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Beauveriabassiana, a Biocontrol Agent against the Red Palm Weevil, *RhynchophorusFerrugineus* Larvae under Laboratory and Field Conditions.

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Abstract

The presented study was carried out to assay the efficacy of a *Beauveriabassiana*(Balsamo) Vuillemincommercial formulation (Newfar) and its isolates against larvae of the red palm weevil *Rhynchophorusferrugineus*(Olivier). Bioassay experiments took place to estimate theLC_{50}, LC_{90}, LT_{50} and LT_{90}values. By Newfar treatment, theLC_{50}'s against the 1st, 5th and 10th larval instars of *R. ferrugineus* were 0.078, 0.192 and 0.406 g/ml, respectively. LC_{90} values were 0.610, 2.030 and 4.547 g/ml, respectively for the 1st, 5th and 10th instarafter 25 days of treatment. Also, the LT_{50}values after using the concentration $1x10^8$ CFU's / 100mlwere 14.549, 16.167 and 21.022, days, respectively, while those of LT_{90} were23.374, 31.196 and 99.344 days, respectively.Whereas,1×10^8 CFU's / 100ml caused 95% mortality for 1st instar, 85% for 5th instar, and 65% for 10th instar, 25 days after treatment. The concentration 28×10^6 conidia / 100ml caused 85% 65% and 55%mortality for1st, 5th and 10th instar, respectively 25days post-treatment. Additionally, five concentrations of both commercial formulation and isolates of conidial spores (1×10^8CFU's / 100ml and 28×10^6 conidia / 100ml) were evaluated against the 1st,5th and 10th larval instars of red palm weevil under laboratory and field conditions. The results revealed that mortalities among treated larvae were significantly different than control, where no larva died among the control treatments. The current study confirmed that the lethal action of *B. bassiana* was directly proportional to the spore's concentration. This study further confirmed that the earlier larval instars was more affected by *B. bassiana* treatments than older ones.

The field study showed that infested date palm trees injected by New far formulation (*B. bassiana*) at the site of infestation by the red palm weevil caused 80% recovery from infestation after 25 days from treatment.

Key word: Bioassay, Red palm weevil, *Beauveriabassiana*.

Introduction

The red palm weevil (RPW) *Rhynchophorusferrugineus* (Olivier) Coleoptera: Curculionidae) causes large economic losses in cultivated palms worldwide **(Murphy and Briscoe, 1999; Faleiro, 2006and Wakil*et al.*, 2015).** It causes yield losses from 0.7–10 tons/ha**(Singh and Rethinam, 2005).**Also, its distribution is reported in Oceania, Asia, Africa and Europe and was found in Curaçao and Marruecos in 2008, and USA in 2010 **(EPPO, 2010).** It affects a wide range of palms **(Dembilio andJaques, 2015)** including economically important species such as the date palm (*Phoenix dactylifera* L.), Canary Islands date palm (*P. canariensis* Hort.), coconut (*Cocosnucifera*L.), African oil palm (*ElaeisguineensisJacq.*) and chusan palm (*Trachycarpusfortunei*) (Hook.).

Intensive chemical control caused the evolution of insects'resistance, residue persistence, hazards to employees and to the environment and harm to non-target organisms have urged researchers to explore safe alternatives for RPW control **(Hussain *et al.*, 2013andJalinas*et al.*, 2015).**The biological control agents involving entomopathogenic bacteria, fungi and nematodes were laboratory assayed for control of this pest (**Salama*et al.*, 2004; Manachini *et al.*, 2009; Dembilio*et al.*, 2010;El-Hindi, 2016 and Muhammad *et al.*, 2019)**.

To improve management options against the weevil, the efficacy of the entomopathogenic nematode *Steinernemacarpocapsae* Weiser (Nematoda: Steinernematidae) and the potential of the entomopathogenicstrain of the fungus *Beauveriabassiana*(Ascomycota: Clavicipitaceae) were evaluated in laboratory, semi-field and field assays by **Dembilio and Jaques, (2013)**. The use of these entomopathogenic microorganisms proved highly efficient against *R. ferrugineus.* Also, **Muhammad *et al.*, (2019)** confirmed that the isolates recovered from *R. ferrugineus* dead cadavers gave higher mortality rates compared to the other sources. In order to ensure safe control of RPW, the present study was carried out to assay the effectiveness of *B. bassiana*(either its commercial product, namely Newfar) or the recovered isolates of fungi from different dead larval instars of *R. ferrugineus*in laboratory. The efficacy of New far against this insectpest in the field was, also, evaluated. It is hoped that this study could add a new beam of light for better understanding the use of entomopathogic fungi for the RPW control.

