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Isolation, Production and Use of Entomopathogenic Fungi for Controlling the Sugar Beet Insect Pests in Egypt

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ABSTRACT

Out of 1491 soil samples from different governorates, *i.e.,* Giza (418 samples), Dakahlyia (683 samples) and Kafr El-Sheikh (390 samples) only 16 samples (1.07%) contained entomopathogenic fungi, *i.e.,* 9 isolates from *Beauveria bassiana,* 10 isolates from *Metarhizium anisopliae*, and 5 samples from *Paecilomyces lilacinus.* The fungi were propagated on PDA medium fortified with nutritional sources of plant origin, e.g., crushed grains / seeds or molasses. The addition of 100g crushed rice grains/1L of PDA gave the highest conidiospore production by *Beauveria,* crushed wheat grains for *Metarhizium* and crushed corn for *Paecilomyces.* Bioassay for determination of LC_{50} values took place versus larvae of the greater wax moth, *Galleria mellonela.* Conidia were prepared in EC formulation for field application at the concentration of $1x10^6$ spores/ml. Results showed successful control against larval population of the cotton leafworm, *Spodoptera littoralis,* the beet worm, *S. exigua,* the semi-loopers, *Autographa gamma, A. circumflexa,* and *A. ni.* Although the laboratory treatment resulted high mortality rates, but it was not successful in the field when sprayed against immature stages and adults of the green stink bug, *Nezara viridula,* and the tortoise beetle, *Cassida vittata,* jassids and white fly inhabiting the lower leaf surfaces where they escape the sprayed material. Also, boring larvae of the beet moth, *Scrobipalpa ocellatella,* and the European corn borer, *Ostrinia nubilalis* as well as larvae of the beet leafminer fly *Pygomia hyoscami* were not significantly affected by the sprayed conidiospores for the same reason. Meanwhile, soil treatment with formulated granules applied as beat controlled nymphs and adults of the gryllotalpid mole cricket, *Gryllotalpa gryllotalpa* successfully.

Key Words: Sugar beet, *Beauveria, Metarhizium,* insect pests, microbial control

INTRODUCTION

As an alternative to chemical control or as part of IPM programs, there is a resurgence of interest in the use of microbial insecticides for biological control of insect pests (Castillo *et al.*, 2000). Particularly, the entomopathogenic fungi have long been known to cause epizootics among certain insects under both laboratory and field conditions (Barson *et al.*, 1994; Watson *et al.*, 1996; and Reithinger, 1997). Traditionally, chemical pesticides control the sugar beet insect pests in Egypt. The sugar beet planted area is expanding and new fabrics for production of the beet sugar are constructed in the new cultivated areas. A project for controlling the sugar beet insect pests with entomopathogenic fungi was funded by the Ministry of Agriculture in 2001 contributing to the policy in supporting IPM strategies in different economic crops.

The present paper represents the obtained results in this project concerning isolation of entomopathogenic fungi from soil samples as well as from naturally infected insects. Also, PDA medium was improved by adding different crushed grains and seeds to reach the highest production of conidiospores. The produced conidia of *Beauveria bassiana* and *Metarhizium anisopliae* were applied in 5 sprayings in sugar beet fields at seasons 2002 and 2003 at Sakha, Kafr El-Sheikh governorate.

MATERIALS AND METHODS

Isolation of Entomopathogenic Fungi

1. Isolation from soil samples

1491 soil samples were collected from Giza (418 samples), Kafr El-Sheikh (390 samples), and Dakahlia (683 samples) for trapping the entomopathogenic fungi following the technique created by Zimmermann (1986) using larvae of the greater wax moth, *Galleria mellonella* L. as highly sensitive trapping insect for pathogenic fungi and entomogenous nematodes. Also, trapping with larvae and adults of the flour beetle,

Tribolium confusum was followed.

2. Isolation from naturally infected insects

Insect pests were observed under field conditions by direct inspection to detect any suspected mycosis, and then transferred to the laboratory for isolation, identification and bioassay versus larvae of the greater wax moth, *G. mellonella* by direct spray test.

3. Production of the Entomopathogenic Fungi

All the isolated fungi were primarily purified using the mono-spore technique. They were propagated in Petri-dishes (10 cm ø) on potato dextrose agar medium (PDAM) enriched with 1% peptone, 4% glucose and 0.2%yeast and incubated at 26 °C. Seven –days old cultures with well developed spores were harvested by washing with 10 cc sterilized distilled water + 0.03% Tween-80 and used as stock suspension with known spore count and kept in refrigerator at 4 °C, from which the fungi were sub-cultured or produced for laboratory evaluation tests (infectivity and bioassay tests) or adjusted in conidiospore concentration of $1x10^6$/ml and mixed with 1% sunflower oil for field application using the spraying technique. Large amounts of conidiospores were produced by culturing on liquid medium (Rombach *et al.*, 1988) in conical flasks or in 1L cell culture glass bottles or on rice grains in 0.5 kg marmalade jars.

4. Trials for Developing Modified Medium for High Conidia Production

Aim of these experiments is to increase the production of spores usually harvested from a certain surface area of the standard Potato-Dextrose-Agar-Medium (PDAM). Thus, PDAM was fortified with additional nutritional sources of plant origin *i.e.,* soy been powder, crushed grains of corn, wheat, barley, rice, and lentil or molasses. Each source was tested in two added amounts, *i. e.,* 50 and 100 g/L of PDAM. The tested media were poured in petri-dishs (Ø12 cm), and each was inoculated with $1x10^7$ conidiospores of the target fungus and incubated at 26°C; each treatment was replicated five times.

5. Bioassay Techniques

Seven concentrations in spore suspensions of 10, $1x10^2$, $1x10^3$, 1x104, 1x105, 1x106, and $1x10^7$ spores/ml were tested versus *G. mellonella* larvae of L_4 by direct spray technique using 4 replicates each of 25 larvae, and versus immature feeding stages of the sugar beet key insect pests in 4 replicates each of 20 individuals. LC_{50} and LC_{90} values were calculated.

