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Assessment of the effectiveness of *Metarhizium anisopliae* and *Beauveria bassiana* in controlling *Scr obipalpa ocellatella* (Boh.), A damaging insect that affects sugar beet crops

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Abstract
Sugar beet (Beta vulgaris) is an important crop in Egypt, contributing significantly to the economy. To meet the local demand for sugar production, farmers are encouraged to increase their sugar beet cultivation. However, the presence of the pest Scrobipalpa ocellatella poses a threat to sugar beet plantations worldwide, leading to economic losses. In order to obtain fungal isolates (*Beauveria bassiana* and *Metarhizium anisopliae*), spores were collected by rinsing them with sterilized 0.5% Tween 80 after 14 days the ancient culture (PDA) media was cultivated at a temperature of 25±2ºC. Then, the spores of *B. bassiana* and *M. anisopliae* were utilized to treat the fourth instar larvae and pupae of S. ocellatella, using concentrations of $1x10^6$, $1x10^7$, and $1x10^8$ spores/ml. After 9 days of treatment, the larvae exhibited a mortality rate of 100% when exposed to a concentration of $1x10^8$ spores/ml of *B. bassiana*. On the other hand, the same concentration of *M. anisopliae* resulted in 100% mortality after 10 days. *B. bassiana* demonstrated higher virulence against the pupae of *S. ocellatella* in comparison to *M. anisopliae*. The LC_{50} value for infected larvae was $2.33x10^6$ for *M. anisopliae* and $0.45x10^6$ for *B. bassiana*, while for infected pupae it was $1.98x10^7$ and $2.97x10^6$, respectively.

Keywords: *Metarhizium anisopliae, Beauveria bassiana, Scrobipalpa ocellatella,* sugar- beet

Introduction

Sugar beet, scientifically known as *Beta vulgaris*, holds great economic importance in Egypt. Therefore, farmers are being encouraged to boost their sugar beet production to meet the increasing local demand for sugar. Nevertheless, there is a serious threat to sugar beet plantations worldwide posed by the sugar beet mining moth, scientifically named *Scrobipalpa ocellatella*. Researchers in the Alexandria district of Egypt have identified the presence of this moth in sugar beet fields. These moths can be found within tunnels in the midrib, leaf stalk, or roots of the sugar beet plant, with a single tunnel potentially housing up to eight larvae. The infestation caused by these moths can result in the death of affected plants. The severity of *S. ocellatella* infestation was found to be particularly high in the Behera province of Egypt [1-3]. These moths' larvae attack the leaf petioles and midribs of all chenopodiaceous plants, and infestations occur throughout the year. Various studies conducted by researchers have emphasized the harmful effects of S. ocellatella on sugar beet plants during different sowing periods.

The eggs of the sugar beet mining moth are typically deposited individually or in clusters on the foliage, with a preference for the stem. Each female that has mated has the capacity to lay anywhere from 15 to 80 eggs, which then undergo hatching after a four-day incubation period. Once emerged, the larvae penetrate the petiole, expand their mining activities to the midrib, and eventually reach the roots. It has been observed that the mining larvae sometimes conceal themselves amidst fallen leaves, utilizing a silk web that they spin to secure the leaves together. The pupal stage of *S. ocellatella* has a duration of approximately 7 to 8 days, while the entire life cycle of the moth is completed within a span of around 30 to 35 days. As per Abdel-Raheem [4], the eggs are usually laid in clusters, with 2-12 eggs arranged in one or two rows.

The majority of the eggs are laid on the petioles, although some can be found on the surfaces of the leaves or the root collar. The average length of time it takes for these eggs to hatch is 4.39 days, and the rate of successful hatching is 93.33%. On average, each female lays 49.43 eggs. Abdel-Raheem [6] conducted a study on the use of entomopathogenic fungi, specifically *Metarhizium anisopliae* and *Beauveria bassiana*, as a method of controlling *S. ocellatella* and *Cassida vittata*. In a similar investigation, entomopathogenic fungi were examined for their effectiveness in controlling cabbage aphids, *Brevicoryne brassica* [7]. Another study focused on the impact of different fertilization rates on the management of *Bemisia tabaci* in potato crops through the use of *Virticillium lecanii* and *Beauveria bassiana* [8].