Materials and Methods

Collection and rearing of red palm weevil (RPW)

Males and females of red palm weevil (*R. ferrugineus*) were collected from date palm groves in Al

Qassaseen, Ismailia Governorate, Egypt during 2018. Collected adults were bred on pieces of sugar cane stems.The females laid eggs below the upper surface of the sugar cane slices. Deposited eggs were separated by a fine brush and transferred into Petri-dishes containing a filter paper wetted with water. Freshly hatched larvae were reared on pieces of sugar cane stems, and the dissection process of sugar cane stems was monitored until pupation inside the cocoon still emergence of insect adults.

Rearing of RPW occurred under 27 ± 2 ºC and 70 ± 2% R.H. in the insectary of Insect Biology, Plant Protection Department., Faculty of Agriculture, Benha University.

The bio insecticide

The commercial bio-insecticide New faris produced by the Pesticide Production Unit of the Plant Protection Research Institute at Dokki. This product contains the entomopathogenic fungi, *B. bassiana*at a concentration of $1x10^8$ CFU's / mg for insect control. Therecommended concentration to be applied is 10gm / 1 L of water.

Bioassay experiments with New farversus larvae of RPW

In order to investigate the efficacy of the commercial formulation Newfar to control red palm weevil,three larval instars (first, fifth and tenth) of red palm weevil were treated with five concentrations [$1x10^8$, $0.5x10^8$, $0.25x10^8$, $0.125x10^8$ and $0.0625x10^8$ CFU's /100 ml distilled water].Each concentration was replicated five times, four larvae from each instar / replicate, *i.e.* a total of 20 larvae / treatment. Treated larvae and those of the control were checked daily and mortalities were recorded for 25 days. The concentrations were prepared in half-life way, where a volume of 2ml of the suspension were added to 10gm of grated sugar cane plant for treatment of the first instar; 5mlwere added to 15gm of grated sugar canefor the 5^{th} larval instar and 10ml were distributed on 25gm of grated sugar cane for the 10^{th} larval instar treatment. Subsequently, the first instar larvae were kept in glass bottles(3cm diameter and 7cm height)with treated diet and covered with cotton cope to allow respiration of larvae, while larvae of the 5^{th} and 10^{th}instarswere kept in small jars (5cm diameter and 10cm height)contained noted weight of treated sugar cane pieces and covered by perforated metal cover for respiration. Larvae in each treatment were checked daily and the larvae that showed no movement were considered dead. With every inspection date, the number of dead larvae was counted and recorded.

Also, the dead larvae were collected, placed in sterile Petri-dishes with 75 – 80% humidity and incubated at 30 ± 1 ºC in order to retrieve fungal spores, to be used later in the needed experiment.

Fungal isolates against larvae of RPW

B.bassiana isolates were obtained from laboratory infected RPW larvae collected from the above mentioned experiment. All the dead cadavers of RPW were placed in sterile dishes with 75 – 80% humidity and incubated at 30 ± 1ºC for a period of seven days until the appearance of any fungal outgrowths and spores, then spores were transferred to Petri-dishes containing Sabouraud Dextrose Agar (SDA). The plates were incubated at 30 ± 1 ºC.

When more than one fungal colony were present on the medium, the colonies at the age of 10 days were suspended in 100 ml of distilled water containing 2ml of the Tween solution 2.0%, then the concentration of spores was calculated using a specific slide under the microscope. The conidial concentration of the suspension was 28 x 10^6 Conidia / ml. Five conidial concentrations [1% ($28x10^6$), 0.5% ($14x10^6$), 0.25% ($7x10^6$), 0.125% ($3.5x10^6$) and 0.0652% ($1.75x10^6$) conidia /100ml]were added each to 100 ml of distilled water to be assayed against 1^{st}, 5^{th} and 10^{th} instar larvae. The treatment was performed with the same sequence and under the same conditions that were used in the previous experiment. The control individuals were treated with only distilled water. Each replicate consisted of four individuals and five replicates in each treatment. A total of 20 larvae / treatment were used. Mortalities among larvae were checked daily for 25 days and no corrected mortality percentages were needed due to zero mortality among all the control replicates.

***B. bassiana* treatment against red palm weevil in the field**

These treatments were performed upon occurrence of infestation by RPW to palm trees at Al Kassasen region in Ismailia Governorate in 2019. Five RPW infested palm trees were randomly chosen in the field, each represented one replicate for this experiment. The fungal suspension was prepared at one concentration (10g Newfar / liter of water) (1 x 10^8 CFU's/mg)in each injection. The bioinsecticide was applied through the holes which were made around the site of infestation using a large iron pin then injected by the fungicide using plastic piping (Plate 1). Trees received the same treatment 7 days after the first one. The chosen date palm trees were inspected for evaluation of injury after 10, 15, 20 and 25 days after the date of the first application by skimming and cleaning the affected places looking for the dead RPW individuals in all its stages.