2.5.1. Standard Bioassay Technique

Evaluating the entomopathogenicity of the isolated fungi could be determined through different methods. The technique developed by Ignoffo (1984) and modified by the Station de lutte biologique, La Miniere, France, was followed in the present studies. It depends on placing and distributing a known dose of conidiospores on plant leaves or diet cut into rounded discs of known surface, and confined in deep suitable wells bored in a plate of plexy-glass material of 1 cm in thickness. After setting the test, the plate is covered with another perforated one of 2 mm in thickness. Two days later, the test insects were offered daily-untreated diet, and mortality rates were, also, recorded daily for 2-3 weeks.

2.5.2. The Spraying Technique

Using a small hand atomizer, the spore suspensions are directly sprayed onto larvae (L_4) of the greater wax moth, *G. mellonella* as test insect. Small perfume atomizers were used to apply the desired spore suspension for the laboratory bioassay tests.

2.6. Field Application Techniques and Dates

One feddan planted with sugar beet was available at Sakha Experiment Station, Kafr El-Sheikh governorate in season 2002, and half feddan in season 2003 beside 8 feddans in Fayoum in season 2003 to carry out the first application trials for controlling the sugar beet insect pests with the entomopathogenic fungi. Twelve visits to the experimental fields in Sakha were arranged from the beginning of 7/11/2001 to 11/5/2002 for monitoring the populations of the different insect pests on sugar beet leaves. During this period, 5 applications by different spraying techniques were carried out as follows:

Date	Spraying apparatus	Fungi ($1x10^6$/ conidia/ml)
7/11/2001	5 L – Sprayer	*Beauveria bassiana* (Nr.6)
25/3/2002	5 L – Sprayer	*Beauveria bassiana*
01/4/2002	10 L – Motor	*Beauveria bassiana*
13/4/2002	10 L – Motor	*Metarhizium anisopliae*(Nr.6)
20/4/2002	600 L – Motor	*Beauveria bassiana*

The first application occurred using a 5L – sprayer to insure spaying of both upper and lower surfaces of the sugar beet plants, where most of the insects were found on lower surfaces of leaves. This technique was very difficult and time consuming, accordingly it is not applicable for large areas of the crop. The third and fourth applications were carried out using a 10L – motor sprayer, aiming that the pressure carrying out the sprayed spore suspension could bend the sugar beet leaves and reach the insects on the lower surfaces of leaves. It was; also, time consuming as we use 200 L of water/feddan. Thus, a 600L–motor was used for the last spraying, which was the practical technique to overcome the previously mentioned difficulties concerning time and effort.

In the last season 2002/03, field application of the entomopathogenic *M. anisopliae* isolate M8D and *B. bassiana* isolate B7D as effective isolates were prepared for application in the two governorates, *i.e.* Kafr El-Sheikh and El-Fayoum. In the first governorate, 0.5 feddan was rent from the farm of Family Mesbah, and 2 feddans from the family Azzam in the second governorate. The experimental field in Fayoum governorate had an area of 8 feddans; one feddan was treated with the isolate M8D of the entomopathogenic fungus *M. anisopliae* and another feddan with the isolate B7D of *B. bassiana* isolated from soil samples collected from Dakahlia governorate. Five feddans were traditionally treated with chemical pesticides, and one feddan was left without any treatment as a control check. Five samples each of 20 plants were inspected per feddan in the four fields to test the microbial control of the sugar beet insect pests under the different weather conditions in these two governorates where the dominant insect pests are also different. Usually, the stink bug *Nezara viridula,* the tortoise beetle, *Cassida vittata,* and the European corn borer, *Ostrinia nublalis* are dominant in Kafr El-Sheikh; while the sugar beet moth, *Scrobipalpa ocellatella* is the main pest on sugar beet in Fayoum. Meanwhile, the cotton leafworm, *Spodoptera littoralis* and the beet worm, *S. exigua* are widely spread in both governorates. Applications with the entomopathogenic fungi in Fayoum fields occurred, also, with the concentration of 1×10^6 conidiospores/ml. Four applications were carried out on January 25th, 2003; March 1st, 2003; March 14th, 2003 and March 20th, 2003.

Monitoring of all insect pests in the crop was done regularly in the experimental fields in both governorates to determine the desired population for the control action using the entomopathogenic fungi. Suppression or control rates were calculated according to the formula of Hendreson and Tilton (1955).

RESULTS AND DISCUSSIONS

1. Isolation of Entomopahogenic Fungi

As seen in Table (1), recovery percentages for the fungus *B. bassiana* from soil samples were 0.47, 0.29, and 0.25%; for *M. anisopliae* 0.47, 0.29, and 0.51%; and for *P. lilacinus* 0.47, 0.29, and 0.25% among those collected from Giza, Dakahlia, and Kafr El-Sheikh governorates, respectively. The total recovery percentages for the three successive fungi were 33, 42, and 33%, representing a total recovery rate of 1.07% from all collected soil samples.

Table (1): Numbers of positive entomopathogenic fungi recovered by trapping with larvae of *G. mellonella* from soil samples collected from three governorates and their rates of incidence (%).

Locality	Number of samples	Fungi positive samples and incidence (%)		
		Beauveria	*Metarhizium*	*Paecilomyces*
Giza	418	2 (0.47%)	2 (0.47%)	2 (0.47%)
Dakahlia	683	2 (0.29%)	2 (0.29%)	2 (0.29%)
Kafr El-Sheikh	390	1 (0.25%)	2 (0.51%)	1 (0.25%)
Total	1491	5 (0.33%)	6 (0.42%)	5 (33%)
	16 (1.07%)			

According to recorded isolates in our laboratory collection, there were four isolates of *B.bassiana, i.e.,* B-1, B-2, B-3, and B-4. Thus, the present isolates of *B. bassiana* were coded as B5G and B6G from Giza; B7D and B8D from Dakalia; and B9K from Kafr El-Sheikh. Similarly, the new isolates of *M. anisopliae* were

coded as M5G and M6G from Giza; M7D and M8D from Dakahlia; and M9K and M10K from Kafr EL-Sheikh. Isolates of *P. lilacinus* were coded as P1G and P2G from Giza; P3D and P4D from Dakahlia; and P5K from Kafr El-Sheikh.

2. Isolation from Insects

During soil sampling for larvae and pupae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) from Egyptian clover fields, *Trifolium alexandrinum* L. at Giza governorate in March 2002, two adult specimens of the mole cricket, *Gryllotalpa gryllotalpa* L. were found dead with mycosis in tunnels under soil. One was showing the typical developed appearance of the white muscardine disease isolated and identified as *B. bassiana* (Bals.)Vuillemin, and coded as isolate Nr.A-101; and the other showed that of the green muscardine caused by the entomopathogenic fungus *M. anisopliae* var. *niger* (Metsch.) Sorokin that was coded as isolate Nr.B-101.

