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# Potency of *Metarhizium anisopliae*, and *Beauveria bassiana* as a biocontrol on the sugar– beet Rib miner, *Scrobipalpa ocellatella* boyd. at Egypt

**Abdel-Raheem M A[1], M M Ismael[2], Huda I Abdel-Aliem[2]**
[1] Department of Pests & Plant Protection, Agricultural and Biological Research Institute, National Research Centre, Dokki, Giza, Egypt
[2] Department of Biological Control Research, Plant Protection Research Institute, Agricultural Research Center, Egypt

**Abstract**
The sugar – beet rib miner, *Scrobipalpa ocellatella* Boyd**.**, is a pest that causes economic damage to sugar- beet plantations in different regions of the world. Experiments carried out at laboratory of Plant Protection Research Institute branch Sakha and laboratory of pests & plant Protection Department, National Research Centre, Egypt. Spores of fungal isolates harvested by rinsing with sterilized 0.5 % Tween 80 from 14 day old culture (PDA) media grown at 25±2ºc for *B. bassiana* and *M. anisopliae.* The 4$^{th}$ instar larvae and pupae of *S. ocellatella* were treated with the spores of *B. bassiana* and *M. anisopliae* using the concentrations of $1x10^6$, $1x10^7$, and $1x10^8$ spores / ml. The egg deposited in groups, each of a single layer consisting of 2-14 eggs arranged in one or two rows. The highest mortality occurred in larvae by concentration of $1x10^8$ was 100 % after 9$^{th}$ day from treatment when infection with *B. bassiana* but reached to 100 % mortality after 10 days with the same concentration from *M. anisopliae***.** *B. bassiana* was the highest virulence against the pupae of *S. ocellatella* than *M. anisopliae*. $LC_{50}$ when infected larvae with *M. anisopliae* and *B. bassiana* was $2.27x10^6$ and $0.4\ x10^6$ respectively while when infected pupae was $1.92\ x10^{7}$ and $2.99\ x10^{6}$ respectively.

**Keywords:** *Metarhizium anisopliae*, *Beauveria bassiana***,** *Scrobipalpa ocellatella*

## Introduction

The sugar – beet rib miner, *Scrobipalpa ocellatella*, is a pest that causes economic damage to sugar- beet plantations. In Egypt, [1] found the sugar – beet rib miner, S. *ocellatella* in Alexandria. About 8 larvae found in one tunnel. Its larvae attack all the chenopodiaceae plants, mining their leaf petioles and mid – ribs. Infestation occurred throughout the year. In Russian, [3] recorded that *S. ocellatella* is one of the most important insects for sugar – beet plants [4- 6] studied the infestation levels, by *S. ocellatella*, of sugar – beet a different sowing period. Hammad [2] stated that eggs of *S. ocellatella* are laid singly or in groups on the leaves, especially on the stem. The mated female lays about 15 – 80 eggs, which hatch after 4days. Soon after hatching, larvae bore inside the petiole. The mine extends into the mid-rib and reaches the roots. The mining larvae may hide between fallen leaves fastened together with silken web which they spin. The pupal stage lasts about 7-8 days. Abdel–Raheem, [6] using of *M. anisopliae* and *B. bassiana* against *S. ocellatella* and *Cassida vittata*. Also, [7] Using of Entomopathogenic Fungi on Cabbage Aphids, *Brevicoryne brassica*, [8] studied the effect of different fertilization rates on control of *Bemisia tabaci* (Genn.) by *V. lecanii* and *B. bassiana* in potato crop. Abdel-Raheem and Zakia A. Ragab, [9] using Entomopathogenic Fungi to Control *Aphis gossypii* Glover on Sugar- beet. Abdel-Raheem, [10] Use of *Verticillium lecanii* and *Beauveria bassiana* against Tomato leaf miner, *Tuta absoluta* (Meyrick) and *Bemisia tabaci* (Genn.) in Tomato Crop. Abla and Abdel-Raheem, [11] using entomopathogenic Fungi to control the tortoise beetle, *Cassida vittata*, Mohamed Abdel-Raheem [12] Isolation entomopathogenic fungi from insect pests.

The aimed of this study to clarify the efficiency of Entomopathogenic Fungi to control one of the most serious pests of agriculture crop, *Scrobipalpa ocellatella*

## Materials and Methods

### Biology of sugar- beet rib miner, *S. ocellatella*

The culture started from larvae, we collected it from the field and put it in glass jars (2 litre) with sugar- beet leaves. The adult moths were transferred into glass cage with sugar-beet plant as an oviposition site. Eggs laid on leaves were collected and transferred to petri – dishes under laboratory condition 26 ± 2 ˚ C and 65 ± 5% R.H.

### Rearing of test insect

In the present work sugar-beet rib miner, *S. ocellatella* insects were reared inside wooden box (100 x 50 x50 cm) on the sugar-beet plants.

