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INTEGRATED CONTROL OF RED PALM WEEVIL *Rhynchophorus ferrugineus* (OLIVIER) (COLEOPTERA : CURCULIONIDAE)

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ABSTRACT

The compatibility of essential oils and antimoulting hexaflumuron with Egyptian isolates of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) was examined against the red palm weevil *Rhynchophorus ferrugineus* (Olivier) under laboratory, greenhouse and field conditions . Effectiveness of fungus with the addition of essential mint oil at a concentration of 2.5 ml/l toward red palm weevil was increased in the laboratory. The results indicated that the impact of the combination of fungus and antimoulting hexaflumuron insecticide was variable, ranging from synergism to antagonism depending on the concentration of hexaflumuron ano larval instars. Significant larval mortality in palm trees injected with a combination of fungus (10^8 spores/ml), mint oil (1.0 ml/l) and antimoulting hexaflumuron (0.1%) was evident in greenhouse. In the field, using the combination applied by injection was an effective method for controlling *R. ferrugineus* in naturally infested palm trees in two weeks .The success of the treatments was 98.3 %, 95.1 %, and 86.2 % within two weeks after injection in light, median and heavy infestations, respectively. The study suggests that curative control of *R. ferrugineus* in infested palm trees is highly too possible with a combination of fungus *B. bassiana*, essential mint oil and antimoulting hexaflumuron.

Keywords : entomopathogenic fungi , *Beauveria bassiana* , red palm weevil, *Rhynchophorus ferrugineus* , essential oil , antimoulting, hexaflumuron.

INTRODUCTION

The date palm , *Phoenix dactylifera* L. , is cultivated since prehistoric times. Date is the most important fruit crop in Egypt as well as in the Middle East. In the mid 1990's a dread pest of palms, the red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera : Curculionidae), was reported to cause serious damage to date palms in Egypt (Cox , 1993) . Since, this time, the weevil has spread widely and now it is the most economically important pest of the date palm in Egypt . Eggs of *R. ferrugineus* are laid on the trunk of the palm and the larval stage feed on the trunk , causing tunnels inside the palm. Frequent and heavy attacks weaken and eventually kill the tree . Present control measures are largely based on insecticide applications by injection into palms to recover those diagnosed as having light to medium infestation and by regular spraying of palms in infested areas (Oehlschlager, 1996 and El Ezaby, 1997).

Now there are deep concerns about environmental pollution and health risks associated with use of chemical insecticides , therefore the development of integrated pest management for the red palm weevil based on pheromone traps and biological control rather than insecticides is needed (Murphy and Briscoe, 1990). Many entomopathogenic fungi offer promising biocontrol agents against various pests (Bing and Lewis, 1991; Lüth , 2003;

Tkaczuk and Labanowska, 2003 and Tounou *et. al.* , 2003). The fungus *Beauveria bassiana* (Balsamo) Vuillemin is a cosmopolitan Deuteromycetes with a broad range of insect hosts , and is currently being developed worldwide as a mycoinsecticide (William and Cogbum , 1999 , and Lord , 2001). *B . bassiana* has been successfully used as a bio-control agent for the management of a number of coleopteran insects including the Colorado potato beetle , *Leptinatarsa decemlineata* Say (Anderson *et. al.*1988), Lesser mealworm , *Alphitobius diaperinus* Panzer (Geden *et. al.* , 1998), blister beetle , *Lytta nuttalii* Say (Miranpuri *and Khachatourians* , 1994) and maize weevil, *Sitophilus zeamais* Motschulsky (Meikle *et. al.*, 2001) . In Egypt , it was reported infecting the pink borer , *Sesamia cretica* (lederer) , European corn borer , *Ostrinia nubilalis* (Hibner) , whitefly *Bemisia tabaci* (Gennadius) and Egyptian alfalfa weevil *Hpyera brunneipennis* , Boheman (El-Sufty and Hrer, 1989 , Sewify , 1999, and Ezz, 2004). The susceptibility of all *R. ferrugineus* stages to *B. bassiana* infection was different under laboratory conditions (personal communication). The efficacy of the entomopathogenic fungus, *B. bassiana* for red palm weevil contrcl may be improved by combining it with other control agents.

