Virulence of four Entomopathogenic Fungi on Some Cotton Pests with Special Reference to Impact of Some Pesticides, Nutritional and Environmental Factors on Fungal Growth

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ABSTRACT

Role of some entomopathogenic fungi against some economic insect pests under laboratory and field conditions was evaluated. Results showed that, the pink bollworm (PBW) *Pectinophora gossypiella* (Saund.) and the cotton leaf worm (CLW) *Spodoptera littoralis* (Boisd.) were susceptible to the fungi; *Beauvaria bassiana* (*B.b.*), *Metarhizium anisopilae* (*M.a.*), *Paecilomyces fumosoroseus* (*P.f.*) and *Verticillium lecanii* (*V.l.*). LC_{50} values obtained were; $154x10^4$, $159x10^4$, $179x10^4$ and $157x10^4$ spores/ml for PBW larvae treated with different concentrations of (*B.b.*); (*M.a.*); (*P.f.*) and (*V.l.*) fungi, respectively. Respective LC_{50} values for CLW were: $163x10^4$, $175x10^4$, $199x10^4$ and $187x10^4$ spores/ml. Under field conditions, the tested fungi showed significant infestations' decrease in the plots treated with *B. bassiana*, followed by *M. anisopliae*. In general, PDA was the best medium for the growth of *B. bassiana* and 30°C was the optimum temperature, followed by 25°C. Concerning R.H., the growth of *B. bassiana* was much enhanced by high R.H., as it increased. Linear growth increased also to reach its maximum (88.25 mm) at 100% R.H. Results revealed that the isolate of *B. bassiana* was able to grow and to utilize any carbohydrate added to the growth medium. Sucrose was the best substrate for supporting growth (87.25 mm) and followed by glucose (56.25 mm). Calcium nitrate was generally the best suitable nitrogen source, followed by glycin, sodium nitrate and then ammonium nitrate. Meanwhile, ammonium phosphate was found to be the lowest one. Benlate was the most toxic fungicide to *B. bassiana*, even at lower concentrations. Growth of the fungus was completely inhibited (100%) at 6.25 ppm. Thymol was the most destructive insecticide, followed by Pyrethrum with a significant difference ($p<0.05$), while Cylone was the least one against *B. bassiana*.

Key words: Environmental factors, nutritional factors, fungi, insect pests, insecticides, fungicides.

INTRODUCTION

The entomopathogenic fungus, *Beauvaria bassiana* is one of several fungi that are of particular research interest due to its potential as commercial bioinsecticides. Some studies had focused on identifying nutrient substrates that *B. bassiana* can utilize with application to industrial production, while others focused on the pathogenic processes of *B. bassiana* and interactions with insect cuticle (Bidochia *et al.*, 1990).

Entomopathogenic fungi are found worldwide associated with insects and phytophagous mite populations, contributing to biological control of these arthropods on several economically important crops (Sabbour and Sahab, 2007). Commercial products have been developed with entomopathogenic fungi (Alves and Pereira, 1998). Quintela and McCoy (1998) reported that fungal concentrations of 10^6 and 10^7 conidia/ml of *B. bassiana* affected the larval development, movement and mobility of *Agrotis ipsilon* and *Spodoptera littoralis* during the seedlings and vegetative stages of cotton plant under laboratory and greenhouse conditions.

Success of a pest control program using *B. bassiana* however depends on conidia survival in the field environment (Benz, 1987). Conidia survival may be affected either by environmental factors (Furlong and Pell, 1997) or chemical products used to protect plants (Anderson and Roberts, 1983). Abdel-Rahman, *et al.* (2006) controlled the cereal aphids with the fungus *B. bassiana* and found that the infestation was reduced after fungal applications under laboratory and field conditions.

As reported by Walstad *et al.* (1970), *B. bassiana* required RH above 92.5% and temperatures between 15 and 35°C for mycelial growth. Optimum growth occurred at 100% RH and 25-30°C. Also, Hallsworth and Magan (1999) reported that the temperature ranges for growth of *B. bassiana* was 5-30°C and the optimum temperature was 25°C. Campbell *et al.*, (1987) reported that *B. bassiana* produces greater mycelial mass by glutamine and KNO_3 as nitrogen source.

