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Efficacy of Local Isolate of *Beauveria bassiana* and Commercial Product of *B. Bassiana* Mixed with *Metarhizium anisopliae* on the Greater Wax Moth

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ABSTRACT

The efficiency of local isolate of *Beauveria bassiana* indigenous in Egypt, was estimated compared with the bio-pesticide (mixture of *B. bassiana* and *Metarhizium anisopliae*) on the greater wax moth (GWM), *Galleria mellonella* L. Five concentrations of *B. bassiana* (1×10^7, 2.5×10^7, 5×10^7, 7.5×10^7 and 1×10^8 conidia/ml) and the biopesticide, (2, 4, 6, 8 and 10g/L) against *G. mellonella* larvae were evaluated under storage conditions. *B. bassiana* was reared in two media (PDA and mummies of GWM larvae). The fungal isolate, *Beauveria bassiana* at all tested concentrations affect the mortality of *G. mellonela* larvae, especially at the highest concentration. The obtained data obviously indicated that, fungal isolate (grown on mummies of GWM) exhibited a relatively high effect on *G. mellonella* larvae in comparison with that reared on PDA media. However, after 21 days of treatment *B. bassiana* reared on GWM larvae caused, 46.33± 17.9, 63.33±11.6, 70± 00, 70± 20 and 86.66± 11.6% mortality , while, *B. bassiana* grown on PDA caused, 40 ± 12.5, 50± 16.0, 73.33± 24.3, 53.33±24.2 and 80 ±26.3 % mortality of the treated larvae at the concentrations of 1×10^7, 2.5×10^7, 5×10^7, 7.5×10^7 and 1×10^8 conidia/ml, respectively .At the highest concentration (10 g/L) of the bio-insecticides the mortality percentages of treated *G. mellonela* larvae were 40±16.4, 66.66±24.2, 76.66±27.6 and 83.33±27.2%, after 6, 9, 12 and 15 days of treatment , respectively.

Keywords; *Galleria mellonella*, entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, control.

INTRODUCTION

The greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) is considered a trouble in honeybee colonies. This pest is usually appeared in combs of weak or dead bee colonies and in stored combs. This pest can't attack combs in active honey bee colonies (Ellis *et al*., 2013). Besides, damaging wax combs, *G. mellonella* larvae fed on stored pollen, destroying frames and wooden parts in the hive. The moth larvae caused galleriasis (The bee pupae in the cells are rarely damaged, but sometimes become trapped in the cells by the silk threads and die). Adult wax moths and larvae may also transfer pathogens of serious bee diseases, e.g. foulbrood. However, in colonies infected with this disease, feces of the wax moth contain large amounts of spores of the causative bacteria *Paenibacillus* (Krams *et al*., 2015) and the potential of transmitting honeybee viruses has raised legitimate concern. Most of investigated by transversal sections in the mid-gut to entomopathogenic fungi belong to Deuteromycetes. *B. bassiana* infected successfully larvae, pupae and adults of many insects and at the time of insect death nearly all of the internal organs of the insect are utilized by the fungus (Tanada and keya 1993). *Beauveria bassiana* is ubiquitous fungus which has been found and isolated from a wide variety of insects of different orders and is the most widely used fungal species available commercially (Mansour, 1991; Zimmermann, 2007; Goettel *et al*., 2010 and Ibrahim *et al*., 2016). According to Vey *et al*., (2001) *B. bassiana* produces several toxic compounds in vitro and in vivo. So, the present study aims to evaluate the efficiency of local isolate of *B. bassiana* compared with the commercial product (Care Protector) of fungi mix (*B. bassiana* and *M. anisopliae*) against the wax moth in the storage.

MATERIALS AND METHODS

The efficiency of local isolate of entomopathogenic fungi (i. e. *Beauveria bassiana*), indigenous in Egypt, previously isolated and identified by (El Sheikh, 2003) from the greater wax moth (GWM), *Galleria mellonella* L. larvae at different regions of the Nile-Delta was estimated compared with the commercial product (fungi mix) on the larvae of GWM. These experiments were carried out under storage conditions in the apiary of Beekeeping Research section at Sakha Agriculture Research Station, Kafr El-Sheikh.