The present work aims to investigate, under greenhouse and field conditions, the possibility of injecting the entomopathogenic fungus, *B. bassiana* combined with essential oils and an antimoulting agent to increase its efficacy and reduce the latency period of lethal infection to the red palm weevil.

MATERIALS AND METHODS

Insects :

R. ferrugineus stages were obtained from the "red palm weevil rearing unit" at the Biological Control of Red Palm Weevil Project , El - Kassasin , Ismailia Govemorate , and kept at 29°±1°C, 60 – 70 % R.H. and 14 h photoperiod . The *R. ferrugineus* stages were reared on one year old palm trees which were kept under plastic screen nets.

Fungal preparations :

The used entomopathogenic fungus *B. bassiana* was originally isolated from red palm weevil *R. ferrugineus* at Ismailia Governorate, Egypt. This fungus was grown on autoclaved Sabourd dextrose yeast agar (SDAY) *containing*, 1 % peptone, 0.2 % yeast extract, 4 % dextrose and 1.5 % agar in distilled water for two weeks at 26 °C ± 1. Conidiospoeres were harvested and suspended in 0.02 % tween 80 solutions (Vernala Devi and Prasad, 1996).

The combined effect of *B . bassiana* and essential oils on red palm weevil:

To determine the effect of the combination of fungus *B. bassiana* and essential oils on *R. ferrugineus* , the following essential oils were used; almond, camphor, castor, fenugreek, caraway, cumin, mint, lemon, orange and bitter orange . Adults of red palm weevil were immersed for 5 sec. in one of the following treatments :

1) fungal conidiospores suspended in 0.02 tween 80 at a concentration of 10^8 spores / ml alone.
2) Emulsified essential oils in 0.02 tween 80 solution at a concentration of 4.0 ml/l (v/v).
3) Emulsified essential oils in 0.02 tween 80 solution at a concentration of 2.5 ml/l (v/v).
4) Emulsified essential oils in 0.02 % tween 80 solution at a concentration of 2.5 ml/l (v/v) containing 10^8 spores / ml of spores suspension.

The treated *R. ferrugineus* adults were transferred individually to plastic containers (10 adults/treatment) provided with sugar cane pieces as food. The treated insects were incubated at 26 °C ± 1. Mortality was assessed on a daily basis during the following 9 days. Cadavers were incubated at high R. H. to confirm fungal outgrowth .

The combined effect of *B. bassiana* and antimoulting agent on red palm weevil :

The antimoulting compound hexaflumuron [Trade name: Consult 10 % EC, Common name: hexaflumuron, IUPAC name: 1-[3,5 – dichloro – 4 – (1 , 1 , 2 , 2 – tetrafluoroethoxy) phenyl] - ٣ - (2,6- difurobenzoyl) urea] was used to study the combined effect of this antimoulting and fungus *B. bassiana* on *R. ferrugineus*.

Experiment 1 : To determine the lethal concentration value (LC_{50}) of the antimoulting hexaflumuron, five concentrations (0.0 , 0.25 , 0.5 , 0.75 and 1.0 %)were used. Sugar cane pieces were immersed for 5 min in each concentration and then transferred to plastic containers (5 g/container) . For each concentration, ten larvae of each 3^{rd} (L3), 6^{th} (L6) and 10^{th} (L10) instars were released individually in plastic containers. Three replicates were used per concentration. The mortality percentages were assessed daily.

