Preliminary Investigations into the Biological Control of Red Palm Weevil *Rhynchophorus ferrugineus* by using *three isolates of the fungus Lecanicillium (Verticillium) lecanii in Egypt*

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Abstract: *When the different stages of Red Palm Weevil (RPW) treated with the different three isolates of fungi Lecanicillium (Verticillium) lecanii different concentrations , the LC50 obtained of isolate (1) were, 244 x10^4, 236 X 10^4, 207 X 10^4, 200 X 10^4, 316 X 10^4 and 305 X 10^4 spores / ml for 2nd, 3rd, 4th, 5th, and adult ♀ and ♂ respectively., When RPW treated with laboratory isolate (2) of L. lecanii the corresponding LC50 obtained, 210 X 10^4, 280 X 10^4, 270 X 10^4, 267 X 10^4 , 241X 10^4 , 350 X 10^4, and 321 X 10^4 spores/ml., respectively. Results detect that the target insect pests affected by isolate (3) of L. lecanii and isolated L. lecanii and the percentage of larval mortality were significantly increased to 87, 97% and 88% after treated with isolated and L. lecanii as compared to 1% in the control. The percentage of moth hatching were significantly decreased to 9% as compared to 100% in the control The result show that the palm weight were significant increase in El-Kassaseen (Ismailia) than in El-Esraa (Nobarya) to 4997±65.32 kg/ Feddan as compared to 1981±80.54 kg/ Feddan during season 2012. During season 2012 the yield loss obtained 56 and 60% in El-Esraa (Nobarya) and El-Kassaseen (Ismailia)which decreased to 22 and 22% in borth corresponding regions. The same results obtained during season 2013. The yield weight were significantly increased to 5875± 34.35 Kg/feddan in El-Esraa (Nobarya) as compared to 1999± 36.81 Kg/ feddan in the control. In El-Kassaseen (Ismailia), the yields were significantly increased to 5997±65.31 Kg/ feddan as compared to 1791±80.44 Kg/ feddan in the control. The yield loss ranged between 70 and 35%.*

Keywords: Lecanicillium lecanii, Rhynchophorus ferrugineus, date palm biocontrol

1. Introduction

Rhynchophorus ferrugineus Olivier (Coleoptera: Curculionidae) is the most important pest of the date palm (*Phoenix dactylifera*) in the world. It is native to southern Asia and Melanesia, where it is a serious pest of coconuts (*Cocosnucifera*), and is a regulated and is a pest within the EU. Since the 1980s it has rapidly expanded its geographical range westwards. It reached Saudi Arabia and the United Arab Emirates in about 1985, spreading throughout the Middle East and into Egypt. In 1994 it was detected in Spain and in 1999 in Israel, Jordan and the Palestinian Authority Territories. It has since spread to the Balearic Islands (2006), Canary Islands (2005), Cyprus (2006), France (2006), Greece (2006), Italy (2004) and Turkey (2007). The two main palm species of concern in the Mediterranean region are date palm and Canary Island date palm (*P. canariensis*), the main crop and ornamental species, but it also attacks several other ornamental palms that are regularly imported into Britain, such as chusan palm (*Trachycarpus fortunei*). The European Commission has introduced emergency measures to prevent the further spread of *R. ferrugineus* within the community. Since it had been introduced to Egypt at1992 (Cox, 1993) thousands of healthy trees were damaged or lost (El-Sebaey, 2004). In infested plantations, yields have been estimated to drop from 10 tonnes to 0.7 tonnes per hectare (Singh and Rethinam, 2005). Furthermore, *R. ferrugineus* is a strong flyer. This increase the weevil's ability to disperse, colonize and breed at new sites (Murphy

and Briscoe, 1999). However, longevity, activity and behavior of adult weevils are greatly affected by humidity. RBM controlled by chemical insecticides which pollute the environments .Unlike other insect pathogens, fungi infect the host by contact, penetrating the insect cuticle. The host can be infected both by direct treatment and by transmission of inoculum from treated insects or cadavers to untreated insects or to subsequent developmental stages *via* the new generation of spores (Lacey et al (2004), Qesada-Moraga et al (2004)). These unique characters make entomopathogenic fungi especially important for the control of concealed insects. Sabbour, 3013 a,b &c control this pest by different entomopathogenic fungi. In the case of *Rhynchophorus* spp., most of the life cycle passes within the tree trunk, making the pest inaccessible to direct-contact treatment. Adults are the only exposed stage and can be infected upon emergence. The questions remain of whether the treated adult will effectively convey the infection onwards and, if so, how and where it can best be treated. This work aims to evaluate the microbial control agents against the BRW under field and laboratory conditions.

