, while reached to 90% in the 1^{st}, 80% in the 5^{th} and 75% in the 10^{th} instar with $1x10^9$ CFU's/100ml concentration from Metmite. Where the highest values of LC_{50} and LC_{90} were obtained with the 10^{th} instar and the lowest value with the 1^{st} instar *i.e.* the 10^{th} instar larvae were the highest resistant, while the 1^{st} instar were susceptible. Newvar and Metmite treatment to RPW larvae caused sharp decreases in larval protein content, while caused increases in carbohydrate content. From field treatment, it cleared that the injury recovery rate was 80% for palm trees that were treated with Newvar (*B. bassiana*) and 60% for trees treated with Metmite (*M. anisopliae*), *i.e.* Newvar appeared more efficient than Metmite on RPW stages.

List of Contents

INTRODUCTION	**1**
REVIEW OF LITERATURE	**4**
1- Taxonomy and distribution of red palm weevil.	**4**
2-Life cycle and mass rearing of red palm weevil.	**6**
3-Evaluation of the fungus as a bio-control agent against RPW.	**12**
3-1-Evaluation of the fungus, *B. bassiana* (Commercial product and isolates).	**13**
3-2-Evaluation of the fungus, *M. anisopliae* (Commercial product and isolated).	**18**
4-Pathogenicity bioassays of *B. bassiana* and *M. anisopliae* on red palm weevil.	**22**
5-Life table of red palm weevil *R. ferrugineus.*	**27**
6-The entomopathogenic efficacy of commercial product of *B. bassiana* and *M. anisopliae* under filed conditions.	**30**
MATERIALS AND METHODS	**33**
1- Collection and rearing of Red Palm Weevil.	**33**
1-1- Determination of some biological aspects of *R. ferrugineus.*	**34**
A- Duration of egg stage.	**34**
B- Larvae Longevity (larvae and adult development times).	**35**
C- Pupal duration.	**37**
D- Adults stage.	**38**
2- Application of commercial products of *B. bassiana* (Newvar) and *M. anisopliae* (Metmite) on larvae of RPW.	**39**
2-1- Determination of the entomopathogenic efficiency of fungal isolates against RPW larvae.	**41**
3- Application of entomopathogenic fungi formulation against red palm weevil in the field.	**42**
4- Biochemical assays.	**43**
5- Bioassay experiment by using two bioinsecticides [Newvar (*B. bassiana*) and Metmite (*M. anisopliae*)] in order to record the data of insect (life stages).	**44**

RESULTS AND DISCUSSION	**46**
1- Effect of the tested diets on the biological aspects of the red oalm weevil.	**46**
1-1-Life cycle of the red palm weevil.	**46**
1-1-1-The immature stages.	**46**
A- The egg- stage.	**46**
B- Larval and pupal durations.	**49**
1-1-2- The adult stage.	**54**
A- Longevity of adult.	**54**
B- Ovipositional periods.	**54**
C- Sex-ratio.	**55**
2-Effect and toxicity of different concentrations of two commercial products of *B. bassiana* and *M. anisopliae* against the red palm weevil larvae.	**56**
2-1- Effect of different concentration of a commercial product (Newvar) of *B. bassiana* against RPW larvae.	**56**
A- Toxicity of Newvar (commercial product of *B. bassiana*) against larvae of RPW.	**59**
B- Efficiency of conidial suspension (*B. bassiana*) of fungal isolate from RPW dead larvae.	**64**
C- Toxicity of conidial suspension of *B. bassiana* conidia against larvae of RPW.	**66**
D- Field application of Newvar (*B. bassiana*) on palm trees for RPW control.	**72**
2-2- Effect of entomopathogenic fungal formulation Metmite (*M. anisopliae*) at $1x10^9$CFU's / 100 ml on the RPW larvae.	**73**
A- Toxicity of commercial product Metmite (*M. anisopliae*) against larvae of RPW.	**76**
B- Effect of a conidial suspension of *M. anisopliae* suspension (24×10^6 conidia / 100 ml sterile distilled water) of fungal isolate from dead larvae on RPW larvae.	**81**
C- Toxicity of conidial suspension of *M. anisopliae* against larvae of RPW.	**84**
D- Effect of Metmite (*M. anisopliae*) against the red palm weevil infestations to palm trees in the field.	**89**

3- Total Contents of homogenate lipids, proteins and carbohydrates to larval treatments with *B. bassiana* and *M. anisopliae*.	**90**
4-Effect of two tested bioinsecticides Newvar (*B. bassiana*) and Metmite (*M. anisopliae*) on *R. ferrugineus*.	**91**
A- Eggs deposited by treated adults.	**91**
B- Durations of larval and pupal stages.	**93**
C- Ovipositional periods.	**95**
D- Eggs reproductivity in relation to fungi infections.	**97**
E- Longevity of *R. ferrugineus* adults.	**98**
F- Effect of *B. bassiana* and *M. anisopliae* on larval, pupal and female adults mortality.	**100**
G- Means of pre-oviposition, oviposition and post-oviposition periods and total number of eggs deposited / female adult and longevity of male and female adults of F2 generation under influence *B. bassiana* and *M. anisopliae* compared to control.	**102**
SUMMARY	**104**
CONCLUSION	**112**
REFERENCES	**114**
ARABIC SUMMARY	**-**

List of Table

No.	Title	Page
1.	Incubation period of *R. ferrugineus* eggs, the percentage of hatching and the percentage of mortality among eggs after rearing on two different diets.	**48**
2.	Difference in time spent on each larval instars a comparison between two different diets and pupal duration.	**53**
3.	Ovipositional periods, fecundity, longevity and sex-ratio of *R. ferrugineus* and its life cycle.	**56**
4.	Mean cumulative mortality percentages among larvae of *R. ferrugineus* treated at 3 instars with commercial product of *B. bassiana* (at different concentrations). (Total number of 20 larvae / treatment).	**58**
5.	Toxicity (LC_{50} and LC_{90} values), 25 days after treatments by the commercial product of *B. bassiana* tested against larval instars of *R. ferrugineus.*	**60**
6.	LT_{50} and LT_{90} values of commercial product of *B. bassiana* tested against 3 larval instars of *R. ferrugineus*. Results were calculated using concentration $1x10^8$ CFU's / 100ml.	**60**
7.	Mean cumulative mortality percentages among *R. ferrugineus* larvae treated with different concentrations of conidial suspension of *B. bassiana* isolate. (Data from treatment of 20 larvae).	**66**
8.	Toxicity (LC_{50} and LC_{90} values), 25 days after treatments by the conidial suspension of *B. bassiana* conidia tested against larval instars of *R. ferrugineus.*	**68**
9.	Time until mortality of 50 and 90% (LT_{50} and LT_{90}) among RPW larval instars after treatments by the conidial suspension of *B. bassiana*.	**68**

10.	Field treatments of date palm trees with Newvar (*B. bassiana*) at concentration (10 g / liter of distilled water) at Al-Kasasen region in July 2019.	**73**
11.	Mean cumulative mortality percent among R. *ferrugineus* larvae treated with commercial product of *M. anisopliae.* (Data from 20 larvae / treatment).	**75**
12.	Toxicity (Lethal concentration) of commercial product of *M. anisopliae* tested against larval instars of *R. ferrugineus.*	**77**
13.	Toxicity (Lethal time) of commercial product of *M. anisopliae* tested against larval instars of *R. ferrugineus*.	**78**
14.	Mean cumulative percentages of mortality of larvae of *R. ferrugineus* treated with conidial suspension of *M. anisopliae* (different concentration) (Data from treatment of 20 larvae for each concentration).	**83**
15.	Toxicity (Lethal concentrations) of conidial suspension (*M. anisopliae*) causing 50 and 90% mortalities among larval instars of *R. ferrugineus.*	**85**
16.	Toxicity (Lethal time) of conidial suspension of *M. anisopliae* tested against larvae instars of *R. ferrugineus*.	**86**
17.	Field treatments of date palm trees with bioinsecticide concentration 10 g per liter of distilled water at Al Kasasen region in the Governorate of Ismailia in July 2019.	**90**
18.	Total contents of homogenate lipids, proteins and carbohydrates (mg/ml) of red palm weevil larvae after treatment with entomopathogenic fungi.	**91**
19.	Effect on *R. ferrugineus* eggs resulted from female adults fed on treated diet with *B. bassiana* and *M. anisopliae***.**	**92**

20.	Duration of *R. ferrugineus* larvae fed on Newvar and Metmite treated diet compared to control. (100 larvae/treatment at 27 ± 2 ºC and 70 ± 5 R.H.).	**94**
21.	Explains the effect of transactions on Pre-oviposition, Oviposition and Post-oviposition Periods.	**96**
22.	Effect of the treatments Newvar (*B. bassiana*) and Metmite (*M. anisopliae*) on the fecundity female shows the number of eggs per female and the percentage of hatching.	**97**
23.	Explains the effect of treatment with fungi product (Newvar and Metmite) on the longevity of male and female duration.	**99**
24.	Mortality percentages among larval, pupal and female adult stages in relation to entomopathogenic fungi treatments.	**101**
25.	Ovipositional periods, eggs reproductivity and longevity of RPW adults (days) for F2 generation in relation to treatments by entomopathogenic fungi	**103**

List of Figures

No.	Title	Page
1.	Mean cumulative mortality percentages among *R. ferrugineus* larvae 25 days after treatment with commercial product of *B. bassiana* (at different concentration).	59
2.	Ldp-line of commercial product of *B. bassiana* used at concentrations against larval instars (1^{st}, 5^{th} and 10^{th}) of *R. ferrugineus*.	62
3.	Period until mortality of *R. ferrugineus* larval instars after treatments by the commercial product of *B. bassiana* for indicating the LT_{50}'s and LT_{90}'s.	63
4.	Mean cumulative mortality percentages among larvae of *R. ferrugineus* treated with conidial suspensions at different conidial concentrations of *B. bassiana* isolate, 25 days after treatment.	65
5.	Ldp-line of conidial suspension of *B. bassiana* conidia tested against larval instars of *R. ferrugineus.*	69
6.	Lethal times (LT's) until mortality of 50 and 90% of RPW larvae after treatment by conidia of *B. bassiana* at $28x10^6$ Conidia/100ml.	71
7.	Mean cumulative mortality percent of *R. ferrugineus* larvae, 25 days after treatment with commercial product of *M. anisopliae* (different concentrations).	76
8.	Ldp-line of Metmite (commercial product of *M. anisopliae*) tested against RPW larval instars of *R. ferrugineus* at 25 days, after treatment.	79
9.	Toxicity (Lethal time) of commercial product of *M. anisopliae* tested against larval instars of *R. ferrugineus*.	80
10.	Mean cumulative mortality percent of *R. ferrugineus* larvae treated with conidial suspensions of *M. anisopliae* (different concentrations) 25 days after treatment.	84

11.	Ldp-line of conidial suspension of *M. anisopliae* tested against larvae instars of *R. ferrugineus* after 25 days.	**87**
12.	Toxicity (Lethal time) of conidial suspension of (*M. anisopliae*) tested against larval instars of *R. ferrugineus*.	**88**
13.	Mortality percentage, hatching, and incubation period for eggs from fungi treatments compared to control.	**93**
14.	Duration of *R. ferrugineus* larval and pupal stages under the efficiency of *B. bassiana* and *M. anisopliae* compared to the control.	**95**
15.	Pre-oviposition, oviposition and post-oviposition periods under the influence of *B. bassiana* and *M. anisopliae* compared to the control.	**96**
16.	The fecundity of adult females and the percentage of hatching in eggs under influence *B. bassiana* and *M. anisopliae* compared to control.	**98**
17.	Longevity of male and female under influence *B. bassiana* and *M. anisopliae* compared to control.	**99**
18.	Mortality Percentages to larval, pupal and female adult stages under influence *B. bassiana* and *M. anisopliae* compared to control.	**101**

List of Plates

No.	Title	Page
1.	Natural and artificial diets in plastic containers for feeding of RPW larvae.	35
2.	Glass jars holding *R. ferrugineus* pairs of sexed adults for mating and oviposition.	36
3.	Petri-dish used to hatch egg.	36
4.	Glass jars used to hatch egg.	36
5.	Collection of pupae of *R. ferrugineus* from pieces of sugarcane stems.	37
6.	Glass bottles used for laboratory treatment for 1st larval instar of RPW with *B. bassiana* and *M. anisopilea*.	40
7.	Glass jars used for laboratory treatment for 5th and 10th larval instar of RPW with *B.bassiana* and *M.anisopliae*.	40
8.	A = Sample showing growth of the fungus on the larval body post treatment. B = Sample showing incubation of fungus in Petri-dish.	42
9.	Field injection of bioinsecticide (Newvar or Metmite) at 10gm / liter of Distilled water in infested date palm trees.	43
10.	Life cycle of *R. ferrugineus.*	47
11.	Egg-laying holes in the sugar cane stem pieces by RPW adults.	47
12.	Larva of *R. ferrugineus* (2x)	49
13.	RPW larval tunnels: A: In sugar cane stem. B: In artificial diet (normal size).	51
14.	RPW larval feeding on natural diets: A: sugar cane. B: palm tree.	51
15.	Cocoon of *R. ferrugineus*.	53
16.	Pupa of *R. ferrugineus*.	53

Introduction

There is no doubt that date palm trees *Phoenix dactylifera* L. are playing an important role in the Egyptian agriculture and represents a significant part in the reclamation programme. Besides the nutritional values and health benefits of the fruits, the date palm products are daily used by Egyptians. Adaptation of date palm to water stress made it as one of the first fruit trees distributed and taken into cultivation in arid and semi-arid regions of Egypt. Although there are many cultivars of dates in Egypt, only about twenty are widely commercial distributed. Besides, there are a great number of seedling date palms, as a result of sexual reproduction; some of them are highly desirable for fruit qualities **(Hussein *et al.,* 1979)**. Also, it is mentioned in the holy Quran and the Bible. It has a special consideration in our hearts as Muslims and Arabs. This tree is an important component of Arab World Flora. Date palm tree is considered one of the fruit trees that belong to Arecaceae. The genus consists of fourteen species distributed in the tropical and sub-tropical regions **(Al Antary and Sharaf, 1994)**.

Date palm is attacked by a large number of pests, including fungi, insects, and nematodes **(Carpenter and Elmer, 1978).** Some of these pests are serious and difficult to control such as red palm weevil (*Rhynchophorus ferrugineus* Olivier, (Coleoptera: Curculionidae) **(El-Sufty *et al.,* 2007 and Arab and El-Deeb, 2012).** The red palm weevil (RPW) became the most dangerous insect pests which is distributed in many Arab countries includes Egypt, Saudi Arabia, United Arab Emirates (UAE), and introduced to Qatar from Saudi Arabia in 1989 **(Al-Khunji and Al-Turaihi, 2000).** The red palm weevil, *Rhynchophorus ferrugineus* causes heavy losses to farmers. Larva attacked plant and tissue feeding to turn into pupa. The adult insects either stay inside the cavities, causing damage to the palm. Where are destroying the stem and continue to grow and thus produce multiple and overlapping generations (**Gomez and Ferry, 1999 and Polana and Saleh, 2011).**

Synthetic pesticides remained the mainstay of red palm weevil, *R. ferrugineus* control over 50 years. However, insecticide resistance, pest resurgence and concerns over human health and environmental pollution by insecticides have encouraged researchers for the development of environmentally benign strategies for pest control including the use of entomopathogenic fungi **(Hussain *et al.*, 2013)**. Many researches and studies focusing only on the use of pathogens such as entomopathogenic nematodes, bacteria and entomopathogenic fungi in controlling RPW. Naturally occurring bio-control agents are alternative to reverse the use of hazardous synthetic insecticides. Among these microorganisms, the use of entomopathogenic fungi was found to be promising alternate for insect's control **(Dembilio and Jacas, 2012)**. The potential of two strains of the entomopathogenic fungi *Beauveria bassiana* (Ascomycota: Clavicipitaceae) and *Metarhizium anisopliae* evaluated in laboratory, semi-field and field assays. So, the aim of this study was to determine:

1- The pathogenicity of two commercial formulations of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Sorok) against different larval instars of red palm weevil (RPW) in laboratory.

2- The pathogenicity of isolates of *B. bassiana* and *M. anisopliae* from cadavers of infected RPW larvae.

3- The LC_{50} & LC_{90} and LT_{50} & LT_{90} values of commercial products of *Beauveria bassiana* and *Metarhizium anisopliae* to confirm infection against RPW under laboratory conditions.

4- The effect of *B. bassiana* and *M. anisopliae* on total protein, carbohydrate and lipid of RPW larvae.

5- The effect of entomopathogenic fungi on population demographic parameters (life table) of RPW.

6- The efficacy of the commercial formulations of *B. bassiana* and *M. anisopliae* to control the RPW under field conditions.

Review of Literature

1-Taxonomy and distribution of red palm weevil:-

Red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) was first described in India as a serious pest of coconut palm **(Lefroy, 1906)** and later recorded on date palm **(Lal, 1917 and Buxton, 1918).**

The taxonomy has changed multiple times in the past. Recent molecular research suggests that *R. ferrugineus* may be a species complex composed of two or more species **(Rugman *et al.*, 2013).**

Rhynchophorus signaticollis Chevrolat, 1882, *Curculio ferrugineus* (Olivier), 1790, *Calandra ferruginea* (Fabricius), 1801 **(CABI, 2009),** *Rhynchophorus vulneratus* (Panzer), 1798 **(Hallett *et al.*, 2004 and El-Mergawy and Al-Ajlan, 2011).**

The origin of this pest was likely South East of Asia, mainly in Pakistan, India, Burma, Bangladesh and Indonesia, as it has been reported by International Institute of Entomology in London **(Liver, 1969)** and spread later in many other countries where date palms are grown such as Iran and Arabian countries; Iraq, Saudi Arabia, Emirates and recently in Egypt.

For the first time in Saudi Arabia was recorded in 1986 in Al-Katif Region **(Al-Abdulmohsin, 1987),** the United Arab Emirates in 1986, and from the Republic of Iran in 1992. It spread to North Africa in Egypt in 1993 **(Cox, 1993).**

During the last decade of the 20th century, a new most serious insect pest, namely the red palm weevil, *R. ferrugineus* was firstly recorded in date palm plantations in Egypt **(Saleh, 1992 and Saleh and Gouhar, 1993).**

R. ferrugineus was introduced in Spain mainland in 1995 and then spread to all palm growing areas in the Mediterranean and recently also to the Canary Islands. It attacks more than 20 palm species worldwide **(Barranco *et al.*, 2000),** including date and coconut palm **(Malumphy and Moran, 2007).** And in 1999 in, Jordan and the Palestinian Authority Territories. This weevil has been reported on 19 palm species belonging to 15 different genera **(Barranco *et al.*, 2000; Eppo, 2008 and Dembilio *et al.*, 2009).**

Worldwide *R. ferrugineus* is currently reported from all the continents, attacking 40 palm species worldwide of which coconut, date palm and the canary island palm are important **(Anonymous, 2013 and Faleiro, 2006).**

Red palm weevil was first described as a harmful insect on the Indian coconut palm in 1906 **(Lefroy, 1906)**, and the date palm tree in 1917 **(Brand, 1917).** It was then discovered in the Gulf Region in the mid-1980s, from where it spread rapidly to several date producing countries through infested planting material that is mainly transported for ornamental gardening **(Faleiro *et al.*, 2012).**

Adult weevils are mainly active during the day and are capable of long-distance flight (900 meters) from the location of hosts or breeding sites (USDA-APHIS, Marked and released weevils migrated up to 7 km during a period of 3 to 5 days **(Abbas *et al.*, 2006).**

The RPW is attracted by kairomones of damaged palms, in the trunk of which larvae develop. As a result, the central tissue of the palm is destroyed and the tree eventually collapses and dies. As RPW is a hidden tissue borer it is difficult to detect its attack at an early stage of infestation **(Faleiro, 2006).**

RPW was officially detected when an adult was recovered from a heavily damaged Canary Island date palm in a private garden **(Hoddle, 2011).**

2- Life cycle and mass rearing of red palm weevil:-

The entire life-cycle of red palm weevil (RPW) takes about 45 to 298 days depending upon weather conditions. Seasonal activity of RPW varied between months and within the same month **(El-Garhy, 1996).**

R. ferrugineus complete its life cycle inside the trunk of the palm. It has four life - stages (complete metamorphosis), which includes eggs, larva, pupa, and adult stage. In general, all types of palm trees support the growth and development of the red palm weevil during all stages of its life. The life cycle begins when the female weevils lay about 300 creamy-white eggs (2.6 mm in length and 1.1 mm of width) inside cracks and crevices on the palm trunk. Gravid female weevils are attracted to young date palms less than 20 years old **(Abraham *et al.*, 1998)** for egg laying which is enhanced due to palm tissue volatiles emitted on fresh injuries on the palm resulting from frond shaving and offshoot removal. The eggs hatch in from 2 to 5 days. The resulting conical legless larvae continue to grow in the palm trunk for 1 to 3 months. They feed primarily on palm tissue and move inwards towards the soft heartwood at the centre of the palm trunk. A complete larva (50 mm in length and 20 mm of width) has a yellowish-white body and a reddish-brown head with strong mandibles that help bore the palm trunks. When fully grown, the larva forms a cocoon about 35 mm long and 15 mm wide. After 14 to 21 days in the pupal stage the insect becomes an adult (imago) 35 mm long and 12 mm wide, reddish-brown in colour with black spots on the thorax, a long proboscis, and a pair of antenna on the front of its head. Adult male weevils are characterized by a tuff of bristles on the dorsal tip of the snout. Adult weevils feed outside the palm trunks and can live an average of 98 days (between 29 to 153 days) **(Wattanapongsiri, 1966; Avand Faghih, 1996 and Abraham *et al.*, 2001).**

A method for laboratory mass rearing of the red palm weevil *R. ferrugineus* (RPW) was developed. Weevils, initially obtained from the field, were maintained on the stems of sugarcane. Befor mass rearing, several artificial diets were formulated and preliminarily evaluated for development of the *R. ferrugineus*. Materials used for preparations of various diets were: oats, coconut cake, coconut fruit pieces, canned and/fresh pineapple, sucrose, molasses, egg yolk, salt, yeast, vegetable oil, potatoes, soybean flours, date palms leaves and palm fibre sheath, sugarcane fibres, bacto-agar, multi-vitamins, preservatives, and water. Oat and white bean diets were preferred by 1^{st} to 3^{rd} larval instars, while oats + fibres preferred by 4^{th} to 5^{th} larval instars. Larvae fully developed on artificial diets and molted four times during their development failed to construct cocoons because of the unavailability of fibres (palm or sugarcane). Facilities, materials required, diet preparation and procedures, and practical difficulties of rearing methods are discussed by **Kaakeh *et al.*, 2001.**

The development of the red palm weevil, *R. ferrugineus* (Olivier) was investigated on a newly developed semiartificial diet as compared with two natural diets namely sugarcane stem and banana fruit. The weevil was successfully maintained on these diets and duration of the life cycle for males and females, respectively were 164.97 and 194.61 days on the semiartificial diet, 192.5 and 186.5 days on banana, and 172.00 and 170.00 days on sugarcane. The average egg production per female was shown to be significantly higher on the semiartificial diet, being 184.00 ± 18.68

eggs compared with an average of 125.00 ± 11.97 and 133.00 ± 15.21 eggs on banana and sugarcane, respectively. The fertility ranged between 94 – 100 % in those eggs deposited by females previously reared on the tested diets. The developed semiartificial diet was shown to be suitable for maintaining laboratory colonies of the red palm weevil, and it can substitute natural diets (**Salama and Abdul Razek, 2002).**

The sex ratio found in a study in the United Arab Emirates was 1 male: 1.5 females **(Abbas *et al.*, 2006),** in Egypt, 1:2 **(El-Garhy, 1996),** and in Palestine, 1:2.5 **(Soroker *et al.*, 2005).**

Life parameters including pre-oviposition period, oviposition period, larval and pupal periods, adult male and female development period, and generation span, were obtained for the red palm weevil, *R. ferrugineus*, reared on artificial diets of oat, potato, pineapple, and palm fibre sheath, and natural diets of sugarcane, palm heart, and palm leaf base. Significant differences in the duration of all life parameters were found when fed on various diets. The pre-ovipositional periods ranged from 3.15 to 3.61 (days), while the oviposition periods ranged from 3.2 d to 3.8 (days). The developmental times of larvae ranged from 70.8 d to 102.2 (d), while the development time of pupae ranged from 16.1 d to 22.2 (d). The developmental time of adults previously reared on natural diets was longer than those fed on artificial diets. Differences in the development time occurred between males and females reared on different diets, except on sugarcane and palm leaf base. The generation span ranged from 93.2 (d) to 131.3 (d). Significant differences in the average number of eggs deposited per female previously reared in their larval stages on various diets, ranged from 68.2 to 185.2 eggs, while the average number of eggs deposited per female per day ranged from 1.28 to 3.03 eggs. The mean total number of eggs laid by females, eggs deposited 30 d after one full copulation with males of similar age, and rate of egg hatch decreased significantly with increasing weevil age and ranged from 65.5 eggs (1 (d) -old female) to 43.5 eggs (45 (d) -old female). The rate of egg hatch also decreased significantly with increasing weevil age and ranged from 75.8% (1 (d) -old weevils) to 47.4% (45 (d) -old weevil). The short copulatory period was adequate for insemination of the female during copulation. Feeding of R. ferugineus on different diets resulted in different life parameters **(Kaakeh, 2005).**

The red palm weevil (RPW) *R. ferruginous* was fully developed and reared on a meridic diet consisting of agar, distilled water, commercial yeast as well as laboratory produced amino and fatty acid rich brewer's yeast (Saccharomyces cerevisiae), wheat meal, corn flour, benzoic acid, ascorbic acid, sorbic acid, vitamin mix and tetracycline hydrochloride. A group of weevils reared on date palm trunk under the same laboratory conditions was used as control. Diet reared female weevils laid fertile eggs which successfully hatched into healthy larvae in 3.6 days. Between the first and last (eighth) larval instars there was a 2311 times increase in the larval body biomass before pupation. Larval development was completed in 43 to 47 days, while the pupa reached the adult stage in 31 to 38 days with an average of 35 days. The whole life cycle of the weevil from egg to adult, was completed in 78 to 85 days. The average adult longevity for male and female weevils was 83.3 and 74.8 days, respectively. Besides the above, important biological parameters including pre-oviposition period, incubation period, percent egg hatchability, fecundity and larval weight gain were recorded. A food-fiber pupation technique was developed with 100% pupation efficiency (**El-Shafie *et al.*, 2013).**

Generally, the red palm weevil takes about three to four months to complete it is life cycle. RPW female chews a hole into the palm tissue by using long beak. Eggs are laid singly into these holes and also in wounds caused by the Rhinoceros beetle in the palm trunk. It was recorded a maximum of 349 eggs laid by a single female during 47 days at 28 ºC **(El-Bokl *et al.*, 2010).** All stages (egg, larvae, pupa and adult) are spent inside the palm itself and the life cycle cannot be completed in some other place.

