

Use of the Entomopathogenic Fungus, *Beauveria bassiana* for the Biological Control of the Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier

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ABSTRACT

The red palm weevil, *Rhynchophorus ferrugineus* Olivier is the major destructive insect pest of date palm trees in Egypt as well as Middle East. Dependence on chemical control constitutes a major constrain to develop an IPM strategy, because of resistance problems, disruptive effects on natural control of secondary pests and pesticide residue on fruits. Therefore, an entomopathogenic fungus constitutes interesting alternative tactic for changing pest status. Indigenous isolate of the fungus, *Beauveria bassiana*, isolated from red palm weevil cadaver was evaluated. Field application was conducted with the fungus against the red palm weevil at Elkassasin, Ismailia Governorate, Egypt through 2007 – 2008. Three methods were carried out: (1) injection of *B. bassiana* in naturally infested palm trees. The success of the treatment was up 90 %, 2) periodical dusting application of fungal spores on palm trees in March and September. A reduction of palm trees infestation following field application was noticed and 3) release of contaminated males of red palm weevil with fungal spores. These methods proved considerable reduction in the palm weevil population. The results suggest that fungus *B. bassiana* is a promising agent for use as bio-insecticide to control the red palm weevil.

Key words: Red palm weevil, *Rhynchophorus ferrugineus*, entomopathogenic fungus *Beauveria bassiana*.

INTRODUCTION

Red palm weevil, *Rhynchophorus ferrugineus* Olive. (Coleoptera: Curculionidae) is a destructive insect pest of palm trees. It invaded Egypt in 1992 (Cox, 1993) where it is now causing severe damage to date palm, *Phoenix dactylifera* L. *R. ferrugineus* can breed in a wide range of climates, and the larvae feed protected within their host palm (Wattanapongsiri, 1966). The weevil is able to complete several generations in a year (Avand Faghieh, 1996), frequently, several generations can pass in the same host tree before the tree collapses. Red palm weevil proved three generations a year in Egypt, the first in Marsh - April, the second in June-July and the third in September-October (Biological control of red palm weevil project, Arab Organization of Agricultural Development in Egypt, unpublished data).

Crowded date palms offshoots are considered preferable breeding sites for the red palm weevil which are very hard to examine and control. Also, *R. ferrugineus* is a strong flyer (Murphy and Briscoe, 1999). In addition, in Egypt, the bulk and quick movement of the date palm offshoots as planting material has led to the rapid spread of the pest (Abraham *et al.*, 1998). All these factors with absence of natural enemies contribute to the weevils' ability to colonize and breed at new sites and for its population to reach outbreak levels. Present control measures are largely based on insecticidal applications through injection into infested palm trees and regular spraying of palms in infested area (Oehlschlager, 1996 and El Ezaby, 1997). However,

there are now deep concerns about environmental pollution and health risks associated with use of chemical insecticides. The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin has gained considerable attention as biological control agent for weevils and other agriculture pests (El Sufty and Borei, 1987 and Meikle *et al.*, 2001). Fungi are especially important for controlling cryptic insects such as red palm weevil which are not accessible to its natural enemies. The use of entomopathogenic fungus *B. bassiana* against red palm weevil has been reviewed by Sewify and Fouad (2006).

Aim of the present work was to evaluate *B. bassiana* as a biocontrol agent for controlling the red palm weevil in Egypt.

MATERIALS AND METHODS

Pest species:

R. ferrugineus adults were obtained from the field at El-Kassasin, Ismailia Governorate, Egypt and kept at 29 °C, 60-70% R.H and 14h photoperiod. The weevils were transferred to plastic boxes containing shredded sugarcane stem for egg-laying. Newly, hatched larvae were transferred to plastic cups provided with a layer of semi artificial diet (Rahalker *et al.*, 1978). The full grown larvae were placed in plastic boxes (20 larvae/box) containing some of the palm bark fibers to pupate. Emerged adults were collected and kept in plastic containers provided with shredded sugarcane stem for feeding.

Fungal inoculums:

The entomopathogenic fungus *B. bassiana* used in the experiments was originally isolated from the red palm weevil, *R. ferrugineus* collected from Ismailia Governorate, Egypt. The fungus was grown on autoclaved Sabouraud and dextrose yeast agar (SDAY), containing 1% peptone, 0.2% yeast extract, 4% dextrose and 1.5% agar in distilled water and incubated for two weeks at 26 ± 1 °C.