2. Material and Methods

RPW collection: Random samples of RPW adults were collected from infested palm trees located at El-kasassen, Ismailia Governorate, Egypt. Insect rearing A RPW colony was established in the laboratory on sugarcane as both food and oviposition substrate, following Rahalkar *et al*., (1985).

Adults were set to mate and oviposit in groups of at least five pairs placed on a substrate of moist sugar cane saw dust or on sugar cane logs. From the first larval stage to adult emergence, the RPWs were reared individually at 27–29º C. For egg harvesting, the adults of both sexes were kept on sugarcane sawdust. Eggs were collected every 2 days.

2.1 Source and Production of fungi

The fungi *Lecanicillium lecanii* laboratory strain isolate (1) were kindly obtained from Prof. Dr Alain Vey, Mycology Unit, Pasture institute, France, and reproduced in the Microbiology Department, National Research Centre, Cairo, Egypt. They were primarily purified using the mono-spore technique. Then, propagated in Petri-dishes (9cm) on potato dextrose agar medium (PDAM) enriched with 1 %, peptone, 4 %. Glucose and 0.2% Yeast and incubated at 26 ºC. Seven days old cultures with well developed spores were harvested by washing with 10 ml sterilized water, then 3 drops of Tween-80 were added and completed to 100 ml with water. It was used as stock suspention and kept in a refrigerator at 4 ºC. From this stock (standared), dilutions with water were adjusted at the needed proposed concentrations. Large amounts of conidiospores, if needed, were produced by culturing the fungus on liquid medium in 1 L cell-culture glass bottles according to Rombach et al (1988) (modified by El-Husseini et al., 2004).

2.2 Isolation of the fungi

The fungus *L. lecanii* was isolated (2) from the diseased RPW insect pests and *L. lecanii* was isolated (3) from Egyptian soil in El kassasin (Ismailia). Isolates were subcultured on nutrient PDA medium. Isolates were identified at National research Centre (NRC) Plant Pathology Department. The spores of *L. lecanii*, were collected from agar surface of the fungus culture in 15cm diameter Petri-dish. Spore suspension in water + 0.1% Tween-80 was prepared. The strength of original culture was $1x10^8$ spore/ml. It was used as stock suspension and kept in a refrigerator at 4°C. From this stock, dilutions with water were adjusted at the needed proposed concentrations. Large amounts of conidiospores, if needed, were produced by culturing the fungus on liquid medium in 1 L cell culture glass bottles according to Rombach *et al*., (1988) and modified by El-Husseini *et al*., (2004).

2.3 Bioassays against target pests

All fungal isolates concentrations of *L. lecanii*, ranged from $1x10^2$ to $1x10^8$ spores/ml were prepared by 1-10 fold dilution from the main stock culture ($1x10^8$) and tested under controlled conditions (25±2ºC and 65±5% RH) against RPW larvae and adults. Ten 3-day-old flies were collected in test tubes, immobilized on ice and carefully transferred to PDA dishes (9 cm diameter) containing the six fully developed fungal colonies. The flies were allowed to walk on the fungal colonies for 5–10 min depending on fly mobility until the flies collected spores on their body. The flies were then removed from the Petri dishes and placed in small cages (10 cm x 10 cm x 10 cm). The same number of flies treated similarly but with uninoculated PDA plates was used as controls. Solid diet and water were offered to flies and kept under rearing conditions. Dead flies were counted and removed from the cages daily for 21 days. Each treatment was replicated five times The percentages of mortality were calculated after seven days and corrected according to Abbott's formula (Abbott, 1925), while the LC_{50} value was calculated through Probit analysis according to Finney equation (Finney, 1971).