Five different diets (small pieces of sugarcane stems, sugarcane stem pieces + sugarcane residues, sugarcane stem pieces + date palm trunk pieces, sugarcane stem pieces + ground frond of date palm and sugarcane stem pieces + food residues of RPW) were tested for rearing the red palm weevil, *R. ferrugineus* and to investigate the effect of diets on some biological aspects under laboratory conditions. Larval durations of different instars were affected by different diets. The larvae fed on (sugarcane stem pieces + ground frond of date palm), slowly developed to exhibit the longest duration of 103.78 days. Meanwhile, the larvae fed on the other diets showed a gradual shortage in larval periods to give means of 89.47 and 74.63 days when fed on small pieces of sugarcane stems and sugarcane stem pieces + food residues of RPW, successively. Feeding on different diets had great effect on the means of the pupal period, which were obtained between the tested diets. Longevity of males and females emerged from larvae fed on the different tested diets varied. The sex-ratio of emerged adults was the highest (more females), when larvae were fed on sugarcane stem pieces + food residues of RPW. Mean number of eggs deposited by emerged females from larvae fed on different diets was highest in case of (sugarcane stem pieces + food residues of RPW). The mean of incubation period of eggs laid from adult fed on three tested diets ranged from 3.78 to 4.54 (sugarcane stem pieces + food residues of RPW and sugarcane stem pieces + ground frond of date palm, respectively. Hatchability percentage proved to be affected by different diets. The superior diet was sugarcane stem pieces + food residues of RPW, because of biology completed and the diet kept moisture and remained fresh all the time (**El-Zoghby and Abdel-Hameid, 2018).**

Life parameters including pre-oviposition, oviposition and larval & pupal periods, adult male and female longevities, and generation period were recorded for the red palm weevil, *R. ferrugineus* Olivier reared on the sugarcane stem pieces (diet 1) and other 3 artificial diets depending mainly on ground sugarcane (diet 2); ground corn (diet 3) and ground mixture of sugarcane and corn (diet 4). In the laboratory at 28±1°C and 75±5% R.H., the effects of all diets on total proteins, carbohydrates and lipids were also determined in the last instar larvae. Larvae fed on diet 2 (ground sugarcane + additives “agar 10g, dried active yeast 15g, sorbic acid 1.25g, L.ascorbic acid 2.50g, sodium benzoate 1.25g and distilled water 150-200ml”) exhibited shortest larval duration (86.30 days), while diet 3 (ground corn + additives) caused the longest larval period (128.35 days). Shortest and longest pupal periods resulted also from feeding on diet 2 and diet 3 (15.3 and 21.75 days, respectively). On the other hand, male and female longevities were the longest and shortest when previous larvae were fed on diet 2 and diet 3, 50.4 and 38.4 days for males and 48.0 and 36.8 days for females, respectively. The sex-ratio was almost 1:1 with all diets, except diet 2 which led to more females (1 male: 1.5 female). Eggs of *R. ferrugineus* hatched after about 4 days, but this period was, significantly, shorter (3.35 days) by rearing on diet 2. Highest hatch ability percentage (93.61 %) resulted from rearing on the same diet. Chemical analyses of last larval instar content indicated that the highest total contents of proteins (51.87 mg / g), carbohydrates (105.0 mg / g) and lipids (21.13 mg / g) were obtained by rearing on diet 2, diet 1 and the control (natural feeding on palm), respectively **(Abdel-Hameid, 2019).**

3- Evaluation of the fungus as a bio-control agents against RPW:-

Because the red palm weevil is a hidden tissue borer and difficult to eradicate, early detection is essential for an effective eradication or management program. When a population of red palm weevil becomes determined, efforts must be put into an (IPM) system. Different strategies have been adopted against different life stages of RPW. The previous investigations have reported the control of adult RPW by adopting different tactics such as the use of Sterile Insect Technique (SIT), insect pheromones and insecticidal applications to prevent the adult entry into the tree trunk. The use of SIT to control RPW was considered for the first time during 1970s, suggested that the 1-2 d exposure of X-rays to the newly emerged male populations of RPW at a dose of 1.5 Krad greatly (90) induced the sterility. In another study, field trials were conducted to investigate the effect of radiations on the growth of RPWs and the viability of eggs laid by the females. They reported that the sterile RPW males remained live till 100 days post exposure. Pheromones usage into the management strategy of RPW started with the identification of aggregation pheromones (ferrugineol {4-Methyl-5-nonanol} and ferrugineone {4-methyl-5nonanone} during 1993. Later on, the work on the use of pheromones to enhance their trapping potential started in different parts of the world **(Dembilio and Jacas, 2012).**

Many researches and studies focusing only on the use of pathogens such as entomopathogenic nematodes, bacteria and entomopathogenic fungi in controlling RPW. Naturally occurring biocontrol agents are alternative to reverse the use of hazardous synthetic insecticides. Among these microorganisms, the use of entomopathogenic fungi was found to be promising alternate for insect's control. According to an estimate, more than 700 species of fungi belonging to different genera are known to infect insects. In the past, the potential of entomopathogenic fungi especially *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* have been valuated against different pests including *Aphis craccivora, Aedes aegypti, Bemisia argentifolii, Coptotermes formosanus, Melanoplus sanguinipes, Ocinara varians* Walker, *Odontotermes obesus, Periplaneta americana, R. ferrugineus, Scolytus scolytus, Thrips tabaci* **(Dembilio and Jacas, 2012).**

Synthetic pesticides remained the mainstay of red palm weevil, *R. ferrugineus* (Olivier) (Coleoptera: Curculionidae) control over 50 years. However, insecticide resistance, pest resurgence and concerns over human health and environmental pollution by insecticides have encouraged researchers for the development of environmentally benign strategies for pest control including the use of entomopathogenic fungi. Entomopathogenic fungi form the largest single group of insect pathogens. Such insect killing fungi are fast growing microorganisms to be recognized as disease causing agents in insects. Recent developments have revealed that successful invasion of pathogens to cause infection among insect populations relied on many fitness factors. Their failure or attenuation led to the development of disease resistance **(Hussain *et al.*, 2013).**

3-1- Evaluation of the fungus, *Beauveria bassiana* (Commercial product and isolates):-

In 2001, the study determined that *B. bassiana* used against RPW during their residence in the trap, each adult has high probability of receiving sufficient *B. spp*. to cause death in approximately 4 days. There was also a significant probability of horizontal transfer from infected individuals to other insects coming into contact with treated individual, but away from the bait site **(Deadman *et al.*, 2001).**

B. bassiana is a common soil-borne fungus that occurs worldwide **(Asensio *et al.*, 2005).**

B. bassiana has a dimorphic mode of growth and in the absence of the specific insect host, *B. bassiana* passes through an asexual vegetative life cycle that includes conidia germination, filamentous growth and sporulation (conidiophores). Molecular and cultural studies have provided insight regarding the phylogenetic position and reproductive biology of *B. bassiana*. An rDNA phylogeny by **Sung *et al.*, (2001)** supported a single evolutionary origin of *Beauveria* within the subfamily Cordycipitoiceae of the Clavicipitaceae. *Cordyceps* species produce *Beauveria* anamorphs (asexual stage), clearly demonstrating that some *Beauveria* species are sexual such as *C. bassiana* **(Li *et al.*, 2001 and Sung *et al.*, 2006).**

Biological control of the red palm weevil, *R. ferrugineus* was studied using the local strain, UAE-B2 of the entomopathogenic fungus *B. bassiana*. For mass production of dry conidia, a new economic simple medium containing granulated rice was evaluated and used. The culture medium yielded 5.2 mg conidia/cm2 with a potentiality of 91.7% on adult weevils. The conidia were stored at 10 °C for 13 months without decrease in its virulence. Preliminary field investigations were carried out in date palm plantations at Ras Al-Khaimah to evaluate the efficacy of the fungus. Spraying date palm trees with an oil formulation containing 5 x 10^7 conidia / ml at a rate of 5 L / tree caused a mortality of 13.7-19.2% in the adult population during the three weeks after application with a monthly delayed mortality of 2.3-12.5% in the following four months. Dusting a date palm tree with 40 g of a powder formulation containing 5% conidia killed 8.9% of adult population during the three weeks after application and caused monthly delayed mortality of 4-5.9% in the following three months **(El-Sufty *et al.,* 2007).**

Fungal pathogenesis mainly starts with the secretion of cuticle degrading enzymes. Known cuticle degrading enzymes are chitinases, proteases and lipases. *B. bassiana* for instance produces extracellular enzymes able to degrade to the major components of insect cuticle, e.g., end proteases, amino peptidases, carboxypeptidases, lipases, esterase's, chitinases, and N-acetylglucosaminidases **(Leger *et al.,* 1986 and Pedrini *et al.,* 2007).** Also, the death of the insect results from a combination of factors: mechanical damage resulting from tissue invasion, depletion of nutrient resources and toxicities **(Bandani, 2008).**

B. bassiana is an ascomycotic filamentous fungus of order hypocreales and genus *Beauveria*. A broad range of *Beauveria* species have been isolated from a variety of insects worldwide that are of medical or agricultural significance. Beauvericin is a famous mycotoxin produced by many fungi, such as *B. bassiana*, which used as an insecticide, the spores are sprayed on affected crops as an emulsified suspension or wettable powder or applied to mosquito nets as a mosquito control agent **(El-Sufty *et al*., 2009).**

The New Saudi Arabia isolates of the entomophathogenic fungi *B. bassiana* (BSA 3 Saudi isolate) showed some result in a field experiment, *B.bassiana* fungi codacide oil suspension was sprayed at a concentration of $5x10^8$ conidia/ ml on infested date palm trees. One month following the first spraying in December the number of weevils was reduced from 16.5 to 6 weevils/ trap giving a reduction of 63.64% **(Hegazy, 2009).**

Recently, **Güerri-Agullo *et al.,* (2010)** showed that *B. bassiana* caused 70-85% *R. ferrugineus* mortality. *B. bassiana* solid formulation with high RPW pathogenicity and persistence could be applied as a preventive as well as curative treatment for RPW control. *B. bassiana* formulation can be a significant component of an IPM strategy for RPW control.The *B. bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin are two of the most commonly studied species of soil-borne entomopathogenic fungi. Both *Beauveria* and *Metarhizium* are cosmopolitan anamorphic genera of facultative necrotrophic arthropod-pathogenic fungi (**El-Sufty *et al.,* 2009).**

Dembilio *et al.,* (2010) studied the pathogenicity of an indigenous strain of *B. bassiana* isolated from pupae and adults of weevil and they found that most adults died between the first and second weeks after treatment and that young larvae are more susceptible than old ones; this strain can infect weevil eggs, larvae and adults, the fungus efficiently transmitted the disease to untreated adults of both sexes and reduced fecundity and egg hatching.

The *B. bassiana* solid formulation with high weevil's pathogenicity and persistence could be applied both as a preventive treatment and as a curative one for pest control. However, **Abdel-Samad *et al.,* (2011)** reported that a commercial oil formulation of *B. bassiana* has little effect on weevil, and thus is not a good candidate since it is expensive for field application. Moreover, polar extracts from adults inhibited the germination of *B. bassiana* spores obtained from commercial products.

Many researchs showed the *B. bassiana* used an effective method against eggs, larvae and adults of RPW. Among these stages, adults withstand relatively more time (4-5 weeks) upon exposure with spore suspensions, while dried formulations impart 100% mortality **(Hussain *et al.*, 2013).**

Entomopathogenic fungi are ubiquitous natural enemies that decrease pest damage. During 2010 and 2011 seasons, more than 700 red palm weevils were collected from the coastal areas of Syria to study the entomopathogenic efficacy of some fungal isolates against this pest. Four fungal *B. bassiana* isolates were isolated and maintained in the laboratory. The characterization of these isolates showed that the best fungal growth, in vitro, was at 25 ºC, and the highest conidial spores productivity was at 20 ºC. Pathogenicity test of three *B. bassiana* isolates (BBS, RPWSL5 and GHA) against the pest adults showed that complete insects mortality (100%) occurred 14 days after spraying the RPWSL5 isolate. Moreover, the LC_{50} for RPWSL5 isolate was $2.12x10^5$ conidia / ml, and the LT_{50} was 3.5 days. These findings indicated that the RPWSL5 seems to be a promising entomopathogenic agent to be used in an integrated pest management program for the control of red palm weevil along the Syrian coast **(Kadour *et al.*, 2014).**

Strains of *B. bassiana* were isolated from symptomatic insects collected on dead palms, and their pathogenicity against different instars of *R. ferrugineus* was evaluated in the laboratory. The overall percentage of infected insects found in Canary palms was 7%. In laboratory bioassays, hatching of eggs treated with three different isolates of *B. bassiana* was 41.2, 26.8 and 29.9%, significantly lower than the control (62.4%). Larvae and adults were treated with a single isolate in two ways: spraying each insect with a conidial suspension or feeding them with fruit portions previously immersed in the same conidial suspension. At the end of the two trials, the mortality of treated larvae was 88 and 92%, and the mean survival time was 10.4 and 11.8 days, significantly different from the control, where no insect died during the trials. Mortality and survival time recorded in either trial on adults did not significantly differ between treatments and control **(Verde *et al*., 2015).**

The host can be infected by: (a) direct treatment, (b) horizontal transmission from infected insects or cadavers to untreated insects, and (c) vertical transmission to subsequent developmental stages via the new generation of spores **(Sarwar, 2016).**

Saleem *et al*., (2019) studied RPW biology and development under laboratory conditions and survival against sub-lethal doses of *B. bassiana* and bio-rational insecticide (Nitenpyram). Sugarcane sets as alternate to date palm stem were successfully used as diet for rearing RPW. Combinat ion of entomopathogenic fungus (Bb) and Nitenpyram (Nit) were found more lethal to survival of RPW. A reduction in pupation and adult emergence was recorded in the combined treatments. Moreover, decrease in weight gain, frass production and cumulative gain in size was found when larvae were treated with integrated effect of Bb and Nit. Depending on the lethal of treatment, development duration of RPW was disturbed. Integration of Bb and Nit delays the development and diet uptake in RPW which can be used for agent of control for this cryptic insect.

3-2-Evaluation of the fungus, *Metarhizium anisopliae* (Commercial product and isolated):-

M. anisopliae, a formerly known as *Entomophthor anisopliae* (basionym), is a fungus that grows naturally in soils throughout the world and causes disease in various insects by acting as a parasitoid, and is the most intensively studied species of the genus Metarhizium. The reproductive structures of *M. anisopliae* (the anamorph, the most commonly encountered form) comprise conidiophores and conidia. Leveduriform structures or blastospores and appressoria are produced by *M. anisopliae* through mycelia differentiation. The disease caused by the fungus is sometimes called green muscardine disease because of the green color of its spores. When these mitotic (asexual) spores (called conidia) of the fungus come into contact with the body of an insect host, they germinate and the hyphae that emerge penetrate the cuticle. The fungus then develops inside the body eventually killing the insect after a few days; this lethal effect is very likely aided by the production of insecticidal cyclic peptides (destruxins) **(Tiago *et al.*, 2014).**

Recent study showed the successful control of red palm weevils mainly depends on the host pathogen interactions. So, there is a constant struggle between host and pathogen that ultimately lead to the success or failure of pathogens. In case of compatible interaction, the pathogen must have high number of conidia with strong adhesion that ultimately penetrate into the host through directly penetrating structures. Moreover, the invading pathogen must have the capacity to bypass or overcome the host immune system by producing toxins **(Gindin *et al.*, 2006).**

The entomopathogenic fungi, *M. anisopliae* (Metsch.) was evaluated for it's pathogenicity against the larvae and adults of *R. ferrugineus* under laboratory conditions. Red palm weevil stages were collected from the infested palm trees, Al-Hasa Governorate, KSA, and reared on pieces of sugarcane. Three concentrations of fungi spore were prepared, $1x10^7$, $1x10^8$ and $1x10^9$ spores/ml. Adults of *R. ferrugineus* (male and female) and larvae were treated by dipping and injection bioassay techniques. The control treatment was treated with sterilized water. After treatments, insects were transferred to jars containing sugarcane. Insects were observed for three weeks and the percent of mortality recorded. The results showed variation in mortality for different stages. Mortality percentages were 35, 50, and 60 for male, female and larvae, respectively at a concentration of $1x10^7$ spores/ml of *M. anisopliae* when insects were treated by dipping. The mortality reached 70, 60, and 70% for the previous stages when treated by injection at the same concentration after ten days of observation. The percent of mortality increased with increasing concentration of fungus spores. The percent of mortality reached 70 and 80 for female and male when contaminated males were transferred to jars containing untreated females after 17 days of transfer. The percent of mortality reached 10% in the control treatment during that period **(Shawir and Al-Jabr, 2010).**

The red palm weevil (RPW) *R. ferrugineus* Olivier represents also in Italy a very severe threat for ornamental palms. Control strategies of the larval and adult stages of the insect include the use of virulent strains of entomopathogenic fungi. During 2009, **Rumine *et al.*, (2010)** carried out laboratory trials in order to evaluate the efficacy of some local isolate of *M. anisopliae*. The results showed a good control of larvae and adults confirming the possibility of application of microbiological control strategies.

Previous study showed the *M. anisopliae* (M.08/I05), which isolated from Italy, was appeared to be an indigenous virulent strain which provided an effective control against RPW and its efficacy could be supported and/or enhanced by suitable insect host treatment **(Francardi *et al.*, 2012).**

R. ferrugineus is considered the worst pest of palm species, and few natural enemies are reported for the parasite in its area of origin. Here, they reported the first recovery of the entomopathogenic fungus

Metarhizium pingshaense associated with *R. ferrugineus* from Vietnam. The morphological, biochemical, and toxicological features of this strain were studied and compared with those of another Metarhizium strain associated with this weevil in Sicily (Italy), an area of recent introduction. The potential use of these fungi as biocontrol agents was tested against adult insects in laboratory trials and a similar mortality rate was found. Both strains were able to produce toxins and cuticle-degrading proteases, but they showed dissimilar enzymatic and toxicological profiles, suggesting a different virulence activity **(Annarita *et al.*, 2014).**

The lack of biological insecticides based on *M. anisopliae* (Metsch.) Sorokin registered in Italy for the control of the red palm weevil (RPW), *R. ferrugineus* (Olivier), prompted studies to evaluate the efficacy of two commercial products, Met52Reg. and BioStorm TM and of their fungal isolates, *M. anisopliae* (Man52) and (ManBS), respectively, against the adults. The virulence of the *M. anisopliae* strains (Man52) and (ManBS) was compared with that of an indigenous *M. anisopliae* (Man08/I05) strain obtained from *R. ferrugineus* specimens collected in the wild and that showed to be very virulent against the RPW in previous studies. In both tests the sublethal effects of the treatments on female reproductive potential were examined in relation to the infective substratum. Laboratory results indicated that the commercial formulations mixed directly into the soil were not active in transmitting the infection to RPW adults and in reducing female fecundity and fertility **(Francardi *et al.*, 2015).**

Results obtained by **EL-Hindi, (2016)** showed that the *M. anisopliae* exhibited a good biological control agent against larvae and adults of RPW. The pathogenicity of the most virulent isolates and the toxicity assay on larvae showed the highest mortality percentage which reached to 90% after spraying the larvae with *M. anisopliae*, and reaches 43.3% after treated by pesticide. The bioassay on the adults of RPW and the maximum mortality of weevils reaches up to 90% after spraying the adult with *M. anisopliae*. The mortality for the adults treated with pesticide arrives to 50% and the control group 10% at the same time.

Xiao Dong *et al.*, (2016) studied the pathogenicity of *M. anisopliae* strain SD-3 against invasive red palm weevil (RPW), *R. ferrugineus* Olivier (Coleoptera: Curculionidae) larvae in Hainan Province, China. They found that inoculation of $1x10^8$ conidia/mL caused 100% mortality of *R. ferrugineus*, indicating that the conidia of strain SD-3 were highly virulent. The process of invasion mechanism was showed by scanning electron microscopy (SEM) and frozen section as follows. Once *R. ferrugineus* was infected by strain SD-3, *M. anisopliae* hyphae first invaded the cuticular and body cavity of *R. ferrugineus*. Secondly, well-developed muscles, fat, tracheaes and digestive tube tissues in the abdomen of *R. ferrugineus* were then decomposed and absorbed by *M. anisopliae* hyphae, leading to the total destruction of the larvae. Finally, *M. anisopliae* hyphae reproduced, resulting in a large number of conidia in the body of RPW. The SEM and frozen section are convenient tools to observe the mode of action of entomopathogenic fungi and to observe how *M. anisopliae* is able to colonize and infect the host.

4- Pathogenicity bioassays of *Beauveria bassiana* and *Metarhizium anisopliae* on red palm weevil:-

The entomopathogenicity of an indigenous *B. bassiana* strain isolated from soil and of *B. bassiana* and *M. anisopliae* strains isolated in Italy from naturally infected *R. ferrugineus* (RPW) adults, was tested against larvae and adults of RPW in laboratory bioassays by **Francardi**

***et al.*, (2012)**. The individuals were infected via direct contact on sporulated mycelia grown on Sabouraud Dextrose Agar or on wheat substrata. *M. anisopliae* obtained from *R. ferrugineus* showed the highest efficacy against RPW larvae and adults particularly against individuals contaminated on sporulated wheat, which showed values of cumulative larval mortality of 100% and adult mortality of 90%; LT_{50} was obtained in 13.1 days in both larvae and adults. *B. bassiana* strain isolated from soil recorded a lower cumulative mortality on larvae (13%) and adults (13%) treated on inoculated Sabouraud Dextrose Agar. *B. bassiana* strain isolated from RPW showed cumulative mortality values higher than 50% against larvae treated on inoculated wheat (55%) and Sabouraud Dextrose Agar (53%); $LT_{50}$was obtained in 15 days and 21.8 days respectively. Also, in laboratory bioassays, the delivery system successfully attracted, infected and released weevil adults after they contacted cereal substrata inoculated with indigenous strains of *B. bassiana* (Balsamo-Crivelli) Vuillemin and *M. anisopliae* (Metchnikoff) Sorokin. Tests carried out with the experimental traps showed that *M. anisopliae* was the more virulent pathogen, causing 75% cumulative mortality in adults, while *B. bassiana* gave a 45% cumulative mortality. Infectivity of *M. anisopliae* was not affected by different cereal substrata, *i.e.* wheat and rice, since curculionid cumulative mortality (95%) and treatment efficiency **(95% Abbott)** were very high on both of them and red palm weevil $LT_{50}$was reached within the same time (15 days). Conidial persistence and germinability of *M. anisopliae* grown on the rice substratum were examined in field conditions inside traps located in sunny and shady positions in spring, summer and autumn. The results showed that the traps preserved fungal inoculum stability longer in spring and summer than in autumn. No significant difference in *M. anisopliae* conidial persistence was found between sunny and shady traps during the various seasons (**Francardi *et al.*, 2013).**

Palm trees are attacked by a large number of pests that affect date production, quality and quantity. Red palm weevil *R. ferruginus* Olivier is the most important pest that attacks date palm. Entomopathogenic fungi are ubiquitous natural enemies that decrease pest damage. During 2010 and 2011 seasons, more than 700 red palm weevils were collected from the coastal areas of Syria to study the entomopathogenic efficacy of some fungal isolates against this pest. Four fungal *B. bassiana* isolates were isolated and maintained in the laboratory. The characterization of these isolates showed that the best fungal growth, in vitro, was at 25 ºC, and the highest conidial spores productivity was at 20 ºC. Pathogenicity test of three *B. bassiana* isolates (BBS, RPWSL5 and GHA) against the pest adults showed that complete insects mortality (100%) occurred 14 days after spraying the RPWSL5 isolate. Moreover, the LC_{50} for RPWSL5 isolate was $2.12x10^5$ conidia / ml^{-1}, and the LT_{50} was 3.5 days. These findings indicated that the RPWSL5 seems to be a promising entomopathogenic agent to be used in an integrated pest management program for the control of red palm weevil along the Syrian coast (**Kadour *et al.*, 2014).**

In order to evaluate the bio control agent potential of *M. anisopliae and B. bassiana* against RPW, **El-Bokl *et al.*, (2010)** showed that these fungi can be effective against eggs and larvae of RPW. Among these stages, adults withstand relatively more time (4-5 weeks) upon exposure with spore suspensions, while dried formulations impart 100% mortality within 2-3 weeks. Their results further suggested that *M. anisopliae* is more effective in causing 100% mortality of the larvae between 6 and 7 days. A new study show that all of the screened *M. anisopliae* strains exhibited pathogenicity to all development stages of

RPW, causing up to 80–100% mortality of larvae and adult weevils under laboratory conditions. When eggs were exposed to sawdust previously sprayed with *M. anisopliae* spores, the total survival of both the eggs and the hatched larvae was reduced by a factor of approximately two to three, relative to control **(Sabbour and Abdel-Raheem, 2014).**

The entomopathogenic fungal infection of target hosts depends on pathogen fitness and host defence mechanisms. Pathogen-host interactions in red palm weevil, *R. ferrugineus* larvae are poorly understood. In order to explore this interaction, 4^{th}, 8^{th}, and 12^{th} instar red palm weevil larvae were immersed in conidial suspensions of four isolates of *B. bassiana*. Significant differences in the virulence of the tested isolates were revealed by LT_{50} values. Conidia of B8463, a highly virulent isolate, showed 33.53% higher relative hydrophobicity and twice as much Pr1 activity than conidia of B8465. Growth indices, calculated after 72 h of incubation, revealed significant differences in the food utilization efficiencies of all studied larval instars infected with different isolates. Conidial infection with B8463 caused 39-45% reduction in efficacy of consumption of ingested food (ECI) and 55-61% reduction in digested food (ECD). The least virulent isolate, B8465, caused the smallest reduction in ECI (2-4%) and ECD (3-9%). Furthermore, enhanced expression of target antioxidant genes (catalases and peroxidase) was observed in larvae infected with virulent isolates. Similarly, approximate digestibility showed an opposite trend, with the highest values being recorded from samples infected with the most virulent conidia (B8463) at each studied larval instar. In conclusion, isolate B8463 significantly affected the growth and development of red palm weevil larvae and has good potential for use in eco-friendly *R. ferrugineus* management **(Hussain *et al.*, 2015).**

Entomopathogenicity tests on RPW were carried out by **Hajjar *et al.*, (2015)**. The results showed that the LC_{50} of *B. bassiana* (Broadband) was $2.19x10^7$ and $2.76x10^6$ spores/ml at 9 and 23 d of treatment, respectively. The LT_{50} was 13.95 and 4.15 d for concentration of $1x10^7$ and $1x10^8$ spores/ml, respectively, whereas $1x10^9$ spores/ml caused 100% mortality after 24 h. Additionally, a red palm weevil pheromone trap was designed to attract the adults to be contaminated with spores of Broadband, which was applied to the sackcloth fabric that coated the internal surfaces of the bucket trap. The mating behavior was studied to determine direct and indirect infection of the spores from male to female and vice versa. Also, results showed a high efficacy of Broadband suspension at $1x10^9$ spores/ml; 40 ml of suspension at this concentration treated to cloth in a trap caused death of contaminated adults with *B. bassiana* spores directly and indirectly. The 100% mortality was obtained even after 13 d of traps treatment with 40 ml of the suspension at $1x10^9$ spores/ml. On the other hand, **Francardi *et al.*, (2015)** mentioned that the fungal *M. anisopliae* (ManBS), (Man52) and (Man08/I05) strains inoculated on a rice substratum caused over 80% mortality of the phytophagous. In particular, *M. anisopliae* (ManBS) and the *M. anisopliae* (Man08/I05) produced the highest mortality (100%), with LT_{50}, LT_{90} reached in 3 and 6 days respectively. *M. anisopliae* (Met52) strain instead led to 85% mortality of RPW specimens in 28-days but it took longer to reach LT_{50} (6 days) and LT_{90} (12 days).

A new *B. bassiana* based attract and infect device (AID) to control *R. ferrugineus* Olivier (Coleoptera: Curculionidae) was developed. The virulence and persistence of the fungal formulation used in the AID were evaluated in the laboratory. Semi-field and field trials were carried out to validate the results and establish the potential of this device as a control tool. In laboratory conditions, a 50% lethal time (LT_{50}) of 4.33 days was obtained when adults (7-10 days old) were exposed to the inoculation tunnel (IT) containing $1x10^{10}$ conidia / g in an oil-based fungal formulation. This formulation maintained conidium viability at 50% for up to 2 months. Moreover, when adults were exposed to 2.5-month field-aged ITs, mortality still reached 50% 40 days after exposure. In addition, no differences were observed between ITs aged in early spring and those aged in summer, suggesting that the fungal formulation is not strongly affected by environmental factors in Mediterranean basin conditions. Semi-field assays showed that the device allowed an easy transit of weevils through the IT, which were effectively attracted and infected. Using the AIDs in 4-ha plot field trials, a reduction of 50% in the percentage of infested sentinel palms was obtained (**Dembilio *et al.*, 2018).**

5- Life table of red palm weevil *Rhynchophorus ferrugineus*:-

To take the variable developmental rates among individuals and both sexes into consideration, **Chi and Liu, (1985) and Chi, (1988)** developed an age-stage life table theory. Because variation in developmental rate among individuals and between sexes in a natural population is a normal occurrence **(Chi, 1988 and Liu *et al.*, 1997),** an age-stage structured model helps take the variation in the predation rate and the survival rate of individuals of the same age but different stage into consideration.

Life tables are useful tools in the study of population biology and ecology **(Chi 1990, Sakai *et al.*, 2001)** because they provide population demographic parameters, which are important for estimating insect population growth capacity. However, traditional age-specific life tables consider only the female population and ignore the variable development rates among individuals and stage differentiation**.**

Entomopathogenic fungi have been studied as potential biological control agents. Strains of *B. bassiana* were isolated from symptomatic insects collected on dead palms, and their pathogenicity against different instars of *R. ferrugineus* was evaluated in the laboratory. The overall percentage of infected insects found in Canary palms was 7%. In laboratory bioassays, hatching of eggs treated with three different isolates of *B. bassiana* was 41.2, 26.8 and 29.9%, significantly lower than the control (62.4%). Larvae and adults were treated with a single isolate in two ways: spraying each insect with a conidial suspension or feeding them with fruit portions previously immersed in the same conidial suspension. At the end of the two trials, the mortality of treated larvae was 88 and 92%, and the mean survival time was 10.4 and 11.8 days, significantly different from the control, where no insect died during the trials. Mortality and survival time recorded in either trial on adults did not significantly differ between treatment and control. This study shows that the pathogenicity of wild isolates of *B. bassiana* differs among the tested *R. ferrugineus* instars. The low mortality of treated adults supports their use as vectors of *B. bassiana* as a potential tool for reducing *R. ferrugineus* populations **(Verde *et al.*, 2015).**

A study was carried out to investigate the insecticidal properties of *B. bassiana, M. anisopliae* and Heterorhabditis bacteriophora for their virulence against different larval instars of *R. ferrugineus* (Olivier). Both fungi were either applied alone or in combination, with *H. bacteriophora* simultaneously or 1 and 2 weeks after fungal application; EPN were also applied alone. Moreover, assessment of host development, diet consumption, frass production and weight gain were observed at sub-lethal dose rates. In combined treatments, additive and synergistic interactions were observed. Synergism was observed more frequently in *H. bacteriophora* + *B. bassiana* combinations than in *H. bacteriophora* + *M. anisopliae* combinations, and was higher in early instars than old instars. In 2nd and 4th instars, synergy was noted in *H. bacteriophora* + *B. bassiana* combinations at 0, 7 and 14 d intervals and in 6th instar synergy was observed only in *H. bacteriophora* + *B. bassiana* combinations (at 0 and 7 d intervals). A decrease in pupation, adult emergence and egg hatching was enhanced in the combined treatments. Furthermore, reduced weights and variation in duration of insect developmental stages were observed among entomopathogens and enhanced in *H. bacteriophora* + *B. bassiana* combinations. Larvae treated with sub-lethal concentrations exhibited reductions in food consumption, growth and frass production and weight gain (**Wakil *et al.*, 2017).**

The biology of RPW was studied under laboratory conditions and survival against sub-lethal doses of *B. bassiana* and bio-rational insecticide (Nitenpyram). Sugarcane sets as alternate to date palm stem were successfully used as diet for rearing RPW. Combination of entomopathogenic fungus (Bb) and Nitenpyram (Nit) were found more lethal to survival of RPW. A reduction in pupation and adult emergence was recorded in the combined treatments. Moreover, decrease in weight gain, frass production and cumulative gain in size was found when larvae were treated with integrated effect of Bb and Nit. Depending on the lethal of treatment, development duration of RPW was disturbed. Integration of Bb and Nit delays the development and diet uptake in RPW which can be used for agent of control for this cryptic insect **(Saleem *et al.*, 2019).**

6- The entomopathogenic efficacy of commercial product of *B. bassiana* and *M. anisopliae* under filed conditions:-

A field experiment was conducted to evaluate the integrated effect of baited aggregation pheromone traps and entomopathogenic fungus *B.bassiana* or insecticide for controlling the red palm weevil (RPW), *R. ferrugineus* through 2008-2009. Results indicated that the intensity of captured weevils were decreased as a result of using combination of baited pheromone traps and fungus or insecticide compared with using baited pheromone trap only. Statistical analysis revealed significant differences between the mean numbers of captured adults in treated areas with baited pheromone traps alone and combination of traps and fungus or insecticide whereas the total mean numbers were 17.04, 4.93 and 8.02 adults/3 traps, respectively. The total mean reduction of RPW population caused by mass trapping and fungus *B. bassiana* or insecticide were 61.40% and 40.16%, respectively. Considerable reduction of infested palm tree numbers was noticed in treated areas with combination compared with untreated areas. There were significant differences among different field trials and untreated areas on mean numbers of infested palms. They were lower (0.77 and 0.82 palms) when used combination of baited pheromone traps plus fungus and baited pheromone traps plus insecticide, respectively and higher (2.03 and 2.15 palms) when used mass trapping or insecticide alone. However the untreated areas recorded highest infested palm trees whereas mean number was 3.73 palms (**Sewify *et al.*, 2010).**

A trap was designed to allow red palm weevil adults to pass through it so that they come out contaminated with a high density of the fungus conidia for spreading them amongst the red palm weevil population in date palm plantations. A fungus inoculum containing 10% conidia was prepared and used in the trap. An adult was contaminated with

$9.53x10^7$ conidia per a tape visit with a lethal time of 8.25 days. Field trials were carried out using 20 traps in 3 date palm plantations in the period from April 2006 to May 2007 in the Northern Region of United Arab Emirates. Efficacy of the trap was evaluated by assessing the monthly mortality caused with the fungus in the adult population. In the last two months, mortality of adults caused by the fungus in the field population ranged 41.2-51.3% compared with 4.8-4.9% in the control. Results showed that the trap is effective for spreading the fungus *B. bassiana* among *R. ferrugineus* population (**El-Sufty *et al.*, 2011).**

Güerri-Agullo *et al.*, (2011) described the effect of a *B. bassiana* solid formulation on *R. ferrugineus* infesting naturally canary palms in SE Spain in the field. The formulation included a highly pathogenic strain of *B. bassiana* derived from *R. ferrugineus*. The formulation was applied 3 times in 2009 in 2 sites (Catral and El Hondo), at 3-month intervals. *B. bassiana* caused 70-85% *R. ferrugineus* mortality. They also concluded that *B. bassiana* formulation can be a significant component of an IPM strategy for RPW control.