Conidiospores production:

B. bassiana aerial conidia were produced using biphasic culture system (Bradley *et al.*, 2002 and Leland *et al.*, 2005). Flasks (100 ml) of liquid biomalt medium (25 g biomalt and 2 g yeast extract), were incubated for 3-4 days at 25 °C and 150 rpm. The liquid cultures were then used to inoculate autoclaved white rice in sterilized plastic bags. The rice was first soaked in water for 12 hours and autoclaved for 30 min in autoclaveable plastic bags (60x80 cm) in the ratio of 2 kg rice/bag at 103 k pa for 20 min. After cooling, three to four day old liquid culture of *B. bassiana* was mixed by hand with the substrate, under aseptic condition, at the ratio of 50ml /bag. An absorbent cotton plug rolled around tube (15cm long) had been used to plug each bag. The bags were connected with the source of filtered air through air valves. Solid substrate fermentation was kept for 11 days at 26 °C. Culture was observed daily and crumbled by hand within bags to prevent binding of the substrate. Whole culture was then transferred to wooden screen shelves where culture was dried for 7 days at 28°C. Conidia were harvested by mechanical sieving.

Field applications:

Field applications with the entomopathogenic fungus, *B. bassiana* were conducted on 5000 Faddens (= 2000 Hectares) date palm *P. dactylifera* at Elkassasin, Ismailia Governorate, Egypt. Pest damage at these sites was severe and had already led to the loss of many palms. The applications of *B. bassiana* were conducted within Integrated Pest Management (IPM) programs. Implementation of the plan work was achieved as follows:

1- Mechanical control of advanced infestations of date palms and wild plantations:

In the targeted date palms field, wild plantations and severely infested date palms were mechanically up rooted and then buried at one meter depth below the soil. Apparently this step was employed to eradicate the source of infestations and in turn to suppress the population density of red palm weevils in the surrounding.

2- Chemical treatment of advanced infestations of date palms:

Severely infested date palms in the areas were

treated with insecticides recommended by of the Ministry of Agriculture in Egypt. This treatment was carried out to prevent the quick emergency of the adults from their cocoons.

3- Fungal applications:

A- Injection of fungus suspension in palm trees:

Naturally infested palm trees with *R. ferrugineus* were treated with *B. bassiana* conidiospores suspension formulated in mint oil. These infested palm trees were classified into light, median and heavy levels of infestation. Number of active galleries with fresh exudates and frass was determined. Fungal spores at concentration of 10^8 spores/ ml formulated in emulsified mint oil in 0.02% tween 80 at concentration of 1.0 ml/L was injected through PVC tubes (30 cm long and 16 mm thick) inserted in deep holes (20 cm) made at a 45°C angle into the core of an infested palm near the top of the insect infestation level. These holes were made by 1151 w electrical hummer drill equipped 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.

B- Fungus dusting technique:

Field applications by dusting the formulated fungus *B. bassiana* conidiospores, at concentration of 10^8 spores / gm were carried out two times per year, in the second half of March and September. Formulated conidiospores were dusted before evening on palm trunks (80 g / palm) by using Knapsack be motorized (Agrimondo HP 5).

C- Release of fungal contaminated males of red palm weevil:

Healthy and active males of red palm weevil captured from the traps and maintained in the lab. were selected and marked. Males were placed in plastic box (15cmx15cmx30cm) provided with pieces of sugarcane and transported to field. Marked males of red palm weevil were immersed in conidiospores suspension for 5 sec. at concentration of 10^8 spores/ ml in the field prior to their release. The males were released on the ground beside palm or/and palm trunk. Fifty contaminated males were released at weekly intervals in the experimental area.

Monitoring of population densities and fungal infected red palm weevils:

Traps baited with aggregation pheromone were used to monitor, the population densities of red palm weevil and fungal infested weevils in treated and untreated areas. Three locations in treated area containing 1525, 1072 and 827 palm trees, respectively, were chosen to place the traps. Other three control areas, containing 1500, 840 and 350 palm trees, respectively were taken. A trap

receptacle of plastic bucket was prepared with a pack of 700 mg of mixture of aggregation pheromones (4-methyl-5 nonanol) and (4-methyl-5 nonanone) which releases an average of 10 mg of pheromone mixture/day, a small bottle containing 10 ml of ethyl acetate released from small 2 mm hole in the cover of the bottle. Number of used traps varied according to the size of treated area (12-16 traps for each). Weevils were collected from traps twice a week. The traps were supplemented with a fermenting mixture of dates and sugarcane molasses. Water was added to the traps regularly to maintain the fermentation process. All traps were set next to the tree trunks at ground level. Dead collected weevils were placed individually in Petri - dishes (14 cm. diameter) lined with moistened filter paper and provided with pieces of sugarcane and incubated at 25°C. Incubated dead weevils were inspected daily to confirm any fungal outgrowth.