Plate (1): Injection method of *B. bassiana* formulation ininfested date palmtrees in the field.

Statistical analysis:

Cumulative mortality at the end of the experiment was analyzed by ANOVA. The concentration scausing 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

Bioassay of Newfar (commercial product of *B. bassiana*) versus larvae of RPW

Results in Table (1) and Fig.(1)show the mortality percentages among RPW larvae after 5,10,15,20 and 25 days post treatment. The first instar larvae of RPW were highly susceptible to *B. bassiana*, where the assayed concentrations caused 95%, 85%, 80%,60% and 45% mortality after 25 days post treatment with$1x10^8$, $0.5x10^8$, $0.25x10^8$, $0.125x10^8$ and $0.0625x10^8$ CFU's / 100ml water, respectively.

Table 1. Mean cumulative mortality percentages among larvae of *R. ferrugineus* treated with different concentrations of commercial product of *B. bassiana*(total number of 20 larvae / treatment).

Time of inspection after treatment on 1st larval instar										
Concentration	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
(CFU's/100ml)	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**
$1x10^8$	0	0	0.8	20%	1.6	40%	3.2	80%	3.8	95%
$0.5x10^8$	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	0.2	5%	1.6	40%	2.2	55%	2.4	60%
$0.0625x10^8$	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
5th larval instar										
Concentration	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
(CFU's/100ml)	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**
$1x10^8$	0	0	0.8	20%	1.6	40%	2.4	60%	3.4	85%
$0.5x10^8$	0	0	0	0	0.8	20%	1.8	45%	2.4	60%
$0.25x10^8$	0.8	20%	1	25%	1.4	35%	2.2	55%	2.4	60%
$0.125x10^8$	0	0	0	0	0	0	1.6	40%	1.8	45%
$0.0625x10^8$	0	0	0	0	0.6	15%	0.8	20%	1	25%
Control	0	0	0	0	0	0	0	0	0	0
10th larval instar										
Concentration	**5 days**		**10 days**		**15 days**		**20 days**		**25 days**	
(CFU's/100ml)	**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	25%	1.4	35%	2	50%	2.4	60%
$0.25x10^8$	0	0	0.6	15%	0.8	20%	1.4	35%	1.6	40%
$0.125x10^8$	0	0	0	0	0	0	0.8	20%	1	25%
$0.0625x10^8$	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0

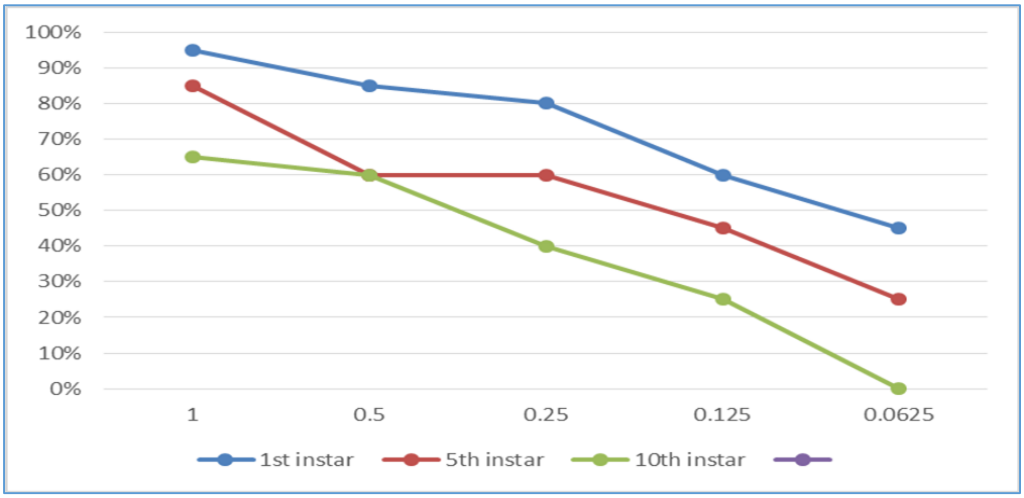

Fig. (1): Mean cumulative mortality percentages among *R. ferrugineus* larvae treated with commercial product of *B. bassiana* (at different concentratione), 25days after treatment.

That was followed by the fifth larval instar which recorded 85, 60, 60, 45, and 25% mortality after 25 days post treatment. While, the 10th larval instar recorded the lowest mortality percentages with most concentrations after 25 days post-treatment (65, 60, 40, 25 and 0.0%), respectively. Thus, indicating that the lowest concentration was, completely, ineffective on the 10th instar larvae. The lowest percentages of mortality were recorded for treatments by the lowest concentration ($0.0625x10^8$ CFU's / 100ml water) in most post-treatment periods.