Many experiments have been carried out aiming to detect side effects of pesticides on *B. bassiana* (Olmert and Kenneth, 1974). Most of them were evaluated for their effects on vegetative growth and sporulation. They emphasized that the inhibition of this initial step affects the plain development of the fungus in the field because the fungal structure is responsible for instability of the disease on insect pest populations.

The present study aimed to estimate the effect of some entomopathogenic fungi against the pink boll worm (PBW), *Pectinophora gossypiella* (Saund.) and the cotton leaf worm (CLW), *Spodoptera littoralis* (Boisd.). Also, to evaluate the effect of some environmental and nutritional factors as well as some pesticides on the growth of *B. bassiana*.

MATERIALS AND METHODS

Tested insects: Samples infested with PBW and CLW were collected from different regions in Egypt and were reared on semi artificial diet according to Salama and Foda (1984) for several generations under laboratory conditions.

Entomopathogenic fungi: The fungi; *B. bassiana* strain (BR3) and *Metarhizium anisopilae* stored in the form of pure conidia in Eppendorf vials at 4°C, were kindly sent by Prof. Dr. Alain Vey, Mycology Unit at Institute National de la Research Agronomique, Montpellier Univ., France. The fungus, *Paecilomyces fumosoroseus* and *Verticillium lecanii* were obtained from Florida Univ., USA. The fungi were reproduced on potato dextrose agar (PDA) plus 0.4% yeast extracts (PDAY) and poured onto sterilized Petri-dishes (Alves *et al.*, 1998). Plating was performed according to the full dish method. The conidia were transferred from the Eppendrof vial to dish containing medium by platinum loop and then streaked. Plates were incubated at 25°C, with 12 hours photo phase for fungus growth and sporulation. After ten days, conidia were scraped and transferred to conical flasks (250 ml) containing 200 ml sterilized distilled water, with 0.02% the speeder sticker (Tween, 80). Conidial concentrations in the suspensions were quantified directly under the optical microscope with a haemacytometer. Then, the suspensions were standardized until the direct concentration $1x10^7$ conidia/ ml was obtained.

Efficacy of entomopathogenic fungi against *P. gossypiella* and *S. littoralis* larvae

Spores of the entomopathogenic fungi; *B. bassiana*, *M. anisopilae*, *P. fumosoroseus* and *V. lecanii* collected from the surface of mycelium growth and spore suspensions with 2 drops of Tween 80 were prepared and adjusted at $1x10^7$ conidia/ ml. Conidial viability was determined by counting germ tubes produced on PDAY medium after 18 hrs, using light microscope at 400X. Conidial viability was 95-100%. The surface of cultures was gently brushed in the presence of 20 ml of sterilized water in order to free the spores and the suspension was filtered through muslin. Six concentrations of spore suspensions were prepared *i.e.*, 10^7, 10^6, 10^5, 10^4, 10^3 and 10^2 conidia/ml. Pieces of castor leaves were dipped in the prepared suspensions and left for drying under laboratory conditions then placed in Petri-dishes (one/dish). For each concentration (4 replicates/ each), ten L_3 larvae of each of the tested insects were transferred into each Petri-dish. Control larvae were fed on untreated castor leaves. Percentages of mortality were calculated according to Abbot, while LC_{50} was calculated throughout probit analysis. The experiment was carried out under laboratory conditions at 26°C± 2 and 60-70 % RH.

Physiological and metabolic characteristics of *B. bassiana*

Growth on different culture media

Tested culture media were PDA; corn meal agar (CMA); glucose peptone agar (GPA); Czapek's agar (Cz.); Czapek's carboxy methyl cellulose (Cz-CMC), Lynch A and Lynch B agar media. Plates were prepared with the different tested media and inoculated with 0.5cm (2%) water agar plugs of *B. bassiana* strain. Plates were incubated in darkness at 25°C± 2 during 10 days. All experiments were carried out in triplicates.

2- Effect of temperature

B. bassiana was inoculated in PDAY medium using 0.5 cm disc and incubated at 10, 15, 20, 25, 30 and 35°C and 100% RH in incubators for 10 days to attain maximum growth. Radial growth (mm) of the fungus was determined.

3- Effect of relative humidity

Six levels of RH were maintained by mixtures of appropriate combinations of concentrated sulphoric acid and distilled water (Table 1), as described by Ayyasamy and Baskaran (2005).