The efficacy of such a treatment was measured by the reduction in red palm weevil population captured by traps and the reduction in infested palm trees according to the formula of Henderson and Tilton (1952).

RESULTS AND DISCUSSION

Treatment of infested palm trees by *B. bassiana* conidiospores

Treatment of red palm weevils in infested palms with fungus *B. bassiana* conidiospores at a concentration of 10^8 spores/ml formulated in mint oil 0.1 ml/l through injection method revealed highly effective method of treating palm trees (Table 1). The successful treatments varied according to the infestation level by the red palm weevil. The light infestation level showed a high response to treatment with fungal conidiospores followed by median and heavy infestations. Thus, the heavily infested palm trees needed more inserted tubes for fungal treatment up to 18 tubes/ palm. The percent of dry galleries was 100, 96.8 and 94.9%, as a result of fungal injection in light, median and heavy infested palms, respectively.

However, the treatments were 100, 95.5 and 87.0% successful cure two weeks post-treatment for the three levels of infestation, respectively. In heavily infested trees, the pupal and adult stages were killed as a result of treatment.

Effect of fungal application by dusting conidiospores and releasing of infected male weevils

Obtained results showed that the number of trapped weevils in treated areas declined after the procedures of mechanical control (Fig 1). The

results presented in (Table 2) and illustrated in (Fig.1) revealed that dusting of fungal conidiospores and release of fungal contaminated males of weevil in the treated area increased significantly the numbers of fungal infected weevils captured by the terrestrial food baited aggregation pheromone trap, compared with untreated area. Fungal infected weevils captured by traps began to appear in May, one month after fungal application, where the percentage of fungal infected weevils was 12%. In control areas, no fungal infected adults were recorded. The percentage of fungal infected weevils captured by traps in treated areas increased up to 45 and 25% in August and September, respectively. The percentage of fungal infected weevils increased again in October (44%), as a result of the second

Table (1): Effect of treating of red palm weevil infested palms with fungus *B. bassiana* at concentration of 10^8 spores/ml formulated in mint oil (low concentrations at 1.0 ml/L) applied by injection method.

No. of recovered (%)	No. of dry galleries (%)	Mean No. of inserted tubes /palm	Mean No. of active galleries/palm	No. of active galleries	No. of treated palms	Infestation level
Light	68	68	1	2.4	68 (100)	68 (100)
Median	68	128	2	3.4	124 (96.8)	65 (95.5)
Heavy	23	79	3-4	11.18	75(94.9)	20(87.0)

Table (2): Mean numbers of red palm weevil *R. ferrugineus* captured by traps (adults /trap) from untreated and treated areas with fungus *B. bassiana* at El-Kassasin, Ismailia Governorate through 2007-2008.

Month after application	Untreated areas			Treated areas				
	Non infected	Infected	Total	Non infected	Infected	Total		
2007	March	5.20	0.00	5.20	2.3	0.0	2.3	
	April	1.30	0.30	1.60	0.62	0.28	0.90	
	May	2.20	0.00	2.20	0.57	0.23	0.80	
	June	0.70	0.00	0.70	0.35	0.15	0.50	
	July	0.70	0.00	0.70	0.22	0.18	0.40	
	August	0.80	0.00	0.80	0.20	0.20	0.40	
	Sept.	0.30	0.30	0.60	0.11	0.09	0.20	
	Oct.	1.50	0.00	1.50	0.39	0.09	0.40	
	Nov.	0.70	0.00	0.70	0.27	0.03	0.30	
	Dec.	0.50	0.00	0.50	0.10	0.00	0.10	
	2008	Jan.	0.30	0.00	0.30	0.10	0.00	0.10
		Feb.	2.00	0.00	2.00	0.20	0.10	0.30
Mar.		2.20	0.30	2.50	0.960	0.64	1.60	
Apr.		4.60	0.00	4.00	0.200	0.60	0.90	
May		2.60	0.00	2.60	0.660	0.24	0.50	
June		1.30	0.00	1.30	0.200	0.30		
Total		0.60	27.20		3.13**	10.50 ^{n.s}		
P-value					0.0038	0.1345		