1-Tested entomopathogenic fungi:

Local isolate of *Beauveria bassiana*

To have colonies of *B. bassiana*, the fungi was grown on *G. mellonella* larvae and PDA media.

On *G. mellonella* larvae *:*

The fungus was cultured on *G. mellonella* larvae as described by Mansour (1999).

On PDA media:-

Potato dextrose agar medium was a suitable media for culturing *B. bassiana* .

For fungal inoculums preparation, inoculations of the fungal isolates were prepared by growing them without shaking in conical flasks (250 ml) containing potato dextrose (PDA) broth medium at 28° C for 15days. The fungal masses were blended and the concentration was adjusted to 10^8 conidia/ml (Mansour, 1991 and Saleh , 2002).

- The bio-pesticide (Care Protector):

The bio- pesticide is a commercial product containing (1-2% *B. bassiana* WP-1×10^9 CFU/gm, *M. anisopliae* WP-2×10^8 CFU/gm, carrier powder 85-90% and Moisture 5-10%).

1. Pathogenicity of *B. bassiana* isolate to GWM under storage conditions:-

This experiment was carried out to evaluate the pathogenecity of *B. bassiana* grown on the larvae of *G. mellonella* and PDA medium to *G. mellonella* under storage conditions in the apiary of Beekeeping Research section at Sakha Agriculture Research Station, Kafr El-Sheikh.

Each prepared inoculums was used with five concentrations (1×10^7, 2.5×10^7, 5×10^7, 7.5×10^7 and 1×10^8 conidia/ml) (the lowest four were prepared by dilution the highest concentration by water). 0.1% Tween 80 was added for each suspension.

Eighteen old wax combs (dark colored) were selected from the apiary store and kept in the oven at 45° C for 72 hours to destroying any infestation with the greater wax moth larvae. Then, selected wax combs were infected with freshly fifth instars larvae of *G. mellonella* (ten individuals/ comb). After three hours, each comb was sprayed on both faces with 10 ml of the fungus suspension using an automizer (three wax combs as replicates for each concentration). In addition, three frames were sprayed with water containing 0.1% Tween 80 (as a check). All wax combs were kept in swarm box and placed in the storage where the temperature ranged from 22°C to 26°C and the relative humidity was 65 to 70% R.H. After 6, 9, 12, 15, 18 and 21 days of treatment, treated and check frames were examined and the number of dead *G. mellonella* larvae was counted and recorded.

2. Pathogenicity of the bio Pesticide containing mixture of *B. bassiana* and *Metarhizium anisopliae*):

Five dilutions of the bio Pesticide, (Care Protector) in distilled water were prepared as follow: (2, 4, 6, 8 and

10g/L). Eighteen old waxcombs infected with GWM larvae was treated as previously mentioned. After 6, 9, 12 and 15 days of treatment, treated and untreated frames were examined and the number of dead larvae was counted and recorded. The efficacy of each treatment was calculated according to the formula described by (Soliman 2005).

$$\text{Percentage of efficacy} = \frac{Ta - Ca}{100 - Ca} \times 100 \text{ where}$$

Ca = Number of dead larvae in the control after treatment.
Ta = Number of dead larvae in the treated bee wax after the application of different treatments.

Statistical analysis

All experiments were repeated twice with three replicates of each concentration or treatment. All data were subjected to one-way analysis of variance (ANOVA) and significant differences between treatment means were determined using Tukey's HSD test at P<0.05. The data were analyzed by SAS (version 9.1, SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

1. Efficiency of *B. bassiana* isolate against *G. mellonela* larvae.

***B. bassiana* reared on PDA.**

The results are summarized in Table (1). The fungal isolate, *Beauveria bassiana* at all tested concentrations affect the mortality of *G. mellonela* larvae, especially at the highest concentration. However, after 6, 9, 12 and 15 days of treatment the mortality percentages of GWM larvae were 20± 6.97, 43.3± 13.54, 70±24.35 and 80± 25.17 % at the concentrations of 1.0×10^8 conidia/ml, respectively. At the concentration of 1.0×10^7 conidia/ml , *B. bassiana* caused a slight mortality of *G. mellonela* larvae.