The entomopathogenic fungus *B. bassiana* was isolated and laboratory tested or applied in the field for controlling different insect pests (Kowalik and Stefaniak, 1986; El-Safty and Borai, 1987 El-Husseini *et al.*, 1996; Abou Bakr, 2002; Agamy, 2002)

3. Modified Media for Higher Conidia Production

The addition of nutritional sources of plant origin in form of crushed grains or seeds to the standard PDA medium gave interesting results concerning the highest number of produced conidiospores as presented in Table (2). *B. bassiana* favored production on PDAM + 100g crushed rice grains (/L) which recorded the highest quantity of spores (0.332 x 10^9 spores/dish); *M. anisopliae* on PDAM + 100g/L crushed wheat grains (13.398 x 10^9 spores/dish); and *P. lilacinus* on PDAM +100g/L crushed corn (8.546 x 10^9spores/dish).These results agree with the conclusion of Li and Holdan (1990) that carbon, nitrogen and vitamin sources influence the growth and sporulation of *M. anisopliae.*

Table (2): Mean numbers of conidiospores (x 10^9) produced on PDAM fortified with different nutritional plant sources in petri-dishs (Ø cm) inoculated with 1x 10^7 conidia.

Added material	*Beauveria bassiana*			*Metarhizium anisopliae*			*Paecilomyced lilacinus*		
	PDA	T1	T2	PDA	T1	T2	PDA	T1	T2
Soybean powder	0.112	0.098	0.670	3.568	3.636	4.505	0.520	1.940	3.138
Crushed corn	0.112	-	-	3.542	8.672	10..528	0.521	5.032	8.546
Crushed wheat	0.112	-	-	3.626	8.432	13.398	0.511	5.290	7.946
Crushed barley	0.112	-	-	3.618	7.622	11..942	0.514	5.270	7.598
Crushed lentils	0.112	-	-	3.756	5.206	6.094	0.516	2.176	2.990
Crushed rice	0.111	0.203	0.334	3.667	4.092	7.294	0.514	2.914	4.788
Molasses	0.112	-	-	3.608	5.280	4.664	0.513	0.651	0.590

T1 = 50 g, T2 = 100g /L

4. Bioassay of isolates

Selected isolates were bioassayed versus lavae (L_4) of the greater wax moth , where calculated LC_{50} and LC_{90} values are presented in Table (3).

Bioassay studies resulted different values in the LC_{50} and LC_{90} between the three genera of the isolated entomopathogenic fungi, *i. e., Metarhizium*, *Beauveria*, and *Paecilomyces*. But no significant differences appeared between efficacy of isolates of the same fungus, except in case of M8D that showed more efficacy at both levels of LC_{50} and LC_{90} and selected for one application in the season. Hluchy (1989) reported similar results, while Draganova (1998) recorded higher efficacy for blast-spores than using conidia when bioassayed versus *G. mellonella* larvae (L_3).

As these results show nearly no differences between the efficacy of the isolates of the same entomopathogenic fungus species versus larvae of the wax moth, *G. mellonella.* It is needed to observe any difference between isolates of the same fungus using electrophoretic techniques for protein or DNA identification.

Table (3): Values of LC_{50} and LC_{90} for the selected entomopathogenic isolates

The fungus	Isolates	LC_{50}	LC_{90}
M. anisopliae	M7D	2094.484	11154E+4
	M8D	1671.687	73354E+3
	M9K	2023.434	23634E+4
B. bassiana	B7D	20787.506	11085E+6
	B8D	25521.384	59289E+5
	B9K	29950.157	57983E+6
P. lilacinus	P7D	14251E+4	19583E+15
	P8D	18293E+4	23037E+15
	P9K	20857E+4	69819E=15

5. Results of Controlling Sugar Beet Insect Pests by Entomopathogenic Fungi

Monitoring of the key pests in sugar beet experimental fields was carried out on regular base at random samples of 20 plants from the experimental plots just before spraying with the isolate and on another 20 plants from the untreated control. The Formula of Hendreson and Tilton (1955) was used to calculate the reduction rates among populations of the target sugar beet pests in the field after application with the entomopathogenic fungi.

5.1.The Cotton Leafworm, *Spodoptera littoralis* (Boisd.)

Data presented on Fig. (1) showed that the 1st application with *B. bassiana* (isolate B7K) decreased the population of *S. littoralis* by 50% after 20 days from application among the so called autumn generation, where the plant leaves are still small in size. During the late winter and early summer generations, four successive applications with one-week intervals were sprayed. The second *B. bassiana* application (25/3/2002) caused reduction of 63.6% among larvae of *S. littoralis* (Fig.1) one week after application that remained stable after the third application (1/4/2002). Using *M. anisopliae* in the fourth application (13/4/2002) decreased larval population by 62.5%, relatively the same effect of *B. bassiana*. The 5th application was not necessary against the cotton leafworm. Many authors recorded successful laboratory control tests with this two fungi against *Spodoptera* spp., *e.g.*, Fargues and Rodrigues-Rueda (1980), Maniana and Fargues (1984), Rajak *et al.* (1990), Hicks *et al.* (2001), and Aponte and Uribe (2001) as well as in field applications, *e.g.*, Kowalik *and* Stefaniak (1986); Siddaramaiah *et al.*(1986) Chen *et al.* (1988) and El-Husseini *et al.*(2003).

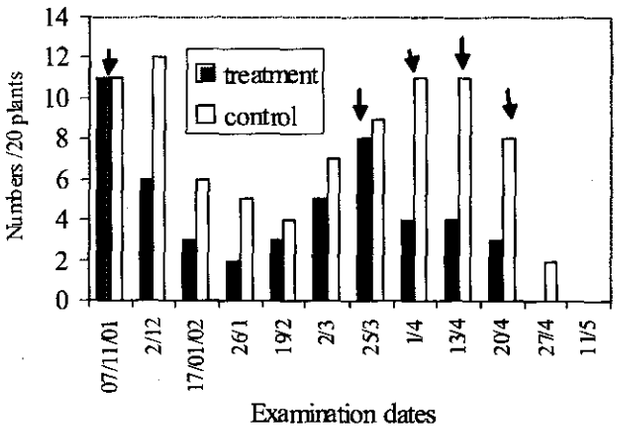

Fig.(1): Numbers of cotton leaf worm larvae, *Spodoptera littoralis* after 5 sprays (arrows) with fungi.