Total from three replicates N. S. not significant at 0.05

** Highly significant at 0.01

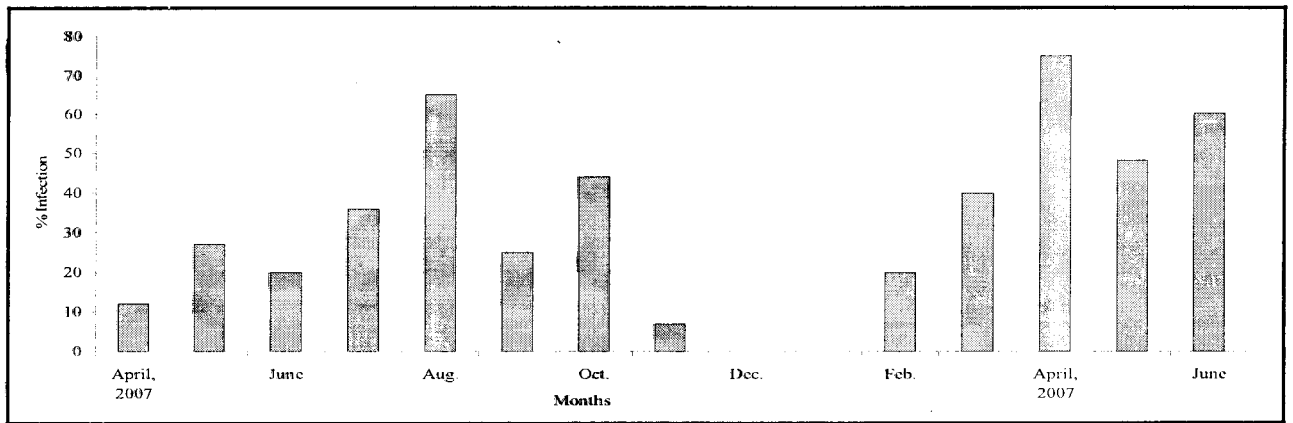


Fig. (1): Percentages of fungal infected adults of red palm weevil captured by traps as a result of application of *Beauveria bassiana* at Elkassasin, Ismailia Governorate, Egypt through 2007 & 2008.

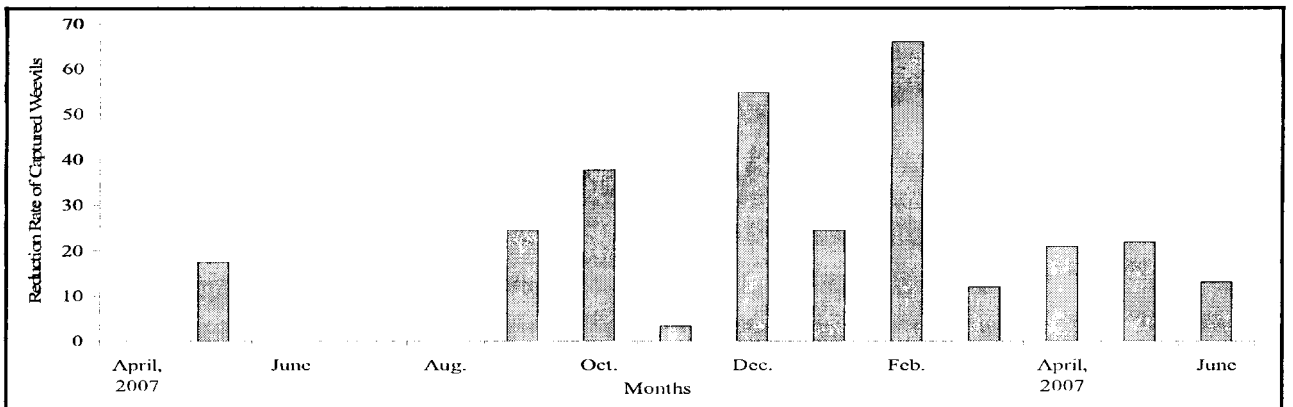


Fig. (2): Effect of *Beauveria bassiana* application on reduction rate of the red palm weevil captured by traps at Elkassasin, Ismailia Governorate, Egypt through 2007–2008.