Table (1): Preparation of solutions for maintenance of different RH levels

Treatment No.	D. water (ml)	Sulphoric acid (ml)	RH %
1	100.0	-	100
2	88.5	11.5	95
3	80.0	20.0	90
4	77.0	23.0	85
5	73.0	27.0	80
6	70.0	30.0	75

Mixtures of sulphoric acid and distilled water were placed in desiccators. Plates of PDAY medium were inoculated at the center with a 5mm diameter disc of *B. bassiana*. Four replicates were used for every treatment and incubated at 25±2°C. Linear growth (mm) was measured.

4- Carbon source assimilation

Capacity of assimilation of different carbon sources with *B. bassiana* was studied. Tested carbon sources were glucose, sucrose, arabinose, mannose and citric acid. Lynch B agar medium ($NH_4H_2Po_4$, 1g ; KCl, 0.2g ; Mg So_4. $7H_2O$, 0.2g; Cu So_4 $5H_2O$, 5mg and Zn So_4. $7H_2o$, 10mg / L) containing 0.05 g/ L of bromocresol purple and 1% (w.v) of the tested carbon source was used. The medium was adjusted to pH 6.5 and autoclaved. Plates of various carbon sources were inoculated with the fungus in the center and incubated at 25±2°C and 100% R.H. Linear growth (mm) was measured.

5-Nitrogen source assimilation

Tested nitrogen sources were: ammonium phosphate, sodium nitrate, glycin, calcium nitrate and ammonium nitrate. Lynch A agar medium (KH_2PO_4, 1g; KCl, 0.5g; Mg SO_4. $7H_2O$, 0.2g; Ca Cl_2. $2H_2O$, 0.1g and sucrose 10 g/ L) containing 0.05 g/L bromocresol purple and 0.2% (w.v) of the tested nitrogen source was used. The medium was adjusted to pH 6.5 and autoclaved. Plates of various sources were inoculated in the center and incubated as mentioned before.

6- *In-vitro* evaluation of pesticides effect

Fungicides:

Benomyl: Methyl 1-(butrylcarbamoyl) benzimid-azol, 1-2-y/ carbamate. (Benlate 50% w.p.).

Rhizolex: o,o-dimethyl-o-(2.6 dichloro- 4 meyhyl phynyl phosphoro thioat).

Kocide: Cupric hydroxide.

Sandofan: N-(2, 6-dimethylphynol)–2-methoxy-N-(2-oxooxazoladin-3-yl) acetamide.

Insecticides:

Pyrithrum: (z)-(s)z-2-methyl-4-oxo 3-(penta-2, 4-dimethyl) cyclopent-2-enyl)3-(2-methoxy prop-1-enyl)-2, 2-dimethyl cyclo propanecarboxylate.

Cyolane: 25% 2-(diethoxy phosphinyl amino 4-methyl 1.3 dithiolane).

Malation: 1.2 bis (ethoxycarbonyl) ethyl-0.0-dimethyl phosphorodithioate).

Thymol: 2 isopropyl–5 methyl phenol 3 hydroxy- p. cymene.

The pesticides were incorporated at different concentrations into PDAY medium at the required amounts according to their active ingredients, while still warm and the Petri-dishes were rotated gently (Subhani *et al.*, 2008). The plates were inoculated at the centre with a 5-mm diameter disc of *B. bassiana* grown on nutritional medium. Four replicates were used for every treatment and incubated at 25±2°C and the linear growth was measured.

Data were analyzed by simple factorial design (Steel *et al.*, 1996). Four replications were used to determine the difference among individual treatments, *i.e.*, pesticides and their doses.

Field trials

Field trials were carried out at Nobaria region (Behera Governorate), Egypt in the two successive cotton seasons 2009 and 2010 to study the effectiveness of the tested fungi on PBW and CLW. Cotton (variety Giza 45) was cultivated by end of March during the two seasons in an area of about half feddan. Fungi were applied as single treatments in randomize plots. Regular agricultural practices were performed and no chemical control was used during the study period. Weeds were removed by hand. Five plots were sprayed by water as control. Samples from each treatment were collected weekly and transferred to the laboratory for investigation. Percentages of infection were estimated.