Experiment 2 : To determine the effect of combination of fungus *B . bassiana* and the antimoulting hexaflumuron on red palm weevil *R. ferrugineus* , the 3^{rd} , 6^{th} , and 10^{th} larval instars were treated with fungus and antimoulting together , fungus alone and antimoulting alone as follows: 1) The *R. ferrugineus* larval instars were immersed in spore suspension at a concentration of 10^8spores/ml for 5 sec. and , transferred individually to plastic containers provided with pieces of sugar cane (5 g /plastic container) treated with antimoulting hexaflumuron at concentrations of 0.1% , 0.5% and 1.0% (10 larvae/concentration). 2) The *R. ferrugineus* larval instars (10 larvae/instar) were immersed in conidiospores suspension at a concentration of 10^8 spores/ml for 5 sec. , then , released individually in plastic containers provided with pieces of sugar cane as food (5g / plastic container) . 3) *R . ferrugineus* larval instars were released individually in plastic containers provided with pieces of sugar cane treated with the antimoulting hexaflumuron at concentrations of 0.1%, 0.5% and 1.0 % (10 larvae / concentration). Three replicates were used for each treatment and the mortality was assessed daily. Cadavers were incubated at high RH. to confirm the infection by fungal outgrowth.

Greenhouse experiment :

A treatment consisted of a combination of fungal spores, essential mint oil and antimoulting hexaflumuron against *R. ferrugineus* was carried out under greenhouse conditions . Eighteen palm trees (3 years old) were transferred into the greenhouse and planted under iron cages (2×2×2 m) covered with plastic net (3trees/cage). These palm trees were infested with different larval instars (20 larvae/ palm) through 20cm deep holes drilled by a hammer drill . Three holes were made in each palm tree stem at a height of 30 cm above the ground. These infested palm trees were treated by one of the eight following treatments; fungal spores alone (10^8 spores / ml), mint oil alone (1.0 ml/l) , antimoulting hexaflumuron insecticide (0.1%) alone, fungal spores + mint oil (1.0ml/l), fungal spores (10^8 spores/ml) + antimoulting hexaflumuron 0.1 % (v/v), fungal spores + mint oil + hexaflumuron , hexaflumuron + oil, and control. Tween 80 (0.05%) was added to all treatments. All treatments were applied by injection through 4 PVC tubes (30 cm long and 16 mm thick) inserted into deep holes (20 cm) made at a 45° into the core of infested date palm trees just above the larval infestation level . These holes were made by 1150W electrical hummer drill equipped with a 50 cm long and 16 mm thick screw . Every application was repeated once a week for two consecutive weeks (3 palm trees / application). After 14 days the palm trees were cut , examined and dead larvae were recorded .

Field trial

This trial was carried out during 2003 – 2004, in El – Kassasin region at Ismailia Governorate. One hundred and fifty palm trees naturally infested with *R. ferrugineus* were chosen for applications . The infestation of these palm trees were classified as light , median and heavy . The number of active galleries with fresh exudates and frass were determined . A combination of *B. bassiana* conidiospores (10^8spores/ml), mint oil (1.0ml/l) and antimoulting hexaflumuron 0.1% (v/v) was injected through PVC tubes inserted into deep holes prepared as above. 50 cm of the combined solution was injected into each tube. The numbers of inserted tubes were connected with the infestation level. The application was repeated weekly for two consecutive weeks, and the numbers of dry and wet galleries were recorded.

RESULTS

Combined effect of fungus *B . bassiana* and essential oils on red palm weevil :

Results indicated that most of the essential oils were toxic to *R . ferrugineus* and it caused 100% mortality at a concentration of 4.0 ml/l within 9 days post treatment . When the concentration of essential oils was 2.5 ml/l, great differences were observed between the effects of *B. bassiana* in combination with essential oils and oils alone on the adult stage of red palm weevil *R. ferrugineus* (Table 1). In general, the combination of fungus with essential oils significantly increased adult mortality. Fungal spores in combination with mint oil or bitter orange (2.5ml/l) caused 100 % mortality in 6 days, while the fungus with cumin or orange caused 100 % mortality in 9 days. It was evident that the combination of fungal spores and essential oils accelerated the mortality time of treated *R. ferrugineus* adults . These results emphasized the existence of the compatibility of the essential oils with the fungal pathogen, *B. bassiana*.