3. Field Experiments

Esraa village- El-Nobaryia region, during the two successive seasons 2012&2013 starting from the first of July till the end of October to evaluate the efficacy of the tested fungi against the target insect pests under field conditions. Three random patches of palm trees were selected, each comprised 12 trees (12 trees for *L. lecanii* isolate (1, 2, 3), and applications and 12 trees for control) to carry out the field experiment. *L. lecanii*, was applied, each as a single treatment at the rate of $1x10^8$ spores/ml. Three applications were made at one week interval at the commencement of the experiment. Treatments were performed at the sunset with a ten litre sprayer. Percentage of infestation/sample was calculated after 20, 50, 90 and 120 days of the application. Each treatment was replicated four times. Four plots were treated with water as control. Random samples of leaves and fruits olives plants were weekly collected from each treatment and transferred to laboratory for examination. The infestation of RPW were estimated in each case.

After harvest, yield of each treatment was weighted as Kg/ Fadden. Yield loss was calculated according to the following equation:

$$Yield\ loss = \frac{Potential\ yield-\ actual\ yield}{Potential\ yield} \times 100$$

Potential yield was that yield obtained after *L. lecanii* isolate, treatment, which gave the best results among the tested pathogens, and was taken as a base for comparing with the other treatments

4. Bioassay Procedures

Selection of most pathogenic isolates. Isolates and standard were selected using larvae in plastic boxes (35 X 20 X 30 mm) containing 2.5 g of moist sugar cane sawdust. Groups of five or six larvae were located in 9-cm petri dishes lined with 5-10 liter paper, sprayed with 2 ml of spore suspension containing 2 X 10^8 spores/ ml. transferred to boxes (one larva per box) 5-10 min later. The control group was treated with an aqueous solution of 0.01% Triton X-100. The boxes were incubated at 27º C in darkness for 7-14 days. The larvae used for the bioassay weighed 60-200 mg. The bioassay was repeated here times and the results were averaged this methods according to Gindin *et al.* (2006).

Eggs exposure. The two isolates of *L. lecanii*, were evaluated in this bioassay. Eggs for bioassay (1–4 days old) were obtained from egg laying cages. The eggs were placed singly in petri dishes each containing 3 g sugar cane sawdust Gindin *et al.* (2006). The petri dishes with eggs were incubated for 10 days at 27º C in darkness. Egg hatching and mortality of emerging larvae were monitored during this period. The egg bioassay was repeated three times with three

different batches of eggs (10–24 eggs per treatment of each test group).

Adult treatment. Also designed according to Gindin *et al.* (2006). Subsequently, each treated or control weevil was placed in an individual box with 50 g of moist sugar cane sawdust, and incubated at 28° C under a 12:12 L:D regime for 2–7 weeks. Fresh substrate was added to the boxes every week. In order to evaluate the possibility of fungal transmission from surface-treated females to their progeny (eggs and larvae) during oviposition, 15 mated females were dusted individually with *L. lecanii* spores in 5 g of a solid rice-based formulation.

The control group comprised 15 untreated females which were incubated under the same regime up to completion of oviposition. Twice a week the sugar cane logs were replaced with new ones, and the old ones with eggs were incubated at 27° C in darkness for 14 days. The logs were then cut up and the eggs or emerged larvae were counted. The latter were placed in individual boxes with moist sugar cane sawdust and incubated for an additional 2 weeks to compare their survival with that of the controls. Analysis of results of the bioassay were recorded as mortality percentages in eggs, larvae and adults, as the hatching percentages of eggs, or as the weight of the surviving larvae. Student's t-test or one-way ANOVA was used to compare the effects of the experimental and control treatments on the eggs and larval mortality, and on larval weight.

5. Results and Discussions

Table 1 show that the LC50 of RPW first larval instars when treated with different concentrations of isolated *L. lecanii* were, $210X\,10^4$ spores/ml. when the different stages of RPW treated with the different fungi concentrations , the LC50 obtained $244\,x10^4$, $236\,X\,10^4$, $207\,X\,10^4$, $200\,X\,10^4$, $316\,X\,10^4$ and $305\,X\,10^4$ spores / ml for 2nd, 3rd, 4th, 5th, and adult ♀ and ♂ respectively., (Table 1). When RPW treated with laboratory standard *L. lecanii* the corresponding LC50 obtained, $210\,X\,10^4$, $280\,X\,10^4$, $270\,X\,10^4$, $267\,X\,10^4$, $241X\,10^4$, $350\,X\,10^4$, and $321\,X\,10^4$ spores/ml., respectively .,(Table 2).