Cumulative mortality reached maximum values 25 days after treatment (Table, 1 and Fig. 1). No control larvae died among either of the concerned three instars. Also, mortality caused by commercial product of *B. bassiana* was lower for 10th than for 1stand 5thlarval instars. In this respect, **Malik *et al.*, 2019** investigated the effect of *B. bassiana* (1.8×10^7 and 1.8×10^8 conidia/ml) alone and in combination against fifth and sixth larval instars of *R. ferrugineus*. The insects were exposed to fungal treatments by diet incorporation method. Results revealed that the application of *B. bassiana* at the rate of (1.8×10^8 conidia/ml) exhibited synergistic effect, Moreover, the mortality data showed that fifth instar larvae were more susceptible to microbial treatments than sixth instar.

Toxicity (LC_{50}&LC_{90} and LT_{50}&LT_{90}) of Newfar (commercial product of *B. bassiana*) against RPW larvae

The lethal concentrations LC_{50}&LC_{90}recorded at 5, 10, 15, 20 and 25 days after treatment of RPW larvae for 1st, 5th and 10thinstarswere assessed (Table, 2 and Fig. 2). The three tested larval instars behaved differently in toxicity. Considering 1stlarval instar, it was the most affected,followed by 5th instar and finally 10thinstar which manifested lowest mortality percentage.The recorded LC_{50} values, 25 days post-treatment were 0.078, 0.192 and 0.406, respectively.Consequently, theLC_{90} values were0.610, 2.030 and 4.547, respectively. Also, it could be easily noticed that the LC_{50} and LC_{90} values of the product's concentration increased as the treated larvae grew elder.

Table 2. Toxicity (Lethal concentration) of commercial product of *B. bassiana* tested against larval instars of *R. ferrugineus.*

Larval instar	LC_{50} (g/ml)*	LC_{90} (g/ml)*	Slope ± SE
1st	0.078 0.041 ± 0.148	0.610 0.321 ± 1.159	1.436 ± 0.142
5th	0.192 0.098 ± 0.374	2.030 1.040 ± 3.963	1.263 ± 0.148
10th	0.406 0.203 ± 0.811	4.547 2.275 ± 9.088	1.225 ± 0.153

*****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}) which were calculated for the treated larvae at a Newfar concentration of $1x10^8$ CFU's / 100 ml. As shown in (Table, 3 and Fig. 3), death of the 1st instar took the shortest time, then the 5th and the 10th instars. Results indicated that the LT_{50}'s were 14.549, 16.167 and 21.022 days, respectively, opposed to 23.374, 31.196 and 99.344 days, for the LT_{90} for the 1st, 5th and 10th larval instars, respectively.