The mortality rate was significantly increased by time. The highest effect was recorded after 15 days of treatment at all concentrations. At 5×10^7 con./ml., the mortality percentage reached to 73.33± 24.31 after 21 days of treatment.

Table 1. Pathogenicity of *Beauveria bassiana* isolate grown on PDA to the fifth larval instars of *G. mellonella* under storage conditions at different inoculums densities (concentrations) of fungi.

Inoculums densities	Mortality % at different inoculums densities after					
	6 days	9 days	12 days	15 days	18 days	21
$1x10^7$	6.66± 4.7bc	20± 9.8b	23.33 ± 13.7cd	33.33± 15.5c	36.66±6bca	40 ± 12.5b
$2.5x10^7$	10± 3.7abc	30±10.0ab	36.66± 13.3bc	43.33±14.6bc	46.66±15.2ba	50± 16.0ab
$5x10^7$	13.33± 4.9ab	30±10.7ab	53.33± 18.4ab	63.33± 20.6ab	70±22.6bca	73.33± 24.3a
$7.5x10^7$	13.33± 5.2ab	30 ± 10.8ab	60±22.2ab	50±21.2bc	53.33±23.2ba	53.33±24.2ab
$1x10^8$	20± 7.0a	43.3± 13.5a	70±24.4a	80± 25.2a	80±25.8ca	80 ±26.3a
Control	0.00±0.0 c	0.00±0.0c	0.00±0.0d	0.00±0.0d	00.0±0.0 d	0.00±0.0 c
LSD	10.27	16.77	28.13	28.13	29.94	31.38

Means followed by different letters are significantly different according to LSD (P=.05).

***B. bassiana* reared on *G. mellonella* larvae.**

The obtained data, as shown in Tables (2), obviously indicated that, fungal isolate (grown on mummies of GWM) exhibited a relatively high effect on *G. mellonella* larvae in comparison with that reared on PDA media. However, after 21 days of treatment *B. bassiana* caused, 46.33± 17.9, 63.33±11.6, 70± 00, 70± 20 and 86.66± 11.6% mortality of the treated larvae at the concentrations of 1×10^7, 2.5×10^7, 5×10^7, 7.5×10^7 and 1×10^8 conidia/ml, respectively.

Table 2. Pathogenicity of *Beauveria bassiana* isolate grown on GWM larvae to the fifth larval instars of *G. mellonella* under storage conditions at different inoculums densities (concentrations) of fungi.

Concentration ./ml	Mean no. of dead GWM larvae after time intervals (days)					
	6	9	12	15	18	21
$1x10^7$	3.33± 4.9ab	10± 4.4c	20 ±9.1c	40 ±14.9c	43.33± 15.8c	46.33± 17.9bca
$2.5x10^7$	3.33±4.5ab	13.33± 5.2bc	26.66±9.1c	50±16.5bc	53.33±18.4bc	63.33±11.6bc
$5x10^7$	3.33±4.4ab	10± 4.4c	30± 9.4c	56.66± 17.6b	66.66±20.7ab	70± 00ab
$7.5x10^7$	6.66± 4.4ab	20±6.9ab	43.33±12.7b	56.66±18.1b	63.33± 21.2b	70± 20ab
$1x10^8$	10 ±4.5a	26.66±8.7a	56.66±17.0a	76.66±21.6a	83.33±24.12a	86.66± 11.6a
Control	0.00±0.0 d	0.00±0.00 d	0.00±0.0 d	0.00±0.0 d	0.00±0.0 d	0.00±0.0d
LSD	8.39	9.38	12.58	14.53	18.75	21.788

Means followed by different letters are significantly different according to LSD (P =.05).

As shown in Table (1 and 2), the mortality percentage of GWM larvae was significantly increased as the inoculums densities of *B. bassiana* increase. Similar results were obtained by Mansour (2003), that there was a positive correlation between *B. bassiana* conidia concentrations (2×10^7 ,4×10^7 ,8×10^7,1.6×10^8 and 3.2×10^8 con./ml) and the mortality of *G. melonella* larvae in laboratory and storage condition. Who, added that at (1.6×10^8) concentration of *B. bassiana* , 96% and 82% mortality was recorded after 20th days of treatment under laboratory store conditions against *G. melonella* larvae, respectively. The present data are agree with Saleh *et al.* (2016) that *B.bassiana, M. anisopliae* and *V. lecanii*. were virulence of isolated fungi which tested against larvae of *G. mellonella*. *B. bassiana* caused the highest mortality in larvae as compared with other tested fungal isolates.