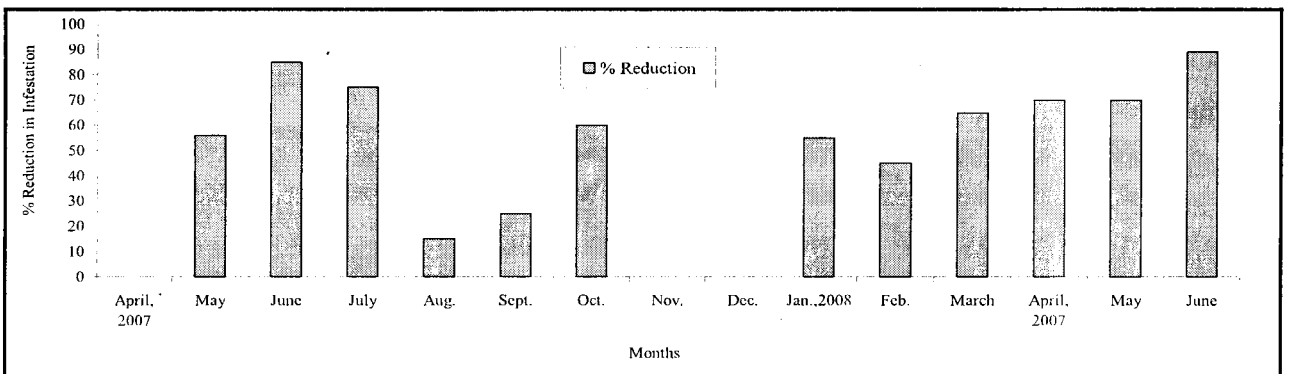


Fig. (3): Reduction rates of infested palm trees in *Beauveria bassiana*-treated areas at Elkassasin, Ismailia Governorate, Egypt during 2007 & 2008.

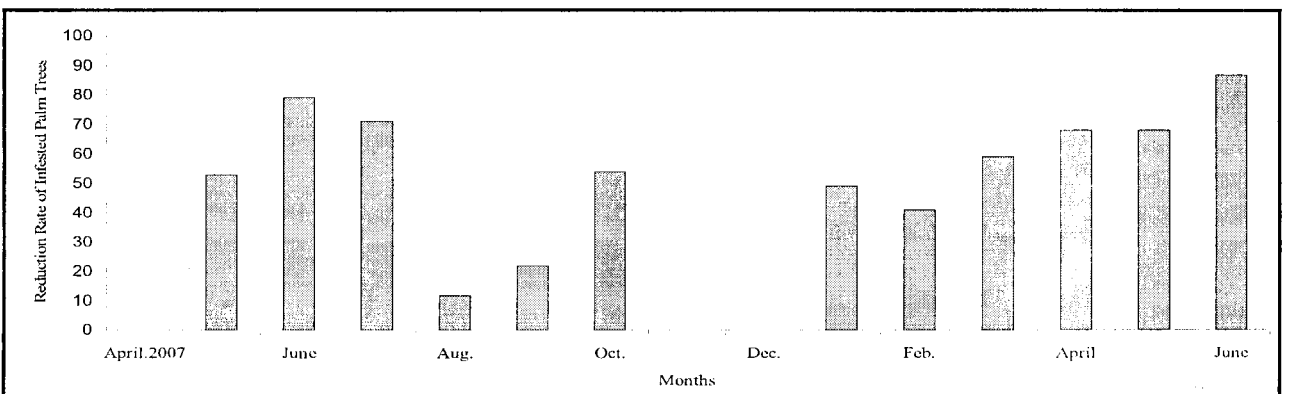


Fig. (4): Effect of *Beauveria bassiana* application on reduction rate of infested palm trees with the red palm weevil at Elkassasin, Ismailia Governorate, Egypt through 2007 – 2008.

dusting of fungal conidiospores in September. Subsequently, the percentage of trapped fungal infected weevils declined to zero% during December and January. However, increase in the percentage of fungal infected weevils occurred in the next year (from January to June 2008) in treated areas compared with the control. Percentage of fungal infected weevils recorded 20% in February, increased sharply to 75% in April after fungal dusting application in March 2008.