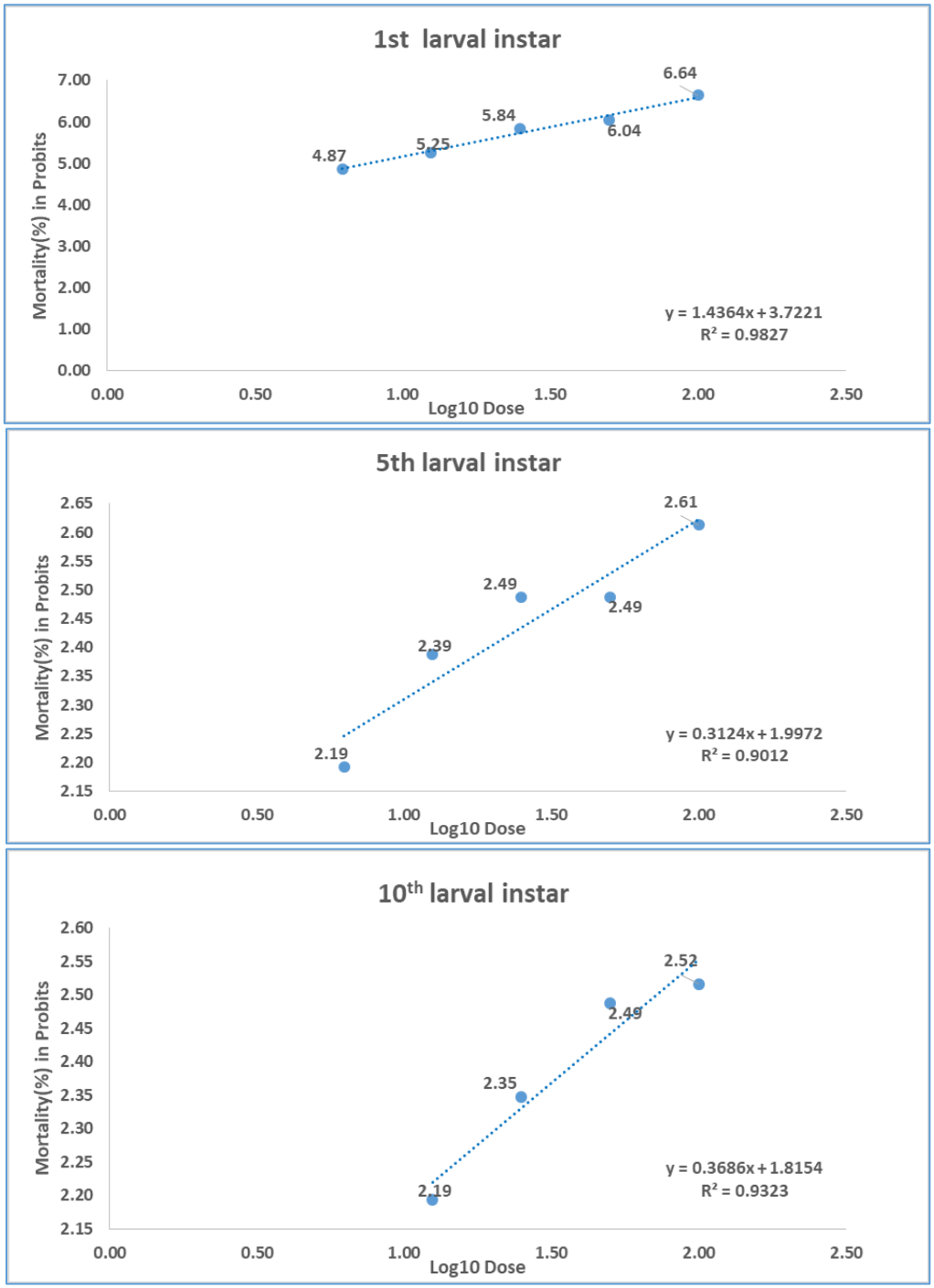

Fig.(2): Toxicity (Lethal concentration) of Newfar(*B. bassiana*) tested against larval instars of *R. ferrugineus*.