To evaluate the pathogenicity of *B. bassiana* isolate reared on PDA and *G. mellonella* larvae to GWM against time, regression analysis has been done between the mortality % and time (days). Data illustrated in Figure (1a and b) showed the regression of the mortality of each concentration to GWM larvae over 21 days). Data obviously indicated that *B. bassiana* reared on GWM larvae caused an approximately higher rate of mortality than that of *B. bassiana* reared on PDA.

Regression analysis illustrated that the pathogenicity of the tested *B. bassiana* isolate varied according to the rearing methods and concentration. However, the efficiency of all concentrations of *B. bassiana* reared on GWM larvae sharply increased by the time (Fig.1 a).

On the contrary, the efficiency of all concentrations of *B. bassiana* reared on PDA was slightly increased by the time (Figure, 1 b). However, the slope of regression line was 3.19, 4.22, 5.047, 5.35 and 5.46 (for *B. bassiana* reared on GWM larvae) and 1.91, 2.22, 3.7 and 3.84 (for *B. bassiana* reared on PDA at 1×10^7, 2.5×10^7, 5×10^7, 7.5×10^7 and 1×10^8 conidia/ml, respectively.

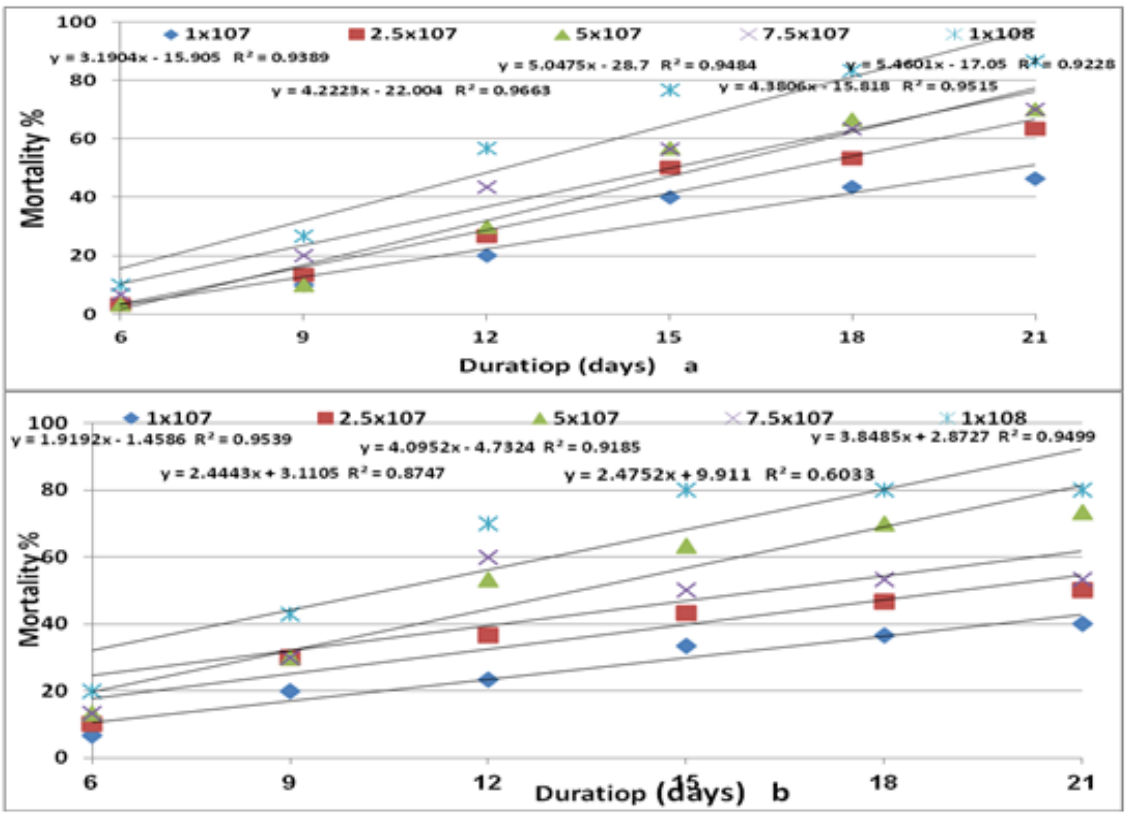

Figure (1 a and b). Stability of different inoculums densities of *B. bassiana* isolate reared on GWM larvae (a) and PDA media (b) in their pathogenecity to *G. mellonella* larvae over 21 days under store conditions.

From above mentioned results it could be concluded that, *G. mellonella* larvae is the best media for mass production of *B. bassiana*, while the best concentration of *B. bassiana* was $1x10^8$ spore/ ml.

2. Efficiency of the bio-pesticide (mixture of *B. bassiana* and *Metarhizium anisopliae*) on *G. mellonela* larvae.

The effectiveness of commercial bio-insecticide (*B. bassiana* mix with *M. anisopliae*) (Care Protecto) was evaluated to determine the most effective concentration against *G.mellonella* larvae. The bio-insecticides at all tested concentrations (2, 4, 6, 8 and 10 g/L) affect the mortality of *G. mellonela* larvae, especially at the highest concentration. However, after 6, 9, 12 and 15 days of treatment the mortality percentages of GWM larvae were 40±16.4, 66.66±24.2, 76.66±27.6 and 83.33±27.2% at the concentrations of 10 g/l, respectively (table 3). At the concentration of 2 g/l , the bio-insecticide caused a slight mortality of *G. mellonela* larvae, represented by 30±14.9% after 15 days.