Abdel-Raheem and Zakia [9] investigated the potential of entomopathogenic fungi for the control of the cotton aphid, *Aphis gossypii* (Glover), on sugar beet crops. Abdel-Raheem [10] and Abla and Abdel-Raheem [11] conducted studies on the efficacy of *Verticillium lecanii* and *Beauveria bassiana* in controlling the tomato leaf miner (*Tuta absoluta*) and *Bemisia tabaci* in tomato plants. Lastly, Mohamed Abdel-Raheem [12] focused on the isolation, mass production, and application of entomopathogenic fungi for the management of insect pests. The primary goal of this study was to assess the effectiveness of Entomopathogenic Fungi in the management of *Scrobipalpa ocellatella*, a significant agricultural pest.

Materials and Methods

Cultivation of Fungi

The fungi were grown and maintained on a substance known as Potato Dextrose Agar (PDA), which consisted of 250 g of potatoes, 20 g of agar, and 1000 ml of distilled water. This substance was sterilized by autoclaving at a temperature of 120 ºC for a period of 20 minutes, then poured into Petri dishes that measured 9 cm in diameter and 1.5 cm in height. The fungal isolates were re-cultivated every 14-30 days and kept at a temperature of 4 ºC for storage. In order to restore the virulence of the isolates, they were either passed through their natural host or through wax moth larvae known as *Galleria mellonella*.

Rearing of Test Insect

The research involved cultivating *Scrobipalpa ocellatella*, a type of sugar-beet mining moth, within a wooden box with dimensions of 100 x 50 x 50 cm. The moth larvae were provided with sugar-beet plants as their primary food source in a laboratory at the Pests & Plant Protection Department, NRC, in 2024.

Laboratory Application

Fungal Inoculants

The spores of fungal strains were obtained by washing a 14-day old culture, which was grown on PDA medium at a temperature of 25±2ºC, with a sterilized solution containing 0.5% Tween 80. To prevent the mycelium from forming clumps, the suspensions were then filtered. The haemocytometer, specifically the Hirscmann 0.1 mm x 0.0025 mm 2 model, was used to determine the concentration of spores. Three different concentrations were prepared: c1, which consisted of $1x10^6$ spores per milliliter, c2, which had $1x10^7$ spores per milliliter, and c3, which contained $1x10^8$ spores per milliliter.

To treat the 4th instar larvae and pupae of *S. ocellatella*, *B. bassiana* and *M. anisopliae* spores at concentrations of $1x10^6$, $1x10^7$, and $1x10^8$ spores per milliliter were used. There were 4 replicates for each treatment, each containing twenty insects grouped in sets of five individuals. The insects were placed on a wetted filter paper inside a Petri dish after being dipped in the spore suspension. They were then fed with parts of sugar-beet leaves. The treatments were kept in an incubator at 25 ± 2 ºC and 85 ± 5 % relative humidity, with daily monitoring. Distilled water was used on the control leaves.

Statistical Analysis

The death rate information was modified by employing a comparator, which was explained in reference [13]. Afterward, the information underwent probit analysis [14], and the Statistical Package for Social Sciences (SPSS) software program was utilized to calculate the Median Lethal Concentration (LC_{50}).

Results

When *S. ocellatella* was exposed to *M. anisopliae* and *B. bassiana* in its fourth instar, there was no mortality after two days at any concentration. This is evident from the data in table (1) and figures 1 and 2. However, after nine days of treatment with a concentration of $1x10^8$ of *B. bassiana*, 100% mortality was observed. Similarly, with the same concentration of *M. anisopliae*, 100% mortality was reached after ten days of treatment. Therefore, it can be concluded that *B. bassiana*, the entomopathogenic fungus, demonstrated higher virulence in comparison to *M. anisopliae* against the fourth instar of *S. ocellatella*.