The invasive red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is one of the most destructive pests of palms in the world. To date, the control of this pest has been mainly based on the use of insecticides. However, this control method can result in environmental, social and economic problems. To improve management options against the weevil, the efficacy of the entomopathogenic nematode *Steinernema carpocapsae* Weiser (Nematoda: Steinernematidae) and the potential of a strain of the entomopathogenic fungus *Beauveria bassiana* (Ascomycota: Clavicipitaceae) were evaluated in laboratory, semi-field and field assays. The use of these entomopathogenic microorganisms resulted highly efficient against *R. ferrugineus* **(Dembilio and Jacas, 2013)**.

A field experiment to evaluate integrated effect of baited aggregation pheromone traps and entomopathogenic fungus *B. bassiana* or insecticide for controlling the red palm weevil (RPW), *R. ferrugineus* Olivier (Coleoptera: Rhynchophoridae) was conducted by **Sewify *et al.*, (2014)** in Ismailia Governorate, Egypt in 2008/09. Results indicated that the population of captured weevils decreased as a result of using a combination of baited pheromone traps + the fungus or insecticide compared with using baited pheromone trap only. Statistical analysis revealed significant differences between the mean numbers of captured adults in treated areas with baited pheromone traps alone and combination of traps and fungus or insecticide whereas the total mean numbers were 17.04, 4.93 and 8.02 adults/3 traps, respectively. Total mean reduction of RPW population caused by mass-trapping and the fungus *B. bassiana* or insecticide was 61.40 and 40.16%, respectively. Considerable reduction of infested palm tree numbers was noticed in treated areas with the combinations compared with the control. There were significant differences in mean numbers of infested palms among different field trials and untreated areas. They were the least (0.77 and 0.82 palms) at the combination of baited pheromone traps + the fungus and baited pheromone traps + insecticide, respectively and the highest (2.03 and 2.15 palms) at the mass-trapping or insecticide alone. However, untreated areas recorded highest infested palm trees whereas the mean number was 3.73 palms.

MATERIALS AND METHODS

1- Collection and rearing of Red Palm Weevil:-

Various stages of RPW used in this study were originally obtained from infested palm trees at Al-Qassasin district (Ismailia Governorate) during 2018، where those were collected by hand after the splitting the sites of injury. Each developmental stage was placed individually in rectangular plastic boxes with press-on tight-fitting lids (30 x 20 x 15 cm.) **(Plate, 1).** Portable wood saw was used to facilitate collecting weevils from heavily infested palm trees.

The insects were cultured in a rearing room of the Insect Research Laboratory at the Plant Protection Department, Fac. of Agri., Benha University. The room was maintained at (27±2ºC, 70±5% R.H.). The photoperiod was approximately 12:12. The room contained three large working benches, electrical outlets and sideboards. The room was also used as a media room for handling and preparing materials of natural diets. Larvae and adults of *R. ferrugineus* were provided with sugarcane stem pieces for feeding.

R. ferrugineus rearing on sugarcane stem pieces were previously reported by **El-Zoghby and Abdel-Hameid, (2018).** Natural diets were prepared for mass rearing of *R. ferrugineus* because of the availability of sugarcane in Egypt in culturing this insect. Diets were also developed to avoid the use of expensive artificial diets for the culture of weevils. All plastic boxes were stored at room temperature until required. Larvae were placed on diets after total coolness.

Diet 1, is mainly sugarcane stems, this diet proved hardly succeeded because of sugarcane stem damage by drought. The phases were separated because the ages were not completed and put on new sugarcane stems. This diet was found expensive and not easily handled.

Diet 2, *R. ferrugineus* rearing on artificial diet were previously reported by **Abdel-Hameid (2019).** The diet ingredients are as follows:

Grated sugar cane	250 gm.
Dried active yeast	15 gm.
Agar	10 gm.
Sorbic acid	1.25 gm.
Ascorbic acid	2.50 gm.
Sodium benzoate	1.25 gm.
Distilled water	150 – 200 ml

1-1- Determination of some biological aspects of *R. ferrugineus*:-

A- Duration of egg stage:-

Cocoons of red palm weevil (RPW) were put in containers as described before, these cocoons were kept till adults' emergence. The freshly emerged adults (15 pairs of freshly emerged male and female weevils) were collected and reared in the glass jars (1 litre) with different diets **(plate, 2)**.

The insects were left for one week to copulate, the eggs laid by females were removed daily by peeling the fibrous tissues, picking up eggs using a soft hairbrush no. zero (camel's hairbrush). Eggs were transferred with the camel hair brush and placed on wet filter papers to avoid dryness inside the Petri-dishes for further studies, where eggs were monitored until hatching (**Plate, 3)**.

Many freshly deposited eggs almost of the same age (0.0-24 hours old) were available to initiate the insect first generation. The Petri-dishes were put in an incubator in the laboratory (27±2ºC, 75±5%R.H.) and inspected daily to observe eggs hatching.

The viability of the eggs was determined by counting the number of hatched eggs per 100 eggs. After 1 to 5 days, larvae from hatched eggs were translocated to separate glass jars **(**7cm. height and 3cm. diameter; **Plate, 4**) and provided with pieces of different diets which were prepared for feeding.

Sugarcane stems were cut off from the middle and divided into pieces. Food was replaced every 3 days and the old pieces were confined in separate containers for seven days before examination for weevil eggs and/or larvae. The stages of weevils were examined daily for mortality.

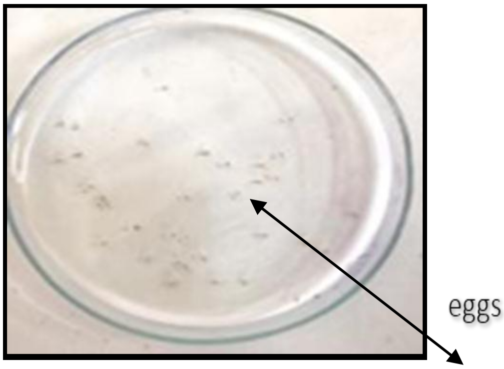
Plate, 1: Natural and artificial diets in plastic containers for feeding of RPW larvae.

B- Larval period and adult Longevity development period:-

Freshly hatched larvae of *R. ferrugineus* (number of eggs = 30) were transferred with a camel's hair brush to different diets. Last instar larvae fed on two diets, were transferred to sugarcane stem pieces to make cocoons and pupation. The size of sugarcane stem pieces was dependent on the size of larvae at different developmental stages. Last instar larvae made their cocoons from the fibres inside the sugarcane stems. The following developmental parameters were estimated: total larval period, pupal period and adult male & female longevities. Containers were stacked side by side or on the top of each other on laboratory benches. Few pores were made on all lids of boxes and jars for ventilation (**Plate, 1).**

Plate, 2: Glass jars holding *R. ferrugineus* pairs of sexed adults for mating and oviposition.

At the end of the larval stage and entering the pupal inside the cocoon. The formed cocoons were transferred to glass jars (height 10cm and 5cm diameter) and providing the proper temperature and humidity until the pupal stage has been finished and adults emerged.



Plate, 3: Petri-dish used for eggs hatching.

Plate, 4: Glass jars used for eggs hatching.

To study the number of larvae, as well as the duration of each individual, 30 newly hatched larvae, were used by introducing each larva in a glass jar (7 cm in height and 3 cm in diameter) containing grated sugarcane stems, and tightly covered with a perforated cover. Larvae were inspected daily by using lens during the first three instars to record the date of moulting by observing exuvia, whereas older larval instars were larger and their exuviae were easily distinguished by naked eyes.

Plate, 5: Collection of *R. ferrugineus* cocoons from sugarcane stems pieces.

C- Pupal duration:-

When larva become full-grown, it starts to spin a cocoon from fibres mixed with an adhesive saliva. Inside the formed cocoon, the larva passes through the pupal stage. The stem pieces of sugarcane were split open and cocoons were collected**.** Cocoons of *R. ferrugineus* were placed in a plastic container, the mist of water was supplied as needed, and closed with lids. After collecting the cocoons, those were checked daily until adult emergence (**Plate, 5).**

D- Adults stage:-

Adults were sexed after emergence from cocoons depending upon with a characteristic long and curved rostrum which comprises about one-third of the total body length. On the dorsal side of the thorax, the weevils exhibit dark spots. In males, the anterior dorsal half of the rostrum has short brownish setae (hairs). By contrast, the female rostrum lacks any hair and is comparatively narrower, more curved and longer than that of the male rostrum and kept separately in small 1-liter glass jars **(**one pair/ box; **Plate, 2**) to start observations about mating and egg- laying. These jars were provided with rasped shreds of sugarcane stem pieces and inspected daily to record the date of laying the first egg as well as the daily number of eggs deposited till the end of female's life time. In order to determine female fecundity, the hatching percent and male & female longevities were determined. When the first newly emerged female began to lay eggs, the second generation was initiated on this date, and so on. Boxes and jars were placed side by side on the laboratory benches. Pores were made on all lids of boxes and jars for ventilation.

The bio insecticides used:

The commercial bio-insecticide Newvar is a product produced by the Pesticide Production Unit of the Plant Protection Research Institute at Dokki. This product contains the entomopathogenic fungi, *Beauveria bassiana* at a concentration of ($1x10^8$ CFU's / g) for insect control, it is recommended to be applied at 10g / L of water.

The commercial bio-insecticide Metmite is produced by the Pesticide Production Unit of the Plant Protection Research Institute at Dokki. This product contains the entomopathogenic fungi, *Metarhizium anisopliae* at a concentration of ($1x10^9$ CFU's / g) for insect control, the recommended concentration to be applied is 10g / L of water.

2- Application of commercial product of *Beauveria bassiana* (Newvar) and *Metarhizium anisopliae* (Metmite) on larvae of RPW:-

In order to investigate the efficiency of the commercial formulations of *B. bassiana* and *M. anisopliae* to control RPW. Three red palm weevil larval instars (first, fifth and tenth) were treated with five concentrations ($1x10^8$, $0.5x10^8$, $0.25x10^8$, $0.125x10^8$, $0.0625x10^8$ CFU's / 100ml distilled water) in case of Newvar, and ($1x10^9$, $0.5x10^9$, $0.25x10^9$, $0.125x10^9$, and $0.0625\ x10^9$ CFU’s / 100 ml distilled) water in case of Metmite. Each concentration was assayed on 4 larvae with 5 replications, thus twenty larvae were treated with each concentrations. Another 20 larvae, divided in 5 replicates, received distilled water treatments as control. After treatment, the larvae were, daily, examined for 25 days and the dead larvae were counted and consequently percentages were calculated. Concentrations were prepared using a half-life method by adding 2 ml of the suspension to be absorbed in 10 g of grated sugar cane with the first instar, 5 ml of solution added to 15 g of grated sugar cane with the fifth instar larvae and 10 ml of solution to 25 g of grated sugar can for the tenth instar larvae. After that, the first instar larvae were kept in glass jars (3 cm diameter and 7 cm height) with a treated diet, than covered with suitable pieces breathable cotton, while the fifth and tenth larval were kept in small bottles (5 cm diameter and 10 cm height) (covered with a perforated metal cover for respiration **Plate, 6-7**) containing a weight of the treated sugar cane and placed in the incubator at 27 ± 2 ºC & 70 ± 5 R.H.. The larvae in each treatment were daily examined and considered dead if they were immobile.

Also, the dead larvae were collected in sterile Petri-dishes with 80 ± 3 R.H. humidity and incubated at 30 ± 1 ° C in order to recover fungal spores, for use in the subsequent experiment.

Plate, 6: Glass bottles used for laboratory treatment for RPW 1st larval instar with *B. bassiana* or *M. anisopliae.*

Plate, 7: Glass jars used for laboratory treatment for 5th and 10th larval instars of RPW with *B. bassiana* and *M. anisopliae.*

2-1- Determination of the entomopathogenic efficiency of fungal isolates against RPW larvae:-

The *B. bassiana* and *M. anisopliae* isolates were obtained from laboratory infected RPW larvae collected from the aforementioned experiment. All the dead cadavers were placed in sterile Petri-dishes with 80 ± 3 R.H. Humidity and incubated at 30 ± 1ºC for few days until the appearance of any fungal mycelia out growth and spores. These spores were transferred to Petri-dishes containing sabouraud Dextrose Agar (SDA). The plates were incubated at 30 ± 1ºC**. (Plate, 8)**

When more than one fungal colony was present on the medium, the colonies at the age of 10 days were dissolved in 100 ml of distilled water containing 2ml of the tween solution 20%, then the concentration of spores was calculated using a specific slide under the microscope. The conidial concentration of the solution, in *B. bassiana* was 28×10^6 Conidia / 100ml and 24×10^6 conidia / 100ml in *M. anisopliae*. Five conidial concentrations were prepared $28x10^6$, $14x10^6$, $7x10^6$, $3.5x10^6$ and $1.75x10^6$ conidia / 100ml distilled water for *B. bassiana,* and $24x10^6$, $12x10^6$, $6x10^6$, $3x10^6$ and $1.5x10^6$ conidia / 100ml distilled water for *M. anisopliae*. The obtained suspension concentrations were assayed against 1^{st}, 5^{th} and 10^{th} instar larvae. The treatment was performed with the same tools and under the same conditions that were used in the previous treatment. The control individuals were treated with distilled water. Each treatment consisted of four individuals that were repeated in five replicates; i.e., a total of 20 larvae were used / treatment. Mortality was checked daily for 25 days and the dead larvae were counted in order to calculate the mortality percentages.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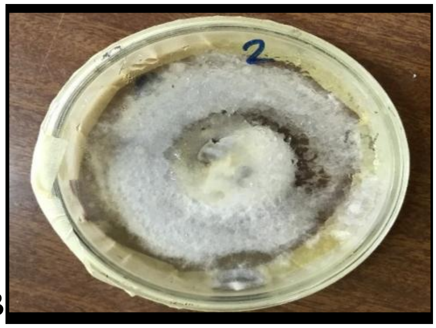


Plate, 8: A = Sample showing growth of the fungus mycelia on the larval body post treatment.

B = Sample showing incubation of fungus in Petri-dish.

3- Application of entomopathogenic fungi formulation against red palm weevil in the field:-

Two experiments were carried out in July 2019 on infested date palms at Al-Qassasin district, Ismailia Governorate. Five RPW infested date palm trees were, randomly chosen to represent five replicates. Each of those trees received an injection of the commercial preparation Newvar (*B. bassiana*) or Metmite (*M. anisopliae*) depending on the experiment (5 trees for each experiment) using a large iron pin for making a hole around the site of infestation then injection through plastic piping. The concentration of bioinsecticide used was 10 g of (1 x 10^8 CFU's/g *B. bassiana*) and (1 x 10^9 CFU's/g *M. anisopliae*) per one litre of distilled water in every place has an infection in the tree. Injection of bioinsecticide at the same rate repeated a week after the first treatment. Successive field observation on date palm trees were evaluated for the injury after 10,15,20 and 25 days after the date of the second application by doing skimming and cleaning the injured places and noting the dead individuals of RPW, then rating the treated trees as either infested or recovered.

Plate, 9: Field injection of bioinsecticide (Newvar or Metmite) at 10gm / liter of distilled water in infested date palm trees.

4- Biochemical assays:-

Chemicals used:-

Bovine albumin standard was purchased from Stanbio laboratory (Texas, USA). Commassie brilliant blue G-250 was from Sigma (Sigma chemicals co.). P- Nitroanisole (purity 97%) was obtained from Ubichem Ltd. (Hampshire), while reduced from of nicotinamideademine dinucleotide phosphate (NADPH) was from BDH chemicals Ltd. (Poole, England). The remaining chemicals were of high quality and purchased from commercial local companies.

Apparatus:-

Insects (larvae) of *R. ferrugineus* were homogenized in a chilled glass Teflon tissue homogenizer (ST – 2 Mechanic - Preczyina, Poland). After homogenization, supernatants were kept in a deep freezer at -20 °C until used for biochemical assays. Double beam ultraviolet / visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was used to measure the absorbance of colored substances or metabolic compounds.

Preparation of full – grown larvae *R. ferrugineus* for analysis:-

The larvae were prepared as described by **Amin (1998).** Those were homogenized in distilled water (50 mg /1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min. at 2 °C in a refrigerated centrifuge. The deposits were discarded and the supernatants, which are referred to as enzyme extract, could be stored for at least one week without appreciable loss of activity at 50 °C.

Determination of total lipids:-

Total lipids were estimated according to the method of **Knight *et al.*, (1972)** using phosphor vanillin reagent.

Determination of total carbohydrates:-

Total carbohydrates were estimated in the acid extract of the sample by the phenol- sulphuric acid reaction of **Dubois *et al.*, (1956).** Total carbohydrates were extracted and prepared for assay according to **Crompton and Birt (1967).**

Total Proteins:-

Total proteins were determined according to the method of **Bradford (1976).**

5- Bioassay experiment by using two bioinsecticides [Newvar (*B. bassiana*) and Metmite (*M. anisopliae*)] to record the data of insect (life stages):-

In order to conduct this experiment, two concentrations of each of the two biopesticides (*B. bassiana* and *M. anisopliae*) were prepared by dissolving 1 g of (Newvar or Metmite) in 100 ml of distilled water and the second by dissolving 0.5 g of (Newvar or Metmite) in 100 ml distilled water. Two freshly emerging insect pairs were used for each concentration per biopesticides with two pairs were used as control.

These insects were dealt with by submersion in the biopesticides solution. On the first day, the feeding process on a diet containing biopesticides solution under the 27 ± 2 ° C and 70 ± 5 R.H.. After two days, the processed food was replaced with pure foods.

Then pairs of insects were placed inside the glass jars (5cm diameter and 10cm height) in order to calculate the pre-oviposition, oviposition, post-oviposition periods, number of eggs, and percentage of eggs hatching, incubation period of eggs, larval and pupal duration, longevity of the male and female and sex-ratio. Rearing extended to the subsequent generation and the percentage of mortality among eggs, larvae, pupae and adults were estimated for each generation.

Statistical analysis:

A probit computer program of **Noack and Reichmuth (1978)** and Finney (1971).

Cumulative mortality at the end of the experiment was analyzed by ANOVA. The concentrations causing 50 and 90% mortalities, (LC_{50} & LC_{90}) and time needed for causing 50 and 90% cumulative mortalities (LT_{50} & LT_{90}) were determined using the probit analysis program LPD-line **(Bakr, 2005)**.

Results and Discussion

1- Effect of the tested diets on the biological aspects of the red oalm weevil.

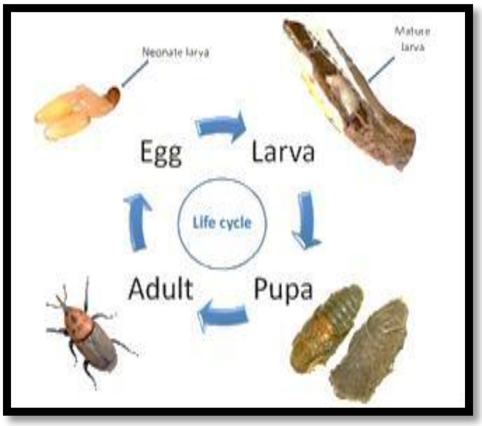
1-1- Life cycle of the red palm weevil.

1-1-1- The immature stages:-

A- The egg- stage:-

The egg of *R. ferrugineus* measures 1-3 mm. It is oval in shape, creamy white in colour, glossy and turns oblong in shape before hatching. At 27 ± 2 ºC and 70 ± 5 % R.H., the incubation period of eggs showed a little insignificant difference between those deposited by females resulted after feeding the previous larvae on the artificial diet (3.62 days) being insignificantly longer than the (3.53 days) estimated for eggs obtained after larval feeding on sugar cane stem pieces **(Table, 1a).** Among eggs deposited by fertilized females, the hatchability percent varied, insignificantly according to the diet on which the larvae were reared. This percentage was higher (84%) among eggs in case of rearing on the artificial diet, while it was lower (80%) for eggs from rearing of previous larvae on sugar cane stem pieces **(Table, 1b).** Whereas, the pre-oviposition period ranged between 9.00 days in females which were fed on artificial diet and 11.40 days in females which were fed on sugar cane stems. As for the oviposition period, those ranged between 25.00 days in females which were fed on artificial diet and 29.30 days in females which were reared on sugar cane stems, and the post-oviposition period ranged between 5.50 days in females which were fed on artificial diet and 6.70 days in females which were fed on Sugar cane stems **(Table, 3a).**

These results are consistent with those obtained by **Abdel-Hameid (2019)** who found that larvae fed on ground sugarcane + additives exhibited shortest larval duration 86.30 days, while the diet ground corn + additives caused the longest larval period 128.35 days. Shortest and longest pupal periods resulted also from feeding on the two diets (15.3 and 21.75 days, respectively). On the other hand, male and female longevities were the longest and shortest when larvae were fed on the two diets (50.4 and 38.4 days for males and 48.0 and 36.8 days for females respectively. The sex – ratio was almost 1:1 with all diets, except the diet of ground sugar cane + additives which led to more females (1 male: 1.5 female). Eggs of *R. ferrugineus* hatched after about 4 days, but this period was, significantly, shorter (3.35 days) by rearing on the former diet. Highest hatchability percentage (93.61 %) resulted from rearing on the same diet.



Plate, 10: Life cycle of *R. ferrugineus*.

Plate, 11: Egg-laying holes in the sugar cane stem pieces by RPW adults.

Table (1): Incubation period of *R. ferrugineus* eggs, the percentage of hatching and mortality percentage among eggs after rearing on two different diets.

Table (1a): Incubation period (per 25 eggs / replicate)

Incubation period (day)	Source of diet	
	Sugar cane	Semi-artificial
3	5.75 ± 0.48^{dB}	7.50 ± 0.29^{dA}
4	14.25 ± 0.25^{cA}	14.50 ± 0.29^{cA}
5	17.50 ± 0.29^{bB}	18.50 ± 0.29^{bA}
6	20.00 ± 0.00^{aB}	21.00 ± 0.58^{aA}
Mean	14.38 ± 1.40^{B}	15.38 ± 1.33^{A}
Mean of incubation period	3.53 ± 0.05	3.62 ± 0.06
LSD at 0.05 for	Period (P)	Diet (D)
	0.71	0.51

a, b & c: There is nonsignificant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B & C: There is nonsignificant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

Table (1b): Hatchability (%)

Incubation period (day)	Source of diet		Mean
	Sugar cane	Artificial	
3	23.00 ± 1.91^{dB}	30.00 ± 1.15^{dA}	26.50 ± 1.68^{d}
4	57.00 ± 1.00^{cA}	58.00 ± 1.15^{cA}	57.50 ± 0.73^{c}
5	70.00 ± 1.15^{bB}	74.00 ± 1.15^{bA}	72.00 ± 1.07^{b}
6	80.00 ± 0.00^{aB}	84.00 ± 2.31^{aA}	82.00 ± 1.31^{a}
Mean	57.50 ± 5.58^{B}	61.50 ± 5.32^{A}	
LSD at 0.05 for	Period (P)	Diet (D)	P*D
	2.86	2.02	4.04

a, b & c: There is nonsignificant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B & C: There is nonsignificant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

Table (1c): Un-hatchability (%)

Incubation period (day)	Source of diet		Mean
	Sugar cane	Artificial	
3	77.00±1.91^{aA}	70.00±1.15^{aB}	73.50±1.68^{a}
4	43.00±1.00^{bA}	42.00±1.15^{bA}	42.50±0.73^{b}
5	30.00±1.15^{cA}	26.00±1.15^{cB}	28.00±1.07^{c}
6	20.00±0.00^{dA}	16.00±2.31^{dB}	18.00±1.31^{d}
Mean	42.50±5.58^{A}	38.50±5.32^{B}	
LSD at 0.05 for	Period (P)	Diet (D)	P*D
	2.86	2.02	4.04

a, b & c: There is nonsignificant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B & C: There is nonsignificant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

B- Larval and pupal durations:-

After hatching of eggs, the freshly hatched larvae of *R. ferrugineus* were gently translocated, using affine (camel) brush, larva of the RPW is creamy white in color with a brownish head. It is a podous having a body comprised of 13 segments. The head capsule is brown russet red to brilliant brown-black in color with strong showing mouthparts (**Plate, 12).**

Plate, 12: Larva of *R. ferrugineus* (2x)

As shown in (**Table, 2),** *R. ferrugineus* has 12 larval instars. Larval durations were estimated in the laboratory at 27 ± 2 ºC and 70 ± 5 % R.H. when larvae were reared on sugar cane small pieces, and also when fed on the artificial diet. The shortest duration was that of the first instar (4.87 and 4.80 days, respectively), followed by the 3rd instar (5.73 and 4.93 days by rearing on sugar cane stem pieces and artificial diet, respectively). While, the longest duration for larvae fed on sugar cane pieces (13.47 days) was that of the 4th instar, while for those fed on the artificial diet was that of the 5th instar (12.47 days). Generally, the durations of 8th to 12th instars [8.60 to 11.27 days (sugar cane) and 9.03 to 9.73 days (artificial diet)] were longer than those of the early instars. The total larval period lasted longer (103.73 days) for larvae reared on sugar cane pieces, than that recorded larvae reared on the artificial diet (98.30 days). The obtained data on larval durations gave the impression that fed on sugar cane stem pieces grew slowly to exhibit longer duration of 103.73 day, compared to those reared on artificial diet (98.30 days) indicating faster development and consequently shorter total larval period.

Freshly emerged larvae seek their way into the sugarcane by boring through tissues and making tunnels in sugarcane stalks **(Plate, 13),** subsequently damage increases as the larvae grew to subsequent instar. Young larvae have the tendency to feed on soft tissues, while older larvae move towards feeding and grinding the harder tissues for feeding and to spin a cocoon enwrapped in palm fibers (**Plate, 14).**

A

B

Plate, 13: RPW larval tunnels. A: In sugar cane stem.

B: In artificial diet (normal size).

A

B

Plate, 14: RPW larval feeding on natural diets. A: sugar cane.

B: palm tree.

Concerning pupal periods of the red palm weevil, statistical analysis of results showed significant differences between the two tested diets. As tabulated in **Table, 2,** the pupal period was shorten (17.10 days) when rearing took place on small pieces of sugarcane stems, but longer (20.10 days) by rearing on artificial diet. During this period the pupae remain in their cocoons **(Plate, 15)** and after the pupa period is finished, the RPW pupa is transferred to the adult stage which finds its way out of the cocoon to repeat its life-cycle. The cocoon has an average size of 80 × 35 mm. just formed pupa is creamy, and then, gradually, turns brown'sh in color (**Plate, 16).** The pupa is greatly furrowed with a shiny surface. Sugarcane stem was suitable for pupation because larval stages require fiber and failed to construct cocoon in any other diet.

El-Zoghby and Abdel-Hameid, (2018) tested rearing of *R. ferrugineus* on five different diets (small pieces of sugarcane stems, sugarcane stem pieces + sugarcane residues, sugarcane stem pieces + date palm trunk pieces, sugarcane stem pieces + ground frond of date palm and sugarcane stem pieces + food residues of RPW) and investigated the effect of diets on some biological aspects of RPW under laboratory conditions. Larval duration of different instars were affected by different diets. The larvae fed on (sugarcane stem pieces + ground frond of date palm), slowly developed to exhibit the longest duration of 103.78 days. Meanwhile, the larvae fed on the other diets showed a gradual shortage in larval periods to give means of 89.47 and 74.63 days when fed on small pieces of sugarcane stems and sugarcane stem pieces + food residues of RPW, successively. Feeding on different diets had a great effect on the means of the pupal period, which was obtained between the tested diets. Longevity of males and females emerged from larvae fed on the different tested diets varied. The sex-ratio among emerged adults was the highest (more females), when larvae were fed on sugarcane stem pieces + food residues of RPW. Mean number of eggs deposited by emerged females from larvae fed on different diets was highest in case of (sugarcane stem pieces + food residues of RPW). The mean of the incubation period of eggs laid from adult fed on three tested diets ranged from 3.78 to 4.54 (sugarcane stem pieces + food residues of RPW and sugarcane stem pieces + ground frond of the date palm, respectively. Hatchability percentage proved to be affected by different diets. The superior diet was sugarcane stem pieces + food residues of RPW, because of biology completed and the diet kept moisture and remained fresh all the time.

Table (2): Difference in time spent (days) on each larval instar a comparison between two different diets and pupal duration.