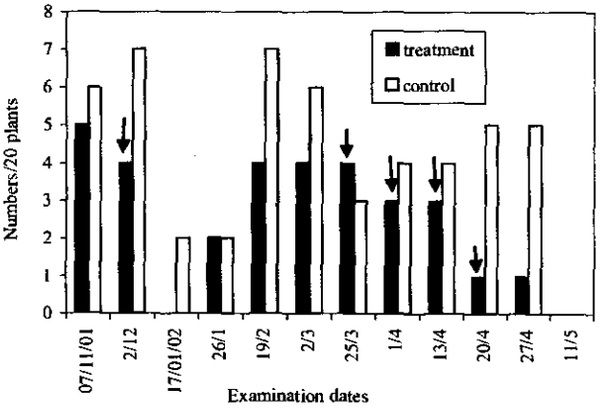

Fig. (2): Numbers of semi-loppers larvae after 5 sprays (arrows) with fungi.

5.2. The Semi-looper Pests:

They belong to more than one species, *i.e., Trichoplusia ni, Syngrapha circumflexa,* and *Autogapha gamma;* which are not differentiable from each other during the larval stage. They are considered here as one group of larvae: "semi-loopers". As seen in Fig.(2), the 1st spraying (7/11/2001) with *B.bassiana* resulted a control rate of 42.8%.

Results of controlling the semi-loopers with fungi in sugar beet, Sakha, Kafr El-Sheikh, season 2001/02 recorded 42.8%, 20 days after treatment among the young larvae (L_1-L_3). Meanwhile, the 2nd application (25/3/2002) caused only 25% reduction among treated larval population that was more developed (L_3-L_5) than at the time of the 1st application. This control rate (25%) remains also the same after the 3rd

5.3. The European Corn Borer, *Ostrinia nubilalis* (Hbn.) :

In the last few years, infestation with the European corn borer was noticed in sugar beet fields. It is a major pest on corn and few other crops; and is of a potential economic damage for sugar beet in the future. The feeding larvae damage the base of core leaves, and the wounds spill large amounts of sap allowing growth of the black fungus to grow in the heart of the sugar beet plant. The feeding location of larvae inside tunnels in the leaf stem protects them from contact with the sprayed conidiospores of the entomopathogenic fungi. Fig. (3) presents the results obtained from the field experiment in Sakha, Kafr El-Sheikh. All applications with both *B. bassiana* and *M. anisopliae* fungi caused no acceptable difference among larval population of *O. nubilalis* between treated and untreated control plots. Phoofolo *et al.* (2001) recorded between 0 – 21% control in corn fields. Meanwhile, the present results are completely the opposite of that presented for the laboratory bioassay experiments showing the high susceptibility of this pest to the tested entomopathogenic fungi (Feng *et al.* 1985; Kagan and Uhlik, 1999 Agamy, 2002 and El-Husseini *et al.*, 2003).

5.4. Sugar-Beet Moth, *Scrobipalpa ocellatella* (Boyd.)

Field results in relation to application of *B. bassiana* and *M. anisopliae* presented in Fig.(4) showed low efficacy of treatments against the larvae of *S. ocellatella;* being little bit less in the treated than in the control plots after 20 days post the 1st treatment ($1x10^6$ spores/feddan)and all over the season.

Such low efficacy in field application could be attributed – as in case of *O. nubilalis* – to the factor that feeding location of larvae in tunnels inside the leaf stem protects them from contacting the fungal spores of the sprayed material.

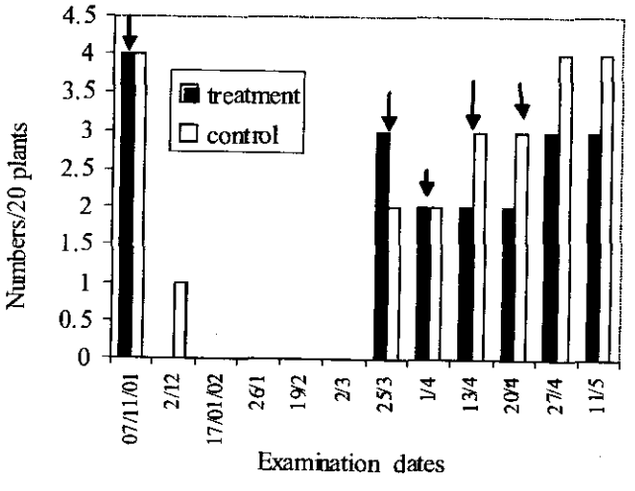

Fig.(3): Numbers of European corn borer larvae, *Ostrinia nubilalis* after 5 sprayings with fungi.

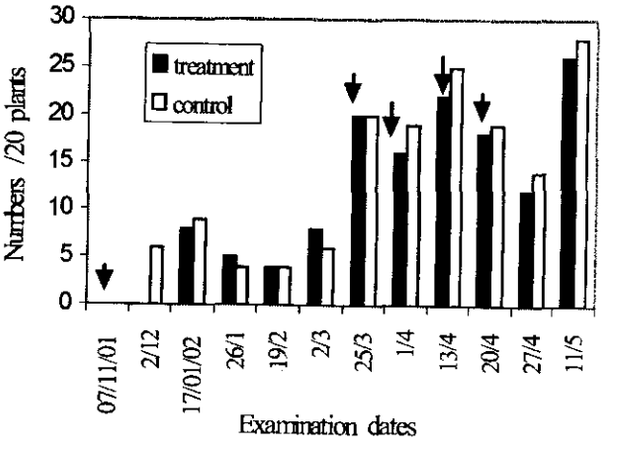

Fig. (4):.Numbers of sugar beet moth larvae, *Scrobipalpa ocellatella* after 5sprays (arrows) with fungi

5.5. The Green Pentatomid Bug, *Nezara viridula* L.

Although *N. viridula* had showed high susceptibility to *B.bassiana* El-Zoghby (2003) and *M.anisopliae* in laboratory tests when directly sprayed with conidiospore suspensions (as in case of *O. nubilalis* and *S. ocellatella*), on the other hand, results of the field application presented in Fig.(5) showed no such high efficacy. The latter result might be firstly due to the mobility of nymphs and adults from one side; and

secondly, the presence of both feeding stages (nymphs and adults) of the pest on the under side of plant leaves. Thus, the insects do not receive or be contacted with enough conidiospores from the sprayed suspensions.