Table 1: Effect of *B. bassiana*[a] in combination with essential oils (2.5 ml/l) on adults of *R . ferrugineus.*

Treatment	Percentages of adult mortality at indicated days after treatment		
	3	6	9
Almond	10.0	10.0	10.0
Almond + fungus	60.0	66.7	86.7
Camphor	0.0	0.0	26.7
Camphor + fungus	0.0	10.0	80.0
Fenugreek	13.3	13.3	13.3
Fenugreek +fungus	73.3	83.3	86.7
Caraway	0.0	6.7	20.0
Caraway + fungus	30.0	63.3	63.3
Cumin	0.0	0.0	6.7
Cumin + fungus	56.6	93.3	100.0
Mint	0.0	40.0	40.0
Mint + fungus	60.0	100.0	100.0
Lemon	0.0	0.0	0.0
Lemon + fungus	0.0	53.3	56.6
Orange	0.0	6.7	6.7
Orange + fungus	10.0	63.3	100.0
Bitter orange	13.3	30.0	30.0
Bitter orange + fungus	33.3	100.0	100.0
Fungus	0.0	40.0	50.0
C.V.			16.33
L.S.D.			1. 40

a : The concentration was 10^{8} spores/ml

Susceptibility of *R. ferrugineus* larval instars to antimoulting hexaflumuron 10% EC:

The probit – Log concentration regression lines of hexaflumuron are illustrated in (Fig. 1). The estimated LC_{50} (0.22 , 0.75 and 1.57 ml) and the slopes assessed the presence of differences in mortality among the three instars treated with antimoulting hexaflumuron. The third larval instar (L3) was more susceptible to hexaflumuron insecticide than the other treated two instars (L6 and L10).

Effect of combination of fungus *B. bassiana* and antimoulting hexaflumuron on red palm weevil larval instars:

The larvae of red palm weevil were severely affected by the combinations of *B. bassiana* conidiospores and antimoulting hexaflumuron comparing to the larvae treated with fungal spores alone or the antimoulting alone (Table 2). The obtained results indicated that the effect of combination was variable depending on larval instars and concentrations of the antimoulting . Differences in mortality percentages among tested instars were obvious.

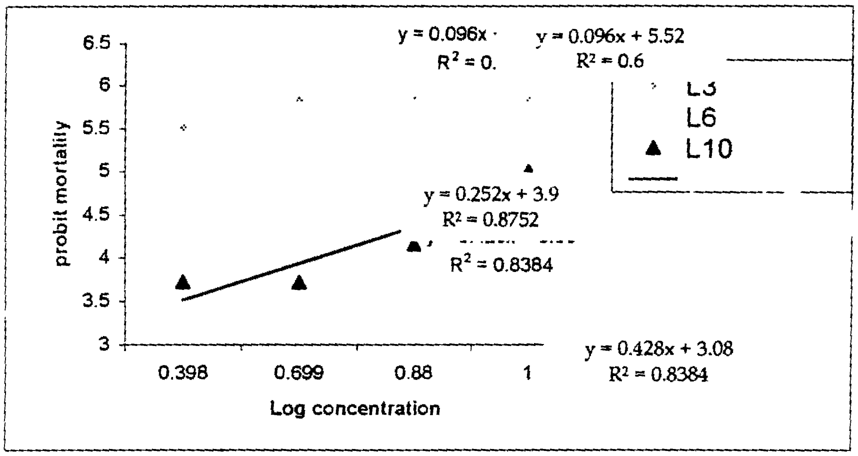

Fig. 1 : Mortality response of *R. ferrugineus* larval instars (L3 , L6 and L10) to different concentrations of hexaflumuron antimoulting.

Table 2: Effect of *B. bassiana*[a] combined with antimoulting hexaflumuron on three larval instars of *R. ferrugineus*.