Yield assessment

Yield data in treated and untreated plots in the cotton harvest seasons (2009 and 2010), represented by weight in kgs were determined. Yield loss was estimated according to the following equation:

$$\text{Yield loss} = \frac{\text{Potential yield} - \text{Actual yield}}{\text{Potential yield}}$$

Potential yield of the *B. bassiana* treatment (the best result among the tested pathogens) was considered the standard for comparison with the other ones.

RESULTS AND DISCUSSION

Effect of entomopathogenic fungi on the target insects

Data in table (2) show that under laboratory conditions, the LC_{50} obtained was $154x10^4$, $159x10^4$, $179x10^4$ and $157x10^4$ spores/ml for PBW larvae, after treatment with *B. bassiana, M. anisopilae, V. lecanii and P. fumosoroseus,* respectively.

Table (2): Effect of some entomopathogenic fungi against the pink bollworm, *P. gossypiella* larvae under laboratory conditions

Fungi	LC_{50}	Slope	Variance	95% confidence limited
B. bassiana	$154x10^4$	0.01	0.002	131-175
M. anisopliae	$159x10^4$	0.01	0.002	141-197
V. lecanii	$179x10^4$	0.02	0.001	146-193
P. fumosoroseus	$157x10^4$	0.02	0.001	135-178

Sabbour and Abdel-Rahman (2007) reported that under laboratory conditions results showed that the LC_{50} values versus *Phyllotreta cruciferaem, Pegomyia hyoscami* and *Cassida vittata* for the tested fungi *Verticillium lecanii (V.l.), Nomuraea rileyii (N.r.)* and *Paecilomyces fumosoroseus (P.f.)*, ranged between $5.4x10^6$ and $1.43x10^7$ spores/ml. Satisfactory results with the entomopathogenic fungi were reported by Sharaf El-Din (1999) and Sabbour and Ismail (2001).

Data in table (3) show that under the laboratory conditions, the LC_{50} obtained was $163x10^4$, $175x10^4$, $199x10^4$ and $187x10^4$ spores/ml for CLW larvae after treatment with different concentrations of *B. bassiana, M. anisopliae;* and *P. fumosoroseus*, respectively. Similar results were obtained by Sabbour and Abd El-Aziz (2002), who reported reduced LC_{50} values for the fungi; *B. bassiana* and *M. anisopliae* versus *S. littoralis* under laboratory conditions.

Table (3): Effect of some entomopathogenic fungi against cotton leafworm *S. littoralis* larvae under laboratory conditions

Fungi	LC_{50}	Slope	Variance	95% confidence limited
B. bassiana	$163x10^4$	0.01	0.002	154-195
M. anisopliae	$175x10^4$	0.01	0.002	146-197
V. lecanii	$199x10^4$	0.01	0.003	166-219
P. fumosoroseus	$187x10^4$	0.01	0.003	165-211

Effect of some environmental and nutritional factors

In-vitro effects of media, temperature, RH, pH, carbon and nitrogen sources on the linear growth of *B. bassiana* were studied.

1 -Culture media

B. bassiana was grown on five different solid media. As shown in table (4), growth of the tested fungi varied depending on the type of medium. In general, PDA was the best medium; the fungus gave its maximum linear growth as 89.5 mm within 7 days, followed by GP and Cz. Media, reaching 82.5 and 80.75 mm, respectively, with significant difference at $p<0.05$. However, PDA is considered a general medium for growth due to its high nutritional value (Trindade, 1994). Present results do not support the statement of Bidochia *et al.* (1987) but agree with the findings of Ayala (1996) and Santa *et al.* (2005), since PDA induced the best linear growth for *B. bassiana.*

Table (4): Effect of different media on the linear growth (mm) of *B. bassiana*

Media	Growth (mm)
Potato dextrose agar (PDA)	89.5
Glucose peptone (GP)	82.5
Czapek's	80.75
Corn meal (CM)	29.75
Carboxy methyl cellulose	7.75
L.S.D. at 0.5%	5.162

- Each figure represents an average of 4 replicates at 25°C ±2 or 6 days

2- Temperature and RH

B. bassiana isolate was able to grow at a wide range of temperature and RH. Data in table (5) indicated that 30°C was the optimum temperature for the growth, followed by 25°C, with a significant difference at ($p<0.05$). On the other hand, there was a very sharp decline in fungal growth above 35°C and completely inhibited at 40°C.