Fig. (5) showed a reduction rate in numbers of the green pentatomid bug 20 days after application with $1x10^6$ conidia/ml from *B. bassiana*. The pest population increased in March, and the 2nd application led to a reduction rate of 32.3% among individuals (nymphs and adults) one-week post treatment. The 3rd application recorded a reduction rate of 50%. When *M. anisopliae* was applied, it caused 55% reduction after one week.

Using the high volume spraying (600 L/feddan) by the 5th application, higher reduction rate of 77% was recorded one week after application (Fig.5). Thus, in the field, entomopathogenic fungi showed a moderate control level against nymphs and adults of *N. viridula* ranging between 8.3 and 77% according to spraying technique and frequency of the fungi application.

Leite *et al.* (1987) obtained 66.7% mortality in the laboratory when nymphs of L_5 were sprayed with $1x10^7$ spores/ml 14 days post treatment associated with LT_{50} of 10.7 days. Meanwhile, Soza-Gomez and Moscardi (1998) recorded LT_{50} of 13.9 days and stated that, due to the low mortality rates obtained in the field, management of pentatomid adults using *B. bassiana* could be difficult. Also, Shimaxu *et al.* (1994) recorded 45-65% mortality among nymphs of L_2 treated with *B. bassiana*. Also, El-Zoghby (2003) reported high efficacy with *B. bassiana* against this pest in laboratory tests. Although strains or isolates of *B. bassiona* virulent to *N. viridula* were selected by different authors, their efficiency in applications under the field conditions remains to be improved.

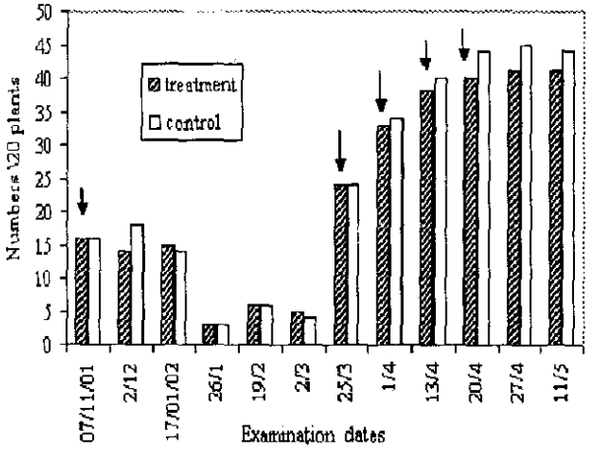

Fig.(5): Numbers of *N. viridula* (nymphs and adults)/20 sugar beet plants after 5 sprays (arrows) with *B. bassiana* (1x106 spores/ml).

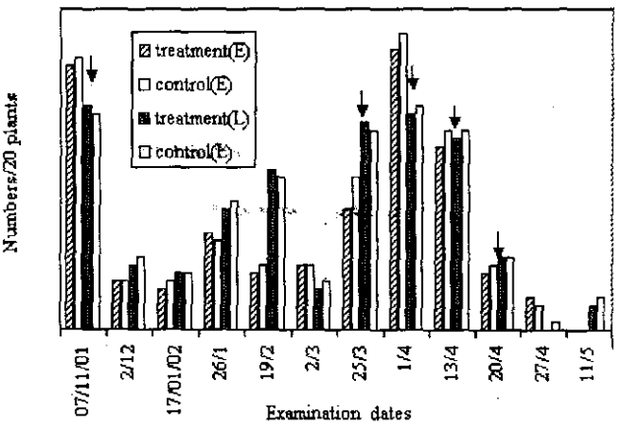

Fig. (6): Numbers of eggs (E) and larvae (L) of the sugar beet leaf miner, *Pygomia hyoscami* after 5 sprays with *condiospores*.

5.6. The Leafminer Fly, *Pegomya hyoscami* Panz.

As seen in Fig. (6), the fungi did not affect this pest. The presence of larvae inside the leaf tissues protects them from getting in contact with the sprayed conidiospores of both *B. bassiana* and *M. anisopliae*. Results revealed, also, no effect on eggs of the fly after each of the five sprayings with the fungi. Meanwhile, a very slight reduction of 11% was recorded among the larvae 20 days post the 1st treatment with *B. bassiana;* followed by 3, 5, zero, and 11% one week after the 2nd, 3rd, 4th, and 5th applications, respectively. Thus the use of entomopathogenic fungi is yet not effective in controlling eggs and larvae of the sugar beet leafminer, *P. hyscami*

5.7. The Tortoise Beetle, *Cassida vittata* Vill.

The tortoise beetle, *C. vittata* became a major pest on the newly introduced sugar beet crop in Egypt (Ebieda *et al.*, 1996) that traditionally controlled by chemical insecticides (Saleh, 1994a and 194b; Ayala and Dominguez, 1996). It is believed that the intensive use of chemical insecticides in this crop had negatively suppressed the population of its natural enemies, especially its trichogrammatid egg parasitoid *Monorthochaeta nigra* Blood and Kryger reported by Awadalla (1996). No records for biological control measurements in sugar beet are available against this pest. Thus, the present results could be considered as first record for microbial control of *C. vittata* in sugar beet. As seen in Fig.(7a), the use of entomopathogenic fungi, *B. bassiana* and *M. anisopliae* sprayed in the field had resulted unsatisfactory control of the beetles eggs, because they were laid on the underside of the leaves, and accordingly they did not receive enough

from the sprayed conidiospores. The reduction in eggs found on the treated sugar beet plants One week after the 2nd application with *B. bassiana* recorded only 7.8% that increased to 18% after another spraying. The spraying of *M. anisopliae* (at 13/4/2002) induced only 4.1% reduction after one week.