To evaluate the pathogenicity of the bio-insecticide to GWM larvae against time, regression analysis has been done between the mortality % and time (days).

Table 3. Mean mortality % of *G. mellonela* larvae treated with mix of *Beauveria bassiana* and *Metrahizium anisopliae* under storage conditions..

Concentrations Gram/L.	Mean no. of dead GWM larvae after time intervals (days)			
	6	9	12	15
2 g/l	0.00±0 c	13.33±10.6b	16.66±11.4c	30±14.9c
4 g/l	20 ±11.4b	43.33±18.5a	50±19.6b	53.33±19.4b
6 g/l	30 ±15.3ab	50±19.8a	66.66±24.7ab	70±23.8ab
8 g/l	33.33±15.5ab	56.66± 21.9a	73.33±26.7a	73.33±25.4a
10 g/l	40±16.4a	66.66±24.2a	76.66±27.6a	83.33±27.2a
Control	0.00±0.0 c	0.00±0.0 b	0.00±0.0 c	0.00±0.0d
LSD	16.77	24.45	19.66	17.78

Means followed by different letters are significantly different according to LSD (P =.05).

Data illustrated in Figure (2) showed the regression of the mortality of each concentration to GWM larvae over 15 days. Data obviously indicated that the efficiency of all concentrations of the bio-insecticide was sharply increased by the time, especially at the highest concentrations (Figure, 2). However, the slope of regression line was 3.11, 3.56, 4.55, 4.56 and 4.67 at 2, 4, 6, 8 and 10 g/L, respectively.

The significant times recorded which achieved the high mortality of larvae were 9th and 12th days after treatment (Table 3). Results indicated that the insecticidal activity of Care Protecto against *G. mellonella* larvae was effective as a biological control agent against larvae in the store (apiary room). These results agree with Klingen *et al.*, (2002) that *M .anisopliae and B. bassiana* were highly virulent to *G. mellonella* larvae and caused 100% mortality. Also, Abdel-Raheem *et al*,. (2016) In Egypt ,bioassyed *B. bassiana* and *M. anisopliae on G.mellonlla* in the laboratory. The mortality percentages of *G. mellonella* larvae treated with *B.bassiana* isolate from Elbehira reached to 100% when treated with the concentration (2×10^4 spores/ ml) after 7th day and 100 % mortality after 9th day when treated with the concentration (2×10^3 and 2×10^4 spores/ ml) from *M. anisopliae*. Also, Care Protecto are effective against larval and pupal stages of wide pests of Lepidoptera , *Tutta absoluta*, etc.