### Laboratory application

### Fungal inoculate

Spores of fungal isolates harvested by rinsing with sterilized 0.5 % Tween 80 from 14 day old culture (PDA) media grown at 25±2º C for *B. bassiana* and *M. anisopliae.* The spore was counted in the suspension using haemocytometer. The concentrations were used $1x10^6$, $1x10^7$, and $1x10^8$ spores / ml.

### Treatment of the 4$^{th}$ instar larvae and pupae of *S. ocellatella*:

The 4$^{th}$ instar larvae and pupae of *S. ocellatella* were treated with the spores of *B. bassiana* and *M. anisopliae* using the concentrations of $1x10^6$, $1x10^7$, and $1x10^8$ spores / ml. each treatment contained 4 replicates and each replicate contained twenty insects transferred in group 5 individuals each in a Petri- dish, placed on a wetted filter paper. After dipping the insects in the suspension, the insects were transmitted on parts of sugar – beet leaves to feed. Each treatment was incubated at 25 ± 2 ºC and 85 ± 5 % R.H. and observed daily and the control leaves were treated with distilled water.

**Statistical analysis**

Mortality data was corrected with that in control by using [13]. The data was then analyzed by probit analysis [14] and the Median Lethal Concentration ($LC_{50}$) was computed by using statistical computer programme, Statistical Package of Social Sciences (SPSS).

## Results

**Sugar- beet rib miner, *Scrobipalpa ocellatella***

**The egg**

The egg is usually deposited in groups, each of a single layer consisting of 2-14 eggs arranged in 1-2 rows. The egg is oval shaped creamy yellow in colour and darkening to yellowish green before hatching.

The incubation period of egg between 3 - 5 days with an average of 4.39 ± 0.66 days, (Table 1).

**The larva**

The newly hatched larva is creamy in colour which changes to yellowish green after feeding. The head capsule is light brown. The 1st instar larval bores its tunnel into mid –rib and to the roots. The 2nd larval instar is pale yellow and often remains in the tunnel or hides in the heart leaves. The 3rd & 4th larval instars are pale yellow with shiny pink tergites. The head capsule is dark brown upon reaching full maturity, (Table 1).

**Table 1:** An average duration of the immature stages of sugar- beet rib miner, *S. ocellatella* at 26 ± 2 ˚ C and 65 ± 5% R.H.

| Stages | *Eggs incubation period (days) | % Hatch | **Larval duration (days) | | | | | Pre-pupal stage (days) | *** pupal stage (days) | Total duration (days) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1st | 2nd | 3rd | 4th | Total | | | |
| Average | 4.39±0.66 | 93.33 | 4.22±0.49 | 2.58±0.49 | 2.79±0.49 | 4.42±0.49 | 13.78±0.61 | 1.50±0.5 | 6.18±0.52 | 26.06±0.76 |
| Range | 3-5 | | 3-5 | 2-3 | 2-4 | 4-5 | 13-16 | 1-2 | 5-7 | 25-27 |

Total no. of 100 (eggs*, larvae**, and Pupae*** )

**The pupal**

The pre-pupal stage appears pale brown in colour and usually takes place inside the larval tunnels in the leaves or root. A small number of individuals pupate among the fallen dry leaves. The duration of the pre-pupal stage was 1-2 days with an average of 1.5 ± 0.5 days. Pupa is dark brown and is rectangular oval in shape with narrow end.

**The adult**

The adults are blackish grey, and whitish grey in the hind wings. The sex is differentiated by the abdominal sternites is blackish in the male and white in the female.

**Fecundity and longevity**

An average period were 4.2 ± 0.78, 7.34 ± 0.72 and 3.83 ± 0.56 days, of pre-oviposition, oviposition, and post – oviposition, respectively. The male lives shorter than the female. The longevity was ranged from 13-16 days with an average of 14.86± 0.63 days. The number of eggs / female was ranged from 42 – 59 eggs with an average of 49.43 ± 3.67 eggs / female, (Table 2).

**Table 2:** An average duration of adult of, *S. ocellatella* at 26 ± 2 ˚ C and 65 ± 5% R.H.

| Pre-oviposition period | oviposition period | post-oviposition period | Female longevity | Average number of eggs/ female |
|---|---|---|---|---|
| 4.2±0.78 | 7.3 ± 0.72 | 3.83±0.56 | 14.86 ± 0.63 | 49.43±3.67 |
| 3-5 | 5-8 | 2-4 | 13-16 | 42-59 |

*Total number of 100 adults were tested

**Control of sugar- beet rib miner, *S. ocellatella***

**The Fourth instar of *S. ocellatella* treated with *M. anisopliae* and *B. bassiana***

Table (3) indicated the mortality was occurred in the second day with all concentrations. The highest mortality occurred by concentration of 1x10 8 was 100 % after 9th day from treatment when infection with *B. bassiana* but reached to 100 % mortality after 10 days from treatment with the same concentration from *M. anisopliae*. So, *B. bassiana* was the most effective than *M. anisopliae* against the 4th instar of *S. ocellatella*.