Treatment	Larval instars	Conc. Of hexaflu-muron %	% mortalities at indicated Post-treatment days 3	4	5	6	7
Fungus alone	3rd	-	0.0	0.0	0.0	60.0	70.0
hexaflumuron alone		0.1	20.0	30.0	50.0	60.0	66. 7
		0.5	20.0	50.0	80.0	80.0	80.0
		1.0	20.0	76.7	76. 7	80.0	80.0
Fungus+hexaflum-		0.1	26.7	40.0	60.0	73.3	100.0
		0.5	76.7	80.0	86. 7	86. 7	86. 7
		1.0	43.3	60.0	60.0	60.0	63.3
Fungus alone	6th	-	0.0	0.0	10.0	10.0	26. 7
hexaflumuron alone		0.1	16.7	20.0	20.0	20.0	26. 7
		0.5	13.3	13.3	23.3	26. 7	36. 7
		1.0	16.7	36.7	36. 7	40.0	40.0
Fungus+ hexaflum-		0.1	20.0	36. 7	46. 7	83. 3	93.3
		0.5	33.3	36. 7	63.3	76. 7	93.3
		1.0	23.3	60.0	83.3	83.3	86. 7
Fungus alone	10th	-	0.0	0.0	0.0	16. 7	26. 7
hexaflumuron alone		0.1	0.0	16.7	36. 7	40.0	46. 7
		0.5	0.0	20.0	23.3	23.3	23.3
		1.0	0.0	10.0	10.0	10.0	10.0
Fungus+ hexaflum-		0.1	0.0	6. 7	16. 7	20.0	63.3
		0.5	10.0	10.0	10.0	16. 7	56. 7
		1.0	0.0	0.0	6.7	16. 7	53.3
C.V.							21.41
L.S.D.							1.94

a:.The concentration was 10^8 spores/ml

The third larval instar (L3) was the most affected one by the combinations of fungus and hexaflumuron. The mortality of all instars was maximized when they were treated with a combination which had the lowest concentration of hexaflumuron (0.1%); the mortality percentages of L3, L6 and L10 instars were 100%, 93.3% and 63.3% at 7 days post treatment, respectively.

Greenhouse experiment :

In this experiment, the separate effect of *B. bassiana* (10^8spores/ml), mint oil (1.0ml/l), hexaflumuron antimoulting (0.1%) and the gathered effect of the three agents were evaluated against *R. ferrugineus* larvae. In comparison with the control, all treatments caused significant mortalities to *R. ferrugineus* larvae (fig 2). The highest mortality was resulted from the combinations of fungus + mint oil + hexaflumuron (100%) and fungus + hexaflumuron (100%) followed by fungus + oil (87.5%) , hexaflumuron + oil (85%), hexaflumuron (80%), fungus (66.25 %) and oil (47.5 %).

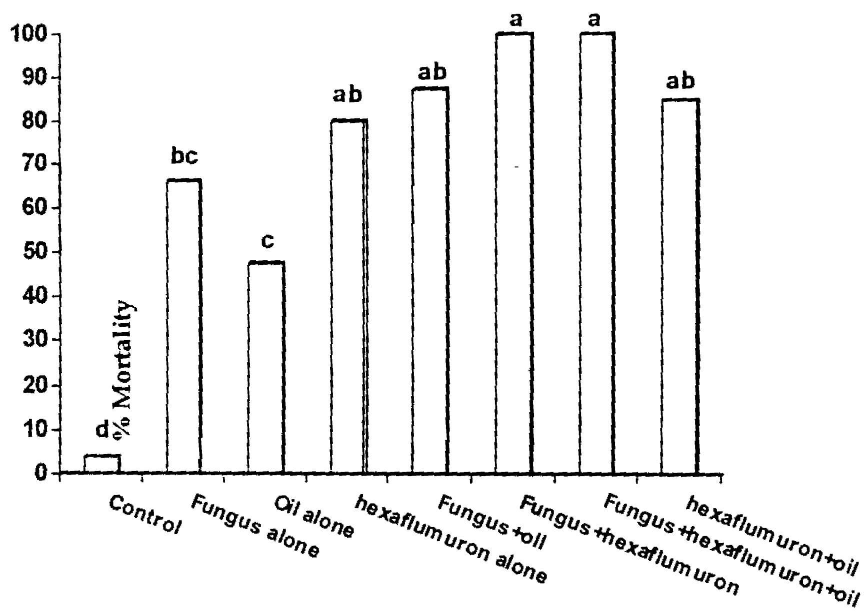

Fig. (2) : **Effect of the combination of *B . bassiana* (10^8spores/ml) , mint oil (1.0 ml/l) and hexaflumuron (0.1%) applied by injecting palm trees infested with *R. ferrugeneus larvae* under greenhouse conditions. Columns not followed by the same letter are significantly different as determined by the Duncan's test at $P < 0.05$.**