Table 3 show the effect of the third isolate of the fungus *L. lecanii* , which cleared that the fungus affected on the target insect pest in all life cycle stages .

The same results obtained by Gindin *et al.* (2006) when found that fungi, *Metarhizium anisopliae* and *Lecanicillium lecanii,* strains of the former were found to be more virulent than those of the latter, achieving 100% larval mortality within 6.7 days. The same results obtained by Shaiju *et al* 2003, Gindin *et al.* (2006). The screening of fungi known to be pathogenic to RPW was aimed at evaluating the pathogenicity of *M. anisopliae* and *L. lecanii* to various development stages of RPW, and to develop a method for fungus implementation. The present investigation showed that the tested *M. anisopliae* and *L. lecanii* isolates infect the larvae and fully completed their life cycles by forming conidiophores with conidia on RPW cadavers. The red pigmentation of larvae killed by *L. lecanii* indicates the

presence of a red pigment, oospore in, produced by *Lecanicillium* spp. (Gupta, *et al* 1995and Vining, et al 1992).

The effect of the fungi *L . lecanii* were evaluated on the RPW eggs. Results show that the isolated *L. lecanii* affected on the RPW eggs hatching the percentage of eggs hatching were significantly decreased to 4 % when the newly eggs treated with the isolated *L. lecanii* as compared to 100% in the control. The percentage of larval mortality were significantly increased to 100 as compared to 1% in the control (Table 4). The same results obtained by Gindin *et al.* (2006) who reported that, the pathogenicity of the two most virulent isolates of *M. anisopliae*, selected in the initial screening on larvae, was tested against *R. ferrugineus* eggs. Both isolates fungi killed the eggs quickly, without preliminary colonization on the egg surface. Gindin *et al.* (2006) reported that, all of the screened *M. anisopliae* strains exhibited pathogenicity to all development stages of RPW, causing up to 80–100% mortality of larvae and adult weevils under laboratory conditions. When eggs were exposed to sawdust previously sprayed with *M. anisopliae* spores, the total survival of both the eggs and the emerged larvae was reduced by a factor of approximately two to three, relative to control. Moreover, larvae developing in sprayed sawdust with fungal spores had a significantly reduced weight in comparison with larvae which emerged in the control treatment. The phenomenon could result from lower food intake by infected insects and/or from the energetic cost of confrontation with the infection. There are a number of examples of reduction in food consumption in insects infected by pathogenic fungi (Ekesi, 2001,Tefera and Pringle (2003) and Soroker *et al.* (2005).

Table 5 show the effect of the three *L. lecanii* tested against RPW under laboratory conditions. Results detect that the target insect pests affected by laboratory standard *L. lecanii* and isolated *L. lecanii* and the percentage of larval mortality were significantly increased to 87, 97% and 88% after treated with isolated and standard *L. lecanii* as compared to 1% in the control.at the same time the pupal mortality were affected by the three treatments. The percentage of moth hatching were significantly decreased to 9% as compared to 100% in the control (Table 5). Magalhaes, *et al* 2001), found that, larvae of lower weight lower have a shorter life span. Gindin *et al.* (2006) reported that the direct effect on adult mortality, fungal treatment reduced female fertility. Females contaminated with the dry spore formulation had a shorter oviposition time than the controls (Furlong and Pell (2001) – 11 days compared with 30–45 days) and, moreover, the total number of eggs laid by infected females was three times less than that by control females. However, the daily numbers of eggs laid by each female, and the survival and weight of emerged larvae, did not differ significantly between treated and control insects. This may suggest that females did not transmit fungal spores to the oviposition tunnels. Thus, the main contribution of the fungus to RPW control was found to be through the premature death of the infected females.