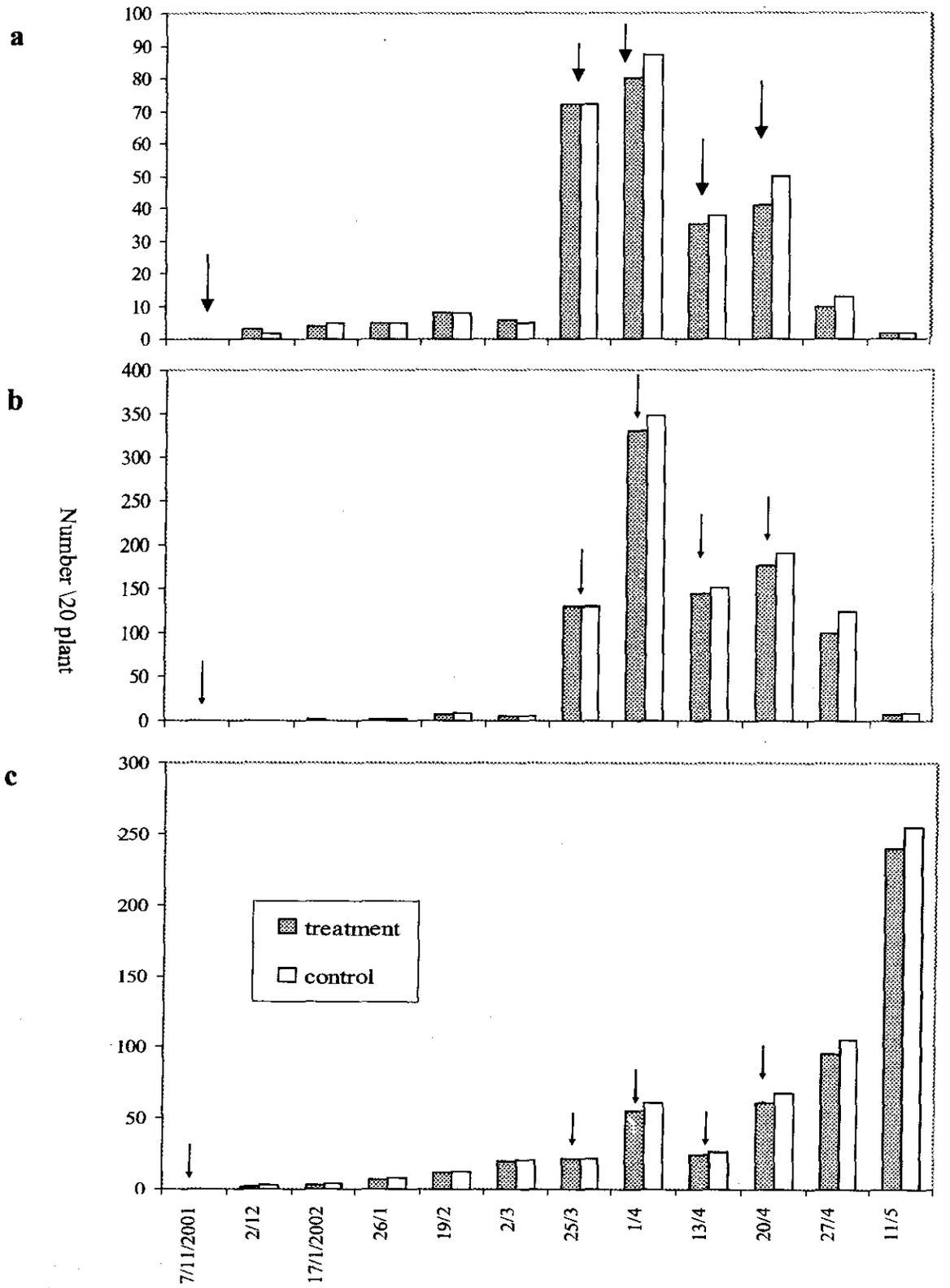

Fig.(7): Numbers of *Cassida vitata* after 5 sprays (arrows) with fungi.
a) Eggs, b) Larvae and pupae, and c) Adults

Concerning the control of *C. vittata* larvae and pupae (Fig.7b), the reduction rates recorded one week post treatment with two successive spraying of *B. bassiana* were 5.1 and 4.6%, followed by 7.3% after treatment with *M. anisopliae* that increased to 20% after an additional treatment (at 20/4/2002) with *B. bassiana*. Also, because their presence on the underside of the leaves, the sprayed conidiospores did not reach them at this location as in the aforementioned case of the eggs.

Although adults of *C. vittata* are mobile than the larvae, but they prefer feeding on the underside of the leaves. Thus, it could be expected that the treatment with fungi using the spraying technique whatever the machine used, may explain the obtained low reduction rate among population of the adult beetles (Fig.7c). Reduction rates of 7.4, 10.2, 9.5, and 5.8% were recorded one week after 4 successive applications with *B. bassiana* and *M. anisopliae* at the mentioned spraying dates.

Although the laboratory bioassay of tested fungi proved highly infective to larvae, pupae and adults of *C. vittata*, the application in the field was not successful (El-Husseini *et al.*,2003). The main two reasons for this result are the location of the different insect stages on the underside of leaves; and that the spraying technique with the different spraying apparatus and motors whatever in low or high volume sprayings is not proper to reach the underside of the leaves. Thus, the dusting technique by which the dusted material could easily be carried by air reaching the underside of leaves and accordingly contaminating the insects found there might be the proper application technique for fungi conidiospores in the sugar beet crop simulating what is really happening in nature for infection with fungi spores.

6. The Yield in the Experimental Field

Although the crop received no chemical insecticide treatment, but only 5 treatments with entomopathogenic fungi (*B. bassiana* and *M. anisopliae*), the harvested yield in Kafr El-Sheikh experimental field was equal to that of the traditional fields at Sakha Experiment Station(12 tons/feddan). Moreover, size of the sugar beet roots was extremely larger than in other fields.

Comparing the yield in the fields treated with the entomopathogenic fungi to those traditionally treated with the recommended chemical insecticides in Fayoum, an increase of one ton/feddan was reached in fields of the microbial pest control as presented in Table (4). The biological control fields yielded 13 tons/feddan compared to the chemical pesticide treatment that yielded 12 tons/feddan. Meanwhile, the untreated control field yielded only 10 tons/feddan.

Table (4): Yield of sugar beet roots (tons/feddan) in the three treatments.