Effect of fungal application on population density of *R. ferrugineus*

Obtained results in Table (2), indicated that the intensity of captured weevils decreased as a result of fungal application by dusting of conidiospores and releasing the fungal contaminated weevil males. Illustrated data (Fig.2) show a reduction of captured weevils that was induced by fungus application in May (2007) recording 17%, then, declined to reach 0% until the end of August. Actually, establishment of fungal efficacy was noticed in weevil's population beginning with September till the end of application in June 2008. The reduction rate of captured weevil was 24.6% in September increased to 39.7 in October. Reduction rate of captured weevils of red palm reached its maximum in February 2008, whereas it recorded 66%, then, declined between 12 and 22%.

Effect of fungal application on rate of infested palm trees

Considerable reduction of infested palm trees was noticed in areas treated with *B. bassiana* compared to untreated ones (Fig. 3). The numbers of infested palm trees decreased during 2008 comparing with 2007. Illustrated data in (Fig. 4) revealed that the reduction rate of infested palm trees reached 35 and 79% in May and June, respectively. The reduction rates of infested date palms decreased to 0% in December 2007. In 2008, the reduction rates increased to reach its maximum in June (87.5%). Generally, the total suppression of palm trees infestation reached 46.9% at the end of the investigation.

In conclusion, obtained results encourage using *B. bassiana* against the pest in a frame of IPM program. The obtained results proved that mechanical control by removing and burning date palms advanced infestation as well as wild plantation which considered breeding sites had reduced the population of the weevil. The successful treatment of infested palm trees was achieved by using *B. bassiana* in combination with mint oil at low concentration. Sewify and Fouad (2006) mentioned that *B. bassiana* in combination with mint oil at low concentration injected into palm trees was effective method for controlling *R. ferrugineus*

within two weeks, but the satisfying control varied according to infestation levels. The results indicated that both mechanical control and fungal treatment of the infested palm trees decreased the population of weevils to low levels. Fungal dusting and releasing fungal contaminated marked males showed superior effect because using biological control is dependent on the presence of low population densities of target pest. Dusting of fungal conidiospores and releasing of fungal contaminated males of weevil increased significantly the fungal infected weevil captured by pheromone traps. The fungal dusting application increased fungal infected weevil captured by traps. Increase in the percentage of fungal infected weevil occurred in the second year of fungal application. The results indicated that the dusting of fungal conidiospores and releasing the fungal contaminated males of red palm weevil decreased the population of captured weevil in treated compared to untreated area. Field observation proved that in most cases, adult weevils didn't fly directly from palm to another but it drops on ground then, walks and hides behind the target palm frond axils. These observations confirm that the dusting of *B. bassiana* conidiospores on palm trunks and ground surrounded correspond to those of the target's habitat.

Application methods can influence the infectivity of *B. bassiana* (Gold *et al.*, 2003). Spraying weevils with *B. bassiana* resulted in higher mortality (56-62%) after 4 weeks. Exposing weevils to dry maize-and rice-based *B. bassiana* formulations resulted in nearly 100% mortality within 3 weeks (Nankinga and Orenge-Latigo, 1996). Fungal application as dry conidiospores will preserve them in an infective state for long time (Prior and Arura, 1984). Latch and Falloon (1976) found that the fungus remained infective in *Oryctes* breeding sites for up two years when released in an oat grain substrate. Fungal application by releasing fungal contaminated males of red palm was able to reduce the pest population within a certain time (Dowd and Vega, 2003). Kreutz *et al.*, (2004) mentioned that the auto dissemination and transmission of *B. bassiana* among the sap beetles and bark beetle, *Ips typographus* were successful and effective method for controlling these insects. This auto dissemination strategy has been already attempted against other pest insects, which are generally difficult to control by chemical means, such as tsetse flies (Kaya & Okech, 1990 and Maniania, 1998), houseflies (Renn *et al.*, 1999), the damson-hop aphid (Hartfield *et al.*, 2001) and the diamondback moth, *Plutella xylostella* (Furlong and Pell, 2001).

The present results showed considerable reduction of infested palm trees in treated areas with fungus *B. bassiana*. This reduction was

distinguished in the second year of application whereas it reached to 87.5%. This reduction was explained that the fungus was established in treated areas. It also suggests that no single control strategy will provide complete control of red palm weevil. Therefore, a well defined IPM approach depending on biological control with the fungus *B. bassiana* might offer the best chance for success.

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