Larvae instars	Sugar cane	Artificial diet
First	4.87 ± 0.20^{gA}	4.80 ± 0.19^{gA}
Second	8.80 ± 0.30^{cA}	7.37 ± 0.21^{deB}
Third	5.73 ± 0.24^{fA}	4.93 ± 0.24^{fgB}
Fourth	13.47 ± 0.43^{aA}	5.67 ± 0.18^{fB}
Fifth	11.37 ± 0.41^{bB}	12.47 ± 0.45^{aA}
Sixth	7.57 ± 0.28^{dB}	11.30 ± 0.47^{bA}
Seventh	6.73 ± 0.23^{eB}	8.10 ± 0.25^{dA}
Eighth	7.40 ± 0.20^{deA}	7.10 ± 0.20^{eA}
Ninth	8.60 ± 0.25^{cA}	8.17 ± 0.21^{dB}
Tenth	9.07 ± 0.14^{cB}	9.63 ± 0.34^{cA}
Eleventh	8.87 ± 0.11^{cB}	9.73 ± 0.32^{cA}
Twelfth	11.27 ± 0.16^{bA}	9.03 ± 0.25^{cB}
Total larval period	103.73±1.74	98.30±1.74
LSD at 0.05 for		
Period (P)	**Diet (D)**	**P*D**
0.77	0.31	1.09
Pupal duration	17.10±0.82	20.10±0.85

a, b & c: There is nonsignificant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B & C: There is nonsignificant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

Plate. (15): Cocoon of *R. ferrugineus* **Plate. (16): Pupa of *R. ferrugineus***

1-1-2- The adult stage:-

A- Longevity of adult:-

After the pupal development has been completed, the adult weevil emerges and find its way out through the cocoon. Adults of RPW are large, rusty red in color measuring 3.43 ± 0.03 cm. long and 1.19 ± 0.02 cm width with a characteristic long and curved rostrum which comprises about one-third of the total body length. On the dorsal side of the thorax the weevils exhibit dark spots. In males, the anterior dorsal half of the rostrum has short brownish setae (hairs). By contrast, the female rostrum lacks any hair, and is comparatively narrower, more curved and longer than that of the male rostrum. The adult weevils have well- developed wings, so weevils are capable to undertake long flights **(Lepesme, 1947)**, and to cover long distances of 500–800 m **(Wattanapongsiri, 1966).** Data presented in **Table, 3,** show that this period had been influenced by feeding of previous larvae on the different tested diets. As regards, adult longevity of both sexes emerged from larvae fed on the two different tested diets, the present results revealed that the type of diet, significantly, affected the longevity of male and female. The longest means of longevities (47.40 days for males and 51.90 days for females) were obtained with adults emerged from larvae fed on natural diet (sugarcane stem pieces), opposed to 39.50 days for males and 43.60 days for females emerged from larvae fed in the larval stage on artificial diet **(Table, 3a).**

B- Ovipositional periods:-

Mated females were allowed to lay their eggs below the upper surface of the sugar cane slices. Females started egg-laying after 11.40 and 9 days for adults resulted from larvae reared on sugar cane and artificial diet, respectively **(Table, 3a).** Thus showing, significantly, shorter pre-oviposition period for adults resulted from larvae reared on artificial diet. As shown in the same Table, 3a, the oviposition period was significantly longer (29.30 days) for females resulted from larvae fed on sugar cane stem pieces, while this period was shortened (25.00 days) in case of rearing on artificial diet.

After the oviposition period has finished, females after post-oviposition died. The period lasted (6.70 and 5.50 days) for adults resulted from the two diets, respectively, thus indicating the nonsignificant difference between rearing of larvae on either of the two diets.

Generally, females lived for nonsignificant a longer than males. Also, adults resulted after rearing of larvae on sugar cane stem pieces lived longer (47.40 and 51.90 days for males and females, respectively) by rearing on sugar cane stem pieces, than those recorded after rearing on artificial diet (39.50 and 43.60 days, respectively). Throughout the whole oviposition period, females developed from larvae fed on the artificial diet produced 255.60 eggs / ♀, being nonsignificantly higher than the mean number of 248.60 eggs / ♀ deposited by a single female fed on sugar cane stem pieces.

C- Sex-ratio:-

Data presented in **Table, 3a** indicated that the sex ratio (females: male) may be affected by the type of food on which the insect was fed during the larval stage. The ratio indicated increased (60%) percentage of females by rearing on artificial diet showing a sex ratio of 1.5♀:1♂. On the other hand, feeding of larvae on the natural food (sugar cane stem pieces) led to adults of a sex ratio of 1:1 (50% were females opposed to 50% males).

Table (3): Ovipositional periods, fecundity, longevity and sex-ratio of *R. ferrugineus* and its life cycle.

Table (3.a): Ovipositional periods, fecundity, longevity and sex-ratio of *R. ferrugineus*

Adult longevity		Sugar cane	Artificial diet	LSD at 0.05
Pre- oviposition period		11.40 ± 0.70^{A}	9.00 ± 0.52^{B}	1.83
Oviposition period		29.30 ± 1.39^{A}	25.00 ± 1.18^{B}	3.84
Post- oviposition period		6.70 ± 0.56^{A}	5.50 ± 0.34^{A}	1.38
Longevity	Male	47.40 ± 1.44^{A}	39.50 ± 1.11^{B}	3.82
	Female	51.90 ± 1.13^{A}	43.60 ± 0.88^{B}	3.01
No. of egg laid by female		248.60 ± 7.46A	255.60 ± 7.14A	21.70
Sex ratio		Male 50% Female 50%	Male 40% Female 60%	

A, B & C: There is nonsignificant difference (P>0.05) between any two means for the same attribute, within the same row having the same superscript letter.

Table (3.b): Life cycle

	Sugar cane	Artificial diet
Incubation period	**3.53±0.05**	**3.62±0.06**
Total Larval	**103.73±1.74**	**98.30±1.74**
Pupa period	**17.10±0.82**	**20.10±0.85**
Pre-oviposition period	**11.40 ± 0.70**	**9.00 ± 0.52**
Total developmental period	**124.36**	**122.02**
Life-cycle	**135.76**	**131.02**

2- Effect and toxicity of different concentrations of two commercial products of *Beauveria bassiana* and *Metarhizium anisopliae* against the red palm weevil larvae.

2-1- Effect of different concentrations of a commercial product (Newvar) of *Beauveria bassiana* against RPW larvae:-

Results in **Table, 4 and Fig. 1** showed the cumulative mortality percentages among RPW larvae after 5,10,15,20 and 25 days. The first instar larvae of RPW proved as highly susceptible to *B. bassiana*, where the mortality % recorded 95, 85, 80, 60 and 45%, at 25 days post-treatment by Newvar at $1x10^8$, $0.5x10^8$, $0.25x10^8$, $0.125x10^8$ and $0.0625x10^8$ CFU's / 100 ml distilled water respectively. That was followed by the fifth larval instar which recorded 85, 60, 60, 45, and 25% mortality after 25 days post-treatment. While, the tenth larval instar manifested the lowest correspondent mortality percentages, being 65, 60, 40, 25, and zero %, respectively, at 25 days post-treatment by the same concentrations. The lowest percentage of mortality was recorded at $0.0625x10^8$ CFU's / 100 ml concentration in most post-treatments periods.

Cumulative mortality reached maximum values 25 days after treatment **(Table 4 and Fig. 1).** No control larvae died in either 1st, 5th and 10th instar larvae. Lowest mortality rates were recorded when treatments took place on RPW larvae at their 10th instars. It is clear from **Table, 4** that mortality % increased on earlier larval instars increasing the applied concentration and prolongation at the period after treatment.

The results obtained in this study are in line with these of **Malik *et al.* (2009)** who investigated the effect of *B. bassiana* when applied at two concentrations (1.8×10^7 and 1.8×10^8 conidia/ml) against the fifth and sixth larval instars of *R. ferrugineus*. The insects were exposed to fungal treatments by diet incorporation method. His results revealed that the application of *B. bassiana* at the rate of (1.8×10^8 conidia/ml) showed the highest mortality rate among treated larvae for fifth instar compared to the sixth instar larvae. The authors stated that the fifth larvae were more susceptible to *B. bassiana* treatments than the sixth instar. In agreement, also, with the present results, **El-Hindi, (2016)** reported that the toxicity assay on larvae showed the highest mortality percentage which reached to 100% against the larvae with *B. bassiana*, after treatment by entomopathogenic 28 days after spraying.

Table (4): Mean cumulative mortality percentages among larvae of *R. ferrugineus* treated at 3 instars with commercial product of *B. bassiana* (at different concentrations). (Total number of 20 larvae / each conc.).

periods after application (days)										
1st larval instar										
Concentration CFU's /100ml	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**
$1x10^8$	0.0	0	0.8	20%	1.6	40%	3.2	80%	3.8	95%
$0.5x10^8$	0.0	0	0.8	20%	1.4	35%	1.6	40%	3.4	85%
$0.25x10^8$	0.6	15%	0.8	20%	1.8	45%	2.4	60%	3.2	80%
$0.125x10^8$	0.0	0	0.2	5%	1.6	40%	2.2	55%	2.4	60%
$0.0625x10^8$	0.0	0	0.2	5%	0.8	20%	1.6	40%	1.8	45%
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
5th larval instar										
Concentration CFU's /100ml	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**
$1x10^8$	0.0	0	0.8	20%	1.6	40%	2.4	60%	3.4	85%
$0.5x10^8$	0.0	0	0.0	0	0.8	20%	1.8	45%	2.4	60%
$0.25x10^8$	0.8	20%	1.0	25%	1.4	35%	2.2	55%	2.4	60%
$0.125x10^8$	0.0	0	0.0	0	0.0	0	1.6	40%	1.8	45%
$0.0625x10^8$	0.0	0	0.0	0	0.6	15%	0.8	20%	1.0	25%
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
10th larval instar										
Concentration CFU's /100ml	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**
$1x10^8$	0.6	15%	0.8	20%	1.4	35%	1.8	45%	2.6	65%
$0.5x10^8$	0.8	20%	1.0	25%	1.4	35%	2.0	50%	2.4	60%
$0.25x10^8$	0.0	0	0.6	15%	0.8	20%	1.4	35%	1.6	40%
$0.125x10^8$	0.0	0	0.0	0	0.0	0	0.8	20%	1.0	25%
$0.0625x10^8$	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0

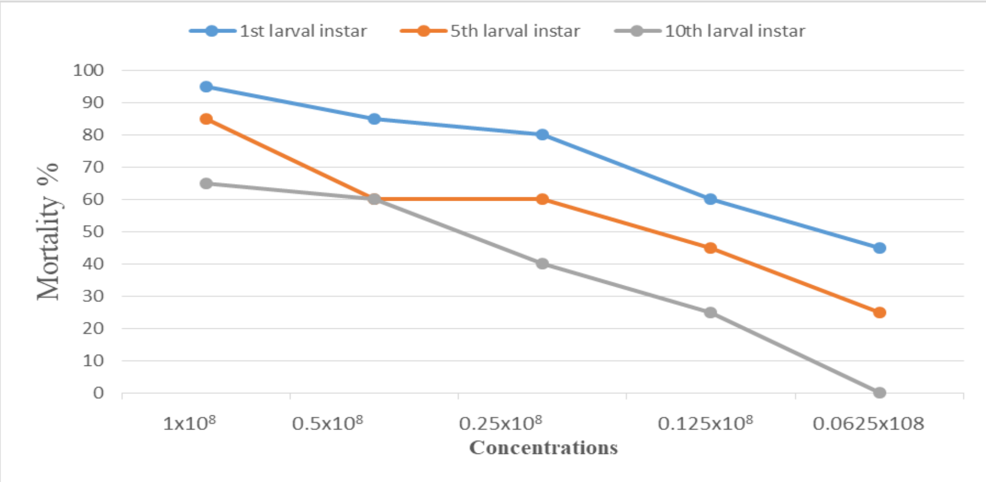


Fig 1: Mean cumulative mortality percentages among *R. ferrugineus* larvae 25 days after treatment with commercial product of *B. bassiana* (at different concentration).

A- Toxicity of Newvar (commercial product of *B. bassiana*) against larvae of RPW:-

The lethal concentrations causing 50 and 90% larvae mortality (LC_{50} & LC_{90}) at the 5, 10, 15, 20 and 25 days after treatment were assessed (**Table, 5 and Fig. 2)**. As a general, the concentration $1x10^8$ CFU's / 100ml highest concentration caused the highest mortality rate and vice versa. The three tested larval instars (1^{st}, 5^{th} and 10^{th}) behaved differently in their reaction to *B. bassiana* treatments. Considering the 1^{st} instar larvae, those were the highest susceptible, showing the lowest LC_{50} (0.077 g/100ml = 0.077x 10^8 CUF's / 100 ml). On contrary, the 10^{th} instar larvae manifested least susceptibility as those showed the highest LC_{50} (0.428 g/100ml = $0.428x10^8$ CUF's / 100 ml). In this respect, the 5^{th} instar larvae showed intermediate position in their susceptibility to *B. bassiana* treatments between the 1^{st} and 10^{th} instars (LC_{50} = 0.191 g/100ml = $0,191x10^8$ CUF's / 100ml distilled water)

(Table, 5 and Fig. 2). As for the LC_{90}'s resulted by assaying *B. bassiana* concentrations on *R. ferrugineus* 1st, 5th and 10th larval instars, those manifested the same trend of susceptibility, as the 1st instars was the highest susceptible (0.630 g/100ml = 0.630 x10^8 CUF's / 100ml), followed by the 5th instar (2.117 g/100ml = 2.117 x 10^8 CFU's / 100ml). While, larvae of the 10th instar LC_{90} (2.448 g /100ml = 2.448 x 10^8CFU's /100ml) **(Table, 5).**

Table (5): Toxicity (LC_{50} and LC_{90} values), 25 days after treatments by the commercial product of *B. bassiana* tested against larval instars of *R. ferrugineus.*

Larval instar	LC_{50} (g/100ml)*	LC_{90} (g/100ml)*	Slope ± SE
1st	0.077 (0.0544 - 0.099)	0.630 (0.478 - 0.932)	1.402 ± 0.154
5th	0.191 (0.150 - 0.237)	2.117 (1.345 - 4.252)	1.227 ± 0.143
10th	0.428 (0.203 - 0.811)	2.448 (1.275 - 5.088)	1693 ± 0.159

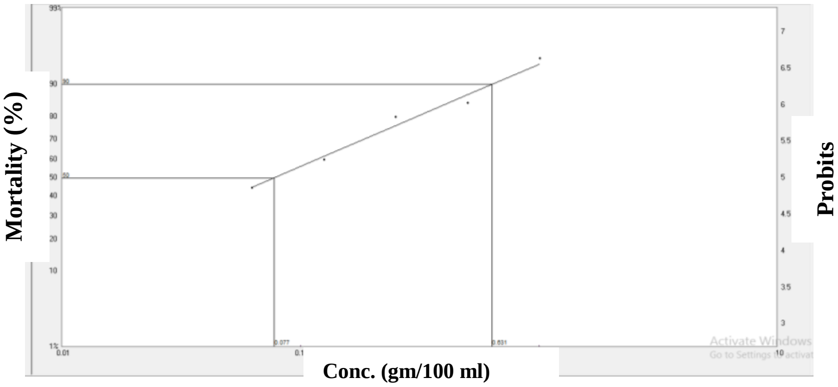
***Results were calculated after 25 days of treatment.**

Table (6): LT_{50} and LT_{90} values of commercial product of *B. bassiana* tested against 3 larval instars of *R. ferrugineus.* Results were calculated using concentration 1x10^8 CFU 's/100ml.

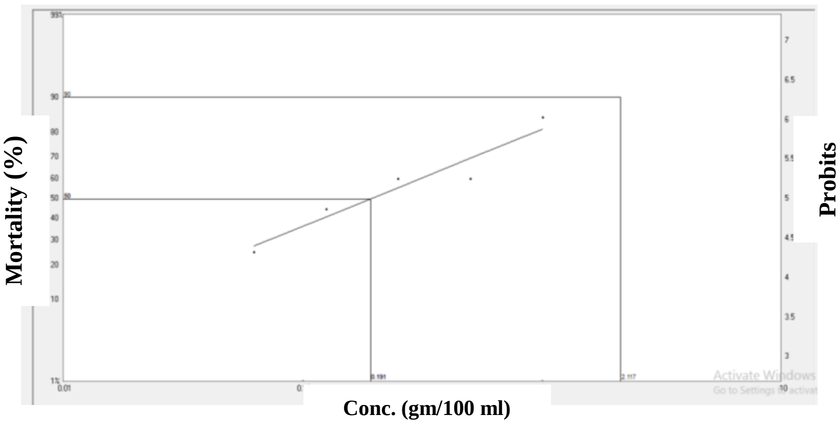
Larval instar	LT_{50} (days)	LT_{90} (days)	Slope ± SE
1st	14.824 (12.307 - 17.238)	23.930 (21.432 - 32.913)	6.162 ± 0.507
5th	16.393 (15.357 - 17.509)	30.682 (27.441 - 35.694)	4.708 ± 0.418
10th	20.758 (13.537 – 32.646)	89.532 (63.971 – 154.277)	2.018 ± 0.269

The days spent till insect died reached 50 and 90% mortalities (LT_{50} & LT_{90}) were calculated for the treated larvae at only the concentration of $1x10^8$ CFU's / 100 ml of water. As shown in **Table, 6 and Fig. 3,** the 1st instar took the shortest time till mortality of 50 or 90% of the treated larvae, then the 5th and the 10th instars which took the longest period until mortality. Results indicated that the LT_{50}'s were 14.824, 16.393 and 20.758 days, respectively, opposed to 23.930, 30.682 and 89.532 days, for the LT_{90}'s.

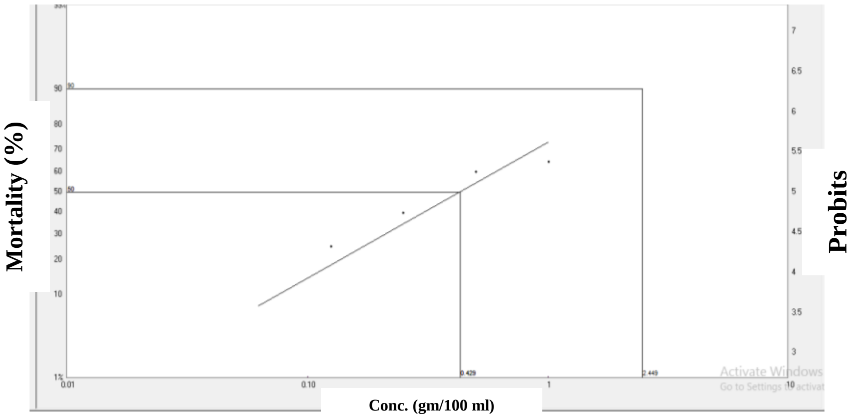
In the same field of study, **El-Sufty *et al*., (2009**) carried out studies, in United Arab Emirates, to estimate the pathogenicity of the entomopathogenic fungus, *B. bassiana* against RPW, *R. ferrugineus* using a local strain "UAE-B2". There, results were in the same line of the present studies. They found that the young instars of larvae were more susceptible than the older ones. They verified that in the adult and larval stages, the fungus remained dormant inside the cadavers and started to continue its saprophytic development when R.H. approached 100%. The same authors estimated the number of *B. bassiana* conidia produced after the mortality of treated individuals. They found that complete mycosed cadaver produced $4.3x10^7$ conidia.



1st larval instar

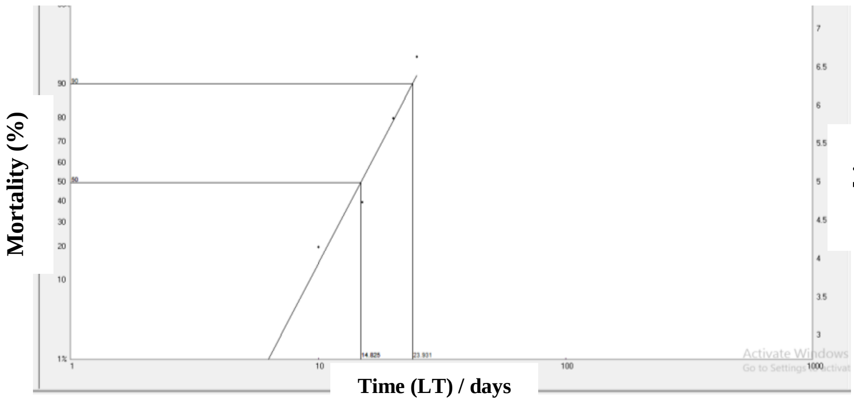


5th larval instar

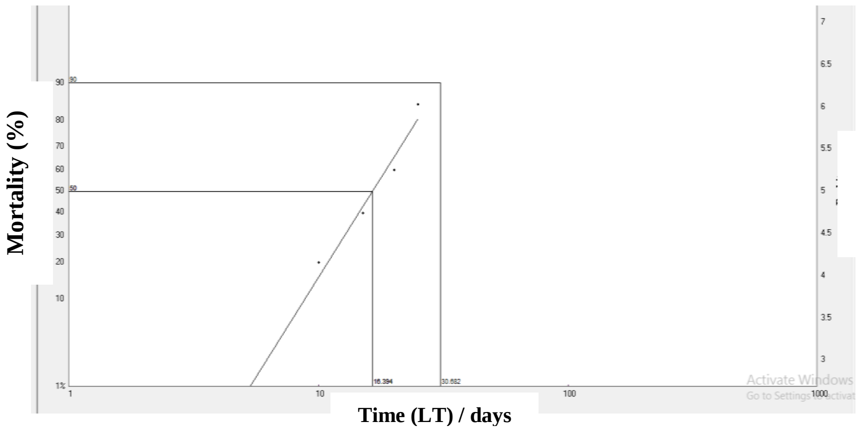


10th larval instar

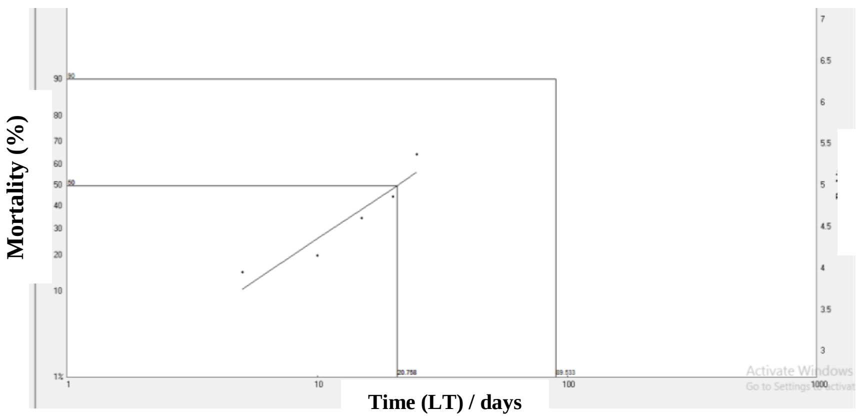
Fig 2: Ldp-line of commercial product of *B. bassiana* used at concentrations against larval instars (1st, 5th and 10th) of *R. ferrugineus*.



1st larval instar



5th larval instar



10th larval instar

Fig. 3: Period until mortality of *R. ferrugineus* larval instars after treatments by the commercial product of *B. bassiana* for indicating the LT_{50}'s and LT_{90}'s.

B- Efficiency of conidial suspension (*B. bassiana*) of fungal isolate from RPW dead larvae:-

Results in **Table, 7 and Fig. 4** showed that cumulative mortality of the 1st instar larvae of RPW were 85, 60, 40, 25 and 20%, while the 5th instar larvae recorded 65, 60, 40, 40 and 20% mortality after 25 days post-treatment by the following concentrations *B. bassiana* conidia; $28x10^6$, $14x10^6$, $7x10^6$, $3.5x10^6$ and $1.75x10^6$ conidia /100ml, respectively. It is also, noticed that the minimum mortality percentage were recorded with the lowest concentration ($1.75x10^6$ conidia /100 ml) after 25 days, those were 20 or 0.0%. The 10th larval instar manifested, generally, the lowest mortality percentages compared to the 1st and 5th larval instars. These mortality percentages after 10th instar larval treatments by $28x10^6$, $14x10^6$, $7x10^6$, $3.5x10^6$ and $1.75x10^6$ conidia / 100ml water were 55, 45, 25, and 0.0%, respectively **(Table, 7).** Both $3.5x10^6$ and $1.75x10^6$ conidia /100ml were, completely ineffective on RPW larvae of the 10th instar, up to 25 days post-treatment. As for the 5th instar larvae, those were ineffective for 20 days after treatment by *B. bassiana* at the concentration $1.75x10^6$ conidia, and up to 10 days for treatment by $3.5x10^6$ conidia / 100ml. While, the 1st instar larval treatments by either of the two concentration $3.5x\ 10^6$ or $1.75x10^6$ conidia didn't show any mortality up to 10 days post-treatment. It could be, generally, concluded that the earlier instars of *R. ferrugineus* larvae were more susceptible to *B. bassiana* conidia, than the older instars. Also, the efficiency of *B. bassiana* against the red palm weevil larvae increased by prolongation of the period after the fungal treatments.

Working on the efficiency of *B. bassiana* on RPW testing palm trees in Canary Island, **Verde *et al.,* (2015)** isolated strains of *B. bassiana* (Balsamo) Vuillemin from symptomatic insects collected from dead palm trees, and their pathogenicity against different instars of *R. ferrugineus* was evaluated in the laboratory. From their results; the overall percentage of infected insects found in Canary palms was 7%. In laboratory bioassays, hatching percentages among eggs treated with three different isolates of *B. bassiana* 41.2, 26.8 and 29.9%, being significantly lower than hatching from the control eggs (62.4%). Larvae and adults were treated with a single isolate in two ways: spraying each insect with the conidial suspension or feeding them with fruit portions previously immersed in the same conidial suspension. At the end of the two trials, the mortality percentages among treated larvae were 88 and 92%, and the means of survival period were 10.4 and 11.8 days, being significantly different from the control, where no insect died during the trials.

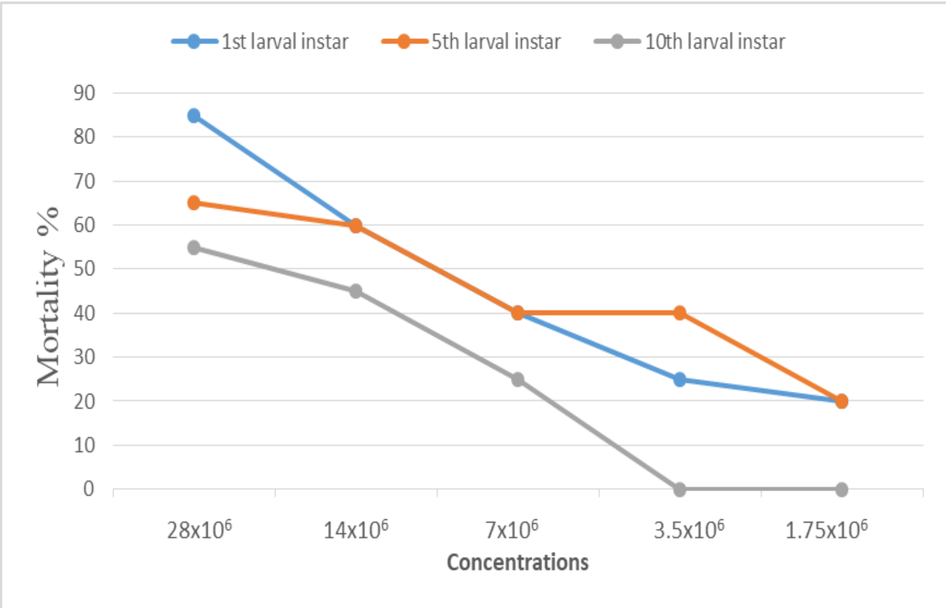


Fig. (4): Mean cumulative mortality percentages among larvae of *R. ferrugineus* treated with conidial suspensions at different conidial concentrations of *B. bassiana* isolate, 25 days after treatment.

Table (7): Mean cumulative mortality percentages among *R. ferrugineus* larvae treated with different concentrations of conidial suspension of *B. bassiana* isolate. (Data from treatment of 20 larvae/ each conc.).

Time of inspection after application (days) 1st larval instar										
Concentration (conidia/100ml)	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**
$28x10^6$	0.0	0	1.0	25%	1.2	30%	2.6	65%	3.4	85%
$14x10^6$	0.6	15%	0.8	20%	1.8	45%	2.0	50%	2.4	60%
$7x10^6$	0.2	5%	0.4	10%	0.8	20%	1.0	25%	1.6	40%
$3.5x10^6$	0.0	0	0.0	0	0.8	20%	1.0	25%	1.0	25%
$1.75x10^6$	0.0	0	0.0	0	0.6	15%	0.8	20%	0.8	20%
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
5th larval instar										
Concentration (conidia/100ml)	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**		**Mean**	**%**	**Mean**	**%**
$28x10^6$	0.0	0	1.0	25%	2.4	60%	2.4	60%	2.6	65%
$14x10^6$	0.8	20%	1.0	25%	1.6	40%	1.8	45%	2.4	60%
$7x10^6$	0.0	0	0.6	15%	0.8	20%	1.4	35%	1.6	40%
$3.5x10^6$	0.0	0	0.0	0	0.8	20%	1.0	25%	1.6	40%
$1.75x10^6$	0.0	0	0.0	0	0.0	0	0.0	0	0.8	20%
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
10th larval instar										
Concentration (conidia/100ml)	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**		**Mean**	**%**	**Mean**	**%**
$28x10^6$	0.0	0	0.0	0	0.8	20%	1.8	45%	2.2	55%
$14x10^6$	0.0	0	0.6	15%	0.8	20%	1.6	40%	1.8	45%
$7x10^6$	0.0	0	0.0	0	0.0	0	0.8	20%	1.0	25%
$3.5x10^6$	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
$1.75x10^6$	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0

C- Toxicity of conidial suspension of *B. bassiana* conidia against larvae of RPW:-

The lethal concentrations causing 50 and 90% larval mortalities (LC_{50} & LC_{90}) at the 5, 10, 15, 20 and 25 days after treatment were assessed (**Table, 8 and Fig. 5).** As a general, the concentration 24 $x10^6$ conidia / 100ml highest concentration caused the highest mortality rate and vice versa. The three tested larval instars (1^{st}, 5^{th} and 10^{th}) behaved differently in their reaction to *B. bassiana* treatments. Considering the 1st instar larvae, those were the highest susceptible, showing the lowest LC_{50} (8.319 conidia /100ml = 0.346 x 10^6 conidia / 100 ml). On contrary, the 10th instar larvae manifested least susceptibility as those showed the highest LC_{50} (19.462 conidia /100ml = 0.810 x 10^6 conidia / 100 ml). In this respect, the 5th instar larvae showed intermediate position in their susceptibility to *B. bassiana* treatments between the 1st and 10th instars (LC_{50} = 9.696 conidia /100ml = 0.404 x 10^6 conidia / 100 ml distilled water) **(Table, 8 and Fig. 5).** As for the LC_{90}'s resulted by assaying *B. bassiana* concentrations on *R. ferrugineus* 1st, 5th and 10th larval instars, those manifested the same trend of susceptibility, as the 1st instars was the highest susceptible (56.241 conidia /100ml = 2.176 x 10^6 conidia / 100ml), followed by the 5th instar (74.672 conidia /100ml = 3.111 x 10^6 conidia / 100ml). While, larvae of the 10th instar LC_{90} (203.524 conidia /100ml = 8.480 x 10^6 conidia /100ml) **(Table, 8).**

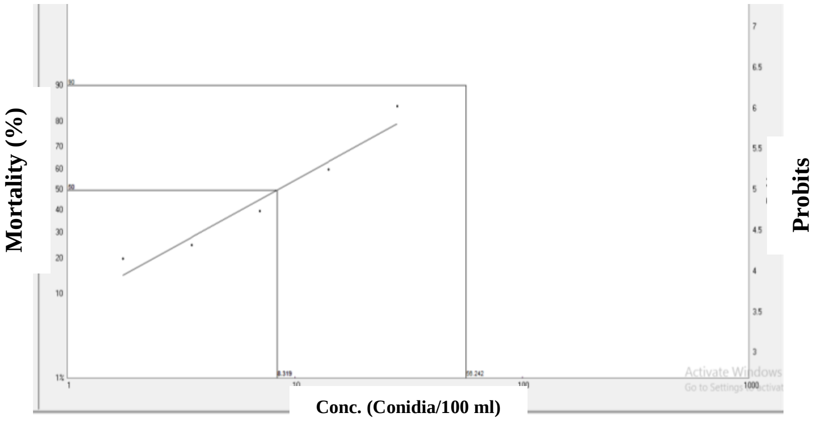
Table (8): Toxicity (LC_{50} and LC_{90} values), 25 days after treatments by the conidial suspension of *B. bassiana* conidia tested against larval instars of *R. ferrugineus.*

Larval instar	LC_{50} (Conidia/100ml)*	LC_{90} (Conidia/100ml)*	Slope ± SE
1st	8.319 (6.697 - 10.026)	56.241 (39.026 - 94.642)	1.544 ± 0.151
5th	9.696 (7.402 - 1.345)	74.672 (47.567 –99.173)	0.969 ± 0.139
10th	19.462 (10.814 – 29.559)	203.524 (94.216 – 780.702)	2.194 ± 0.209

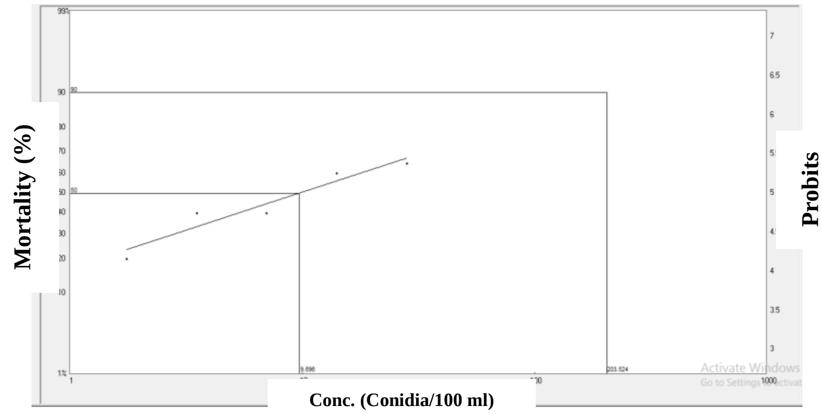
*** Results were calculated after 25 days of treatment.**

Table (9): Time until mortality of 50 and 90% (LT_{50} and LT_{90}) among RPW larval instars after treatments by the conidial suspension of *B. bassiana*.

