PATHOGENICITY OF THE FUNGUS, *BEAUVERIA BASSIANA* TO THE RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* UNDER FIELD CONDITIONS

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Abstract

The present study was carried out at El-Kassasin district Ismailia Governorate, Egypt to evaluate the virulence of entomopathogenic fungus, *Beauveria bassiana* against of the red palm weevil under field conditions. Field trials showed that the injection of *B. bassiana*-9894 in infested palm trees gave successful recovery at light and median infestation level. On the other hand, dusting a date palm trees with *B. bassiana*-9894 caused an increase in adult weevil infestation and reduction of the palm trees infestation. The obtained results also showed that the chemical insecticides Chlorzan 48% EC and Diazinox 60% EC were highly effective against red palm weevil.

Key words: *Rhynchophorus ferrugineus*, microbial control, entomopathogenic fungus, *Beauveria bassiana*.

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) belongs to the family of Curculionidae (Coleoptera). *R. ferrugineus* is a major economic pest of coconut palm, date palm, oil palm and sago palm. Nowadays, *R. ferrugineus* is known as the most destructive pest to date, coconut and oil palms in Arabic region and south East Asia (Hanounik, 1998). It has been known first in south east Asia until it appeared in United Arab Emirates in 1985, Kingdom of Saudi Arabic in 1987 (Murphy and Briscoe, 1999) and Egypt in 1992 (Saleh, 1992).

The use of biological control agents in the management of insect pests has increased in recent years. Due to the deep concerns about environmental pollution and health risks associated with use of chemical insecticides. Among biological control measures are the entomopathogenic fungi which are considered potentially and environmentally safe to invertebrates and plant species in addition to their wide use in control of many insect pests (Chakaravarthy *et al.*, 2008).

R. ferrugineus has been found naturally infected by the entomopathogenic fungus, *Beauveria bassiana* (Berenice *et al.*, 2010). *B. bassiana* is known as an entomopathogenic fungus with a worldwide distribution and abroad spectrum

insecticidal activity (Martin *et al*., 2000). This fungus has proven to be effective for the control of many Coleopteran species, (Magra *et al*., 2004). Gindin *et al*., (2006) and El-Sufty *et al*., (2007) who recommended using the fungi in the management programs of *R. ferrugineus*.

MATERIALS AND METHODS

1.*Beauveria bassiana*.

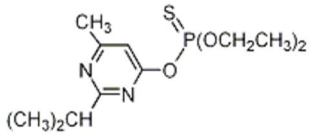
The strain of *B. bassiana* used in this study was isolated from infected cadaver adults of *R. ferrugineus* collected from El-Kassasin area. Isolated fungi were purified and identified in Assuit University Mycological Center (AUMC).

The fungus conidia were cultured on agar Petri dishes of Dox medium (Thom and Raper., 1945). *B. bassiana*-9894 aerial conidia were mass produced using biophasic culture system (Sewify *et al.,* 2009).

2. Chemical insecticides.

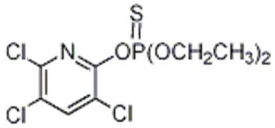
Two insecticides recommended for the red palm weevil by the Ministry of Agriculture in Egypt were tested.

A: Diazinox 60% EC (Diazinon).



Chemical name: *O,O*-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate.

B: Chlorzan 48% EC (Chlorpyrifos).



Chemical name: *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate.

3. Evaluating the efficiency of the effectiveness of *B. bassiana*-9894 against the red palm weevil, *R. ferrugineus* in the field.

Field applications were conducted on 17 Fadden's containing 1134 date palm, *P. dactylifera*. The field plantation included naturally RPW infested and non-infested palm trees ranging in age between 7-12 years (5x6m between palms) at El-Kassasin area, Ismailia Governorate, Egypt. Infested date palms were marked at the start of the experiments.