Metarhizium anisopliae	*Beauveria bassiana*	Chemical control	Untreated control
13 tons	13 tons	12 tons	10 tons

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REFERENCES

Abou Bakr, H. E. 2002. First isolation of *Beauveria bassiana* (Bals.) Vuillemin and *Metarhizian anisopliae* (Metch.) Sorokin from naturally infected mole cricket, *Gryllotalpa gryllotalpa* L. (Orthoptera: Gryllotalpidae) in Egypt. Egypt. J. Biol. Pest Cont, 12(1): 63-66.)

Agamy, E. A. 2002. Entomopathogenicity of *Beauveria bassiana* (Bals.) Vuillemin to early larval instars of the European corn borer, *Ostrinia nubilalis* (Hbn.) (Lepidoptera: Pyralidae). Egypt. J. Biol. Pest Control, 12(1): 67-70.

Aponte,L. and D. Uribe 2001. Insecticidal activity of native strains of *Beauveria* spp. and *Metarhizium* spp. against second instar larvae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Revista Colombiana de Entomologia, 27(1-2):67-72.

Awadalla, S. S. 1996. Biological studies on *Monorthochaeta nigra* Blood and Kryger (Hymenoptera: Trichogrammatidae) as egg parasitoid on the tortoise beetle, *Cassida vittata* Vill. (Coleoptera: Chrysomelidae). J. Appl. Entomol., 120(6):353-355.

Ayala, J. and M. Dominguez 1996. Evaluation of insecticide action against *Cassida vittata* (Vill.) (Cleoptera: Chrysomelidae) during the first growth stage of autumn-sown sugar beet. Boletin de Sanidad Vegetal, - Plagas., 22(4):653-657.

Barson, G.; N. Renn; and A. F. Bywater, 1994. Laboratory evaluation of six species of entomopathogenic fungi for the control of the house fly (*Musca domestica),* a pest of intensive animal units. J. Invertebr. Pathol., 64:107-113.

Castillo, Maria-Angeles, P. Moya; E. Hernandez, and E. Primo-Yufera, 2000. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. Biological Control, 19:274-282.

Chen, W. S.; C. C. Tu; and F. I. Cahng 1988. Ecological studies on the new pests of asparagus. Asparagus Res.Newl., 6(1):107-111.

Draganova, S. A. 1998. Virulence of blastospores and conidia of *Beauveria bassiana* (Bals.) Vuill. strains against *Galleria mellonella* L. and *Leptinotarsa decimliniata* Say. Bulgarian J. Agric. Sci., 4(3):287-292.

Ebieda,A. M. M.; M. A. Hassanein, and A. G. Solouma 1996. Studies on sugar beet pests. IV. Biophysiological parameters and new approaches in tortoise beetle, *Cassida vittata* Vill. and beet fly *Pegomya mixta* Vill. Ann. Agric. Sci., Moshtohor, 34(3):1213-1228.

El-Husseini, M. M.; H. E. Abou Bakr, and E. A. Agamy 1996. Could isolation of white muscardine, *Beauveria bassiana* from the hairy rose beetle, *Tropinota squalida* Scop. (Col.: Scarabaeidae) be integrated in control programs in Egypt?. Egypt. J. Biol. Pest control, 6(1): 105-109.

El-Husseini, M. M.; A. H. Mesbah; Amal El-Zoghby and E. A. Agamy 2003. Isolation, propagation and application of entomopathogenic fungi for controlling noctuid and cassidid pests in sugar beet. Egypt. J. Biol. Pest Cont, 13(1&2):95-100.

El-Safty, R. and H. A. Borai 1987. The fungus *Beauveria bassiana* (Balsamo) Vuillemin, a natural pathogen for diapaused adults of *Hypera brunneipennis* (Boheman) (Col.: Curculionidae) in Egypt. Bull. Soc. Entomol. Egypte, 67:141-150.

El-Zoghby, Amal, A. 2003. Studies for Using *Beauveria Bassiana* (Bals.) Vuillemin In Controlling The Green Sting Bug, *Nezara viridula* L. (Hymenoptera: Pentatomoidae) In Sugar Beet Plantations In Egypt. Egypt. J. Biol. Pest Cont 13(2) (In press).

Fargues, J., and D. Rodrigues-Rueda 1980. Sensibilité des larves de *Spodoptera littoralis* (Lep.: Noctuidae) aux hyphomycétes entomopathogènes *Nomuraea rileyi* et *Paecilomyces fumosoroseus.* Entomophaga, 25:43-54.

Feng,Z.; R. I. Carruthers; D. W. Roberts; and D. S. Robson 1985. Age-specific dose-mortality effects of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) on the European corn borer, *Ostrinia nubilalis* (Lepidoptera:Pyralidae). J. Ivertebr. Pathol., 46(3):259-264.

Hendreson, C. G. and E. W. Tilton 1955. Tests with acaricides against the brown wheat mite. J. Econ. Entomol., 48: 157-161.

Hicks, B. J.; A. D. Watt,; and D. Cosens 2001. The potential of *Beauveria bassiana* as a biological control agent against the pine beauty moth, *Panolis flammea* (Lepidoptera:Noctuidae).Forest Ecology and Management, 149(1-3):275-281.

Hluchy, M., Samsinakova. 1989. Comparative study on the susceptibility of adult *Sitophilus granarius* (L.) and larval *Galleria mellonella* L. to the entomogenous fungus *Beauveria bassiana* (Bals.) Vuill., J. Stored Products Res.,25(3):61-64.

Kagan, L. and V. Uhlik 1999. Pathogenicity of *Beauveria bassiana* strains isolated from *Ostrinia nubilalis* Hbn. (Lep.: Pyralidae) and to ladybirds (Col.: Coccinellidae). Plant Protection Science, 35(3):108-112.

Kowalik, J. Napiorkowska and Stefaniak, Z. Machlowicz 1986. Evaluation of parasitic insects and fungi in the regulations of noctuid larvae (Lep.: Noctuidae) occurring in cabbage and sugar beet crops. Polskie Pismo Entomologiczne, 56(3):687-700.

Leite, L. G.; A. I. A. Fraga, and S. B. Alves 1988. Pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Paecilomyces* sp. to *Nezara viridula* L. Ecossistema, 12:20-40.