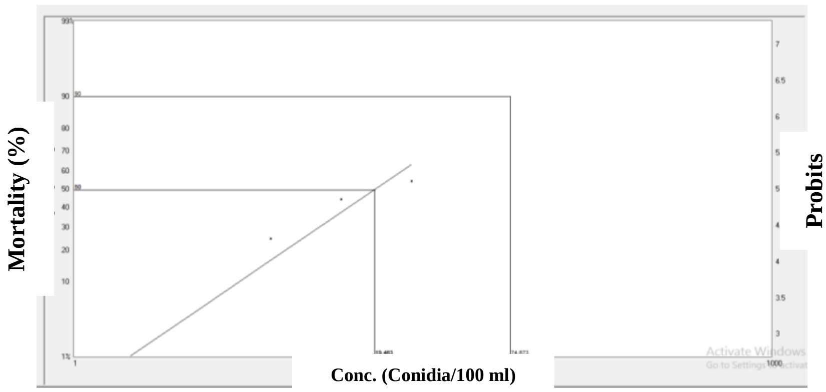
Larval instar	LT_{50} (days)	LT_{90} (days)	Slope ± SE
1st	15.298 (11.136 - 22.027)	31.060 (25.714 - 30.919)	4.601 ± 0.409
5th	16.355 (12.255 – 22.351)	37.066 (36.076 – 46.819)	3.419 ± 0.321
10th	22.351 (20.968 - 24.223)	38.632 (33.205 – 70.607)	5.542 ± 0.620



1st larval instar



5th larval instar

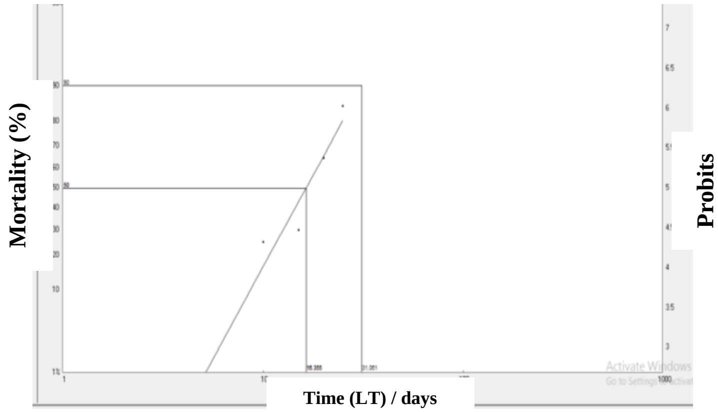


10th larval instar

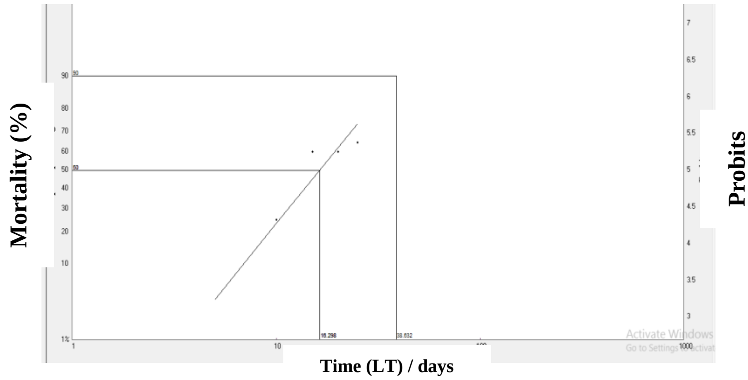
Fig. (5): **Ldp-line of conidial suspension of *B. bassiana* conidia tested against larval instars of *R. ferrugineus.***

The days spent till insect mortality reached 50 and 90% mortalities (LT_{50} & LT_{90}) were calculated for the treated larvae at only the concentration of $24x10^6$ conidia / 100 ml of water. As shown in **Table, 9 and Fig. 6,** the 1st instar took the shortest time till mortality of 50 or 90% of the treated larvae, then the 5th and the 10th instars which took the longest period until mortality. Results indicated that the LT_{50}'s were 15.298, 16.355 and 22.351 days, respectively, opposed to 31.060, 37.066 and 38.632 days, for the LT_{90}'s.

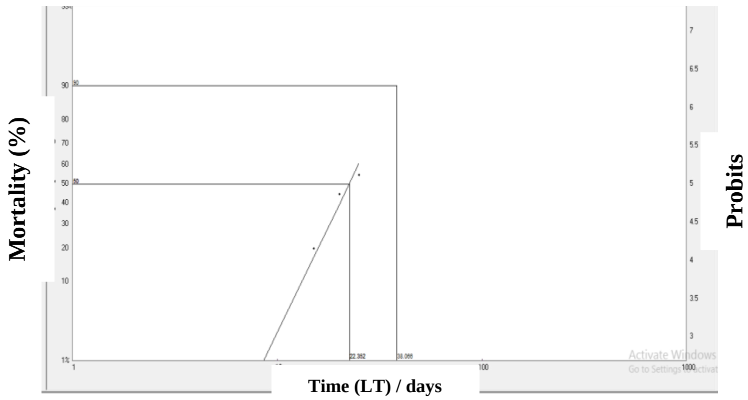
Laboratory and semi-field trials were carried out to evaluate the efficacy of the fungus, *B. bassiana* (Bals.) Vuill against the red palm weevil (RPW), *R. ferrugineus* (Oliv.). At concentrations of $1x10^9$, $1x10^8$, $1x10^7$, $1x10^6$ and $1x10^5$ conidia / ml, mortalities of RPW adults were 86.7, 73.3, 66.7, 46.7 and 40.0%, respectively. The LC_{50} was found to be $7.3x10^5$ conidia / ml and the LT_{50} was 8.87 days at the concentration of $1x10^9$ conidia /ml. Rearing of RPW adults on leaf-axils of date palms at 0, 1, 2, 3 and 4 days post-treatment with *B. bassiana* suspension at a concentration of $1x10^9$ conidia / ml caused 26.7, 6.7, 6.7, 0.0 and 0.0% mortalities, respectively **(Abdel-Samad *et al.*, 2011).**



1st larval instar



5th larval instar



10th larval instar

Fig. (6): lethal times (LT's) until mortality of 50 and 90% of RPW larvae after treatment by conidia of *B. bassiana* at 28 × 10^6 conidia /100ml.

D- Field application of Newvar (*B. bassiana*) on palm trees for RPW control:-

These treatments were performed upon infections in at Kasasen region in the Governorate of Ismailia in 2019, where five replicates of an infested date palm tree each was, randomly chosen. Infested trees were treated with the fungal suspension through a crescent shape holes which were made around the site of infestation by using a large iron pin, then the fungicide was injected by using plastic piping **(Plate, 9)**. The same process was repeated one week later after the first treatment. The concentration of Newvar used was 10g (1 x 10^8 CFU's / g) per liter of distilled water. Field observations were photographed and recorded by naked eyes after 10, 15, 20 and 25 days from the second treatment. From data in Table (10), it could be concluded that the infection began to stop 20 days after treatment. Also, the treated date palm trees manifested 80% recovery after 25 days from treatment.

In similar studies, **El-Sufty *et al.*, (2009**) assayed the effect of field application of *B. bassiana* for control of RPW in the United Arab Emirates. The authors used two methods for application of the entomopathogenic fungi in date palm plantation. Their results indicated that treatments caused 12.8, 21.2 and 23.47% mortalities among adults population in 2005 and 2006, respectively. In another study, **Sewify *et al.*, (2014)** carried out a field experiment in 2008 and 2009 to evaluate the integrated effect of baited aggregation pheromone traps and entomopathogenic fungus *B. bassiana* or insecticide for controlling *R. ferrugineus* in Ismailia Governorate, Egypt. Total mean reduction of RPW population caused by mass-trapping and the fungus *B. bassiana* or insecticide was 61.40 and 40.16%, respectively, to baited pheromone traps + the fungus and baited pheromone traps + insecticide.

Table (10): Field treatments of date palm trees with Newvar (*B. bassiana*) at concentration (10 g / liter of distilled water) at Al Kasasen region in July 2019.

Date of inspection	Time of inspection after application / days				
	1^{st} Tree (5 Holes)	2^{nd} Tree (4 Holes)	3^{rd} Tree (6 Holes)	4^{th} Tree (5 Holes)	5^{th} Tree (6 Holes)
10 days	X	X	X	X	X
15 days	X	X	X	X	X
20 days	D	X	X	X	D
25 days		D	X	D	

N.b: X= still infested & D= recovered

2-2- Effect of entomopathogenic fungal formulation Metmite (*M. anisopliae*) at $1x10^9$ CFU's / 100 ml on the RPW larvae:-

Data presented in **Table, 11 and Fig. 7** showed that the RPW 1^{st} instar larvae were highly susceptible to *M. anisopliae* as the highest mortality rate (90%) was recorded, 25 days after treatment by concentration $1x10^9$ CFU's /100ml, opposed to 85, 65, 45 and 40% for the concentrations $0.5x10^9$, $0.25x10^9$, $0.125x10^9$ and $0.0625x10^9$ CFU's/100ml, respectively after the same period of treatment. Whereas, treatment of the fifth larval instar with concentrations $1 x 10^9$ and $0.5 x 10^9$ CFU's / 100 ml caused, also high mortality rates, 25 days after treatment (80 and 60% respectively). Larvae of the tenth instar manifested their highest resistant to Metmite (*M. anisopliae*) treatments **(Table, 11 and Fig. 7).** After 25 days of 10^{th} larval treatments at the highest concentration used (1g / 100ml.= $1 x 10^9$ CFU's / 100 ml), mortality among the treated larvae reach 75%, being the lowest compared to the 5^{th} (80% mortality) and the 1^{st} (90% mortality) larval instars. No mortality was noticed among the control (untreated) larvae. Generally, the obtained data confirmed that the efficiency of *M. anisopliae* against different instar larvae of *R. ferrugineus* was dependent upon the applied concentration (increased as the applied concentration was increased), larval instar at the time of treatment (higher efficiency on earlier larvae) and the period after treatment (efficiency increased as the period after treatment was prolonged; *i. e.*, highest mortality rates were recorded 25 days after treatment **(Table, 11 and Fig. 7).** As a general, treatments to defferent larval instars by *M. anisopliae* at higher concentrations caused higher mortality rates and vice versa.

M. anisopliae (Hypocereales: Clavicipitaceae) which was recorded infecting the stages of red palm weevil (RPW), *R. ferrugineus*. That was clear from the laboratory screening tests with 10 x 10^{10} spores / ml of *M. anisopliae* and the three larval instars of *C. carnea* undertaken by **Merghem, (2015)** against stages of the red palm weevil and the apple stem-borer. Data confirmed that the susceptibility of RPW stages to the infection with *M. anisopliae* spores resulted in an average mortality reached 63.4 and 45.9% for larval and adult stages, respectively.

Table (11): Mean cumulative mortality percent among *R. ferrugineus* larvae treated with commercial product of *M. anisopliae.* (Data from 20 larvae / each conc.).

Time after treatment / (days) 1st larval instar										
Concentration (CFU's /100ml)	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**
$1x10^9$	0.0	0	0.8	20%	2.6	65%	3.4	85%	3.6	90%
$0.5x10^9$	0.0	0	0.8	20%	1.6	40%	3.0	75%	3.4	85%
$0.25x10^9$	0.6	15%	1.0	25%	1.2	30%	1.6	40%	2.6	65%
$0.125x10^9$	0.6	15%	0.8	20%	0.8	20%	1.0	25%	1.8	45%
$0.0625x10^9$	0.0	0	0.0	0	0.6	15%	1.4	35%	1.6	40%
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
5th larval instar										
Concentration (CFU's /100ml)	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**		**Mean**	**%**	**Mean**	**%**
$1x10^9$	0.6	15%	0.8	20%	1.6	40%	2.4	60%	3.2	80%
$0.5x10^9$	0.0	0	0.0	0	1.6	40%	1.8	45%	2.4	60%
$0.25x10^9$	0.0	0	0.8	20%	1.4	35%	1.6	40%	2.2	55%
$0.125x10^9$	0.6	15%	0.8	20%	1.0	25%	1.6	40%	1.6	40%
$0.0625x10^9$	0.0	0	0.0	0	0.6	15%	0.6	15%	1.0	25%
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
10th larval instar										
Concentration (CFU's /100ml)	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**		**Mean**	**%**	**Mean**	**%**
$1x10^9$	0.8	20%	1.0	25%	1.6	40%	2.4	60%	3	75%
$0.5x10^9$	0.0	0	0.0	0	0.6	15%	1.4	35%	2.4	60%
$0.25x10^9$	0.0	0	0.6	15%	1.0	25%	1.4	35%	1.6	40%
$0.125x10^9$	0.0	0	0.0	0	0.6	15%	0.6	15%	0.8	20%
$0.0625x10^9$	0.0	0	0.0	0	0.0	0	0.6	15%	0.6	15%
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0

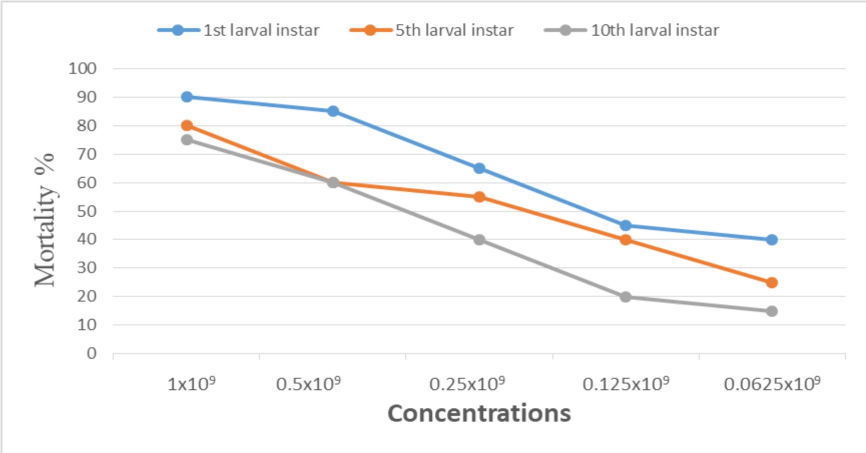


Fig. 7: Mean cumulative mortality percent of *R. ferrugineus* larvae, 25 days after treatment with commercial product of *M. anisopliae* (different concentrations).

A- Toxicity of commercial product Metmite (*M. anisopliae*) against larvae of RPW:-

The lethal concentrations of 50 and 90% of treated larvae (LC_{50} & LC_{90}) after 5, 10, 15, 20 and 25 days of treatment were assessed (**Table, 12 and Fig. 8).** The three tested larval instars behaved differently in their susceptibility to *M. anisopilae* treatments. The LC_{50}'s after different concentration treatments to the 1^{st}, 5^{th} and 10^{th} larval instars *R. ferrugineus* were 0.117, 0.223 and 0.361 g/100ml, opposed to 0.973, 2.530 and 2.838 g/100ml, respectively (**Table, 12 and Fig. 8**). Also, results of the present study revealed that LC_{50} and LC_{90} values of the product of entomopathogenic fungi were increased by increasing the treated larvae were older at the time of treatment indicating higher resistance of these larvae, while, these values of LC_{50} and LC_{90} decreased (indicating higher susceptibility) for larvae of earlier instars.

Table (12): Toxicity (Lethal concentration) of commercial product of *M. anisopliae* tested against larval instars of *R. ferrugineus.*

Larval instar	LC_{50} (g/100ml)*	LC_{90} (g/100ml)*	Slope ± SE
1st	0.117 (0.090 - 0.145)	0.973 (0.701 - 1.568)	1.397 ± 0.153
5th	0.223 (0.175 - 0.281)	2.530 (1.644 - 4.499)	1.161 ± 0.142
10th	0.361 (0.301 - 0.443)	2.838 (1.693 - 6.416)	1.517 ± 0.151

***Results were calculated after 25 days of treatment.**

Values between brackets indicate the range between lowest-highest values.

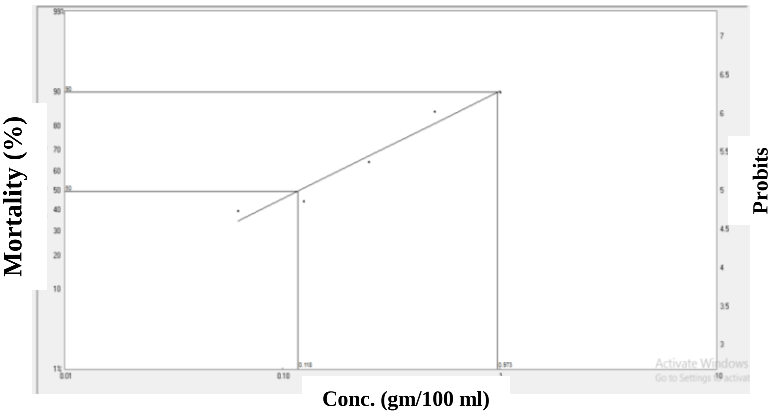
The days spent until mortality of 50 and 90% of *R. ferrugineus* 1st, 5th and 10th instars, (LT_{50} & LT_{90}) were, also, calculated after the treatment of larvae by the highest concentration of 1g / 100ml ($1x10^9$ CFU's / 100ml) of Metmite (*M. anisopliae*). As shown in **Table, 13 and Fig. 9**, the 1st instar took the shortest period until mortality of 50 and 90% (13.627 and 22.748 days) respectively. The correspondent values for the 5th and 10th instars were 15.855 and 46.981 days for the former instar, and (21.496 and 60.776 days), respectively for larvae of the latter instar.

Table (13): Toxicity (Lethal time) of commercial product of *M. anisopliae* tested against larval instars of *R. ferrugineus*.

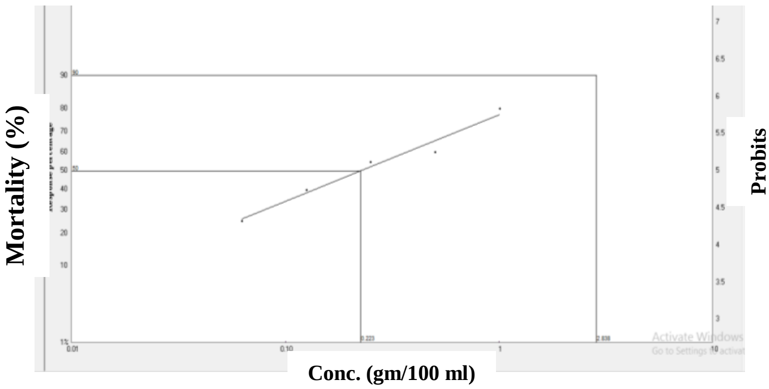
Larval instar	LT_{50} (days)*	LT_{90} (days)*	Slope ± SE
1st	13.627 (12.818 - 14.419)	22.748 (21.089 - 25.018)	5.759 ± 0.445
5th	15.855 (12.036 – 20.263)	46.981 (26.075 – 62.531)	2.716 ± 0.281
10th	21.496 (13.547 – 34.112)	60.776 (48.660 – 89.893)	2.186 ± 0.260

***Result were calculated in concentration 1g/100ml.**

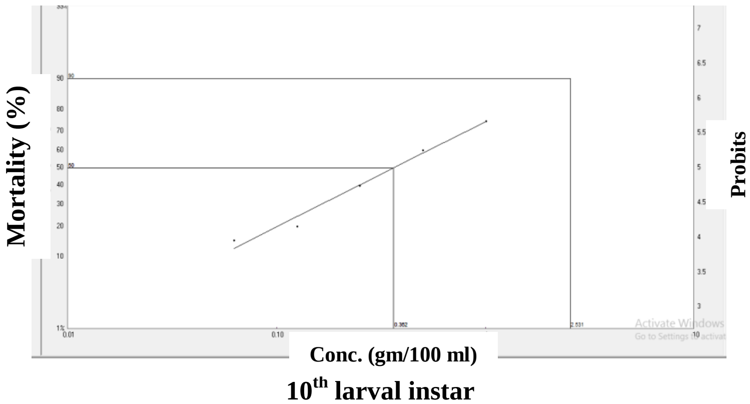
Francardi *et al.,* (2014) isolated *M. anisopilae* strain M. 0.8/105 from larval stage of *R. ferrugineus* to be laboratory tested against adults of this curculionid. Infection of adults took place on different inoculated substrata. This strain of *M. anisopilae* on wheat substratum proved the highest virulent against RPW adults as it led to 90% of the exposed adults and LT_{50} of 13.1 days. *M. anisopilae* (M. 0.8 / 105) showed, also, conidial stability when used as inoculated rice, in the field, as the conidia remained more constant in spring and summer inside traps used for attracting RPW adults than autumn.



1st larval instar

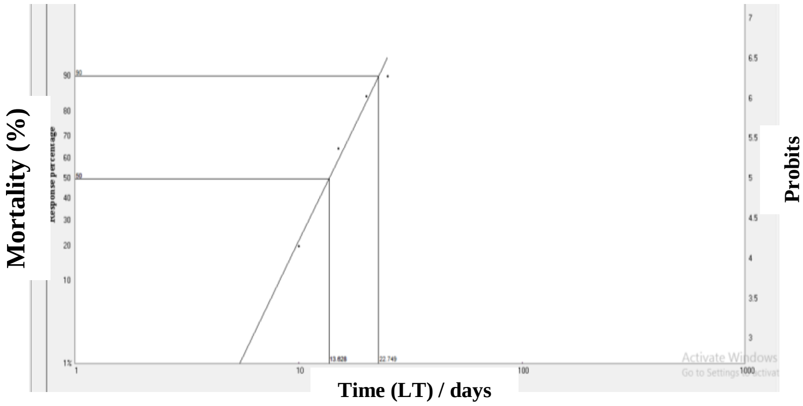


5th larval instar

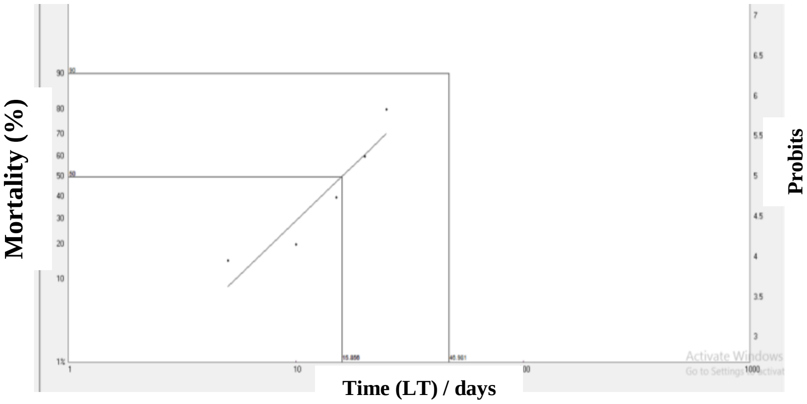


10th larval instar

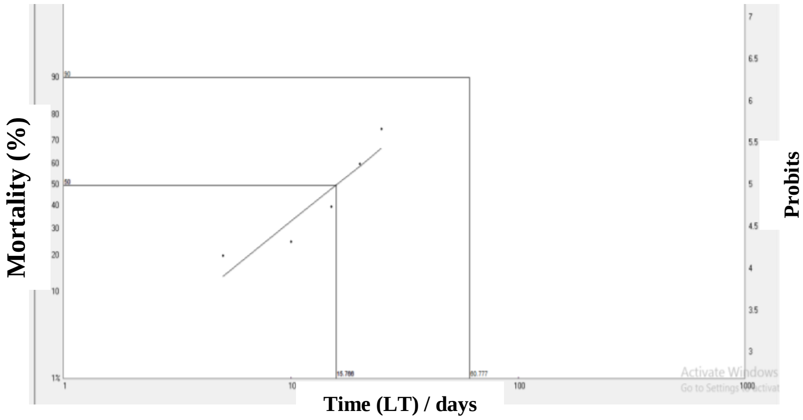
Fig. 8: Ldp-line of Metmite (commercial product of *M. anisopliae*) tested against RPW larval instars of *R. ferrugineus* at 25 days, after treatment.



1[st] larval instar



5[th] larval instar



10[th] larval instar

Fig. 9: Toxicity (Lethal times = LT's) of commercial product of *M. anisopliae* tested against larval instars of *R. ferrugineus*.

B- Effect of a conidial suspension of *M. anisopliae* suspension (24×10^6 conidia / 100ml sterile distilled water) of fungal isolate from dead larvae on RPW larvae:-

In this experiment, *R. ferrugineus* larvae were infected with *M. anisopliae* through the commercial preparation Metmite in the laboratory. Diseased larvae were allowed to survive until full-growth of the fungus mycelium in the larval bodies, hyphal growth to outside the host larvae and the fungal conidia, appear. The conidia were, gently, swept and the fungal suspension was prepared in pure distilled water to be obtained at the concentration 24 x 10^6 conidia / 100ml. water the basic suspension. From this concentration, the remaining dilutions needed for experiments were, easily, prepared (12 x 10^6, 6 x 10^6, 3 x 10^6 and 1.5 x 10^6 conidia / 100ml.). Each of the five obtained concentrations of conidia suspension was assayed on each of the 1^{st}, 5^{th} and 10^{th} larval instars of the red palm weevil. Obtained results on mortality percentages amongst treated larvae are shown in **Table, 14 and Fig. 10**. Data confirmed that the 1^{st} instar larvae were the highest susceptible to *M. anisopliae*, where the highest mortality rate (70%) was recorded 25 days after treatment for the highest concentration (24 x 10^6 conidia / 100ml.). this mortality rate decreased, successively, when the applied concentration decreased to 12 x 10^6 and 6 x 10^6 conidia / 100ml. (65 and 55%, respectively). The least mortality rate among the 1^{st} instar larvae reached it is the lowest value (35%) when larvae received the lowest concentration (1.5 x 10^6 conidia / 100ml.). As for the 5^{th} instar larval treatment, the highest mortality rate (65%) occurred by treatment with the highest concentration (24 x 10^6 conidia / 100ml.), then mortality % decreased as the applied concentration decreased, until reached the lowest percentage of mortality (20%) in correlation with the lowest concentration of *M. anisopliae* conidia (1.5

x 10^6 conidia). Treatments of the 10^{th} RPW larval instar showed the same trend of mortality rates, but with lowest rates, being 60% for the highest concentration applied (24 x 10^6 conidia / 100ml.), then the mortality percent decreased, successively to 55, 35 and 15% when the conidial suspension concentration applied was decreased to 12 x 10^6, 6 x 10^6 and 3 x 10^6, respectively. Whereas the lowest concentration (1.5 x 10^6 conidia) appeared, completely, ineffective on the 10th instar larvae of *R. ferrugineus* (0.0 mortality up to 25 days after treatment). Obtained results in the present confirmed that the mortality rates occurred among treated larvae was proportional to the tested concentration, treatment of early larval instar and prolongation of the period after treatment.

The obtained results agree with those previously reported by **Muhammad *et al.*, (2019)** who found that *M. anisopliae* caused 88% larval and 75% adult of *R. ferrugineus* when used at it is highest concentration respectively. More sporulation cadavers (mycosis) resulted from a high dose rate compared to low dose on both life stages of *R. ferrugineus*. The authors study confirmed the lethal action of *M. anisopliae* isolates with mortality levels was usually directly proportional to the conidial concentration. Their study further confirmed that the isolates obtained from *R. ferrugineus* dead cadavers gave more mortality compared to the other sources. In another study, **Merghem, (2011)** isolated the green muscardine fungus, *M. anisopliae* from naturally infected red palm weevil *R. ferrugineus* in Egypt. Three concentrations of the isolated fungus (i.e. 10 x 10^{10}, 5x10^{10}, and 2.5x10^{10} spores/ml) were applied to both full grown larval and adult stages of RPW. Laboratory pathogenicity tests revealed mortality rates of 88.3 ± 2.3 and 79.3 ± 0.4% for larval and adult stages at 10 x 10^{10} spores/ml concentration, respectively. Mortality rates were 81.6 ± 2.4

and 72.5 ± 0.7% for larval and adult stages, respectively in the case of $5x10^{10}$ spores/ml concentration. The concentration of $25x10^{9}$ spores/ml scored average mortality rates of 69.9 ± 5.3 and 60.3 ± 0.7% for both stages, respectively. Field trials were conducted to test the efficacy of *M. anisopliae* against *R. ferrugineus* infestation. Mortality levels of *R. ferrugineus* stages in the field experiments were represented by the recovery percentage of the infested palm trees (RPIPT). The RPIPTs were 58.33, 41.67 and 16.67% for the tested concentrations, respectively.