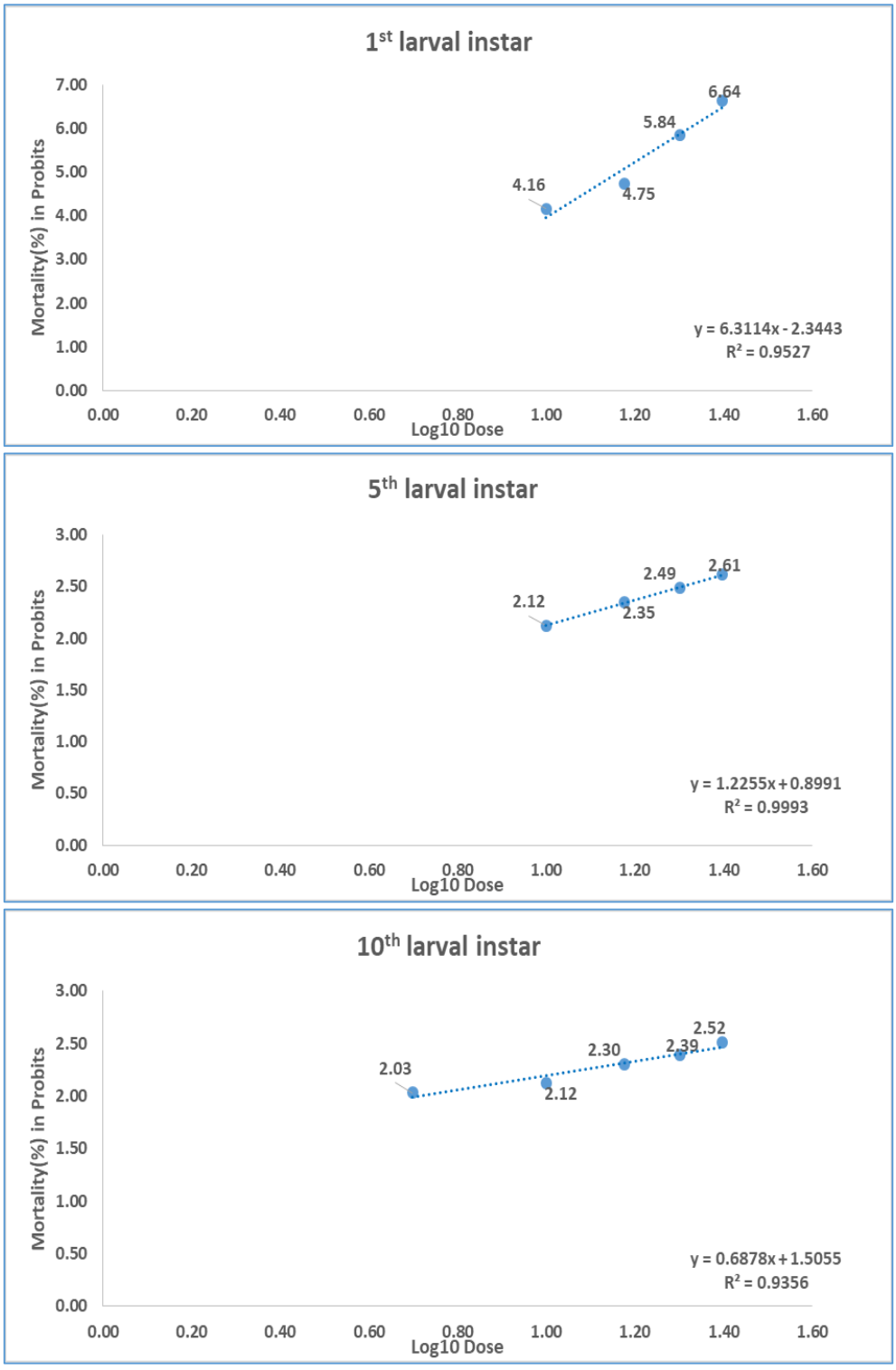

Fig.(3): Toxicity (Lethal time) of Newfar(*B. bassiana*) tested against larval instars of *R. ferrugineus*.

Table 3. Toxicity (Lethal time) of commercial product of *B. bassiana* tested against larval instars of *R. ferrugineus.* Results were calculated using concentration 1gm/100ml.

Larval instar	LT_{50} (days)	LT_{90} (days)	Slope ± SE
1st	14.549 12.314 ± 17.190	23.374 19.783 ± 27.616	6.311 ± 0.037
5th	16.167 13.103 ± 19.947	31.196 25.283 ± 38.490	4.534 ± 0.047
10th	21.022 13.537 ± 32.646	99.344 63.971 154.277	1.935 ± 0.098

Pathogenicity of the entomopathogenic fungus, *B.bassiana* against *R.ferrugineus* was studied by **El-Sufty*et al.*, (2009)**in United Arab Emiratesusing a local strain "UAE-B2". In agreement with present results, they and **El Husseini (2019)** found that the young instars of larvae were more susceptible than the older ones. **El- Sufty*et al.*, (2009)** added that in adult and larval stages, the fungus remains dormant inside the cadavers and started to continue its saprophytic development when R.H. approached 100%. The same authors estimated that complete mycosed cadaver produced $4.3x10^7$ conidia.

Effect of a conidial suspension (28×10^6conidia / 100 ml sterile distilled water) of fungal isolate from RPW dead larvae

Results in (Table, 4 and Fig.4) show that cumulative mortalities among the 1stinstar larvae of RPW were 85, 60, 40, 25 and 20%, opposed to 65, 60, 40, 40 and 20% among larvae of the 5th instar at 25 days post treatment by the $28x10^6$, $14x10^6$, $7x10^6$, $3.5x10^6$ and $1.75x10^6$ conidia /100ml,respectively.

Table 4. Mean cumulative mortality percentages among*R. ferrugineus*larvae treated with conidial suspension (28×10^6 spores / 100ml) of fungal isolate.

Concentration (conidia/100ml)	5 days		10 days		15 days		20 days		25 days	
Time of inspection after treatment on 1st larval instar										
	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
$28x10^6$	0	0	1	25%	1.2	30%	2.6	65%	3.4	85%
$14x10^6$	0.6	15 %	0.8	20%	1.8	45%	2	50%	2.4	60%
$7x10^6$	0.2	5%	0.4	10%	0.8	20%	1	25%	1.6	40%
$3.5x10^6$	0	0	0	0	0.8	20%	1	25%	1	25%
$1.75x10^6$	0	0	0	0	0.6	15%	0.8	20%	0.8	20%
Control	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	1	25%	2.4	60%	2.4	60%	2.6	65%
$14x10^6$	0.8	20 %	1	25%	1.6	40%	1.8	45%	2.4	60%
$7x10^6$	0	0	0.6	15%	0.8	20%	1.4	35%	1.6	40%
$3.5x10^6$	0	0	0	0	0.8	20%	1	25%	1.6	40%
$1.75x10^6$	0	0	0	0	0	0	0	0	0.8	20%
Control	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	0.8	20%	1.8	45%	2.2	55%
$14x10^6$	0	0	0.6	15%	0.8	20%	1.6	40%	1.8	45%
$7x10^6$	0	0	0	0	0	0	0.8	20%	1	25%
$3.5x10^6$	0	0	0	0	0	0	0	0	0	0
$1.75x10^6$	0	0	0	0	0	0	0	0	0	0
control	0	0	0	0	0	0	0	0	0	0