So, Care Protecto (mixture of *B. bassiana* and *M. anisopliae)* can be used as a promising agent in pest control and integrated pest management programs instead to reduce the damage of this pest.

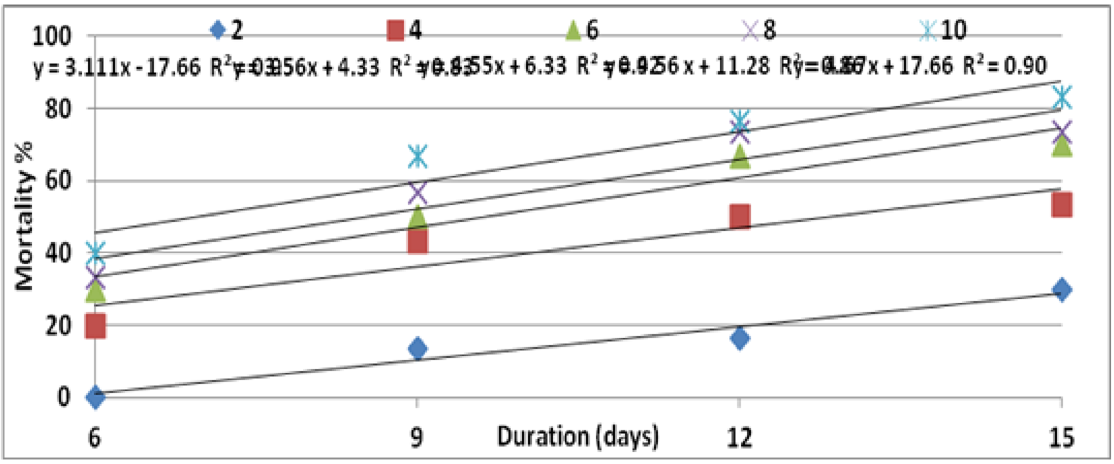

Figure 2. Stability of different concentrations of *B. bassiana* isolate reared on GWM larvae (a) and PDA media (b) in their pathogenecity to *G. mellonella* larvae over 21 days under store conditions.

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قياس فعالية اثنين من المبيدات الحيوية في مكافحة دودة الشمع الكبيرة في المخزن

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أجريت هذه الدراسة لقياس فعالية اثنين من المبيدات الحيوية في مكافحة دودة الشمع الكبيرة في المخزن .وهما *Beauvaria bassiana* ومخلوط *Beauvaria bassiana and Metarhizium anisopliae* (Care Protector). وتم استخدام خمس تركيزات مختلفة لفطر *Beauvaria bassiana* $1X10^7$، $2.5x10^7$، $5X10^7$، $7.5X10^7$، ($1 X10^8$ conidia/ ml) بينما استخدمت خمس جرعات مختلفة 2، 4، 6، 8 و 10 gm / L (Care Protector) مزيج *Beauvaria bassiana* و *Metarhizium anisopliae* على يرقات دودة الشمع الكبيرة في المعمل والمخزن. حيث تم تنميه فطر *B. bassiana* علي بئتين PDA مومياوات من دودة الشمع . أوضحت النتائج أن التركيز $1 x10^8$ conidia / ml من *B. bassiana* قد أظهر أعلى متوسط لليرقات الميتة 3.00 ± 1.00 (86.66٪ معدل موت يرقات دودة الشمع) بعد 12 يوماً. بينما أعطت الجرعة (10 جم /لتر) من الخليط *B.bassiana* و *M. anisopliae* أعلى متوسط لليرقات الميتة 4.66 ± 0.00 بالمقارنة بالجرعات الأخري بعد يوم واحد وقد سجلت هذة الجرعة اعلي نسبة موت (100 ٪) في اليرقات في المختبر. كانت نسبة الموت لمعظم يرقات دودة الشمع ما بين 3 - 9 أيام لجميع الجرعات المستخدمة . وبصفة عامة يمكن استخدام هذان العاملان الحيويان في مكافحة يرقات دودة الشمع لأنهما يتسببان في نفوق 100٪ في المخزن.