3.1. Injection of fungus, *B. bassiana*-9894 suspension in palm trees.

A stock solution of the fungal formulation was prepared (2 g *B. bassiana*-9894 spores suspended in 100ml sunflower oil and 0.02 % Tween-80 and then sterilized distilled water was added to make 1liter and well mixed). The conidia concentration (10^8 spores/ml) in the resulting suspension was determined by the use of a haemocytometer slide and adjusted to the desired concentration.

Thirty five naturally infested palm trees with *R. ferrugineus* (5-9 years old) were treated with *B. bassiana*-9894 spore's suspension formulated in sunflower oil according to (Sewify *et al*., 2009). These infested palm trees were divided into three levels, light, median and heavy level of infestations. Fungal spores at concentration of 10^8 spores/ml formulated in emulsified sunflower oil in 0.02% Tween-80 at concentration of 1.0 ml /L was injected through PVC tubes (30 cm long and 16 mm diameter) inserted in deep holes (20 cm) made at 45˚C angel into the core of an infected palm near the top of the insect infestation level (2 liter/palm). These holes were made with a 50 cm long and 16 mm thick screw. Numbers of inserted tubes depended on infestation level. The application was repeated on weekly bases for two consecutive weeks. The numbers of dry and wet galleries were recorded. Evidence of recovery tree was determined by external signs which were the absence of fresh sap liquid appearing externally on the tree trunk as well as the absence of the bad pungent smell. Also, a thin twig was inserted in the bored holes to verify the dryness of fluid in the inner tissues.

3.2. Dusting of *B. bassiana*-9894.

Field applications by dusting the formulated fungus, *B. bassiana*-9894 spores, at concentration of 10^8 spores /g (Sewify *et al*., 2009) were carried out on red palm weevil-infested trees starting from March 2012 up to May 2013. Formulated spores were dusted before evening on leaf axils and palm trunks (80gm/palm) by using motorized Knapsack sprayer (Agrimondo HP 5)**.** Control palms were left untreated. The *B. bassiana*-9894 formulation was applied 4 times throughout the year (starting in March 2012) at approximately 3- month intervals. Efficacy of treatments was based on monthly mortalities caused by the fungus in RPW adult population. Therefore, adults were weekly collected by pheromone traps a month before application and continued till May 2013. For each sample, cadavers of dead weevils were disinfected by immersing in 1% sodium hypochlorite for 15 seconds, washed in sterile water and exposed to mycosis test. The cadavers were placed on moistened filter papers inside Petri dishes. The dishes were incubated at 25±2ºC and 60-70% R.H for 10 days and

the fungus growth was observed. The cadavers that showed external growth of the fungus were considered killed by the fungus.

4. Effect of Diazinox 60% and Chlorzan 48% EC on the red palm weevil.

The experiment was carried out to test the efficacy of two insecticides (Diazinox 60% and Chlorzan 48% EC) for control of RPW on date palm trees. Sixty infested date palm trees were selected to control using the two insecticides. Date palm orchard was visited thoroughly and all the trees which were being damaged by RPW were identified, marked and tagged. From infested palms a brown fluid was oozing out from the minute holes made by RPW. Three to seven holes were made above the insect attacking point. The holes were drilled at 45° angle downwards the infestation points.

The insecticides were diluted in distilled water (Diazinox 60%EC 3cm/l and Chlorzan 48%EC 3cm/l) before application to the trees and 2 liter per tree of diluted insecticide was injected in the holes. All the trees were observed at 7 and 21 days and thoroughly checked that the oozing has stopped or till loosening.

5.Analysis of results.

Experimental data were analyzed using one way analysis of variance (ANOVA) using SPSS (statistical package for social sciences, ver. 20), and the significance among the samples was compared at $P \leq 0.05$. Results were represented as mean ±SE (n=4).