It was, also, noticed that the lowest mortality percentage occurred when treatment took placewith lowest concentration ($1.75x10^6$ conidia / 100ml). Mean while,the 10th larval instar suffered 55%

mortality, 25 days post treatment with the concentration $28x10^6$ conidia /100ml, followed by 45 and 25% mortality for the $14x10^6$ and $7x10^6$ conidia /100ml concentration, followed by 25% for the 0.25 ml concentration. Both $3.5x10^6$ and $1.75x10^6$ conidia / 100ml concentrations had no effect on 10^{th} larval instar 25days post treatment. No control larvae died during the bioassay experiments. It could be concluded that the first instar larvae were the highest susceptible to the*B. bassiana* treatments compared to thoseof elder instars. Regarding post-treatment mortalities, the lower cumulative mortality percentages occurred throughout the first five days post treatment.

Entomopathogenic fungi have been studied as potential biological control agents, but information on their natural incidence was limited in their studies. **Verde *et al.*, (2015)** isolated strains of *B. bassiana* from symptomatic insects collected from dead palm trees in canary island, and their pathogenicity against different instars of *R. ferrugineus* was evaluated in the laboratory. They recorded 7% infected insects in Canary palms. In laboratory bioassays, 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 among treated larvae were 88 and 92%, and the means of survival time were 10.4 and 11.8 days, being significantly shorter than those in the control, where no insect died during the trials. **El-Hindi, 2016** recorded significant difference in growth between treated and untreated larvae, the toxicity assay on larvae treated with the *B. bassiana* isolate, proved to be the most virulent to the larvae. The mortality of larvae was recorded for 6 days after treatment with spore suspension spraying (Hand Sprayer) by 3.4x108 spores/ml of *B.bassiana*. The highest percentage mortality of the larvae reached 100% by 6 days after spraying with *B. bassiana*.

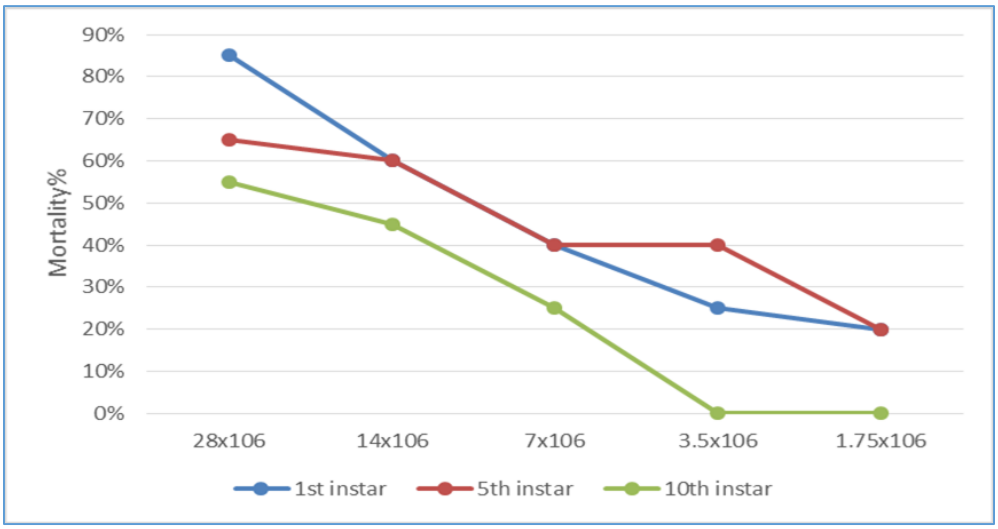

Fig. (4): Mean cumulative concentration mortality percentages among larvae of *R. ferrugineus* treated with conidial suspension (28×10^6 spores / 100ml) of fungal isolate after 25 days of treatment.

Field application of Newfar (*B. bassiana*) on infested palm trees for RPW control.

Results are shown in (Table,5) depending upon the field observations(by naked eyes) on date palm trees which received Newfar(*B.bassiana*) treatment. These field observations were recorded 10, 15, 20 and 25 days after the first treatment.

Obtained results indicated that the infestation by *R. ferrugineus*began to stop 20days after treatment. As two trees proved recovered from infestation, the remaining three trees were found infested. 25days after treatments, one of the two infested palms proved recovered and, only, one palm tree continued as infested by RPW. Thus, indicating that the application method of *B. bassiana* for *R. ferrugineus* control caused 60% success (3 recovered trees from the 5 infested ones) 20days after treatment. While, after 25days of treatment, this method proved 80% success (4 of the 5 treated palms becomecompletelyrecovered).