**Table 3:** The percent Mortality of Fourth instar larva of *S. ocellatella* infected with three concentrations of *M. anisopliae* and *B. bassiana* at 25 ± 2ºc and 85 ± 5 % R.H.:

| Days after treatment | Control | *M. anisopliae* | | | *B. bassiana* | | |
|---|---|---|---|---|---|---|---|
| | | 1x10 6 | 1x10 7 | 1x10 8 | 1x10 6 | 1x10 7 | 1x10 8 |
| 2nd | - | - | - | - | - | - | - |
| 3rd | - | - | - | 10 | 25 | 25 | 30 |
| 4th | - | 10 | 20 | 30 | 30 | 35 | 40 |
| 5th | - | 15 | 25 | 35 | 35 | 40 | 55 |
| 6th | - | 20 | 35 | 45 | 35 | 45 | 65 |
| 7th | - | 25 | 35 | 55 | 40 | 55 | 75 |
| 8th | - | 35 | 55 | 75 | 45 | 75 | 85 |
| 9th | - | 40 | 60 | 85 | 75 | 80 | 100 |
| 10th | - | 65 | 70 | 100 | 85 | 100 | 100 |

**The Pupae of *S. ocellatella* treated with *M. anisopliae* and *B. bassiana*:**

Table 4 showed that no mortality in the Fourth days with all concentrations. The % mortality occurred by concentration of 1x10$^{8}$ was 95 % after 7$^{th}$ days from infection with *B. bassiana* but was 75 % by infection with *M. anisopliae*. *B. bassiana* was the highest virulence on the pupae of *S. ocellatella* than *M. anisopliae*. Table 5 obtained that $LC_{50}$ when infected larvae with *M. anisopliae* and *B. bassiana* was 2.27x10$^{6}$ and 0.4 x10$^{6}$ respectively while when infected pupae was 1.92 x10 10$^{7}$ and 2.99 x10$^{6}$ respectively.

**Table 4:** The percent mortality of pupa of *S. ocellatella* infected with entomopathogenic fungi at 25 ± 2ºC and 85 ± 5 % R.H.:

| Days after treatment | Control | *M. anisopliae* | | | *B. bassiana* | | |
|---|---|---|---|---|---|---|---|
| | | 1x10$^{6}$ | 1x10$^{7}$ | 1x10$^{8}$ | 1x10$^{6}$ | 1x10$^{7}$ | 1x10$^{8}$ |
| 2$^{nd}$ | - | - | - | - | - | - | - |
| 3$^{rd}$ | - | - | - | - | - | - | - |
| 4$^{th}$ | - | - | - | - | - | - | - |
| 5$^{th}$ | - | 20 | 35 | 65 | 30 | 60 | 65 |
| 6$^{th}$ | - | 20 | 35 | 65 | 70 | 75 | 85 |
| 7$^{th}$ | - | 30 | 45 | 75 | 70 | 85 | 95 |
| 8$^{th}$ | - | 30 | 45 | 75 | 70 | 85 | 95 |

**Table 5:** The susceptibility of sugar-beet rib miner, *S. ocellatella* to larvae and pupa infected with *M. anisopliae* and *B. bassiana at* different concentrations

| Treated Stages | $LC_{50}$ | | Fudicial limits 95% | | Slope ±SE | |
|---|---|---|---|---|---|---|
| | *M. anisopliae* | *B. bassiana* | *M. anisopliae* | *B. bassiana* | *M. anisopliae* | *B. bassiana* |
| 4$^{th}$ instar Larva | 2.27x10$^{6}$ | 0.4 x10$^{6}$ | (6.14 x10$^{6}$ – 6.53 x10$^{5}$) | (0.7 x10$^{6}$ – 4.03 x 10$^{6}$) | 0.53 ±0.09 | 0.04 ± 0.14 |
| Pupa | 1.92 x10 10$^{7}$ | 2.99 x10$^{6}$ | (6.14 x10 6 – 9.6 x10$^{7}$) | (0.9 x10$^{6}$ – 5.63 x 10$^{6}$) | 0.88 ± 0.17 | 1.2 ± 0.19 |

## Discussion

The results showed clearly that the biology of *S.ocellatella* was agreed with [15, 16]. *B. bassiana* and *M. anisopliae* are specific entomopathogenic fungi against the *S. ocellatella*. As given above by [17], the insect infection with fungal spores is beginning with the attachment of the infective unit by the host insect, germination, multiplication, production of toxic toxins. These four steps are occurred in the insect heamocoel without giving any outer symptoms. This incubation period which given by [17] is in a full agreement with our results which showed clearly that all the fungal spore concentrations needed about three days to cause the death of the host insect. Preventing feeding followed by insect death could introduce a very good plant protection. This work proved that *B. bassiana* and *M. anisopliae* when used using the fungal spores by the concentration of 1x10$^{8}$ spore/ml water could prevent feeding and cause insect death introducing a very good plant protection method [17-22]. All results in our work agree with [23- 29].

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