Field trial

The used combination of *B. bassiana* (10^8spores/ml) + mint oil (1.0 ml/l) + antimoulting hexaflumuron (0.1%) , was highly effective for treating palm trees which were naturally infested by the red palm weevil *R . ferrugineus* (Table 3). The success of control of red palm weevils in infested trunks was determined by the presence of external symptoms of recovery which varied according to the infestation level. The highest response to the used combination resulted from the light infestation level, then followed by median and heavy infestations . Thus, the heavily infested palm trees needed more injections than the used ones (32 injections / palm tree) . The percentages of dry galleries were 98.5, 97.2 and 94.9 in light , median and heavy infested palms, respectively . The numbers of recovered palms in 2 weeks post treatment were 98.3 %, 95.1 % and 86.2 in light , median and heavy infestations, respectively.

Table 3 :Effect of the combination of fungus *B. bassiana* , mint oil[a] and antimoulting hexaflumuron [b] applied by injection into naturally infested palm trees .

Infestation level	No. of treated palms	No. of active galleries	Mean No. of active galleries/ palm (range)	Mean No. of inserted tubes / palm (range)	No. of dry galleries (%)	No. of recovered palms (%)
Light	60	67	1.12 (1-2)	3.92 (2-6)	66 (98.5%)	59 (98.3%)
Median	61	146	2.39 (3-6)	7.1 (3-13)	142 (97.2%)	58 (95.1%)
Heavy	29	118	4.1 (4-11)	11.7 (6-32)	112 (94.9%)	25 (86.2%)

a. low concentration (0.2 %)
b. sublethal concentration (0.1 %)
c.

DISCUSSION

The present study confirms the additive or synergistic interaction among the entomopathogenic fungus *B. bassiana*, essential oil, and hexaflumuron under laboratory, greenhouse and field conditions and subsequently this interaction affects *R. ferrugineus*. The results showed that most of the high concentrations of the essential oils were toxic to *R. ferruginus*, while the effectiveness of fungus increased when it was combined with a low concentration of mint oil. Mint oil had been reported as the strongest repellent and antifeedants agent against tested curculionid insects (Nadasy and Saringer, 1985; Singh *et. al*., 1989; and Ignatowicz, 1997). Coats *et. al*., (1991) and Karr and Coats, (1992) referred this action to the presence of monoterpenoids which act as neurotoxins, insect growth regulators , and repellent agents. Monoterpenoids affect the sensory nerves of the peripheral nervous system, without inhibiting the acetyl cholinesterase. It causes spontaneous stimulation of sensory nerves and subsequent signaling to motor nerves that results in muscle twitching, convulsion, and then paralysis of insects. The present study indicates that the impact of the combination of *B. bassiana* and hexaflumuron on the *R. ferruginous* larvae was variable, ranging from synergism to antagonism based on the concentration of hexaflumuron and larval instars. The *R. ferrugineus* larval mortality was significantly increased when the two agents were applied together at a low concentration of hexaflumuron.