RESULTS AND DISCUSSION

1. Evaluating the efficiency of *B. bassiana*-9894 against red palm weevil, *R. ferrugineus* in the Filed.

1.1. Effect of *B. bassiana*-9894 application by trunk injection method.

Data in Table(1) and Fig.(1&2), show the treatment of red palm weevil, *R. ferrugineus* in infested palms with spores of fungus, *B. bassiana*-9894 at a concentration of $3x10^8$ spores/ml formulated in sunflower oil (0.1ml/l) through injection method. Three different levels of infestation were considered, light, median and heavy infestation as depicted by visible external symptoms. As shown in Table (1) Fig. (1), the light infestation level showed a high response to treatment with the fungal spores followed by median and heavy infestations. The light level infestation showed recovery from infestation after one week from the injection of the fungus, *B. bassiana*-9894 formulation in drilled holes in the tree trunks. The percent of dry galleries was 81.8, 58.82 and 0.0%, as a result of fugal injection in light, median and heavy infested palm, after one week post-treatment, respectively.

Therefore, in the second week, a second fungus formulation was administered by injection through the fitted perforated rubber tubes only in the tree that still exhibiting symptoms of infestation.

The treatments gave 100, 76.47 and 0.0% successful cure two weeks post-treatment for the three levels of infestation, respectively. Subsequently, after the third week from initial application of *B. bassiana*-9894, results were 100, 88.23 and 0.00% recovery at the three levels of infestation, respectively.

Data in Table (1) and Fig. (1) cleared that the heavily infested palm trees showed no external sign of recovery from infestation. Therefore, the heavily infested palm trees negatively responded with fugal treatment compared to the light and median infestation.

Table 1. Efficiency of *B. bassiana*-9894 (10^8 spores/ml) against three levels of infestation by *R. ferrugineus* on date palm trees applied by trunk injection method.

Type of infestation	Number of treated palm	% Recovry after treatment		
		One week	Two weeks	Three weeks
Light	11	81.8	100	100
Median	17	58.82	76.47	88.23
Heavy	7	0.00	0.00	0.00
Mean recovery of infestation		54.29%	68.57%	74.29%
General average of recovered infestation		65.72 %		

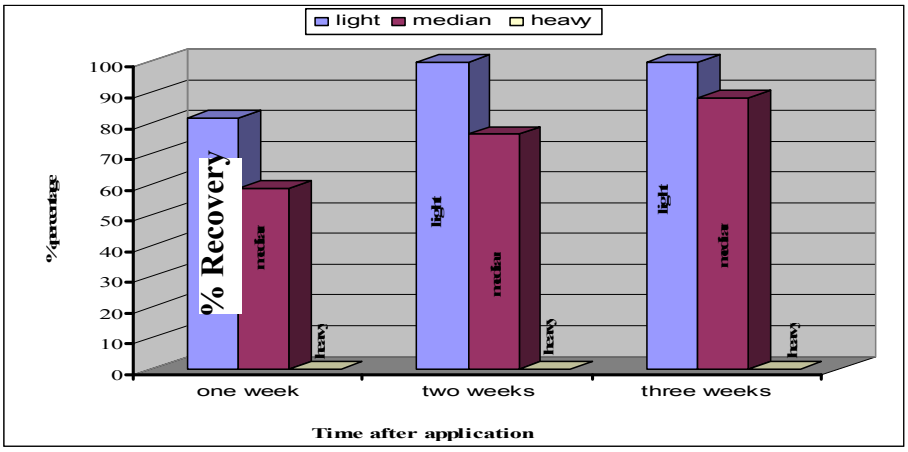


Fig. 1. Percentage of recovered date palms from infestation by the red palm weevil, *R. ferrugineus* after injection with the fungus, *B. bassiana*-9894.

It could be concluded from the obtained results of the present field application that treatment with the fungus, *B. bassiana*-9894 by trunk injection in tree in three different categories of infestation caused 54.29% average recovery after one week (Table, 1 and Fig., 2).