Table 1: Shows the analysis of the mortality rate of fourth instar *S. ocellatella* larvae when subjected to varying concentrations of *M. anisopliae* and *B. bassiana*, under controlled conditions of 25 ± 2ºC and a relative humidity of 85 ± 5%.

Days after treatment	Control	*M. anisopliae*			*B. bassiana*		
		*C_1	C_2	C_3	C_1	C_2	C_3
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

*c1, c2, and c3 each contain spore concentrations of $1x10^6$, $1x10^7$, and $1x10^8$ per milliliter, respectively.

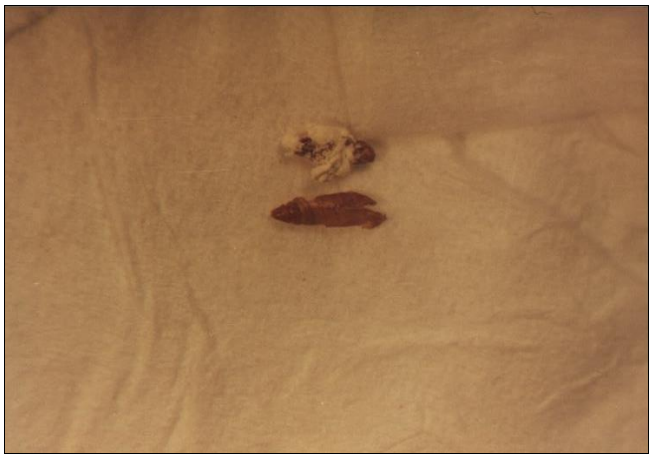

Fig 1: Illustrates a *Beauveria bassiana*-infected larva of *Scrobipalpa ocellatella*

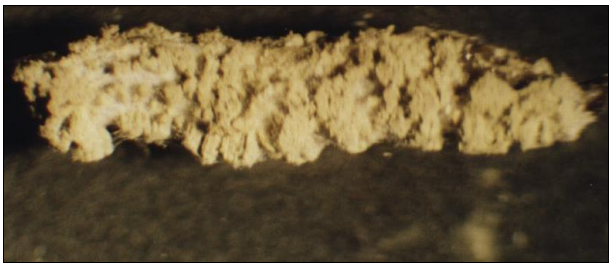

Fig 2: Illustrates the *Metarhizium anisopliae*-infected larvae of *S. ocellatella*

The pupae of *S. ocellatella* were subjected to treatment with *M. anisopliae* and *B. bassiana*. The results presented in table (2) and Fig. (3&4) indicated that no mortality was observed within the first four days across all concentrations. However, after seven days of infection, the mortality rate reached 95% when infected with a concentration of $1x10^8$ with *B. bassiana*, while it was 75% for *M. anisopliae*. *B. bassiana* demonstrated higher potency against *S. ocellatella* pupae when compared to *M. anisopliae*. Based on table (3), the LC_{50} value for infected larvae with *M. anisopliae* and *B. bassiana* was determined to be $2.27x10^6$ and $0.4x10^6$ respectively. Additionally, for infected pupae, the LC_{50} was found to be $1.92x10^7$ and $2.99x10^6$ respectively.

Table 2: Presents an analysis of the mortality rate among S. ocellatella pupae, which were exposed to three distinct concentrations of *M. anisopliae* and *B. bassiana*, under specific conditions of a temperature range of 25 ± 2ºC and a relative humidity range of 85 ± 5%.

Days after treatment	Control	*M. anisopliae*			*B. bassiana*		
		*C_1	C_2	C_3	C_1	C_2	C_3
2nd	-	-	-	-	-	-	-
3rd	-	-	-	-	-	-	-
4th	-	-	-	-	-	-	-
5th	-	20	35	65	30	60	65
6th	-	20	35	65	70	75	85
7th	-	30	45	75	70	85	95
8th	-	30	45	75	70	85	95

*c1, c2, and c3 each contain spore concentrations of $1x10^6$, $1x10^7$, and $1x10^8$ per milliliter, respectively.