Concerning RH, the growth of *B. bassiana* was much enhanced by high RH; as the RH increased. Linear growth also increased to reach its maximum (88.25 mm) at 100% RH (Table 6). This increment was found significant as RH increased from 75 to 95%. In this respect, Walstad *et al.* (1970) found that *B. bassiana* required RH above 92.5% and a temperature between 15 and 35°C for luxuriant mycelial growth. Optimum growth occurred at 100% and 25-30°C. Hallsworth and Magan (1999) reported that the temperature range for growth of *B. bassiana* was 5-30°C and the optimum temperature was 25°C.

Table (5): Effect of different temperatures on the mycelium growth of *B. bassiana*

Temperature ± 1°C	Growth (mm)
15	12.25
20	33.75
25	80
30	88.25
35	12.25
40	0
L.S.D. at 0.5%	4.98

Table (6): Effect of relative humidity (RH) on the mycelium growth of *B. bassiana*

RH %	Growth (mm)
75	43.25
80	51.5
85	62.5
90	79.5
95	82.5
100	88.25
L.S.D. at 0.5%	5.37

- Each figure represents an average of 4 replicates at 25±2°C or 6 days

3- Effect of some nutritional factors

3-a- Effect of different carbon sources

Results in table (7) revealed that the isolate *B. bassiana* was able to grow and to utilize any carbohydrate source added to the growth medium. Sucrose was the best substrate for supporting growth (87.25 cm), followed by glucose (56.25cm), with a significant difference. Mannose had the lowest capability, while other substrates showed moderate effects. Results obtained by Bharati *et al.* (2007) revealed that starch was the best carbon source which recorded maximum growth of the fungus *M. anisopliae,* followed by sucrose and fructose.

Table (7): Effect of different carbon sources on the mycelium growth of *B. bassiana*

Carbon source	Growth (mm)
Glucose	56.25
Sucrose	87.25
Arabinose	33.25
Mannose	22
Citric acid	27.5
L.S.D. at 0.5%	3.63

- Each figure represents an average of 4 replicates at 25°C ±2 or 6 days

3-b Effect of different nitrogen sources

Data in table (8) revealed that all tested nitrogenous compounds were utilized by the fungus *B. bassiana*. Calcium nitrate was generally the best suitable nitrogen source, followed by glycin and sodium nitrate and then ammonium nitrate. Ammonium phosphate was found to be the lowest one. Results obtained by Bharati *et al.* (2007) showed that KNO_3 was the best nitrogen source which recorded maximum growth of the fungus *M. anisopliae,* followed by $NH_4 NO_3$.

4- Effect of different pesticides on linear growth

Tested pesticides showed different effects on the mycelium growth of *B. bassiana* depending on chemical composition and its concentration in the medium (Tables 9 and 10).

a- Fungicides

The fungicide Benlate was the most toxic to *B. bassiana* even at the lower concentration. The growth of the fungus was completely inhibited (100%) at 6.25 ppm. Kocide fungicide had the least effect against *B. bassiana,* while Rhizolix, Kocide and Sandofane showed high toxicity to the fungus at the highest concentration of 400 ppm. Data also showed that Benlate was very effective, followed in a descending order by Rhizolex, Sandofane and Kocide.

b- Insecticides

Tested insecticides showed different effects on the mycelium growth of *B. bassiana* depending on type and concentration in the medium (Table 10). None of the tested insecticides caused death to *B. bassiana* except, Thymol at higher concentrations between 100 and 800 ppm. In some cases, the inhibition was moderate, especially in lower concentration of 50 ppm. Thymol proved to be the most effective insecticide, followed by Pyrethrum with a significant difference at $p<0.05$, while Cyolane was the least effective one against *B. bassiana.*

Many experiments were carried out to detect side effects of pesticides on *B. bassiana* (Olmert and Kenneth, 1974). Most tests evaluated the effects of pesticides on vegetative growth and sporulation. The use of incompatible insecticides may inhibit the development and reproduction of entomopathogenic fungi, and thus affecting IPM (Malo *et al.*, 1993).

Table (11) summarizes the percentage of infestation after treatments with the tested bio-insecticides. The fungi (*B.b.*) and (*M.a.*) showed a high potential effect against (PBW) and (CLW). The infestation percents were; 21±2.4 and 20±4.2 when (PBW) and (CLW) were treated with (*B.b.*), as compared to 97±5.1 and 99±3.3 in the control in 2009 and 2010, respectively.