Meanwhile, the percent of recovered date palm trees from infestation by the red palm weevil after trunk injection with the fungus was 68.57% and 74.29% after two and three weeks when treated for the 2nd and 3d time, respectively.

Therefore, general average of recovered infestation after trunk injection with *B. bassiana* was 65.75% (Table, 1 and Fig., 2).

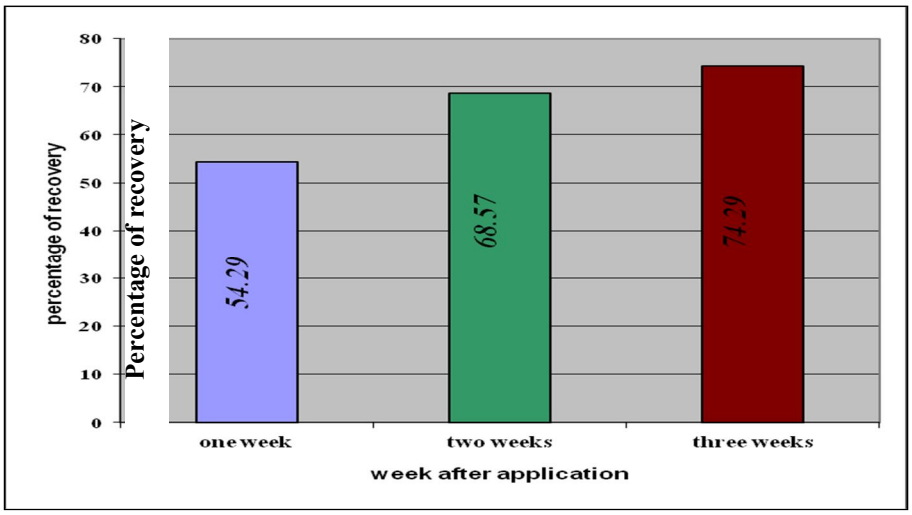


Fig. 2. Mean percentage of recovery date palm trees from infestation by red palm weevil after injection with *B. bassiana*-9894.

According to Yasuda *et al*., (2000) and Consolo *et al*. (2003) the efficiency of *B. bassiana* formulation for controlling many insect species was enhanced when prepared in a corn oil formulation.

Our results agree with Sewify and Fouad (2006) who mentioned that *B. bassiana* in combination with mint oil at low concentration injected into palm trees was an effective method for controlling *R. ferrugineus* within two weeks, but the satisfying control varied according to infestation levels.

2.2. Effect of *B. bassiana* application by dusting method.

Data in Table (2) and illustrated in Fig. (3) revealed that dusting fungus, *B. bassiana*-9894 (10^8 spores/ml) in treated area increased the numbers of fungal infected weevils captured by terrestrial food baited aggregation pheromone trap, compared with untreated area.

Captured red palm weevils by traps were scored as dead or alive and whenever present insects with signs of mycoses were also scored. Palm trees infestation by red palm weevil was scored. The red palm weevil population was larger in untreated palms area than in treated palms area. The largest number of living adults was found in untreated palms area, unlike treated ones. Reduction of red palm

weevil, *R. ferrugineus* in treated palms area could be due to the action of the entomopathogenic fungus, *B. bassiana*-9894.

Table 2. Infection of red palm weevil, *R. ferrugineus* captured by traps from untreated and treated areas with *B. bassiana*-9894 through 2012 – 2013 applied by dusting method.