Fig 3: Illustrates the pupal stage of the *S. ocellatella* species showing an infection induced by *M. anisopliae*

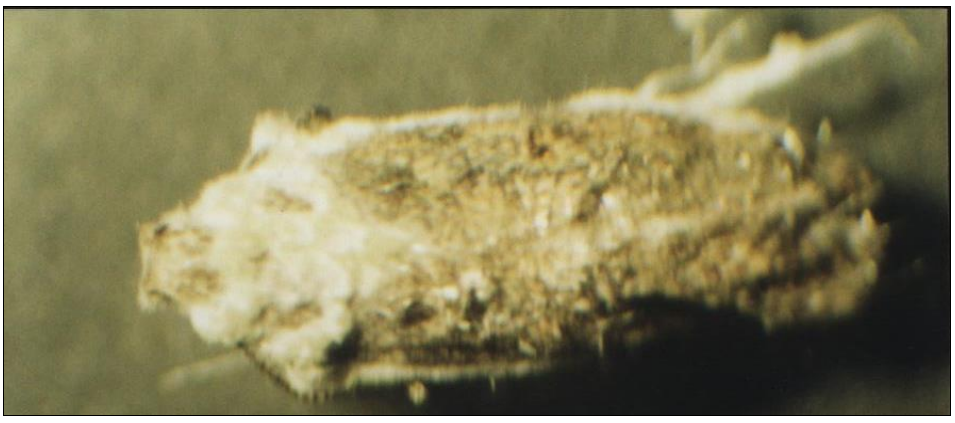

Fig 4: Illustrates an adult *S. ocellatella* that is infected with *M. anisopliae*

Table 3: Presents the susceptibility of the sugar beet mining moth, *S. ocellatella*, to larvae and pupa that are infested 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*
4th instar Larva	$2.33x10^6$	$0.45 x10^6$	($6.19 x10^6 – 6.77 x10^5$)	($0.9 x10^6 – 4.23 x 10^6$)	0.59 ±0.19	0.14 ± 0.22
Pupa	$1.98 x10^7$	$2.97 x10^6$	($6.19 x10^6 – 9.8 x10^7$)	($1.2 x10^6 – 5.68 x 10^6$)	0.99 ± 0.22	1.9 ± 0.27

Discussion

It is evident from the findings that *Beauveria bassiana* and *Metarhizium anisopliae* are specific fungi that induce disease in the *Scrobipalpa ocellatella* insect. According to [15], the infection begins when the fungal spores attach to the insect, followed by germination, multiplication, and the production of toxins. These processes occur internally within the insect's body and do not manifest in any visible external symptoms, except for the insect's refusal to feed. This sequence of events, referred to as the "fungal incubation period, typically lasts approximately three days, which aligns with the research presented in [15].

Our findings clearly show that it takes approximately three days for all concentrations of fungal spores to result in the death of the host insect by stopping feeding. This implies that preventing feeding and inducing insect death could be a

successful approach for protecting plants. Our study exhibits that *Beauveria bassiana* and *Metarhizium anisopliae*, when applied at a concentration of 1x10 ^{8} spore/ml water, can effectively hinder feeding and cause insect death, making them a dependable method for plant protection. Additionally, we have included several photos illustrating infected insects with fungal infective units, providing supporting evidence for the theory proposed in [15-20]. The death of the host insects, the penetration of hyphae from inside the insect to the outside through the cuticle, and the creation of new infective units (conidia) are clearly illustrated in these photographs. Our research findings are consistent with earlier studies [21-25] examining the effectiveness of entomopathogenic fungi in real-world conditions. These studies indicated that the temperature and relative humidity, especially in tropical and subtropical climates, have a substantial impact on the infection process, the time it takes for symptoms to appear, and the survival of these microorganisms.

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