Table 6 and 7 show the effect of the three isolated fungi *L. lecanii* on RPW in two Egyptian regions differ in climatic conditions in El-Kassaseen (Ismailia) clay soil and El-Esraa (Nobarya) sandy soil . The result show that the palm weight were significant increase in El-Kassaseen (Ismailia) than in

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El-Esraa (Nobarya) to 4997±65.32 kg/ Feddan as compared to 1981±80.54 kg/ Feddan during season 2012. During season 2012 the yield loss obtained 56 and 60% in El-Esraa (Nobarya) and El-Kassaseen (Ismailia)which decreased to 22 and 22% in borth corresponding regions (Table 6). The same results obtained during season 2013. The yield weight were significantly increased to 5875± 34.35 Kg/feddan in El-Esraa (Nobarya) as compared to 1999± 36.81Kg/feddan in the control. In El-Kassaseen (Ismailia) the yield were significantly increased to 5997±65.31Kg/feddan as compared to 1791±80.44Kg/feddan in the control. The yield loss ranged between 70 and 35% (Table 7).

Gindin *et al.* (2006). Despite the observed susceptibility of all the development stages of the RPW to the entomopathogenic fungi under laboratory conditions, the practicability of achieving efficient control of RPW in the field seems problematic. The field efficacy of entomopathogenic fungi toward various pests depends on many factors, often related to the behavior of the insect host in its natural habitat. The soil is the natural habitat of fungi and, since the RPW pupae occasionally inhabit the soil, it is theoretically possible to infect them with fungal spores by soil treatment. The spraying of palms and of large areas between them to ensure contact between free living adult RPW and fungal spores also presents a difficulty, because of the large fungal inoculum needed. The best strategy would be to treat only selected areas that are especially likely to attract adults. Adult weevils are usually cryptic, taking refuge between petioles and offshoot bases. They are highly attracted to wounds in palm trees, e.g. that are infected during vegetative production practices that include the removal of offshoots Furlong and Pell (2001) . These areas are often the most attractive sites to females for oviposition and, therefore, may be the best candidates for treatment with a dry fungal formulation. The possibility of infecting the Rhynchophorus adults by this method was discovered by chance, after application of a rice-based formulation of *M. anisopliae* against the *Scarabaeid Scapanes*aus tralis on young palms in New Guinea (Prior,.and Arura, (1985). The treatment of frond axils with this formulation caused infection not only to the target pest but also infected some incidental infection on *R. bilineatus*. The high mortality of adults to dry spores of a selected isolate indicates that there was proper contact between fungi and ovipositing females. Hydrophobicity of fungal spores could play a significant role in the success of the dry rice formulation. The attachment of a fungal spore to the cuticle surface is the initial and thus a crucial event in the establishment of mycosis (Boucias, *et al* 1998). The spores of *M. anisopliae* possess an outer layer composed of interwoven fascicles of *hydrophobic rodlets*, which provided the adhesion to the insect cuticle due to non-specific hydrophobic forces (Boucias, *et al* 1998). Thus, similar to the natural infection process, the application of dry spores could provide better adhesion relative to application of aquatic spore suspension. Ideally, we would like to use artificially inoculated females as vectors of infection to their progeny via egg contamination during oviposition. However, in the present study we were unable to prove that such infection transfer occurred. We believe that the lack of success was due mainly to insufficient inoculum transferred by females to eggs. It is possible that this problem is connected with spore hydrophobicity. This character may interfere with fungus transfer from female hydrophobic surface to the egg under humid conditions during oviposition into the plant tissue. The mechanical transfer of fungal spores between the oviposition sites and even further into the oviposition tunnels would be possible, provided that a suitable fungus formulation could be developed. In light of the high susceptibility of eggs and larvae to fungal infection, we assume that in such a case the contamination of eggs and of the larval habitat would add to the effectiveness of this pest control method. The economic effects resulted from using different pesticides to get control the palm weevil *R. ferrugineus*. The cultivation of date palm spreads in most of the Egyptian governorates as the cultivated land estimated 88. 000 fedans which have 16 million palm trees out of which 12 million palm trees annually produce 1.3 million tons of dates [1]. The palm tree number in one fedan estimates 200-500 kg of the good sorts such as the sort of Barhi [2]. A tree palm production ranges from 100 to 120 kilo per a fedan in the case of currently well-known sorts as the tee palm valued $100-150 US Dollars [3]. The study of the economic effects resulted from using different pesticides requires investigating that effect at the farming level to determine the added value of using the pesticides and studying that effect at the national level