Li,D. P. and D. G. Holdom 1990. Effects of carbon and nitrogensources and vitamins on germination, growth and sporulation of *Metarhizium anisopliae* (Metch.) Sorokin. (Deuteromycotina: Hyphomycetes). Proc.Vth Inter. Colleq. on Invertebr. Pathol. & Microb. Control, Adelaide, Australia, 20-24 August, 1990, p. 154.

Maniana, N. K. and J. Fargues 1984. Specificity of entomopathogenic hyphomycetes to lepidopterous noctuid larvae. Entomophaga, 29(4):451-464.

Phoofolo, M. W.; J. J. Obrycki, and L. C. Lewis 2001. Quantitative assessment of biotic mortality factors of the European corn borer (Lep.: Crambidae) in Field corn. J. Econ. Entomol, 94(3):617-622.

Rajak, R. C.; S. S. Sandhu; A. R. Khan, and G. P. Agarwal 1990. Susceptibility of teak defoliator and teak skletonozer (Lep.: Noctuidae) to *Beauveria bassiana*: Effects of instar, dosage and temperature. Proc.Vth

Inter. Colleq. on Invertebr. Pathol. & Microb. Control, Adelaide, Australia, 20-24 August, 1990, p. 153.
Reithinger, R.; C. R. Davies; H. Cadena ; and B. Alexander 1997. Evaluation of the fungus *Beauveria bassiana* as a potential biological control agent against phlebotomine sand flies in Colombian coffee plantations. J.Invertyebr.Pathol.70: 131-135.
Rombach, M. C.; R. M. Aguda and D. W. Roberts 1988. Production of *Beauveria bassiana* in different liquid media and subsequent conidiatioin of dry mycelium. Entomophaga, 33(3): 315-324.
Saleh, A. Y. 1994a..The joint action of some organophosphorus insecticides against the tortoise beetle, *Cassida vittata* (Vill.). Assiut J. Agric. Sci., 25(4):191-196.
Saleh, A. Y. 1994b. Insecticide tests to control the tortoise beetle, *Cassida vittata* (Vill.) in sugar beet crops. *Ibid.* pp. 197-202.
Shimaxu, M.; R. Teixeira-Alves, and Kishino 1994. Investigation on entomogenous fungi in the Cerrado Region and their utilization for microbial control of pests. Relatorio tecnico do projecto nipo brasileiro de cooperacao em pesquisa agricola 1987-1992, No. 202-2104.
Siddaramaiah, A. L.; T. Narendrappa, and V. S. Panchabhavi 1986. Occurrence and severity of *Metarhizium anisopliae* on *Spodoptera litura* Fb. in groundnut fields. Current Research, University of Agricultural Sciences, Bangalor, 15(5-6):48-49.
Sosa-Gomez, D. R. and F. Moscardi 1998. Laboratory and field studies on the infection of stink bugs, *Nezara viridula, Piezodorup guildinii* and *Euschistus heros* (Hemiptera: Pentatomidae) with *Metarhizium anisopliae* and *Beauveria bassiana* in Brazil. J. Invertebr. Pathol., 71 (2): 115-120.
Wagner, B. L.and L. C. Lewis 2000.Colonization of corn, Zea mays, by the entomopathogenic fungus *Beauveria bassiana.* Appl. & Environ. Microbiol., 66(8);3468-3473.
Wallengren, H. 1930. On the infestation of *Pyrausta nubilalis* Hb. by *Metarhizium anisopliae* (Metch.) Sorokin. II. Intern. Corn Borer Invest. Sci., Rep. III, pp.64-73, Chicago, Illin., U.S.A.
Watson, D. W.; D. A. Rutz; and S. Long 1996. *Beauveria bassiana* and sawdust bedding for the management of the house fly, *Musca domestica* (Diptera: Muscidae) in calf hutches. Biological Control, 7: 221-227.
Zimmermann, G.1986. The "*Galleria* bait method" for detection of entomopathogenic fungi in soil. J. appl. Entomol., 102 (2): 213-215.

عزل وإكثار وإستخدام الفطريات الممرضة للحشرات لمكافحة الآفات الحشرية فى بنجر السكر

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جمعت ١٤٩١ عينة تربة من محافظات الجيزة (٤١٨ عينة)، الدقهلية (٦٨٣ عينة)، كفر الشيخ (٣٩٠ عينة) حيث نجحت إجراءات العزل والتعريف للفطريات الممرضة للحشرات في ١٦ عينة تمثل ٠٧و١% من إجمالى العينات، عبارة عن ٩ عزلات من الفطر *Beauveria bassiana* ، ١٠ عزلات من الفطر *Metarhizium anisopliae* ، ٥ عزلات من الفطر *Paecilomyces lilacinus* . كما أدخلت إضافات نباتية على بيئة هلام البطاطس والدكستروز (PDA) لتختص كل منها بإنتاج أعلى كمية من الجراثيم الكونيدية لكل جنس من هذه الفطريات.وإستخدمت برقات دودة الشمع الكبيرة فى التقديرات الحيوية(bioassay) لتحديد الجرعات القاتلة النصفية(LC_{50})، وبناء عليه جهزت مستحضرات زيتية (EC) من الجراثيم للرش فى الحقل بتركيز ١ x ١٠^{٦} جراثيم/سم^{٣}. أظهرت النتائج الحقلية نجاح مكافحة كل من برقات دودة ورق القطن، الدودة الخضراء، والديدان نصف القياسة بالفطريات *Beauveria* ، *Metarhizium* ، وعدم فعالية الرش فى مكافحة الآفات التى تتواجد على السطح السفلى للأوراق بأطوارها المختلفة مثل الخنفساء السلحفائية، البقة الخضراء، والجاسيد، والذبابة البيضاء علما بأن الإختبارات المعملية قد أظهرت حساسيتها العالية للإصابة بهذه الفطريات. وأيضا، لم يكن الرش فعالا فى مكافحة الآفات الحشرية التى تسلك برقاتها مسلك الثاقبات وهى دودة فراشة البنجر، ودودة الذرة الأوروبية. إلا أن إستخدام الفطريات فى صورة محببات (granules) كطعوم لمعاملة التربة قد أدت للنجاح فى مكافحة الحفار.(الدراسة ضمن مشروع بحثى ممول من وزارة الزراعةالمصرية).