	Months of dusting					Mean	Interaction
	March	June	September	December	March		
Untreated	0.81	1.28	0.00	0.00	1.54	0.726[b]	
Treated	8.71	26.96	37.30	45.85	67.58	37.28[a]	**
Mean	4.76[c]	14.12[b]	18.65[ab]	22.925[ab]	34.56[a]		

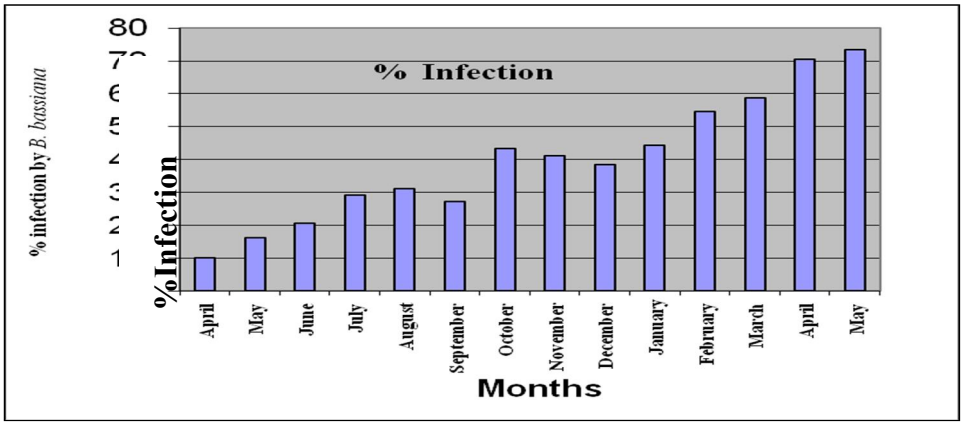


Fig. 3. Percentages of fungal-infected adults of red palm weevil by captured traps as a result of application of *B. bassiana*-9894 (2012-2013).

Three months after the first field application of *B. bassiana*-9894 formulation, the percentage of fungal infected weevils was 10, 16.12% in April and May (2012), respectively. In control area, the recorded fungal infected adults were zero, 2.4% in April and May (2012), respectively. The percentage of the fungal infected weevils after 6 months of *B. bassiana*-9894 treatments increased to 31.03%, in August (2012). This could be due to the large RPW infestation prior to our *B. bassiana*-9894 treatments.

Subsequently, at least one application of the fungus, *B. bassiana*-9894 increased the percentage of trapped fungal infected weevil in treated area to 70.59, 73.33% in April and May (2013), respectively. This would maintain a *B. bassiana*-9894 treatments reduce the impact of red palm weevil infestation, such as in this study.

Data in Table (3) and illustrated data in Fig. 4 show that the induced reduction of captured weevils was due to the application of the fungus, *B. bassiana*-9894 in May (2012) recording 13.77%, then enhanced to reach 57.69%. Reduction rate of captured weevils of red palm increased to 71.48% in May 2013.

On the other hand, results in Table (3) and illustrated in Fig. (5) indicat a reduction of infested palm trees in area treated with the entomopathogenic fungus, *B. bassiana*-9894 in comparison with untreated area. Data in Table (3) revealed that the numbers of infested palm trees decreased during 2013 when compared with 2012. Data in Table (3) reveal a reduction rate of infested palm trees reached 21.33% and 45.58% in May and August 2012, respectively. The reduction rate increased to reach it 66.54% in May 2013.

Table 3. % Infection, reduction of the captured weevils and % reduction of infested palm trees as a result of application of *B. bassiana*-9894 (2012-2013).

Months	% Infection of red palm weevil	%Reduction in captured weevils	% Reduction of infested palm trees
March	0	9.93	0
April	10	12.39	0
May	16.12	13.77	21.33
June	20.68	29.77	35.24
July	29.19	44.84	41.39
August	31.03	36.48	45.58
September	27.27	47.80	42.86
October	43.47	52.37	46.03
November	41.17	61.12	50.84
December	38.46	57.69	49.21
January	44.44	65.58	49.40
February	54.54	72.16	55.18
March	58.82	67.18	60.32
April	70.59	69.74	61.90
May	73.33	71.48	66.54