First: the effect of using different pesticides at the farming level:

Table 8 shows that the cost of fedan using different pesticides to combat palm weevil estimated about L.E. 400/fedan. The pesticide cost per a palm tree estimated about L.E. 6.1 on the basis that a fedan contains 66 palm trees. The study indicated that the production waste rate reduced to about 17% and 26% in relation to the first pesticide. This rate reduced to almost 18% and 25% as to the second pesticide in the areas of Ismailia and Nobaryia respectively as shown in Table 6. In addition, table 8 shows the production waste reduction had contributed to the increase of palm tree production by about 10, 7, 17 and 8 kg. The increase value palm tree expected return and net return by about L.E. 20, 14, 34, and 16 and almost L.E. 13.9, 7.9, 27.9 and 9.9 for both first and second pesticides in the two areas of study as shown in Table 8. The study of the added value of using different pesticides indicates that the pound return spent on different pesticides cost estimates almost L.E. 2.28, 1.30, 2.93 and 1.62 respectively for the first and third pesticides in the two areas of study as shown in Table 8.

Second: The effect of using different pesticides at the national level:

The study of this effect necessitates the overgeneralization of using the different referred to pesticides at the national level which is expectedly leads to the crop production increase at the domestic level as shown in Table 9.

Table 9 indicates that the state adoption of generalizing the use of pesticides to combat the palm weevil at the republic level will lead to production increase by , 0.348, 0.435, and 0.496 million tons after deducting the Table- referred to waste rate for the first and third pesticides in Ismailia and Nobarya respectively. This increase can save to the state a good yield of foreign currency that contributes to revive the

Egyptian economy, particularly after the collapse of the Egyptian economy in the post period of January, 25 revolution in the case of being world-market exporting oriented. This entails chemicals would not be used in the production of different crops. In the case of exporting, the production increase will contribute to save about $ 284, 362, 452 and 516 million Dollars. This will help lessen the deficit rate on the Egyptian trade balance in the case of generalizing the use of the first and third pesticides as shown in Table 9. The aim of this study is the dominancy of Egypt on world dates exporting and will replace Iraq in this industry prior the industry collapse in Iraq after Iraq's invasion in 2003.

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Table 1: Effect of the isolated fungi *L. lecanii* (1)on the Palm weevil *R. ferrugineus* biology

Pathogen	LC50 (spores/ml)	slope	variance	95 % confidence limits
1st	210	1.1	2.3	200-344
2nd	244	0.2	1.4	222-288
3rd	236	1.1	0.3	234-288
4th	207	0.3	1.5	231-289
5th	200	1.2	1.3	267-300
Adults (♀)	316	2.0	1.8	299-323
Adults (♂)	305	0.1	1.1	289-332

Table 2: Effect of the laboratory isolate(2) fungi *L. lecanii* on the Palm weevil *R. ferrugineus*

Pathogen	LC50 (spores/ml)	slope	variance	95 % confidence limits
1st	210 X 10^4	1.1	1.3	121-298
2nd	280 X 10^4	0.2	1.2	121-299
3rd	270 X 10^4	1.1	0.1	131-380
4th	267 X 10^4	0.3	1.4	140-399
5th	241X 10^4	1.2	1.3	209-398
Adults (♀)	350 X 10^4	2.0	1.4	200-321
Adults (♂)	321 X 10^4	0.1	1.1	189-341

Table 3: Effect of the laboratory isolate (3) fungi *L. lecanii* on the Palm weevil *R. ferrugineus*.