Table (14): Mean cumulative percentages of mortality of larvae of *R. ferrugineus* treated with conidial suspension of *M. anisopliae* (different concentration) (Data from treatment of 20 larvae for each concentration).

Time after treatment / (days)										
1^{st} larval instar										
Concentration (conidia/100ml)	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**
$24x10^{6}$	0.0	0	0.8	20%	1.8	45%	2.6	65%	3.6	70%
$12x10^{6}$	0.0	0	0.6	15%	2.2	55%	2.4	60%	2.6	65%
$6x10^{6}$	0.6	15%	0.8	20%	1.0	25%	1.8	40%	2.2	55%
$3x10^{6}$	0.6	15%	0.8	20%	0.8	20%	1.2	30%	1.8	45%
$1.5x10^{6}$	0.0	0	0.0	0	0.8	20%	1.0	25%	1.6	35%
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
5^{th} larval instar										
Concentration (conidia/100ml)	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**		**Mean**	**%**	**Mean**	**%**
$24x10^{6}$	0.0	0	0.8	20%	1.6	40%	2.4	60%	2.6	65%
$12x10^{6}$	0.0	0	0.0	0	0.8	20%	1.8	45%	2.4	60%
$6x10^{6}$	0.6	15%	0.8	20%	1.4	35%	1.8	45%	2	50%
$3x10^{6}$	0.0	0	0.0	0	0.2	5%	1.4	35%	1.4	35%
$1.5x10^{6}$	0.0	0	0.0	0	0.6	15%	0.6	15%	0.8	20%
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
10^{th} larval instar										
Concentration (conidia/100ml)	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
	Mean	**%**	**Mean**	**%**	**Mean**		**Mean**	**%**	**Mean**	**%**
$24x10^{6}$	0.6	15%	0.8	20%	1.4	35%	1.8	45%	2.4	60%
$12x10^{6}$	0.4	10%	0.8	20%	1.4	35%	2	50%	2.2	55%
$6x10^{6}$	0.0	0	0.4	10%	0.8	20%	1.2	30%	1.4	35%
$3x10^{6}$	0.0	0	0.0	0	0.2	5%	0.6	15%	0.6	15%
$1.5x10^{6}$	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
control	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0

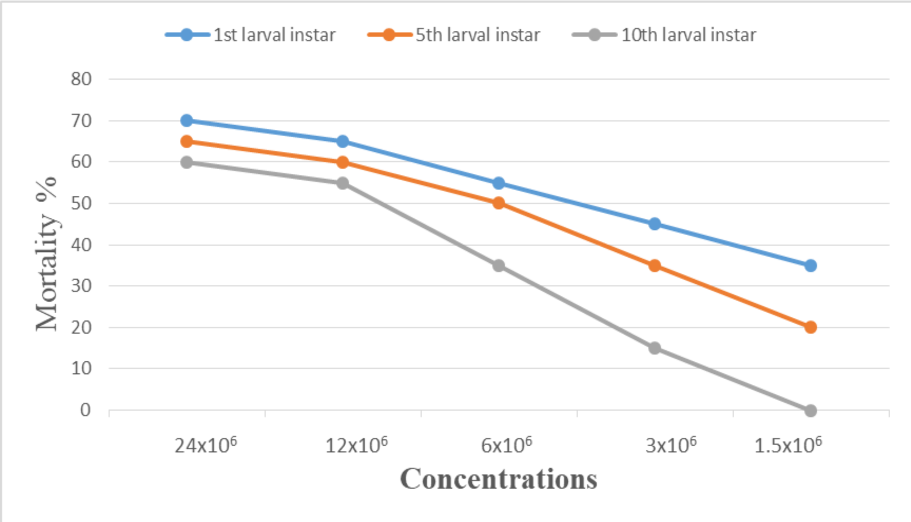


Fig. 10: Mean cumulative mortality percent of *R. ferrugineus* larvae treated with conidial suspensions of *M. anisopliae* (different concentrations) 25 days after treatment.

C- Toxicity of conidial suspension of *M. anisopliae* against larvae of RPW:-

The lethal concentrations LC_{50} & LC_{90} at the 5, 10, 15, 20 and 25 days after treatment were assessed (**Table, 15 and Fig. 11).** As a general, the $24x10^6$ conidia /100ml concentration caused highest mortality percentages and vice versa. The three tested larval instars behaved differently in their toxicity. Considering 1^{st} larval instar, (it was the highest affected (lowest LC_{50} and LC_{90}), then the 5^{th} instar and finally 10^{th} instar). The calculated LC_{50} values were 4.385, 7.728 and 12.694 conidia/100ml, respectively, while those of the according the LC_{90} values were 67.142, 141.412 and 197.528 conidia/100ml, respectively, for the RPW 1^{st}, 5^{th} and 10^{th} instar larvae, respectively **(Table, 15 and Fig. 11).**

Table (15): Toxicity (Lethal concentrations) of conidial suspension (*M. anisopliae*) causing 50 and 90% mortalities among larval instars of *R. ferrugineus.*

Larval instar	**LC_{50} (Conidia/100ml)***	**LC_{90} (Conidia/100ml)***	**Slope ± SE**
1st	**4.385 (2.903 - 6.123)**	**67.142 (75.786 - 1002.258)**	**0.775 ± 0.136**
5th	**7.728 (5.968 - 10.359)**	**141.412 (69.989 - 467.169)**	**1.015 ± 0.139**
10th	**12.694 (8.169 - 36.939)**	**197.528 (76.546 - 1320.713)**	**1.771 ± 0.168**

***Results were calculated after 25 days of treatment.**

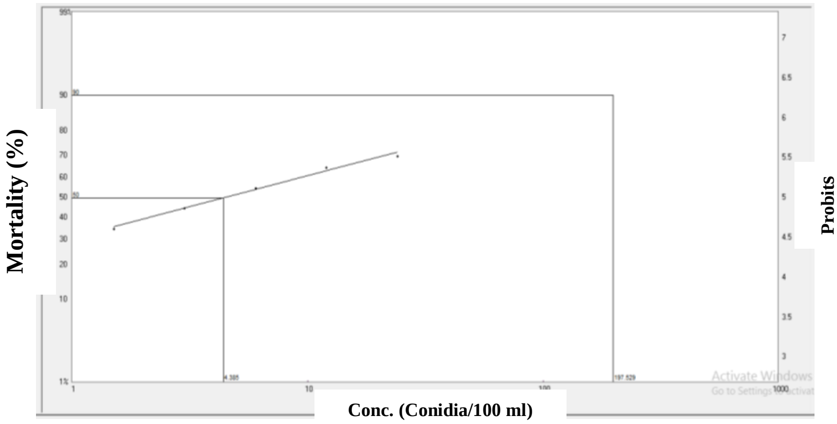
The days spent till insect mortality are calculated at 50 and 90% mortalities, (LT_{50} & LT_{90} values) which treated larvae were calculated for the treated larvae at a concentration of $24x10^6$ conidia / 100 ml. As shown in **Table, 16 and Fig. 12,** the 1st instar took the shortest time, then the 5th and the 10th instars. Results indicated that the LT_{50} values were 16.844, 18.012 and 22.078 days respectively, opposed to 35.167, 39.845 and 105.674 days, for the LT_{90}'s for the 1st, 5th and 10th larval instars.

Table (16): Toxicity (Lethal time) of conidial suspension of *M. anisopliae* tested against larvae instars of *R. ferrugineus*.

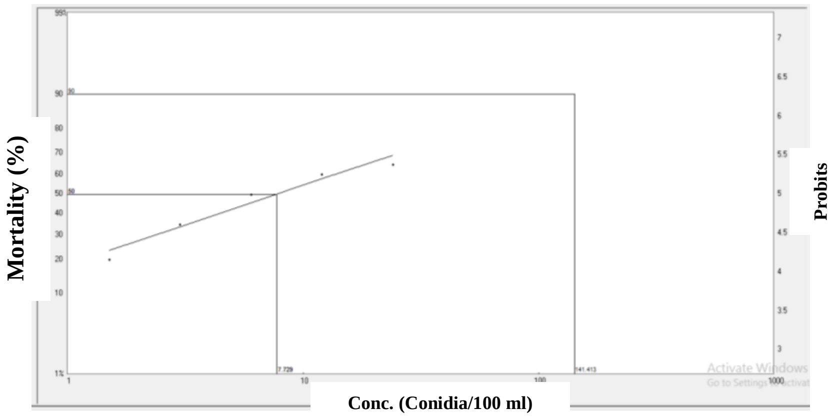
Larval instar	LT_{50} (days)	LT_{90} (days)	Slope ± SE
1^{st}	16.844 (15.652 - 18.196)	35.167 (30.608 - 42.618)	4.009 ± 0.369
5^{th}	18.012 (16.647 - 19.659)	39.845 (33.870 - 50.191)	3.716 ± 0.361
10^{th}	22.078 (18.789 - 27.867)	105.674 (67.270 - 231.682)	1.884 ± 0.267

***Results were calculated in concentration $24x10^6$ conidia /100ml.**

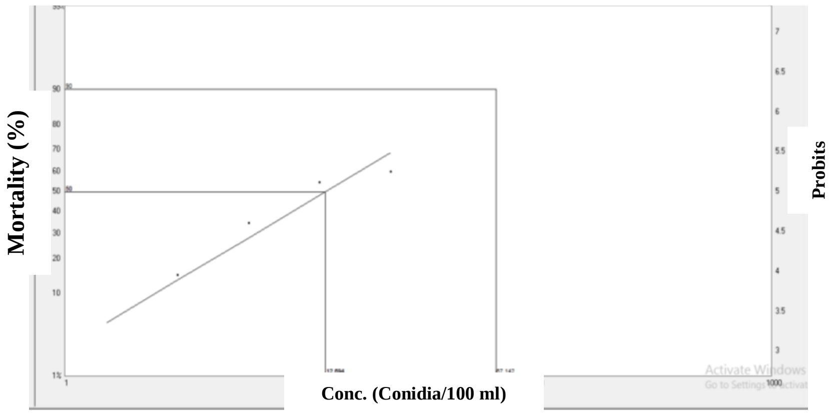
One strain of *Metarhizium* sp. ZJ-1, isolated from Chinese soils, was evaluated for growth characteristics, and screened for its virulence to *R. ferrugineus* larvae in laboratory conditions. An approximately 685-bp fragment was amplified by ITS (ITS1-5.8S-ITS2) PCR from strain ZJ-1, further phylogenetic analysis revealed that 93% similarity to *Metarhizium anisopliae*. Inoculation of $1x10^8$ conidia/mL caused 100% mortality of *R. ferrugineus*, LT_{50} levels of ZJ-1 were 1.66 days ($1x10^8$ conidia/mL), indicating that the conidia of strain ZJ-1 were highly virulent. These results suggest that *M. anisopliae* ZJ-1 has potential as an effective and persistent biological control agent for *R. ferrugineus* (**Xiao Dong *et al.*, 2016).**



1st larval instar

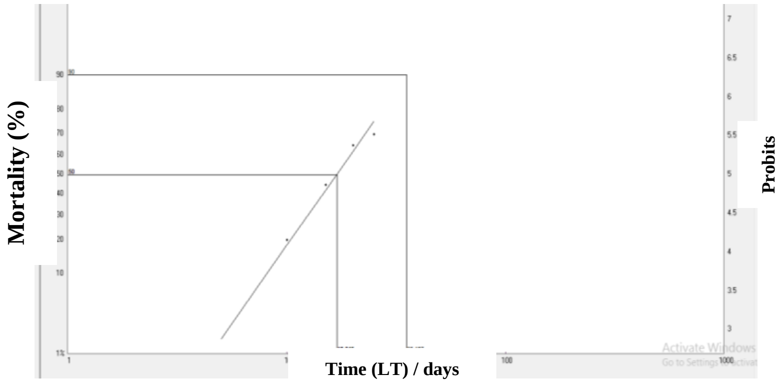


5th larval instar

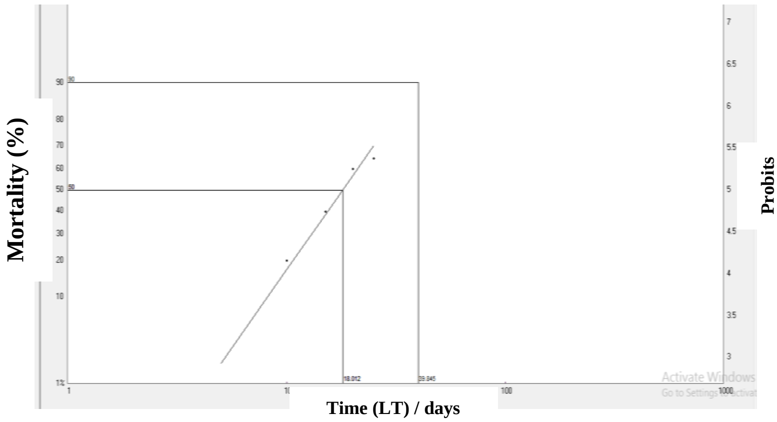


10th larval instar

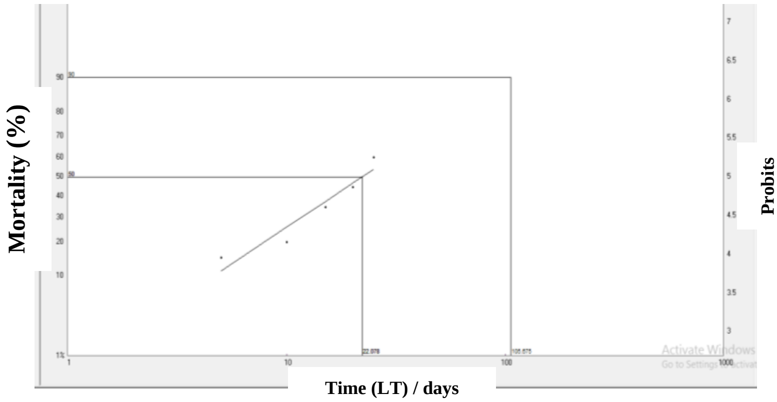
Fig. 11: Ldp-line of conidial suspension of *M. anisopliae* tested against larvae instars of *R. ferrugineus* after 25 days.



1st larval instar



5th larval instar



10th larval instar

Fig. 12: Toxicity (Lethal times = LT's) of conidial suspension of (*M. anisopliae*) tested against larval instars of *R. ferrugineus*.

D- Effect of Metmite (*M. anisopliae*) against the red palm weevil infestations to palm trees in the field:-

These treatments were performed on *R. ferrugineus* infested palm trees at Al-Kasasen region, Ismailia Governorate in July 2019. Five infested palm trees were randomly chosen in a wide area in the field to be considered as 5 replicate (each replicate represented by one infested date palm tree). Infested trees were treated with the fungal suspension at the concentration of 10 g / L (1 x 10^9 CFU's / 100 ml water) through a crescent shape holes which were made around the location of infection by using a large iron pin, then the entomopathogenic suspension was injected bioinsectcide using plastic piping. The same procedure has been repeated one week after the second treatment. (*i.e.*, trees were treated twice at 7 days interval). Results in **Table, 17** demonstrated the field observations on date palm trees that received the fungal suspension. These field observations were recorded by the naked eye after 10, 15, 20 and 25 days after the second treatment. The recorded observations indicated that 2 of the treated trees (40%) became recovered, 20 days after 2^{nd} treatment one (20%) on the day 25^{th}, while the remaining two palm trees (40%) remained infested up to the end of experiment (25 days after second treatment). It is thought that the latter two trees had heaving infestation rate by RPW stages, so those may had the need of one more treatment with the preparation's suspension.

From data of field application, it can be concuded that treatment of infested trees by Newvar (*B. bassiana*) was more effective on RPW stages (80% recovery from infestion) than treatment by Metmite (*M. anisopliae*) which caused 60% recovery.

Table (17): Field treatments of date palm trees with bioinsecticide concentration 10 g per liter of distilled water at Al Kasasen region in the Governorate of Ismailia in July 2019.

Date of inspection	**Time of inspection after application / days**				
	1st Tree (5 Holes)	**2nd Tree (4 Holes)**	**3rd Tree (6 Holes)**	**4th Tree (5 Holes)**	**5th Tree (6 Holes)**
10 days	X	X	X	X	X
15 days	X	X	X	X	X
20 days	X	D	X	D	X
25 days	X		D		X

N.b: X= still infested & D = recovered

3- Total Contents of homogenate lipids, proteins and carbohydrates to larval treatments with *B. bassiana* and *M. anisopliae*:-

The results in **Table, 18** indicated a nonsignificant increase in the total body lipid in RPW larvae due to the effect of the *B. bassiana* treatment compared to control, and a clear while larval treatment with *M. anisopliae* resulted in a nonsignificant decrease in total lipids than control, this decrease was also nonsignificant than *B. bassiana* treatment. Generally, *M. anisopliae* had, evidently, higher efficiency in decreasing lipids in treated larvae. As for the total Protein after entomopathogenic fungi treatments, both *B. bassiana* and *M. anisopliae* resulted in significant decreases in total protein in *R. ferrugineus* larvae after treatments (1.13 and 1.61, mg / ml respectively than control 4.76 mg / ml). The severest efficiency was that of *B. bassiana*. Concerning the total carbohydrates after treatments of *R. ferrugineus* larvae, it is, clearly, evident from **Table, 18** that either of the two fungal species resulted increases in protein content than control

(18.07 mg / ml). This increase was significant in case of *M. anisopliae* (32.80 mg / ml) and nonsignificant (19.31 mg / ml) in case of *B. bassiana* treatment **(Table, 18).**

Table (18): Total contents of homogenate lipids, proteins and carbohydrates (mg/ml) of red palm weevil larvae after treatment with entomopathogenic fungi.

Fungi	Total of		
	Lipid	Protein	Carbohydrate
B. bassiana **($1x10^8$ CFU's/100ml)**	**2.12 ± 0.35[a]**	**1.13 ± 0.18[b]**	**19.31 ± 2.26[b]**
M. anisopliae **($1x10^9$ CFU's/100ml)**	**1.67 ± 0.55[a]**	**1.61 ± 0.31[b]**	**32.80 ± 5.07[a]**
Control	**2.07 ± 0.04a**	**4.76 ± 0.06a**	**18.07 ± 0.81b**

4- Effect of two tested bioinsecticides Newvar (*B. bassiana*) and Metmite (*M. anisopliae*) on *R. ferrugineus.*

A- Eggs deposited by treated adults:-

Results in **Table, 19 and Fig. 13** indicated that feeding of RPW adults on diet treated with *B. bassiana* and *M. anisopliae* produced eggs that showed longer incubation period being 3.75 – 4.75 days opposed to 3.63 days for eggs of the control treatment. With treatments by the two fungal species, the incubation period of eggs was prolonged by increasing the applied concentration. Among the deposited eggs from different treatments, the hatchability percentage was, evidently, decreased for eggs from treated females than control (57 & 61% for *B. bassiana* and 60 & 66% for *M. anisopliae* treatments, opposed to 88% for eggs from the control females; Table, 19 and Fig. 13). Consequently, it was clear that the mortality percentage (unhatched:

eggs) were, clearly, higher among the eggs from treated females (43 & 39% for *B. bassiana* and 40 & 34% for *M. anisopliae* treatments, opposed to 12% among the control eggs; (**Table, 19**).

Table (19): Effect on *R. ferrugineus* eggs resulted from female adults fed on treated diet with *B. bassiana* and *M. anisopliae*:-

Treated by	Concentration CFU's/100ml	Incubation period/ day	Hatchability%	Mortality%
B. bassiana **($1x10^8$ CFU's/100ml)**	**$0.5x10^8$**	**3.75**	**57%**	**43%**
	$1x10^8$	**4.25**	**61%**	**39%**
M. anisopliae **($1x10^9$ CFU's/100ml)**	**$0.5x10^9$**	**4.50**	**60%**	**40%**
	$1x10^9$	**4.75**	**66%**	**34%**
Control		**3.63**	**88%**	**12%**

Data from 100 eggs (25 / replicate)

The potential of *B. bassiana* strain obtained from a naturally infected *R. ferrugineus* pupa as a biological control agent against this weevil was evaluated both in the laboratory and in semi-field assays by **Dembilio *et al.*, (2010).** Laboratory results indicated that this strain of *B. bassiana* can infect eggs, larvae and adults of *R. ferrugineus* (LC_{50} from $6.3x10^7$ to 3.0 $x10^9$ conidia per ml). Also, in pairing combination with fungus challenged males, females as both sexes, treatments with *B. bassiana*, significantly, reduced eggs hatching to (32.8%). The same authors confirmed, also, the efficiency of *M. anisopliae* against *R. ferrugineus* infesting potted 5 year old *Phoenix canariensis* palms in semi-field experiments.

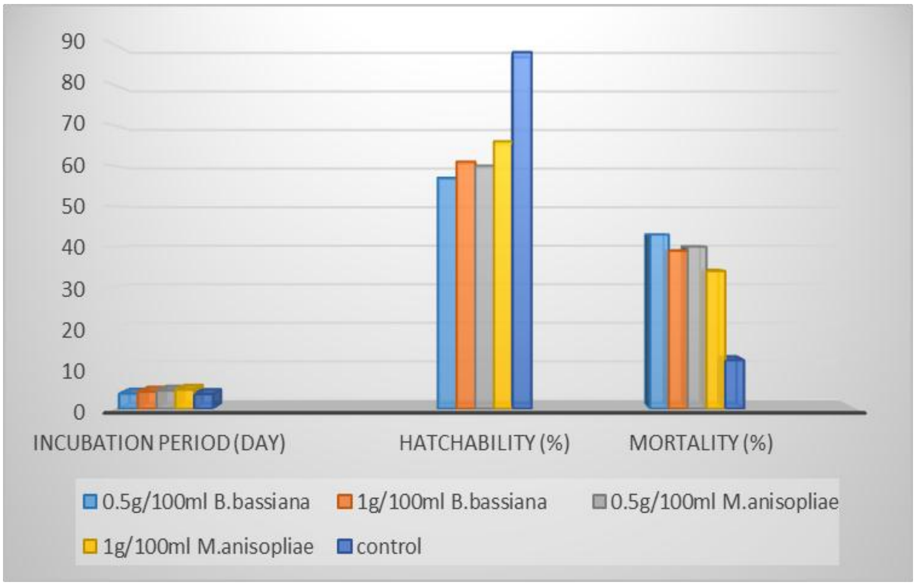


Fig. (13): Incubation period, hatching, and Mortality percentage for eggs from fungi treatments compared to control.

B- Durations of larval and pupal stages:-

Data in **(Table, 20 and Fig. 14)** indicated that the total larval duration shortened by adults treatments by Newvar (*B. bassiana*) or Metmite (*M. anisopliae*) compared to control (untreated adults). Larval period after treatment by the concentration 0.5 g / 100ml was 79.5 and 83.0 days for *B. bassiana* and *M. anisopliae*, respectively, opposed to 72.0 and 76.0 days, respectively for treatment by the concentration 1 g / 100ml. Whereas, the total larval period averaged 91.0 days for the control larvae. As for the RPW pupal stage duration, pupae that developed from *B. bassiana* or *M. anisopliae* treated adults, data in **Table (20)** indicated that the control pupae (resulted from healthy untreated adults) manifested the shortest pupal period (17.5 days). On contrary, pupae from treated adults had longer pupal period; being 23.5

and 20.0 days for *B. bassiana* treatments at 0.5 and 1 g / 100ml, respectively, opposed to 18.0 and 20.5 days for Metmite (*M. anisopliae* treatments) **(Table, 20 and Fig. 14).**

Likewise, 30-35% increase in larval mortality was observed in larvae obtained from eggs from fungus-challenged females or from untreated females coupled with inoculated males, resulting in an overall 78% progeny reduction **(Dembilio *et al*., 2010).**

These results are in agreement with, Treatment also caused a reduction in the pupation and adult emergence percentage. There was also a great reduction in the mean number of eggs/female and the mean number of hatched eggs (**Lotfy *et al*., 2016).**

Table (20): Duration of larval and pupal stage for *R. ferrugineus* larvae fed on diet treated by Newvar and Metmite compared to control. (100 larvae/treatment at 27 ± 2 ºC and 70 ± 5 R.H.).

Concentrations (g/100ml)	Mean duration (days)			
	Larval stage		Pupal stage	
	B. bassiana	***M. anisopliae***	***B. bassiana***	***M. anisopliae***
0.5	**79.5 ± 0.5^{bc}**	**83.0 ± 20^{b}**	**23.5 ± 1.5^{a}**	**18.0 ± 1.0^{b}**
1	**72.0 ± 2.0^{d}**	**76.0 ± 1.0^{cd}**	**20.0 ± 1.0^{ab}**	**20.5 ± 1.5^{ab}**
Control	**91.0 ± 1.0^{a}**		**17.5 ± 0.5^{b}**	
LSD at 0.05	**5.21**		**4.22**	

a, b & c: There is nonsignificant difference (P>0.05) between any two means, within the same column have the same superscript letter.

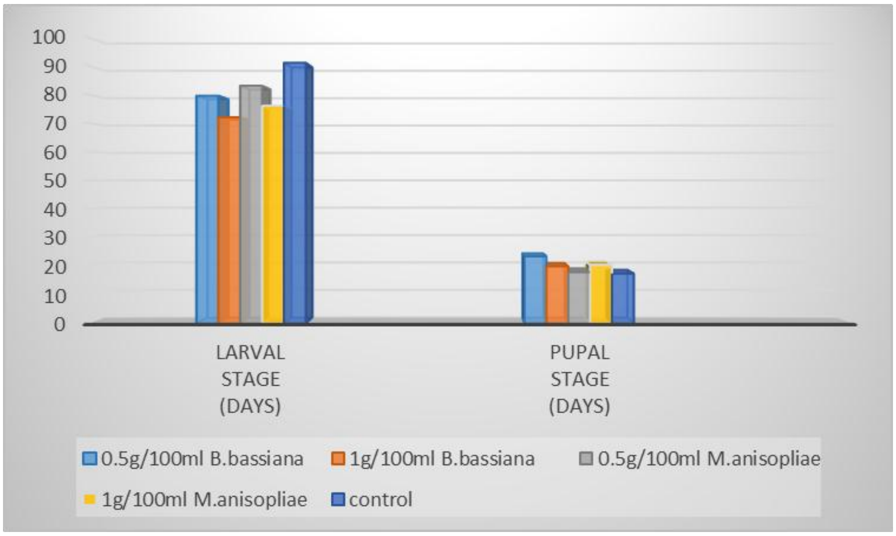


Fig. (14): Duration of *R. ferrugineus* larval and pupal stages under the efficiency of *B. bassiana* and *M. anisopliae* compared to the control.

C- Ovipositional periods:-

The results in **Table, 21 and Fig. 15** indicate the calculation of the average Pre-oviposition period and oviposition period as well as the Post-oviposition period for the freshly emerged female from the cocoon, which were adults treated with entomopathogenic fungi species *B. bassiana* and *M. anisopliae* compared to the control are tabulated in **Table (21)** and presented in **Fig. (15).** Generally, fungi treatments led to shorter oviposition and post-oviposition period 31.0 & 3.5 days for *B. bassiana*, and 29.0 & 2.0 days for *M. anisopliae* treatments at 0.5 g / 100ml the correspondent values for treatments at 1 g / 100ml were 29.0 & 2.5 days for *B. bassiana* and 26.5 & 3.0 days for *M. anisopliae* as oviposition and post-oviposition periods, respectively, opposed to 37.0 and 4.5 days for the control females. On contrary, the fungi treatments caused prolongation of the pre-oviposition period to reach 9.5 and 8.0 days for 0.5 g / 100ml and 11.0 and 9.0 days for the concentration 1 g / 100ml of *B. bassiana* and *M. anisopliae*, respectively, opposed to 5.5 days as a mean pre-oviposition period of the control (untreated) females **(Table, 21 and Fig. 15).**

Table (21): Explains the effect of transactions on Pre-oviposition, Oviposition and Post-oviposition Periods.

Concentration (g/100ml)	**Mean period (days)**					
	Pre- oviposition		**Oviposition**		**Post- oviposition**	
	B. bassiana	***M. anisopliae***	***B. bassiana***	***M. anisopliae***	***B. bassiana***	***M. anisopliae***
0.5	**9.5 ± 1.5^{ab}**	**8.0 ± 2.0^{b}**	**31.0 ± 1.0^{ab}**	**29.0 ± 2.0^{ab}**	**3.5 ± 0.5^{a}**	**2.0 ± 0.5^{a}**
1	**11.0 ± 1.0^{a}**	**9.0 ± 1.0^{ab}**	**29.0 ± 2.0^{ab}**	**26.5 ± 2.5^{b}**	**2.5 ± 1.0^{a}**	**3.0 ± 1.0^{a}**
Control	**5.5 ± 1.0^{b}**		**37.0 ± 1.5^{a}**		**4.5 ± 1.5^{a}**	
LSD at 0.05	**4.95**		**6.80**		**3.54**	

a, b & c: There is nonsignificant difference (P>0.05) between any two means, within the same column have the same superscript letter.

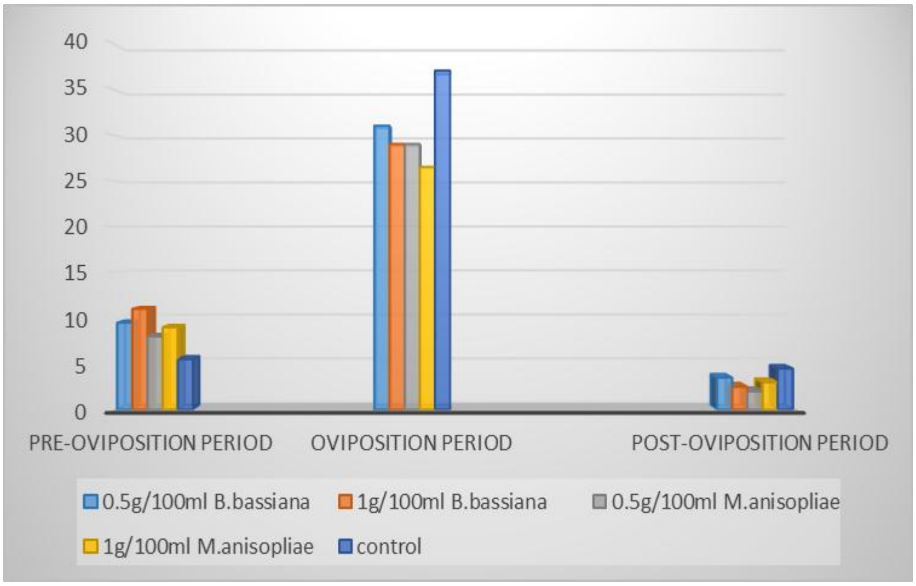


Fig. (15): **Pre-oviposition, oviposition and post-oviposition periods under the influence of *B. bassiana* and *M. anisopliae* compared to the control.**

D- Eggs reproductivity in relation to fungi infections:-

Data in **Table, 22 and Fig. 16** indicated the *B. bassiana* and *M. anisopliae* treatments at 0.5 and 1 g / 100ml of water led to reductions in the total number of eggs deposited by *R. ferrugineus* female and also in the hatchability percentage than control eggs (deposited by healthy untreated female adult). The mean of total number of eggs deposited by single female treated by fungi suspensions at 0.5 g / 100ml were 200.50 and 110.50 eggs from which 66.83 and 52.03 % hatched for *B. bassiana* and *M. anisopliae*, respectively. In case of treatments by 1 g / 100ml suspension of the two fungi species, the correspondent values were 203.00 and 136.50 eggs and the hatchability percentages were 66.25 and 60.07%, respectively. It could be observed that the effect of *B. bassiana* and *M. anisopliae* increased as the applied concentration of fungal suspension was increased. On the other hand, data from the control females showed higher fecundity of untreated females; being 246.50 eggs as mean total number / ♀ and 87.22% as hatching%.