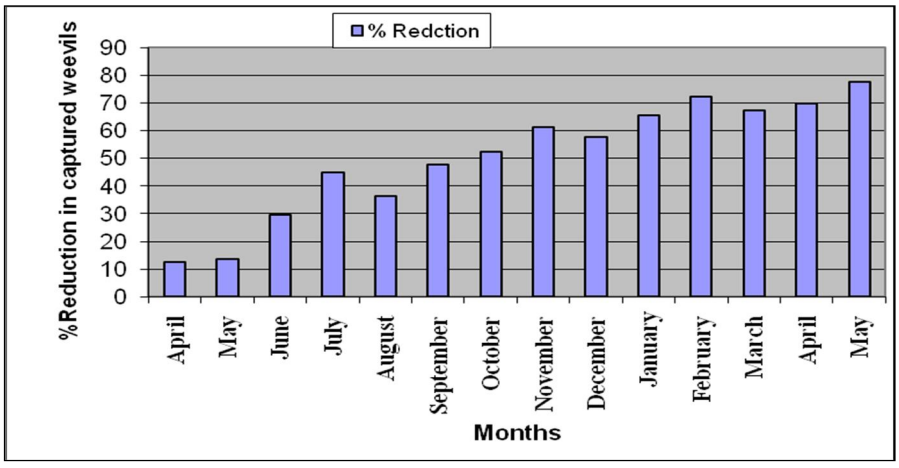


Fig. 4. Effect of *B. bassiana* -9894 application on reduction rate of red palm weevil captured by traps (2012-2013).

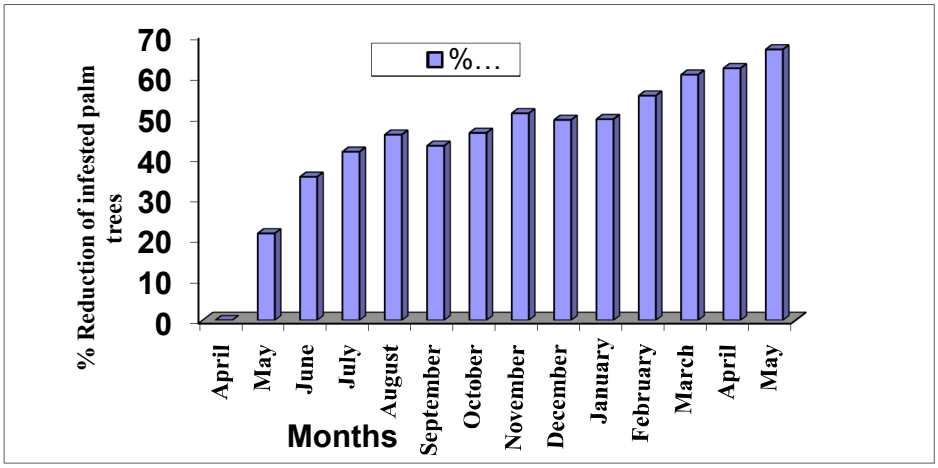


Fig. 5. Effect of *B. bassiana*-9894 application on reduction rate of infested palm trees with red palm weevil, *R. ferrugineus* (2012-2013).

This result agree with El-Sufty *et al*., (2007) and Sewify *et al*., (2009) who concluded that the dusting application increased fungal infected weevil captured by traps. Sewify *et al*., (2009) reported also that the fungal dusting application increased fungal infected weevil captured by traps. They stated that the increment in the percentage of fungal infected weevil was occurred in the second year of fungal application. The authors noticed, in field observation, that in most cases, adult weevils didn't fly directly from palm to another but they droped on ground then, walked and hid behind the target palm frond axils. Mueller-Koegler (1965) stated that the high population density of host insect enhances the development of the fungus disease in the population. The delayed effect of the fungus may be attributed to the development and sporulation of the fungus on cadavers of infected weevils, thereby spreading the fungus inoculums in the population. The results showed that our *B. bassiana* cause RPW mortality, and reduced RPW populations and palm infestation levels. El-Sufty *et al*., (2011) mentioned that the adult weevils themselves act as very efficient carriers of the fungus conidia.