Thus, the combination of the fungus and hexaflumuron at a low concentration induced a synergistic effect. It seems that the antimoulting hexaflumuron under low concentration may act as a stressing agent; increasing the susceptibility of *R. ferrugineus* to the fungus , while hexaflumuron at a high concentration inhibits the mycosis development . It is well known that the antimoulting insecticide acts as a cuticle chitin synthesis inhibitor which facilitates the penetration of fungal spores into the target insect, causing a successful infection. Hassan and Chamley, (1983) and Rabie and Risha (1994) recorded that benzoylphenyl urea diflubenzuron and teflubenzuron act synergistically with the entomopathogenic fungus, *M. anisopliae* against the tobacco hornworm, *Manduca sexta* and the desert locust , *Schistocerca gregaria* (Forsk.). Also, changes in the chemicals of insect cuticle during infection , may affect the outcome of pathogen and antimoulting interaction. Thus, both the mint oil and antimoulting insecticide under low concentrations may act as stressing agents ; increasing the susceptibility of red palm weevil *R . ferrugineus* to the fungus. In greenhouse the use of fungus *B. bassiana* in combination with mint oil and antimoulting hexaflumuron was necessary to increase the larval mortality in injected palm trees. The mechanism of spore penetration in insect host was both physical and chemical functions (Boucias and Pendland , 1998) . Therefore, it might be possible to propose other additives, which may naturally or chemically facilitate the spore penetration . In this respect, oils and antifeedants of plant origin (Vemala Devi and Prasad 1996) and antimoulting (Hassan and Chamley 1983) were used to make the insect host more susceptible to entomopathogenic fungi. Field studies revealed that the utilization of *B. bassiana* combined with mint oil at a low concentration and antimoulting hexaflumuron at a sub lethal concentration injected into palm trees was an effective method for controlling *R. ferruginous* in two weeks. The success of control varied according to the infestation level by red palm weevil. Shams Eldean (2002) treated infested date palm trees with Egyptian nematodes, *Heterorhabditis indicus* and *H. bacteriophora* and he reported complete dry galleries in a month . However, Saleh and Alheji, (2003) cited that the injection of nematodes in infested palm trees caused only 50% dry galleries in three weeks after treatment. Generally, the presence of the essential oil in the combination as a repellent agent may enhance the fungus virulence by increasing the insect movement, while the antimoulting weakens the insect immunity. So, the utilized combinations can substitute broad–spectrum chemical insecticides . In conclusion, this study emphasized that the combination of fungus *B. bassiana*, mint oil (low concentration) and hexaflumuron (sub-lethal concentrations) could be an effective and economically feasible control method of the red palm weevil *R. ferrugineus.*

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المكافحة المتكاملة لسوسة النخيل الحمراء باستخدام الفطر الممرض *Beaveria bassiana* مع الزيوت العطرية ومانع الانسلاخ hexaflumuron

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تم دراسة مدى التوافق بين ثمانية من الزيوت العطرية ، ومانع الانسلاخ hexaflumuron ، والفطر الممرض للحشرات *Beavena bassiana* فى مكافحة سوسة النخيل الحمراء تحت الظروف المعملية والحقلية والصوب السلكية.

فى الدراسة المعملية، زادت كفاءة الفطر فى مكافحة سوسة النخيل الحمراء عندما اضيف له زيت النعناع بتركبز ٢,٥ مل / لتر . دلت النتائج على ان تأثير الجمع بين الفطر ومانع الانسلاخ على سوسة النخيل يتراوح بين التنشيط والتثبيط حسب تركيز مانع الانسلاخ والعمر اليرقى.

تحت ظروف الصوبة السلكية، سجلت زيادة معنوية فى نسبة موت يرقات سوسة النخيل الحمراء الموجودة داخل أشجار النخيل عندما حقنت هذه الأشجار بالفطر ($١٠^{٨}$ / مل) ÷ زيت النعناع (١ مل / لتر) + مانع الانسلاخ (٠,١%) .

أثبتت الدراسة الحقلية أن استخدام الخليط السابق الذكر لحقن اشجار النخيل المصابة طبيعيا بسوسة النخيل وسيلة فعالة لمكافحة هذه الآفة فى خلال اسبوعين فقط و كانت نسب نجاح الجمع بين كل من الفطر وزيت النعناع ومانع الانسلاخ فى مكافحة سوسة النخيل ٩٨,٣ ، ٦٤,١ ، ٨٦,٢ % فى أشجار النخيل المصابة باصابات خفيفة ، متوسطة ، شديدة ، على التوالى.

اقترحت هذه الدراسة أن مكافحة سوسة النخيل الحمراء داخل أشجار النخيل بالجمع بين الفطر وزيت النعناع (تركيز منخفض) ومانع الانسلاخ (تركيز أقل من المميت) طريقة فعالة واقتصادية ، ويمكن استخدامها بدلا من المبيدات الحشرية .