Table (22): Effect of the treatments Newvar (*B. bassiana*) and Metmite (*M. anisopliae*) on the fecundity female shows the number of eggs per female and the percentage of hatching.

Concentration (g/100ml)	Total eggs / ♀		Hatched eggs			
	B. bassiana	*M. anisopliae*	*B. bassiana*	%	*M. anisopliae*	%
0.5	200.50 ± 2.5^{b}	110.50 ± 20.0^{c}	134.00 ± 1.0^{b}	66.83 %	57.50 ± 10.5^{c}	52.03 %
1	203.00± 2.0^{b}	136.50 ± 3.5^{c}	134.50 ± 5.5^{b}	66.25 %	82.00 ± 6.0^{c}	60.07 %
Control	246.50 ± 4.5^{a}		215.00 ± 3.0^{a}	87.22 %	215.00 ± 3.0^{a}	87.22 %
LSD at 0.05	34.21		22.21			

a, b & c: There is nonsignificant difference (P>0.05) between any two means, within the same column have the same superscript letter

In laboratory bioassays carried out by **Verde *et al.*, (2015),** hatching of RPW eggs treated with three different isolates of *B. bassiana* was 41.2, 26.8 and 29.9% being, significantly, lower than the control (62.4%).

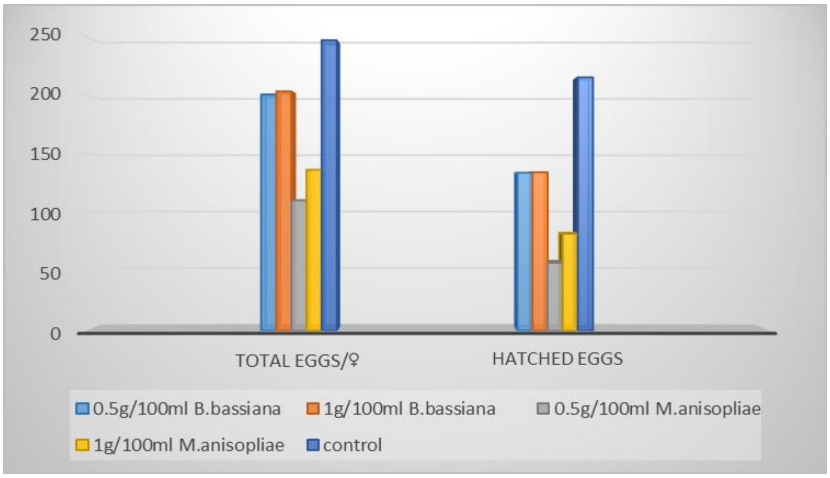


Fig. (16): The fecundity of adult females and the percentage of hatching in eggs under influence *B. bassiana* and *M. anisopliae* compared to control.

E- Longevity of *R. ferrugineus* adults:-

The results in **Table, 23 and Fig. 17** indicated the calculated longevity of the male's and female's life under the influence of the two fungal species *B. bassiana* and *M. anisopliae* compared with the control (untreated). At two tested concentrations 0.5 and 1 g / 100ml where used, the results were 44.0 and 42.5 days, respectively with *B. bassiana* concentrations, and 39.0 and 38.5 days respectively, with *M. anisopliae* concentrations in calculating the longevity female, compared with the control 47.0 days. The results were 37.0 and 34.0 days in two concentrations 0.5 and 1g / 100ml respectively with *B.*

bassiana concentrations compared to the control 49.0 days, and 37.0 and 39.5 days respectively, with the two concentrations 0.5 and 1 g / 100ml of *M. anisopliae* compared with control 49.0 days, in calculating the longevity of male.

Table (23): Effect of treatment with fungi product (Newvar and Metmite) on the longevity of male and female duration.

Concentration (g/100ml)	Longevity Male		longevity Female	
	B. bassiana	*M. anisopliae*	*B. bassiana*	*M. anisooliae*
0.5	**37.0 ± 0.0^{b}**	**37.0 ± 2.0^{b}**	**44.0 ± 0.0^{a}**	**39.0 ± 0.5^{a}**
1	**34.0 ± 1.0^{b}**	**39.5 ± 1.5^{b}**	**42.5 ± 4.0^{a}**	**38.5 ± 0.5^{a}**
Control	**49.0 ± 3.0^{a}**		**47.0 ± 1.0^{a}**	
LSD at 0.05	**6.55**		**6.80**	

a, b & c: There is nonsignificant difference (P>0.05) between any two means, within the same column have the same superscript letter.

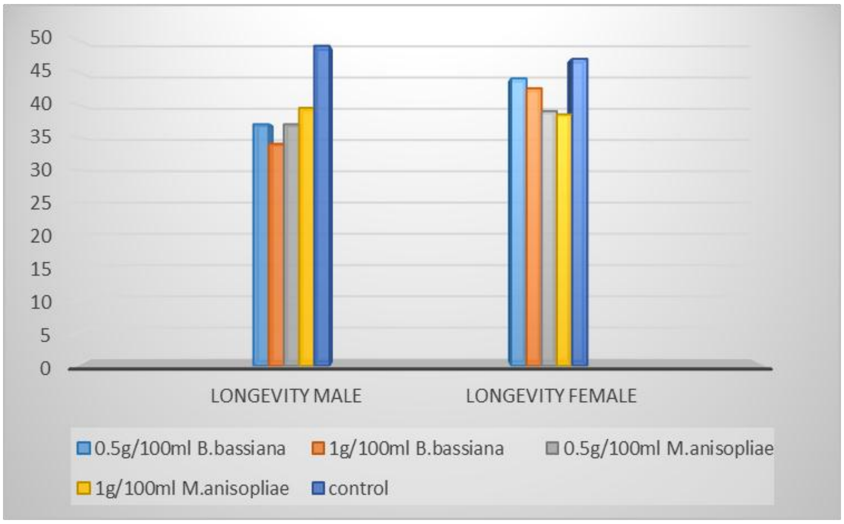


Fig. 17: Longevity of male and female under influence *B. bassiana* and *M. anisopliae* compared to control.

F- Larval, pupal and female adult's mortality:-

The results in **Table, 24 and Fig. 18** showed that the mortality rates after treatments by Newvar (*B. bassiana*) and Metmite (*M. anisopliae*) suspension at 0.5 x 10^8 CFU's or 0.5 x 10^9 CFU's/100ml suspension 48.00 & 23.00%, 56.25 & 27.50 % and 49.00 & 36.50% mortalities among the treated RPW larvae, pupae and female adults, respectively. By raising the fungi suspension concentration to 1 x 10^8 or 1 x 10^9 CFU's/100ml, the correspondent values were 55.00 & 27.50%, 70.00 & 32.50% and 54.00 & 39.00%, respectively, opposed to 20.00, 23.75 and 10.75% among stages of the control treatments, respectively. These results confirmed higher mortality rates among the fungi infected stages than control, one hand, and that the mortality percentages among the treated stages were concentration dependent; *i. e.*, increased as the applied concentration was increased. On the other hand, *B. bassiana* was more effective against RPW stages, *i. e.*, caused higher mortality rates than caused by *M. anisopliae* treatments **(Table, 24 and Fig. 18).**

Verde *et al.*, (2015) treated RPW larvae and adults with a single *B. bassiana* isolate larvae in two ways: spraying each insect with a conidial suspension or feeding them with fruit portions previously immersed in the same conidial suspension. At the end of the two trials, the mortality of treated larvae was 88 and 92%, and the mean survival time of larvae was 10.4 and 11.8 days, significantly different from the control, where no insect died during the trials.

Table (24): Mortality percentages among larval, pupal and female adult stages in relation to entomopathogenic fungi treatments.

Fungi treatment	Concentration CFU's/100ml	Mean mortality percentages		
		Larval stage	Pupal stage	Adult female
B. bassiana	$0.5x10^8$	48.00%	56.25%	49.00%
	$1x10^8$	55.00%	70.00%	54.00%
M. anisopliae	$0.5x10^9$	23.00%	27.50%	36.50%
	$1x10^9$	27.50%	32.50%	39.00%
Control		20.00%	23.75%	10.75%

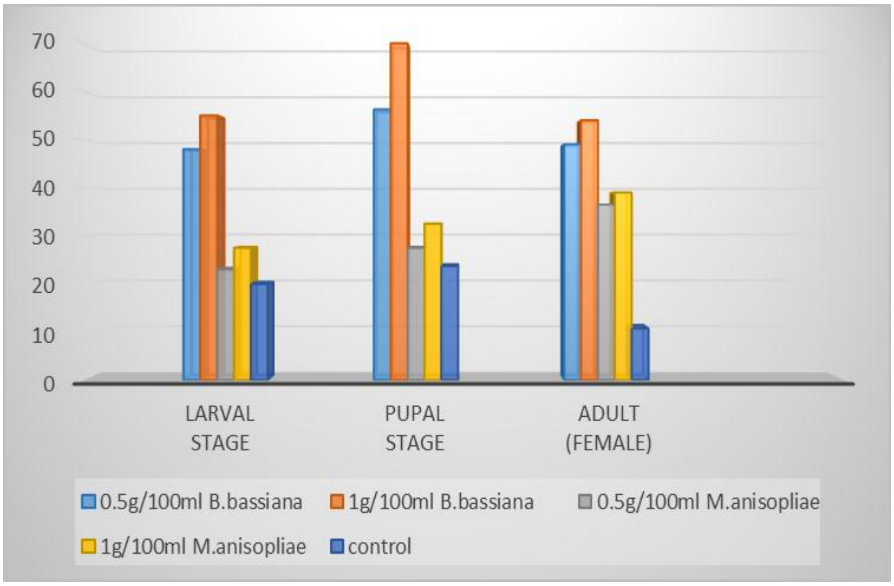


Fig. 18: **Mortality Percentages to larval, pupal and female adult stages under influence *B. bassiana* and *M. anisopliae* compared to control.**

G- Means of pre-oviposition, oviposition and post-oviposition periods and total number of eggs deposited / female adult and longevity of male and female adults of F2 generation under influence *B. bassiana* and *M. anisopliae* compared to control:-

Means of pre-oviposition, oviposition and post-oviposition periods and total number of eggs deposited, female adult and longevity of male and female adults of F2 generation under influence *B. bassiana* and *M. anisopliae* compared to control are tabulated in **Table (25).** From the table, it could be deduced that the effect of treatments to *R. ferrugineus* during F1 extended to the subsequent generation which recorded shorter pre-oviposition, oviposition and post-oviposition periods, fewer total number of eggs / ♀ and shorter female and male adults' longevity **(Table, 25)** compared to those of F1 generation (Table, 21). The pre-oviposition, oviposition and post- oviposition periods and total eggs / female of F2 generation were 7.5 & 8.5, 26.0 & 24.5, 4.0 & 3.5 days and 53.0 & 59.0 eggs / ♀ for Newvar (*B. bassiana*) at 0.5 x 10^8 and 1 x 10^8 CFU's / 100ml respectively. The correspondent values for Metmite (*M. anisopliae*) treatments were 7.0 & 6.5, 29.0 & 23.0, 2.5 & 3.0 days and 50.0 & 56.5 eggs / ♀ for treatments by suspension concentration at 0.5 x 10^9 and 1 x 10^9 CFU's/100ml respectively. While, the control adults showed the ovipositional periods; 5.0, 39.5 and 5.5 days, respectively and 229.0 eggs / ♀.

The longevities of F2 female and male adults were 37.5 and 42.5 days for *B. bassiana* treatments at 0.5 x 10^8 CFU's / 100ml and 36.5 and 47.5 days, respectively for concentration 1 x 10^8 CFU's / 100ml and they were 38.5 and 51.5 days for *M. anisopliae* treatments at 0.5 x

10^9 CFU's / 100ml, and 32.5 and 50.0 days, respectively for concentration 1 x 10^9 CFU's / 100ml.

The correspondent longevities of female and male adults of the control treatment were much longer, being 50.0 and 55.0 days, respectively (Table, 25).

Table (25): Ovipositional periods, eggs reproductivity and longevity of RPW adults (days) for F2 generation in relation to treatments by entomopathogenic fungi.

Fungi treatment	(g/100ml)	2nd progeny					
		Pre-oviposition period	Ovipostion period	Post-oviposition period	Total no. of egg	Longevity of adult ♀	Longevity of adult ♂
B. bassiana	0.5	7.5 ± 1.0[a]	26.0 ± 2.5[a]	4.0 ± 0.5[ab]	53.0 ± 2.0[b]	37.5 ± 1.0[ab]	42.5 ± 0.5[a]
	1	8.5 ± 1.0[a]	24.5 ± 0.5[a]	3.5 ± 1.0[b]	59.0 ± 2.0[b]	36.5 ± 0.5[b]	47.5 ± 1.0[a]
M. anisopliae	0.5	7.0 ± 1.5[a]	29.0 ± 1.5[a]	2.5 ± 2.0[a]	50.0 ± 2.0[b]	38.5 ± 2.0[ac]	51.5 ± 2.5[a]
	1	6.5 ± 1.0[a]	23.0 ±1.5[ab]	3.0 ± 0.5[ab]	56.5 ± 1.5[b]	32.5 ± 2.0[b]	50.0 ± 1.5[a]
Control		5.0 ± 1.0[a]	39.5 ± 1.5[b]	5.5 ± 1.5[b]	229.0 ± 9.0[a]	50.0 ± 2.0[c]	55.0 ± 1.5[b]
LSD at 0.05		4.07	5.92	4.53	15.87	5.92	5.63

Summary

The red palm weevil *Rhynchophorus ferrugineus* is considered a serious pest that affects all palm trees, including date palm trees, and due to the negative impact of chemical pesticides on human health and beneficial elements in the environment, the use of biological elements was used to treat this pest. Two types of fungi were assayed *Beauveria bassiana* and *Metarhizium anisopliae* on the first, fifth and tenth larval instars as well as adults in the field and under laboratory conditions (27 ± 1 ºC and 70 ± 5 % R.H.).

1- **Life-cycle of the red palm weevil**

In order to study the biological characteristics of *R. ferrugineus*, it was reared on two types of diet, (diet, A): consisted of pieces of sugar cane stems and (diet, B) consisted of (grated stalk of sugar cane – Dried yeast - Sorbic acid - Ascorbic acid - Sodium benzoate - Agar-agar and distilled water) with specific weights under laboratory conditions (27 ± 1 ºC and 70 ± 5 % R.H.).

1-1-The immature stages

A-The egg- stage

The mean incubation period of egg stage was 3.53 days when the insect was reared on (diet, A) and 3.62 days when the insect was reared on (diet, B). The percentage of hatching was 80 and 84% for eggs resulted from insects reared on the two diets, indicating 20 and 16% mortalities, respectively.

B- Larval and pupal durations

When reared on (diet, A) the mean durations of the 12 larval instars were 4.87, 8.80, 5.73, 13.47, 11.37, 7.57, 6.73, 7.40, 8.60, 9.07, 8.87 and 11.27 days, respectively, opposed to 4.80, 7.37, 4.93, 5.67, 12.47, 11.30, 8.10, 7.10, 8.17, 9.36, 9.73 and 9.03 days, respectively by

rearing on (diet, B). Consequently, the total larval duration lasted 103.73 and 98.30 days, respectively, being longer on diet (A) than diet (B). Mean of the pupal duration were 17.10 days after larval rearing on diet (A) and 20.10 days for pupae resulted from larvae reared on diet (B).

1-2- The adult's stage

A- Longevity of adult

Female and male longevities by rearing on diet (A) were 51.9 and 47.4 days, respectively, opposed to 43.6 and 39.5 days, respectively by rearing on diet (B). That indicated longer longevity of female than male.

B- Ovipositional periods

The pre-ovipostion, oviposition and post-oviposition periods lasted 11.4, 29.3 and 6.7 days when rearing took place on diet (A), opposed to 9.0, 25.0 and 5.5 days on diet (B). It is means that more females resulted by rearing on diet (B).

C- Sex-ratio

The sex-ratio reached, almost, 1:1 after rearing on diet (A), while it was 1.5:1 (female: male) when rearing occurred on diet (B).

2- Toxicity of Newvar (*Beauveria bassiana*) and Metmite (*Metarhizium anisopliae*) against the red palm weevil larvae

2-1- Effect of different concentrations of Newvar (a commercial product of *Beauveria bassiana*) against RPW larvae

20 days after treatment of 20 larvae of *R. ferrugineus* 1^{st}, 5^{th} and 10^{th} larval instars by each of five concentrations (1×10^8, 0.5×10^8, 0.25×10^8, 0.125×10^8 and 0.0625×10^8 CFU's / 100 ml distilled water) the recorded mortalities were; 95, 85, 80, 60 and 45%, respectively, among the 1^{st} instar; 85, 60, 60, 45 and 25%, respectively,

for the 5th instar; 65, 60, 40, 25 and 0%, respectively, after treatment of the 10th larval instar. No mortality occurred in the control treatment.

A- Toxicity of Newvar against RPW larvae

The LC_{50} and LC_{90} values of Newvar to RPW L_1, L_5 and L_{10} were estimated after 25 days of the three larval instar treatment were 0.077, 0.191 and 0.428 gm/100ml, respectively as LC_{50}'s, opposed to 0.630, 2.117 and 2.448 gm/100ml, respectively as LC'$_{90}$. On the other hand, the time needed for mortality of 50 and 90% of the same larval instars (LT_{50}&$_{90}$'s) after treatment with concentration (1×10^8 CFU's / 100 ml distilled water) were 14.824, 16.393 and 20.758 days, respectively as LT_{50}'s and 23.930, 30.682 and 89.532 days, respectively as LT_{90}'s.

B- Efficiency of *B. bassiana* conidial suspension of fungal isolate from RPW dead larvae

25 days after treatment of 20 larvae of the first, fifth, and tenth larval instars of *R. ferrugineus* with conidial suspention of *B. bassiana* at concentrations (28×10^6, 14×10^6, 7×10^6, 3.5×10^6 and 1.75×10^6 Conidia / 100 ml distilled water), the recorded mortalities reached 85, 60, 40, 25 and 20%, respectively, at the 1st larval instar, 65, 60, 40, 40 and 20%, respectively, in the 5th larval instar and 55, 45, 25, 0 and 0%, respectively, in the 10th larval instar, while no mortality recorded in the untreated check.

C- Toxicity of *B. bassiana* conidial suspension on RPW larvae

After 25 days of treatment of *R. ferrugineus* larvae by *B. bassiana* conidial suspension at the forementioned concentrations, the LC_{50}'s were 8.319, 9.696 and 19.462 conidia/100ml. among L_1, L_5 and L_{10}, respectively, opposed to 56.241, 74.672 and 203.524 conidia/100ml, respectively for the LC_{90}'s. While, by performing treatments by conidial suspention at 28×10^6 conidia / 100 ml distilled water on RPW larvae of 1^{st}, 5^{th} and 10^{th} instars, the LT_{50}**'**s were 15.298, 16.355 and 22.351 days, respectively, opposed to 31.066, 37.066 and 38.632 days, respectively for the LT_{90}**'**s.

D- Field application of Newvar (*B. bassiana*) on palm trees for RPW control

After the treatment was performed for five infested trees with red palm weevil by supplying the fungal suspention injection of bioinsecticide at the same rate repeated a week after the first treatment and follow-up took place after 5, 10, 15, 20 and 25 days of treatment. The percentage of trees recovered from injury was 80% after 25 days of treatment.

2-2- Effect of entomopathogenic fungal formulation, Metmite (*M. anisopliae*) at 1 gm / 100 ml on the RPW larvae

After treatments of 20 larvae of first, fifth, and tenth larval instars by Metmite suspention concentrations of 1 x 10^9, 0.5 x 10^9, 0.25 x 10^9, 0.125 x 10^9 and 0.0625 x 10^9 CFU's / 100 ml distilled water, mortalities among treated larvae after 25 days were 90, 85, 65, 45 and 40%, respectively, for the 1^{st} larval instar, 80, 60, 55, 40 and 25%, in the 5^{th} instar and 75, 60, 40, 20 and 15%, for larvae of the 10^{th} instar, while no mortality occurred among the control larvae.

A- Toxicity of Metmite against RPW larvae

After 25 days of L_1, L_5 and L_{10} treatments at the mentioned concentrations, the LC_{50}'s were 0.117, 0.223 and 0.361 gm /100ml, respectively, opposed to 0.973, 2.530 and 2.838 gm /100ml, respectively, for the LC_{90}'s. While, the mean time spent till to mortality of 50 and 90% of the same larval instars treated with concentration (1 x 10^9 CFU's / 100 ml distilled water) was 13,627, 15,855 and 21,496 days, respectively for the LT_{50}, opposed to 22,748, 46,981 and 60,776 days, respectively, for the LT_{90}.

B- Efficiency of conidial suspension of *M. anisopliae* isolated from RPW dead larvae

After 25 days from treatment of 20 larvae of the 1^{st}, 5^{th} and 10^{th} larval instars with *M. anisopliae* conidial concentrations at 24 x 10^6, 12 x 10^6, 6 x 10^6, 3 x 10^6 and 1.5 x 10^6 conidia / 100 ml distilled water, the mortality rates amongst treated larvae in their 1^{st} instar reached 70, 65, 55, 45 and 35%, respectively, opposed to 65, 60, 50, 35 and 20%, respectively, for the 5^{th} instar and 60, 55, 35, 15 and 0%, respectively, for the 10^{th} larval instar, while no mortalities were recorded in the control (untreated) larvae.

C- Toxicity of *M. anisopliae* conidial suspension against RPW larvae

After 25 days of *R. ferrugineus* larval treatments by *M. anisopliae* conidial suspention at the forementioned concentrations, the LC_{50}'s were 4.385, 7.728 and 12.694 conidia /100ml for the 1^{st}, 5^{th} and 10^{th} larval instars, respectively, opposed to 67.142, 141.412 and 197.528 conidia /100ml, respectively, for LC_{90}'s. While, by treatment of the three larval instars by *M. anisopliae* conidia at (24 x 10^6 conidia / 100 ml distilled water), the LT_{50}'s were 16,844, 18,012 and 22,078 days, respectively, while, those of LT_{90}'s were 35,167, 39,845 and 105.674 days respectively, after treatment of larvae in their 1^{st}, 5^{th} and 10^{th} instars.

D- Field application of Metmite (*M. anisopliae*) on palm trees for RPW control

Treatments were performed to five *R. ferrugineus* infested trees, and supplying the same solution a week later, then follow-up took place after 5, 10, 15, 20 and 25 days of treatment. The percentage of trees recovered from injury was 60% after 25 days of treatment. That improved that treatment of infested trees with RPW stages by Newvar

(*B. bassiana*) was more effective (80% recovery from infestation) than treatment by Metmite (*M. anisopliae*) which caused 60% recovery.

3-The effect of Newvar and Metmite on the content of lipids, proteins and carbohydrates in treated *R. ferrugineus* larval body

After 25 days of the 10th larval instar treatment with Newvar and Metmite at concentrations 1 x 10^8 CFU's / 100 ml and 1 x 10^9 CFU's / 100 ml of Metmite, respectively, the lipid content was 2.12 and 1.67, respectively, compared to 2.07 mg/ml in control, while, those were 1.13 and 1.61, respectively, for proteins, compared to 4.76 mg/ml in control. As for carbohydrates, those recorded 19.31 and 32.80 mg/ml respectively, compared to 18.07 mg/ml in larval of the control treatment. Metmite (*M. anisopliae*) proved more effective in causing considerable decreases in protein and lipid contents, and increase in carbohydrate content in treated larvae.

4-Life table of red palm weevil *Rhynchophorus ferrugineus*

A- Effect on eggs deposited by treated adults

After the adults were treated with concentrations of 1 x 10^8 and 0.5 x 10^8 CFU's / 100 ml of Newvar and 1 x 10^9 and 0.5 x 10^9 CFU's / 100 ml of Metmite, the incubation period of egg was 4.25 and 3.75 days, respectively in Newvar and 4.75 and 4.50 days, respectively, in Metmite, compared to 3,63 days to control. While the percentages of eggs hatching were 61 and 57%, respectively, in Newvar, 60% and 66%, respectively, in Metmite, compared to 88%. Data indicated longer incubation period and higher mortality rates for eggs deposited by adults developed from treated larvae than control.

B- Durations of larval and pupal stages

Treatments of RPW 10^{th} larval insatr with Newvar or Metmite at the formantioned concentrations led to F1 larvae of somewhat recorded shorter total larval period and, subsequently, to pupal of longer duration, compered to control larvae and pupae.

C- Ovipositional periods

Red palm weevil larval treatments with Newvar (*B. bassiana*) or Metmite (*M. anisopliae*) resulted weevil adult females having longer pre-oviposition period and shorter oviposition and post-oviposition periods, compared to control.

D- Eggs reproductivity in relation to fungi infections

There were decreases in the total number of eggs, being 203.0 and 200.5 eggs / treated♀, respectively, with Newvar, 136.5 and 110.5 eggs / treated♀, respectively, in Metmite, compared to 246.5 eggs / control♀, while the total numbers of hatched eggs were 134.5 (66.25%), 134.0 (66.83%) eggs / ♀, respectively, in Newvar; 82.0 (60.07%), and 57.5 (52.03%) eggs / ♀, respectively, in Metmite, opposed 215.0 (87.22%) eggs / control♀.

E- Longevity of *R. ferrugineus* adults

The mean male longevities were 34.0 and 37.0 days, respectively, in the Newvar and 39.5 and 37.0 days, respectively, in Metmite treatments, compared to 49.0 days in the control. Female's manifasted shorter means of life-time, being 42.5 and 44.0 days, respectively, in Newvar and 38.5 and 39.0 days respectively, in Metmite treatments, compared to 47.0 days in the control.

F- Effect of *B. bassiana* and *M. anisopliae* on larval, pupal and female adult's mortality

Throughout the whole period of laboratory rearing (treated and control) RPW from egg to adult, the mortality rates among larvae were 55.00 and 48.00%, respectively in Newvar, while those were 27.50 and 23.00%, respectively in Metmite treatment, opposed to 20.00% among the control larvae.

The mortality rates among the obtained pupae were 70.00 and 56.25%, respectively, in case of Newvar and 32.50 and 27.50%, respectively, in Metmite, compared to 23.75% in control, while those among females were 54.00 and 49.00%, respectively in Newvar and 39.00 and 36.50%, respectively in Metmite, compared to the control that recorded 10.75%.

G- Ovipositional periods, eggs reproductivity and F2 adult's longevity under influence of *B. bassiana* and *M. anisopliae*

The pre-oviposition, oviposition and post-oviposition periods of female emerged after larval treatment with Newvar were 8.5 and 7.5 days, respectively, opposed to 6.5 and 7.0 days, in Metmite, compared to 5.0 days in control for pre-oviposition; 24.5 and 26.0 days, respectively in Newvar, 23.0 and 29.0 days, in Metmite, compared to 39.5 days in control for oviposition period, and 3.5 and 4.0 days, respectively, in Newvar, 3.0 and 2.5 days in Metmite, compered to 5.5 days in control for post-oviposition period female. Throughout the oviposition period, a single female from treatment deposited 59.0 and 53.0 eggs / ♀, respectively in Newvar and 56.5 and 50.0 eggs / ♀, in Metmite opposed to a mean of 229.0 eggs from a single female from control. The means of female's longevity were 36.5 and 37.5 days, respectively, in Newvar and 32.5 and 38.5 days, in Metmite, compared to 50.0 days in control female, opposed to 47.5 and 42.5 days, in Newvar, 50.0 and 51.5 days, respectively, in Metmite, compared to 55.0 days in control male's longevity.

CONCLUSION

For all data presented and explained in this thesis, the following points may be concluded:

1- From data obtained on the biology of the red palm weevil from rearing on Diet A (sugar cane stem pieces) and Diet B (greated sugar cane stalk + Dried yeast + Sorbic acid + Ascorbic acid + sodium benzoate + Agar-agar + distilled water), Diet (B) proved, generally better for successful rearing of RPW.

2- Resulted on toxicity (LC's) and time needed until mortalities (LT's) after Newvar (*B. bassiana*) and Metmite (*M. anisopliae*) treatments confirmed that toxicity and time/mortality were correlated with the following factors:

a- Treated larval instars; as toxicity increased (lower LC's) and time/mortality shortened by treatment of larvae at earlier instar; *i. e.* the 10th instar larvae were the highest resistant, while those of the 1st instar were susceptible.

b- The applied concentration; toxicity increased and time/mortality decreased as the applied concentration was increased.

3- From data on LC's and LT's after treatment by Newvar (*B. bassiana*) and Metmite (*M. anisopliae*); the latter product (Metmite) appeared, generally, more virulent on *R. ferrugineus* larvae than Newvar.

4- Newvar or Metmite treatments to *R. ferrugineus* larvae caused sharp decreases in larval protein content, while treatments resulted increases in carbohydrate content.

5- From field treatments on *R. ferrugineus* infested palm trees, Newvar (*B. bassiana*) appeared more efficient (80% recovery from infestation) than Metmite (*M. anisopliae*) which resulted 60% recovery from infestation after 25 days from treatment.

6- The two bioproducts (Newvar and Metmite) proved valuable if used for biological control against *R. ferrugineus*, in spite of the relatively, higher efficiency of Newvar than Metmite.

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الملخص العربي

تعتبر حشرة سوسة النخيل الحمراء من الآفات الخطيرة التي تصيب جميع اشجار النخيل بما فيها اشجار نخيل التمر، وبسبب التأثير السلبي للمبيدات الكيميائية على صحة الإنسان والعناصر البيولوجية المفيدة في البيئة و تهدف الدراسه الى إستخدام عناصر بيولوجية لمكافحة هذه الآفة، حيث تم استخدام نوعين من المبيدات الحيوية هما نيوفار للفطر الممرض للحشرات *Beauveria bassiana* و ميتاميت للفطر الممرض للحشرات *Metarhizium anisopliae.* على الأعمار اليرقية الاول و الخامس و العاشر وكذلك الحشرات الكاملة لسوسة النخيل الحمراء تحت الظروف المختبرية و في الحقل.