Pathogen	LC50 (spores/ml)	slope	variance	95 % confidence limits
1st	251 X 10^4	1.1	1.3	221-297
2nd	275 X 10^4	0.2	1.2	210-299
3rd	288 X 10^4	1.1	0.1	210-390
4th	372 X 10^4	0.3	1.4	240-399
5th	381 X 10^4	1.2	1.3	290-399
Adults (♀)	355 X 10^4	2.0	1.4	301-348
Adults (♂)	328 X 10^4	0.1	1.1	249-352

Table 4: Effect of the isolate(2) fungi *L. lecanii* on the Palm weevil *R. ferrugineus* eggs

Age	Eggs Hatching %						larval mortality %					
	Isolates(1)		Isolates(2)		Isolates(3)		Isolates(1)		Isolates(2)		Isolates(3)	
	T	C	T	C	T	C	T	C	T	C	T	C
Newly	0	100	1	99	11	00	3	100	2	100	4	100
Two days old	9	100	3	99	31	00	3	100	2	99	3	100
Three days old	12	99	10	100	11	99	3	100	3	100	2	99
Four days old	20	100	18	100	13	99	6	100	7	100	2	99

T. treated C. control (untreated)

Table 5: Effect of the fungi *L. lecanii* three isolates on the Palm weevil *R. ferrugineus* biology.

Treatments	%Larval mortality	% of malformed pupae	pupal mortality	% of moth hatching
Control	1	0	0	100
L.l isolate(1)	87	71	53	10
L.l isolate(2)	97	50	84	4
L.l isolate(3)	88	55	75	7

Table 6: Weight of harvested palm and percentage of yield loss after treatment with the fungi *L . lecanii* against *RPW* during seasons 2012 in two different regions.

Treatments	El-Esraa (Nobarya)		El-Kassaseen (Ismailia)	
	Weight palm (Kg/feddan)	% yield loss	Weight palm (Kg/feddan)	% yield loss
Control	2000± 36.82	56	1981±80.54	60
L.l Isolate(1)	3123± 41.20	26	3345± 40.30	33
L.l Isolate(2)	4560± 34.31	-	4997±65.32	-
L.l . Isolate(3)	3531± 42.57	22	3879±69.33	22
F values	31.22		32.11	
LSD 5%	92		90	

Table 7: Weight of harvested palm and percentage of yield loss after treatment with the fungi *L. lecanii* against RPW during 2013 in two different regions .

Treatments	El-Esraa (Nobarya)		El-Kassaseen (Ismailia)	
	Weight palm (Kg/feddan)	% yield loss	Weight palm (Kg/feddan)	% yield loss
Control	1999± 36.81	65	1791±80.44	70
*L.l*Isolate(1)	3003± 32.40	48	3113± 42.50	48
L.l . Isolate(2)	5875± 34.35	-	5997±65.31	-
L.l Isolate(3)	3473± 42.50	40	3892±69.73	35
F values	33.02		30.30	
LSD 5%	84		80	

Table 8: The added value of using different pesticides to combat palm trees weevil at the farming level

The net of return/cost in pounds	the net of expected return/palm tree in pounds	The expected return/palm tree in pounds	Increase of production/palm tree kg	The cost of using pesticide/palm tree in pounds	The cost of using pesticide/fedan	pesticide	Area
2.28	13.9	20	10	6.1	400	M.a Isolate(1)	Ismalia
1.30	7.9	14	7	6.1	400	M.a Isolate(1)	Noubaria
2.93	27.9	34	17	6.1	400	M.a . Isolate(3)	Ismalia
1.62	9.9	16	8	6.1	400	M.a . Isolate(3)	Noubaria

Source: computed and collected from Tables 6 & 7.

Table 9: The economic effects resulted from the overgeneralization of using different pesticides at the national level:

Expected exporting value (US Dollars)	Ton exporting price (US Dollars)	Expected increase in production (million tons)	Waste amount (million tons)	% of harvest waste	Expected increase in production (million tons)	% production increase	Production (million tons)	pesticide	Area
284	1040	0.273	0.243	47	0.516	38	1.359	M.a Isolate(1)	Ismalia
362	1040	0.348	0.223	39	0.571	42	1.359	M.a Isolate(1)	Noubaria
452	1040	0.435	0.095	18	0.530	39	1.359	M.a . Isolate(3)	Ismalia
516	1040	0.496	0.061	11	0.557	41	1.359	M.a . Isolate(3)	Noubaria

Source: computed and collected from FAO and Tables 6 & 7.