In similar studies, **El-Sufty*et al.*, (2009**) assayed the effect of field application of *B. bassiana* for control of RPW in United Arab Emirates. The authors used two methods for application of the entomopathogenic fungi in date palm plantations. Their results indicated that treatments caused 21.2 and 23.47% mortalities among adult population in 2005 and 2006, respectively. In another study, **Sewify*et al.*, (2014)** carried out a field experiment to evaluate integrated effect of baited aggregation pheromone traps and entomopathogenic fungus *B. bassiana* or insecticide for controlling *R. ferrugineus*in Ismailia Governorate, Egypt in 2008/09. Total mean reduction of RPW population caused by mass-trapping and the fungus *B. bassiana* or insecticide was 61.40 and 40.16%, respectively. Also, considerable reduction of infested

palm tree numbers was noticed in treated areas with the combinations compared with the control. Those 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) occurred at the mass-trapping or insecticide alone, opposed to 3.73 palms in the control.

Table 5. Field treatments of date palm trees with Newfar (*B. bassiana*) at concentration (10 gm / liter of distilled water) at Al Kasasen region in the Ismailia Governorate in 2019.

Period after treatment	Period 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	×	×	×	×	×
15 days	×	×	×	×	×
20 days	dry	×	×	×	dry
25 days		dry	×	dry	

N.b: X= still infected & dry= recovered

Conclusion:

Laboratory and field data obtained in this investigation proved that *Beauvariabassiana*in both the commercial formulation (Newfar) or the fungal conidia are efficient against larvae and adults of the red palm weevil *Rhynchophorusferrugineus*. Accordingly, this entomopathogenic fungus is recommended to be considered when planning for RPW integrated control management program, taking into consideration that for older larval instars the increase of the applied concentration is need to insure satisfactory control.

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أستخدام ال *Beauveriabassiana* كعنصر مكافحة بيولوجية ضد يرقات سوسة النخيل الحمراء تحت الظروف المختبرية والحقلية.

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أجريت هذه الدراسة لفحص كفاءة المستحضر التجاري (نيوفار) لفطر *Beauveriabassiana*(Bals.) Vuil. و كذلك تركيزاته لسلالته المعزولة من جثث اليرقات الميتة لسوسة النخيل, ضد يرقات سوسة النخيل الحمراء *Rhynchophorusferrugineus*(Olivier) . أجريت تجارب الكفاءة الحيوية لتقدير قيم LC_{50} & LC_{90} و LT_{50} & LT_{90} حيث سجلت النتائج من خلال المعاملة بمستحضر (نيوفار) ان LC_{50} ضد يرقات العمر الأول , الخامس و العاشر 0.078, 0.192 و 0.406 جم / مل, على التوالي, بينما كانت قيم ال LC_{90} 0.610, 2.030 و 4.547 جم / مل, على التوالي للاعمار الأول , الخامس و العاشر وذلك بعد 25 يوم من المعاملة. أما, ال LT_{50} بعد المعاملة بتركيز 1×10^{8} CFU / s' 100ملكانت 14.549, 16.167 و 21.022 يوم على التوالي , بينما كانت LT_{90} 23.374, 31.196 و 99.344 يوم على التوالي. ولقد أظهرت النتائج أن التركيز 1×10^{8} CFU / s' 100مل تسبب بموت 95% من يرقات العمر الأول, 85% من يرقات العمر الخامس و 65% من يرقات العمر العاشر, بعد 25 يوم من المعاملة, و تسبب التركيز 28 × 10^{6} كونيديا / 100مل بموت 85% من يرقات العمر الأول, 65% من يرقات العمر الخامس و 55% من يرقات العمر العاشر, بعد 25 يوم من المعاملة. بالأضافة الى ذلك, تم تقييم خمس تركيزات من كل من المستحضر التجاري والعزلآت الكونيدية (1×10^{8} CFU / s' 100مل و 28 × 10^{6} كونيديا / 100مل) على العمر الأول, الخامس والعاشر من يرقات سوسة النخيل الحمراء في المختبر والحقل. أظهرت النتائج إن الوفيات بين اليرقات المعاملة كانت تختلف بشكل ملحوظ عن الكنترول, حيث لم يسجل أي موت لليرقات في الكونترول (بدون معاملة). أكدت الدراسة إن التأثير المميت ل *B. bassiana* يتناسب طرديا مع تعداد الكونيدات. وأكدت أيضا إن يرقات العمر الأول كانت اكثر تاثيراً بعلاجات *B. bassiana* مقارنة بأعمار اليرقات الأخرى.

أوضحت الدراسة الميدانية إن أشجار النخيل المصابة التي تم حقنها بواسطة مستحضر نيوفار (*B. bassiana*) في موقع الأصابة بسوسة النخيل الحمراء تسببت فيشفائها من الاصابة بنسبة 80% بعد 25 يوماً من المعاملة.