١- تأثير تربية حشرة سوسة النخيل الحمراء على نوعين من البيئات الغذائية على دورة حياتها

تم تربية سوسة النخيل الحمراء بأطوارها المختلفة في المختبر على نوعين من البيئات الغذائية، (البيئة A) تتكون من قطع من سيقان قصب السكر، (البيئة B) تتكون من (مبشور سيقان قصب السكر – الخميرة الجافة - سوربك أسد - أسكوربك أسد - بنزوات الصوديوم - الاجار النقي - ماء مقطر) بأوزان معينة تحت الظروف المختبرية (٢٧ ± ٢ °م و ٧٠ ± ٥ % رطوبة نسبية).

١-١- الاطوار غير الكاملة للحشرة

١-١-أ-طور البيضة

بلغت متوسطات فترة حضانة البيض للحشرة ٣,٥٣ يوماً عند تربية الحشرة على (البيئةA) و ٣,٦٢ يوماً عند تربية الحشرة على (البيئة B). وكانت النسبة المؤية لفقس البيض ٨٠% للبيض الناتج من الحشرات التي تم تربيتها على (البيئة A) و ٨٤% للبيض الناتج من الحشرات التي تم تربيتها على البيئة B، هذا يبين ان نسبة الموت بين البيض هي ٢٠% في البيئة A و ١٦% في (البيئة B).

١-١-ب- فترات الأعمار اليرقية وطور العذراء

بلغت متوسطات الاعمار اليرقية التي تم تربيتها على (البيئة A و البيئة B) على التوالي ٤,٨٧ و ٤,٨٠ يوم للعمر اليرقي الاول ،٨,٨٠ و ٧,٣٧ يوماً للعمر اليرقي الثاني، ٥,٧٣ و ٤,٩٣ يوماً للعمر اليرقي الثالث ، ١٣,٤٧ و ٥,٦٧ يوماً للعمر اليرقي الرابع ، ١١,٣٧ و ١٢,٤٧ يوماً للعمر اليرقي الخامس ، ٧,٥٧ و ١١,٣٠ يوماً للعمر اليرقي السادس ، ٦,٧٣ و ٨,١٠ يوماً للعمر اليرقي السابع ، ٧,٤٠ و ٧,١٠ يوماً للعمر اليرقي الثامن ،

٨,٦٠ و ٨,١٧ يوماً للعمر اليرقي التاسع ، ٩,٠٧ و ٩,٦٣ يوماً للعمر اليرقي العاشر ، ٨,٨٧ و ٩,٧٣ يوماً للعمر اليرقي الحادي عشر و ١١,٢٧ و٩,٠٣ يوماً للعمر اليرقي الثاني عشر والاخير، كما كانت طول مدة الطور اليرقي ١٠٣,٧٣ يوماً على (البيئة A) و ٩٨,٣٠ يوماً على (البيئة B).

بلغ متوسط فترة عمر طور العذراء ١٧,١٠ يوماً للعذراء الناتجة من اليرقات التي تم تربيتها على (البيئة A) و ٢٠,١٠ يوماً للعذراء الناتجة من اليرقات التي تم تربيتها على (البيئة B).

١-٢- الطور الكامل للحشرة

١-٢-أ-طول عمر البالغات

بلغ متوسط طول عمر الإناث الناتجة من اليرقات التي تم تربيتها على (البيئة A) ٥١,٩٠ يوماً مقابل ٤٣,٦٠ يوماً للإناث الناتجة من (البيئة B). بينما كان متوسط عمر الذكر ٤٧,٤٠ يوماً على (البيئة A) و ٣٩,٥٠ يوماً على (البيئة B).

١-٢-ب-فترات وضع البيض في الإناث

كان متوسط فترة ما قبل وضع البيض ١١,٤٠ يوماً للإناث الناتجة من التغذية على (البيئة A) و ٩,٠٠ يوماً للإناث الناتجة من التغذية على (البيئة B). بينما فترة وضع البيض كانت ٢٩,٣٠ يوماً في (البيئة A) و ٢٥,٠٠ يوماً في (البيئة B). اما فترة ما بعد وضع البيض فبلغت ٦,٧٠ للإناث الناتجة من التغذية على (البيئة A) و ٥,٥٠ يوماً للإناث الناتجة من التغذية على (البيئة B). وقد بلغ متوسط عدد البيض الذي وضعته الأنثى الواحدة ٢٤٨,٦٠ بيضة / ♀ في الإناث التي تم تربيتها على (البيئة A) مقابل ٢٥٥,٦٠ بيضة / ♀ في الإناث الناتجة من تربيتها على (البيئة B).

١-٢-ت-النسبة الجنسية

كانت نسبة خروج الحشرات الكاملة من العذارى الناتجة من التغذية على (البيئة A) ٥٠% إناث : ٥٠% ذكور اي ان هذه النسبة كانت ١ : ١ بين الجنسين ، بينما كانت ٦٠% اناث : ٤٠% ذكور في البالغات الناتجة من العذارى التي جمعت من التغذية على (البيئة B) اي ان هذه النسبة كانت ١ذكر :١,٥ أنثى بين الجنسين. هذا مما أثبت زيادة نسبة الإناث عند التربية على البيئة (B).

٢- تأثير وسمية التركيزات المختلفة لمنتجين تجاريين؛ (نيوفار من *B. bassiana*) و (ميتاميت من *M. anisopliae*) ضد يرقات سوسة النخيل الحمراء

٢-١-تأثير التركيزات المختلفة للمنتج التجاري نيوفار لفطر ال *B. bassiana* ضد يرقات سوسة النخيل الحمراء

بعد ان تم معاملة ٢٠ يرقة من كل من الأعمار اليرقية الاول والخامس والعاشربكل من التركيزات (١×١٠^٨ ، ٠,٥×١٠^٨ ، ٠,٢٥×١٠^٨ ، ٠,١٢٥ × ١٠^٨ و ٠,٠٦٢٥×١٠^٨ CFU's / ١٠٠مل ماء مقطر) كانت نسبة موت اليرقات بعد ٢٥ يوم من المعاملة، ٩٥، ٨٥، ٨٠، ٦٠ و ٤٥% على التوالي في العمر اليرقي الاول؛ ٨٥، ٦٠، ٦٠، ٤٥ و ٢٥% على التوالي في العمر اليرقي الخامس مقابل ٦٥، ٦٠، ٤٠، ٢٥ و ٠%، على الترتيب في العمر اليرقي العاشر، بينما لم تسجل اي نسبة موت لليرقات في الكونترول (بدون معاملة).

٢-١-١- قياس سمية المنتج التجاري (نيوفار) ضد يرقات سوسة النخيل الحمراء

بلغ متوسط قيم سمية المنتج التجاري (نيوفار) على الاعمار اليرقية المختلفة بعد ٢٥ يوم من معاملتها بالتركيزات (١×١٠^٨ ، ٠,٥×١٠^٨ ، ٠,٢٥×١٠^٨ ، ٠,١٢٥ × ١٠^٨ و ٠,٠٦٢٥×١٠^٨ CFU's / ١٠٠مل ماء مقطر)، اللازمة لقتل ٥٠% من اليرقات المعاملة (LC_{50}) ٠,٠٧٧، ٠,١٩١ و ٠,٤٢٨ جم / ١٠٠مل، للأعمار اليرقية الاول والخامس والعاشر، على الترتيب، بينما سجلت LC_{90} ٠,٦٣٠، ٢,١١٧ و ٢,٤٤٨ جم / ١٠٠مل مع نفس الأعمار اليرقية الثلاث ، على الترتيب.

هذا بينما بلغ متوسط الفترات اللازمة لموت ٥٠ و ٩٠% من نفس الأعمار اليرقية الثلاث المعاملة بالتركيز (١×١٠^٨ CFU's / ١٠٠مل ماء مقطر) ١٤,٨٢٤، ١٦,٣٩٣ و ٢٠,٧٥٨ يوماً لل LT_{50} على التوالي، مع الاعمار اليرقية، وسجلت ٢٣,٩٣٠ ، ٣٠,٦٨٢ و ٨٩,٥٣٢ يوماً لل LT_{90} مع الاعمار اليرقية الثلاث، على الترتيب.

٢-١-٢- فعالية معلق العزلة الفطرية من يرقات سوسة النخيل التي ماتت بتأثير الفطر

تمت معاملة ٢٠ يرقة من كل من الأعمار اليرقية الاول والخامس والعاشربكل من التركيزات الأتية (٢٨×١٠^٦، ١٤×١٠^٦، ٧×١٠^٦، ٣,٥×١٠^٦ و ١,٧٥×١٠^٦ كونيديا / ١٠٠مل ماء مقطر) من معلق يحتوي على كونيديات الفطر النامي على جسم اليرقات الميتة من المعاملة السابقة وقد أوضحت النتائج أنه بعد ٢٥ يوم من المعاملةكانت نسبة موت اليرقات، ٨٥، ٦٠، ٤٠، ٢٥ و ٢٠% على التوالي في العمر اليرقي الاول؛ ٦٥، ٦٠، ٤٠، ٤٠ و ٢٠% على التوالي، في العمر اليرقي الخامس مقابل ٥٥، ٤٥، ٢٥، ٠ و٠%، على

التوالي في العمر اليرقي العاشر، بينما لم يسجل اي حالة موت في افراد الكونترول (بدون معاملة).

٢-١-٣-قياس سمية معلق كونيديات الفطر ضد يرقات سوسة النخيل الحمراء

بلغ متوسط سمية المعلق الفطري الازم لقتل ٥٠ % من الأعمار اليرقية المختلفة بعد ٢٥ يوم من معاملتها بالتركيزات (٢٨×١٠^٦، ١٤×١٠^٦، ٧×١٠^٦، ٣,٥×١٠^٦ و ١,٧٥×١٠^٦ كونيديا / ١٠٠مل ماء مقطر) ٨,٣١٩، ٩,٦٩٦ و ١٩,٤٦٢ كونيديا / ١٠٠مل للاعمار اليرقية الاول، الخامس والعاشر على الترتيب، بينما بلغ متوسط سمية المعلق الفطري الازم لقتل ٩٠% من اليرقات كانت ٥٦,٢٤١، ٧٤,٦٧٢ و ٢٠٣,٥٢٤ كونيديا / ١٠٠مل مع الاعمار اليرقية الثلاث على التوالي.

هذا بينما بلغ متوسط الوقت اللازم لموت ٥٠ % من نفس الأعمار اليرقية المعاملة بالتركيز (٢٨×١٠^٦ كونيديا / ١٠٠مل ماء مقطر) ١٥,٢٩٨، ١٦,٣٥٥ و ٢٢,٣٥١ يوماً للأعمار اليرقية L_1، L_5 وL_{10} ، على الترتيب، بينما بلغ متوسط الوقت اللازم لموت ٩٠% ٣١,٠٦٦، ٣٧,٠٦٦ و ٣٨,٦٣٢ يوماً مع الأعمار اليرقية الثلاث، على الترتيب.

٢-١-٤-التطبيق الحقلي للمركب نيوفار (*B. bassiana*) على أشجار نخيل البلح لمكافحة سوسة النخيل الحمراء

بعد ان تم حقن محلول مركب نيوفار (بمعدل ١٠جم / لتر ماء) لخمس اشجار من النخيل المصابة بسوسة النخيل الحمراء تم إجراء نفس المعاملة مرة أخرى بعد أسبوع من المعاملة الاولى ومتابعة نسبة شفاء الأشجار بعد ٥، ١٠، ١٥، ٢٠ و ٢٥ يوم من المعاملة الثانية، ولقد سجلت نسبة شفاء الاشجار من الإصابة ٨٠% بعد ٢٥ يوم من المعاملة.

٢-٢- تأثير التركيزات المختلفة للمنتج التجاري (ميتاميت) لفطر *M. anisopliae* ضد يرقات سوسة النخيل الحمراء

بعد ان تم معاملة ٢٠ يرقة من الأعمار اليرقية الاول والخامس والعاشر بالتركيزات الاتية (١×١٠^٩، ٠,٥×١٠^٩، ٠,٢٥×١٠^٩، ٠,١٢٥×١٠^٩ و ٠,٠٦٢٥×١٠^٩ CFU's / ١٠٠مل ماء مقطر) أوضحت النتائج أنه بعد ٢٥ يوم من المعاملة كانت نسبة موت اليرقات، ٩٠، ٨٥، ٦٥، ٤٥ و ٤٠%، على التوالي بين يرقات العمر اليرقي الاول؛ ٨٠، ٦٠، ٥٥، ٤٠ و ٢٥% على التوالي للعمر اليرقي الخامس مقابل ٧٥، ٦٠، ٤٠، ٢٠ و ١٥%، على التوالي للعمر اليرقي العاشر، بينما لم تسجل أي نسبة موت لليرقات في الكونترول.

٢-٢-١-قياس سمية المنتج التجاري (ميتاميت) ضد يرقات سوسة النخيل الحمراء

تم تقدير متوسطات سمية المنتج التجاري (ميتاميت) الذي يسبب نسبة موت ٥٠ و ٩٠% من الاعمار اليرقية المختلفة وذلك بعد ٢٥ يوم من معاملتها بالتراكيزات سالفة الذكر حيث بلغت ٠,١١٧، ٠,٢٢٣ و ٠,٣٦١ جم / ١٠٠مل لل LC_{50} على الأعمار اليرقية الأول والخامس والعاشر على التوالي بينما سجلت لل LC_{90}؛ ٠,٩٧٣، ٢,٥٣٠ و ٢,٨٣٨ جم / ١٠٠مل على الاعمار اليرقية الثلاث على الترتيب.

هذا بينما بلغ متوسطات المدة اللازمة لموت ٥٠ و ٩٠ % من نفس الأعمار اليرقية المعاملة بالتركيز (1×10^{9} CFU's / ١٠٠مل ماء مقطر) ١٣,٦٢٧، ١٥,٨٥٥ و ٢١,٤٩٦ يوماً، لل LT_{50} وسجلت لل LT_{90} ٢٢,٧٤٨، ٤٦,٩٨١ و ٦٠,٧٧٦ يوماً على الأعمار اليرقية الأول والخامس والعاشر، على التوالي.

٢-٢-٢- فعالية معلق كونيدات الفطر *M. anisopliae* المستخلصة من يرقات سوسة النخيل الميتة

بعد أن تم معاملة ٢٠ يرقة من الأعمار اليرقية الاول والخامس والعاشربكل من التركيزات (24×10^{6}، 12×10^{6}، 6×10^{6}، 3×10^{6} و 1.5×10^{6} كونيديا / ١٠٠مل ماء مقطر) من معلق يحتوي على كونيديات الفطر النامي المستخلص من على جسم اليرقات الميتة من المعاملة السابقة كانت نتائج نسب الموت بعد ٢٥ يوم من المعاملة، ٧٠، ٦٥، ٥٥، ٤٥ و ٣٥%، على يرقات العمر الاول؛ ٦٥، ٦٠، ٥٠، ٣٥ و ٢٠%، على يرقات العمر الخامس، مقابل ٦٠، ٥٥، ٣٥، ١٥ و ٠%، على التوالي في يرقات العمر اليرقي العاشر، بينما لم يسجل اي حالة موت بين يرقات افراد المقارنة (بدون معاملة).

٢-٢-٣-قياس سمية معلق الفطر *M. anisopliae* ضد يرقات سوسة النخيل الحمراء

تم تقدير متوسط التركيزات السامة للمعلق الفطري التي سببت نسبة موت ٥٠ و ٩٠% من الأعمار اليرقية المختلفة وذلك بعد ٢٥ يوم من معاملتها بالتركيزات (24×10^{6}، 12×10^{6}، 6×10^{6}، 3×10^{6} و 1.5×10^{6} كونيديا / ١٠٠مل ماء مقطر) حيث بلغت ٤,٣٨٥، ٧,٧٢٨ و ١٢,٦٩٤ كونيديا / ١٠٠مل لل LC_{50} للأعمار اليرقية الاول والخامس والعاشر على التوالي، بينما سجلت لل LC_{90}؛ ٦٧,١٤٢، ١٤١,٤١٢ و ١٩٧,٥٢٨ كونيديا / ١٠٠مل مع الأعمار اليرقية الثلاث على الترتيب.

أما متوسطات الوقت اللازم لموت ٥٠ و ٩٠ % من نفس الأعمار اليرقية المعاملة بالتركيز (24×10^{6} كونيديا / ١٠٠مل ماء مقطر) فبلغت لل LT_{50}، ١٦,٨٤٤، ١٨,٠١٢ و

٢٢,٠٧٨ يوماً على الأعمار اليرقية الثلاث، على التوالي، وسجلت لل LT_{90}؛ ٣٥,١٦٧، ٣٩,٨٤٥ و ١٠٥,٦٧٤ يوماً مع الاعمار اليرقية الأول و الخامس والعاشر على الترتيب.

٢-٢-٤- التطبيق الحقلي للمبيد الحشري البيولوجي ميتاميت (*M. anisopliae*) على أشجار النخيل لمكافحة سوسة النخيل الحمراء

بعد ان تم إجراء المعاملة لخمس اشجار (حقن الأشجار بمحلول المبيد بمعدل ١٠جم/ لتر ماء) من النخيل المصابة بسوسة النخيل الحمراء وأعادة نفس المعاملة بعد اسبوع من المعاملة الاولى ومتابعة نسبة شفاء الأشجار المصابة بعد ٥، ١٠، ١٥، ٢٠ و ٢٥ يوماً من المعاملة الثانية كانت نسبة شفاء الاشجار من الاصابة ٦٠% بعد ٢٥ يوم من المعاملة. هذا مما أثبت أن معاملة أشجار النخيل المصابة بسوسة النخيل بالمبيد الحيوي نيوفار كانت أكثر كفاءة حقلياً (٨٠% شفاء من الأصابة) عن المبيد الحيوي ميتاميت (٦٠% شفاء من الأصابة).

٣- تأثير المعاملة بالمستحضرين نيوفار وميتاميت على معدل نسب الدهون واالبروتينات والكربوهيدرات في جسم اليرقات

وجد إن بعد معاملة يرقات العمر العاشر بتركيز ١×١٠^٨ CFU's / ١٠٠مل من المستحضر التجاري النيوفار و تركيز ١×١٠^٩ CFU's / ١٠٠مل من الميتاميت، أصبحت نسبة الدهون ٢,١٢ و ١,٦٧ على التوالي، مقارنة ب ٢,٠٧ ملجم / مل للمقارنة، بينما كانت نسبة البروتينات ١,١٣ و ١,٦١ على التوالي، مقارنة ب ٤,٧٦ ملجم / مل للكونترول، بينما سجلت نسبة الكاربوهيدرات ١٩,٣١ و ٣٢,٨٠ على التوالي، مقارنة ب ١٨,٠٧ ملجم / مل ليرقات الكونترول وذلك بعد ٢٥ يوم من المعاملة، مما أثبت انخفاضاً حاداً في نسبة البروتين الكلية نتيجة للمعاملتين، بينما زادت نسبة الكربوهيدرات الكلية عن المقارنة.

٤- دراسة جدول الحياة لسوسة النخيل الحمراء *Rhynchophorus ferrugineus*

٤-١- مدة حضانة البيض ونسبة الفقس والموت في البيض نتيجة للمعاملات

تم معاملة الحشرات الكاملة لسوسة النخيل الحمراء بالتركيزات ١×١٠^٨ و ٠,٥×١٠^٨ CFU's / ١٠٠مل من مركب النيوفار و ١×١٠^٩ و ٠,٥×١٠^٩ CFU's / ١٠٠مل من مركب الميتاميت و لقد أوضحت النتائج أن مدة حضانة البيض ٤,٢٥ و ٣,٧٥ يوماً، على التوالي مع النيوفار و ٤,٧٥ و ٤,٥٠ يوماً، على التوالي مع الميتاميت، مقارنة ب ٣,٦٣ يوماً للكونترول، و لقد سجلت النسب المئوية لفقس البيض ٦١% و ٥٧% على التوالي مع النيوفار و ٦٠% و ٦٦% على التوالي، مع الميتاميت، مقابل ٨٨% للكونترول، وهذا يوضح

إن نسبة الموت في البيض كانت ٣٩% و ٤٣% على التوالي ، مع النيوفار و ٤٣% و ٤٠% على التوالي مع الميتاميت، مقارنة ب ١٢% للكونترول.

٤-٢-طول فترة العمر اليرقي و عمر العذراء

بلغ متوسط فترة الطور اليرقي ٧٢,٠ و ٧٩,٥ يوماً على التوالي بعد المعاملتين بال نيوفار، بينما كانت ٧٦,٠ و ٨٣,٠ يوماً، على التوالي مع تركيزي الميتاميت مقارنة مع الكونترول ٩١,٠ يوماً، وسجلت طول مدة طور العذراء ٢٠,٠ و ٢٣,٥، يوماً، على التوالي مع تركيزي النيوفار و سجلت ٢٠,٥ و ١٨,٠ يوماً، على التوالي مع تركيزي الميتاميت بينما سجلت ١٧,٥ يوماً. للكونترول.

٤-٣-فترات وضع البيض في الاناث :-

سببت المعاملات بالتركيزات السابقة لكل من نيوفار و ميتاميت إطالة فترة ما قبل وضع البيض لتصبح ١١,٠ و ٩,٥ يوماً، على التوالي مع النيوفار و ٩,٠ و ٨,٠ يوماً، على التوالي مع الميتاميت مقارنة ب ٥,٥ يوماً في الكونترول، بينما قصرت فترة وضع البيض إلى ٢٩,٠ و ٣١,٠ يوماً، على التوالي مع النيوفار و ٢٦,٥ و ٢٩,٠ يوماً على التوالي، مع الميتاميت مقارنة ب ٣٧,٠ يوماً في الكونترول، أما فترة ما بعد وضع البيض فقد قصرت إلى ٢,٥ و ٣,٥ يوماً، على التوالي في النيوفار و ٣,٠ و ٢,٠ يوماً، على التوالي في الميتاميت مقارنة ب ٤,٥ يوماً الكونترول.

٤-٤- العدد الكلي للبيض /♀ وعلاقته بالعدوى بالفطر

لوحظ إن هناك انخفاض في اجمالي عدد البيض الذي وضعته الإناث حيث وضعت ٢٠٣,٠ و ٢٠٠,٥ بيضة / ♀، على التوالي عند معاملتها بال نيوفار و ١٣٦,٥ و ١١٠,٥ بيضة / ♀، على التوالي مع الميتاميت مقارنة مع ٢٤٦,٥ بيضة / ♀ للكونترول، بينما كان إجمالي عدد البيض الفاقس ١٣٤,٥ (٦٦,٢٥%) و ١٣٤,٠ (٦٦,٨٣%)، على التوالي مع النيوفار و ٨٢,٠ (٦٠,٠٧) و ٥٧,٥ (٥٢,٠٣%)، على التوالي مع الميتاميت مقارنة ب ٢١٥,٠ بيضة / ♀ (٨٧,٢٢%) في الكونترول.

٤-٥- طول عمر الحشرات الكاملة

بلغ متوسط عمر الذكر ٣٤,٠٠ و ٣٧,٠٠ يوماً، على التوالي بعد المعاملة بال نيوفار و ٣٩,٥٠ و ٣٧,٠٠ يوماً، على التوالي مع الميتاميت مقارنة مع ٤٩,٠٠ يوماً للكونترول، بينما كانت الاناث أقل عمراً حيث سجلت ٤٢,٥٠ و ٤٤,٠٠ يوماً، مع النيوفار و ٣٨,٥٠ و ٣٩,٠٠ يوماً، مع الميتاميت على التوالي مقارنة ب ٤٧,٠٠ يوماً في إناث المقارنة

٤-٦-النسبة المئوية للموت في مختلف الأطوار (اليرقات، العذارى و إناث البالغات)

خلال الفترة الكلية لتربية سوسة النخيل الحمراء بعد المعاملة (من طور بيضة حتى طور حشرة كاملة) بلغت نسبة الموت الكلية بين اليرقات ٥٥,٠٠% و ٤٨,٠٠%، مع النيوفار و ٢٧,٥٠% و ٢٣,٠٠%، على التوالي مع الميتاميت مقارنة ب ٢٠,٠٠% بين يرقات المقارنة، وكانت نسبة الموت خلال طور العذراء ٧٠,٠٠% و٥٦,٢٥% مع النيوفار و ٣٢,٥٠% و ٢٧,٥%، على التوالي مع الميتاميت مقارنة ب ٢٣,٧٥% للكونترول، أما نسبة الموت في الاناث البالغة ٥٤,٠٠% و ٤٩,٠٠%، على التوالي في النيوفار و ٣٩,٠٠% و٣٦,٥٠%، على التوالي في الميتاميت مقارنة مع الكونترول الذي سجل ١٠,٧٥%. هذا مما أثبت أن المعاملات بالمبيدين سببت أرتفاعاً كبيراً في نسبة الموت بين اليرقات، العذارى والإناث الكاملة.

٤-٧- قياس التأثير الممتد للمبيدين البيولوجيين بين أفراد الجيل الثاني :-

بلغت فترة ما قبل وضع البيض في الإناث حديثة الخروج من الجيل الثاني ٨,٥ و ٧,٥ يوماً، في النيوفار و ٦,٥ و ٧,٠ يوماً على التوالي، في الميتاميت مقارنة ب ٥,٠ يوماً بين إناث الكونترول، بينما سجلت فترة وضع البيض ٢٤,٥ و ٢٦,٠ يوماً، في النيوفار و ٢٣,٠ و ٢٩,٠ يوماً، على التوالي في الميتاميت مقارنة ب ٣٩,٥ يوماً لإناث الكونترول، إلا إن فترة ما بعد وضع البيض كانت الأقصر حيث سجلت ٣,٥ و ٤,٠ يوماً على التوالي في النيوفار و ٣,٠ و ٢,٥ يوماً على التوالي في الميتاميت مقابل ٥,٥ يوماً للكونترول، وكان عدد البيض منخفضاً حيث وضعت الاناث ٥٩,٠ و ٥٣,٠ بيضة / ♀، عند المعاملة بمبيد النيوفار و ٥٦,٥ و ٥٠,٠ بيضة / ♀ على التوالي عند المعاملة بمبيد الميتاميت مقارنة ب ٢٢٩,٠ بيضة / ♀ للكونترول. و لقد بلغ متوسط طول عمر الاناث ٣٦,٥ و ٣٧,٥ يوماً، على التوالي مع النيوفار و ٣٢,٥ و ٣٨,٥ يوماً، على التوالي مع الميتاميت مقارنة بالكونترول الذي بلغ متوسط عمر إناثه ٥٠,٠ يوماً بينما كان طول عمر الذكر ٤٧,٥ و ٤٢,٥ يوماً، مع النيوفار و ٥٠,٠ و ٥١,٥ يوماً، على التوالي مع الميتاميت مقارنة مع ٥٥,٠ يوماً سجلت كمتوسط فترة حياة الذكر مع الكونترول.

إختبارات حيوية لبعض الممرضات الحشرية على سوسة النخيل الحمراء

الموجز

أصبحت سوسة النخيل الحمراء *Rhynchophorus ferrugineus* (RPW) من أخطر الآفات الحشرية التي تصيب جميع أشجار النخيل وتتسبب في خسائر فادحة للمزارعين ، وبسبب التأثير السلبي للمبيدات الكيميائية على صحة الإنسان والكائنات المفيدة ... إلخ في البيئة، تم استخدام العناصر البيولوجية لعلاج هذه الحشرة. أجريت الدراسات المختبرية والميدانية لإختبار كفاءة نوعين من الفطريات *Beauveria bassiana* و *Metarhizium anisopliae* على اليرقات RPW والبالغين. تمت دراسة دورة حياة سوسة النخيل الحمراء على نوعين من النظام الغذائي ، النظام الغذائي A (سيقان قصب السكر) والنظام الغذائي B (مبشور سيقان قسب السكر + الإضافات). ثبت أن النظام الغذائي المشار إليه (B) أفضل بشكل عام للتربية الناجحة لـ RPW.

بعد معالجة الأعمار اليرقيه الأول والخامس والعاشر لسوسة النخيل بالمعلق الفطري لـ Newvar (*B. bassiana*) و Metmite (*M. anisopliae*) ، وصلت الوفيات المسجلة إلى ٩٥٪ في العمر اليرقي الأول و ٨٥٪ في العمر اليرقي الخامس و ٦٥٪ في العمر اليرقي العاشر عند معالجته بـ ١×$١٠^{٨}$ CFU's /١٠٠مل ماء مقطر من Newvar ، بينما وصل إلى ٩٠٪ في العمر اليرقي الأول و ٨٠٪ في العمر اليرقي الخامس و ٧٥٪ في العمر اليرقي العاشر مع تركيز ١×$١٠^{٩}$ CFU's /١٠٠مل ماء مقطر، من Metmite حيث تم الحصول على أعلى قيم LC_{50} و LC_{90} مع العمر اليرقي العاشر وأقل قيمة مع العمر اليرقي الأول.

تسببت معاملة يرقات سوسة النخيل بالمستحضرات Newvar و Metmite في انخفاض حاد في محتوى بروتين اليرقات ، بينما تسبب في زيادة محتوى الكربوهيدرات.

من المعاملات الحقلية ، إتضح أن معدل الشفاء من الإصابة كان ٨٠ ٪ لأشجار النخيل التي تم علاجها باستخدام Newvar (*B. bassiana*) و ٦٠ ٪ للأشجار المعالجة Metmite. (*M. anisopliae*)

إختبارات حيوية لبعض الممرضات الحشرية على سوسة النخيل الحمراء

رسالة مقدمة من

أحمد عبد الصمد بردان

بكلوريوس العلوم الزراعية - قسم وقاية النبات - كلية الزراعة

جامعة الموصل – جمهورية العراق ٢٠١٦

للحصول على درجة

الماجستير في العلوم الزراعية

حشرات اقتصادية

(مكافحة بيولوجية)

لجنة الفحص والمناقشة:-

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تاريخ المناقشة / / ٢٠٢٠

وكيل الكلية للدراسات العليا والبحوث	عميد الكلية
أ.د. محمد حسن رفعت	أ.د. محمود مغربي عراقي

إختبارات حيوية لبعض الممرضات الحشرية على سوسة النخيل الحمراء

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للحصول على درجة

الماجستير في العلوم الزراعية

حشرات اقتصادية

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٢٠٢٠

إختبارات حيوية لبعض الممرضات الحشرية على سوسة النخيل الحمراء

مقدمة من

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جامعة الموصل– جمهورية العراق ٢٠١٦

رسالة مقدمة للحصول على درجة

الماجستير في العلوم الزراعية

حشرات اقتصادية

(مكافحة بيولوجية)

قسم وقاية النبات – كلية الزراعة – بمشتهر

جامعة بنها

٢٠٢٠