These results suggest that fungus *B. bassiana*-9894 is a promising agent for use as biological control agent to control the red palm weevil, *R. ferrugineus.*

2. Field evaluations of the chemical insecticides by trunk injection of palm trees.

The effectiveness of two pesticides (Chlorzan 48% EC and Diazinox 60%EC) on red palm weevil, *R. ferrugineus* was assessed after one week to three weeks from treatment under field conditions and the obtained results were tabulated in Table(4).

The results indicated that the chemical insecticides were more effective on red palm weevil than the tested bioagents. The results in Table (4) showed that Chlorzan 48%EC was the most efficient treatment to control red palm weevil infestation, as the

recovery reached 100,100 and 83.33% at light, median and heavy infestation date palm trees, respectively after one week.

Table 4. Efficiency of chemical insecticides against three levels of *R. ferrugineus* infestation on date palm trees applied by injection method.

treatments	Type of infestation	Number of treated palms	% of palm recovery after treatment		
			One week	Two weeks	Three weeks
Chlorzan 48%EC (3cm/l)	Light	15	100	100	100
	Median	11	100	100	100
	Heavy	6	83.33	100	100
	Recovery infestation		96.88%	100%	100%
	General average of recovered infestation		98.96%		
Diazinox 60%EC (3cm/l)	Light	12	100	100	100
	Median	9	100	100	100
	Heavy	7	71.43	100	100
	Recovery infestation		92.86%	100%	100%
	General average of recovered infestation		97.62%		

On the other hand, Diaznox 60%EC caused 100, 100 and 71.43% recovery at the same levels after one week. Percentages of recovery of palm trees at the three levels of infestation showed 100% two weeks post-treatment with the two tested insecticides.

It could be concluded that the successful treatment of infested palm trees was achieved by using injection of a formulation of *B. bassiana*-9894 in combination with sunflower oil at the concentration $3x10^8$ spores /ml. The successful was observed in suppressing infestation by the red palm weevil in light and median infested trees as depicted by external symptoms of infestation on the trunk of date palm trees. However, when the fungus formulation was injected at the same concentration in heavy infested trees it had no effect as signs of infestation were even more evident.

Field observation proved that the fungal dusting application increased fungal infected weevil captured by traps in treated area when compared with untreated area. An increase in percentage of fungal infected weevil occurred in the second year of fungal application. Dusting fungus reduced the population of red palm weevil in treated area compared with untreated area. Reduction of red palm weevil, *R. ferrugineus* in treated palms area could be due to the action of the entomopathogenic fungus, *B. bassiana*-9894.

The results indicated that the chemical insecticides (Chlorzan 48% EC and Diazinox 60%EC) were more effective on red palm weevil than the tested bio-agents.

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القدرة المرضية لفطر البوفاريا باسيانا على سوسة النخيل الحمراء (رتبة غمدية الأجنحة – فصيلة السوس) تحت الظروف الحقلية

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تم إنجاز هذا العمل بمنطقة القصاصين– محافظة الإسماعيلية– مصر، لتقييم كفاءة الفطر بوفاريا باسيانا لسوسة النخيل الحمراء تحت ظروف الحقل.

وأظهرت التجارب الحقلية أن حقن الفطر بوفاريا باسيانا أدى إلى حدوث نسبة شفاء فى مستوى الاصابة الخفيف والمتوسط، وعند معاملة الفطر بوفاريا باسيانا عن طريق التعفير أدى إلى زيادة فى أعداد الحشرات الكاملة المصابة بالفطر، وكذا إنخفاض فى أعداد الأشجار المصابة بسوسة النخيل الحمراء، ولوحظ عند حقن مبيد الكلورزان و الدايازينون كانا أكثر فعالية على سوسة النخيل الحمراء.