Table (8): Effect of different nitrogen sources on the mycelium growth of *B. bassiana*

Nitrogen Source	Growth (mm)
Ammonium phosphate	7.75
Sodium nitrate	50.5
Glycin	54
Calcium nitrate	88
Ammonium nitrate	12.5
L.S.D. at 0.5%	3.63

- Each figure represents an average of 4 replicates at 25°C ±2 or 6 days

Table (9): Effect of fungicides incorporated into PDA medium on the linear growth (mm) of *B. bassiana*

Concentration (ppm)	Benlate	Rhizolex	Kocide	Sandofane
0.0 (Untreated)	84.75*	84.75	84.75	84.75
6.25	0.0	53.25	67.25	63.50
12.5	0.0	51.25	47.25	51.75
25.0	0.0	51.00	42.75	47.25
50.0	0.0	33.00	38.50	43.50
100.0	0.0	23.25	24.50	16.25
200.0	0.0	16.25	16.50	11.75
400.0	0.0	10.50	10.50	10.25
Mean	10.59	40.41	41.44	40.84

*Colony diameter/mm L.S.D. (5%) for Fungicides = 16.99, Concentrations = 12.02 and Interactions = 33.99

Table (10): Effect of insecticides incorporated into PDA medium on the linear growth (mm) of *B. bassiana*

Concentration (ppm)	Pyrithrum	Cyolane	Malation	Thymol
(Untreated)	89.0	89.8	86.3	83.8
50.0	38.3	34.8	37.8	43.5
100.0	22.3	29.8	27.5	0.0
200.0	9.3	26.8	21.8	0.0
400.0	9.8	16.5	16.3	0.0
800.0	8.0	7.3	14.5	0.0
Mean	29.42	34.13	34.0	21.22

*Colony diameter/mm L.S.D. (5%) for: insecticides = 8.19, Concentrations = 10.03 and Interactions = 20.05

Table (11): Effect of different treatments on the target insect pests under field conditions

Post 1st application date	Treatments	% of infestation (means)±s.e (PBW) during 2009	(CLW) during 2010
20	Control	65±2.5	69±3.4
50		83±3.4	88±3.4
90		97±5.1	99±3.3
20	(*B.b.*)	31±4.4	24±4.3
50		28±4.7	27±3.4
90		21±2.4	20±4.2
20	(*M.a.*)	38±3.1	30±3.1
50		31±4.4	27±2.2
90		25±2.3	22±3.2
20	(*P.f.*)	41±2.6	40±3.4
50		39±3.5	34±2.3
90		33±4.1	29±1.3
20	(*V.l.*)	45±3.3	44±1.2
50		41±4.2	37±3.5
90		38±4.2	30±2.3
F value=		15.10	
LSD 5%=		1.42	

Data in table (12) show that the weights of the cotton crop treated with *P.f.*–treated amounted 2976 and 2998 kgs/ feddan as compared to 989 and 823 kgs/ feddan in the control plots in 2009 and 2010 crop seasons, respectively. Meanwhile, they ranged between 2321 and 3400 in 2009 and 2546 to 3583 kgs/ feddan in 2010 seasons in the other tested treatments. This led to a significant decrease in the yield loss ranged between 7.7-53 and 14-53% kgs/ feddan in seasons 2009 and 2010 as compared to 80 and 82% in the control plots, respectively (Table 12).

Table (12): Assessments of damage caused in cotton field after treatment with fungi

Treatments	Season 2009		Season 2010	
	Wt of cotton crop (kg/feddan)	yield loss %	Wt of cotton Crop (kg/feddan)	yield loss %
B.b.	3632 ± 54.66	31	3919 ±63.43	34
M.a.	3510 ± 66.71	31	3599 ± 80.12	35
V.l.	2612 ± 48.92	7.7	3731 ± 71.23	14
p.f.	2976±87.4 1	40	2998±66.43	45
Control	989		823	
F value	34.6		32.9	
LSD5%=	122.7		126.5	

Mesbah *et al.* (2004) reported that some microbial control agents were mainly effective as biocides and reduced the infestations of the sugar beet insect pests and increased the yield in Kafer El-Sheikh, Egypt. Seweify (1998) found that the crop yield increased after treatments with fungi. Sabbour (2006) found that the yield loss of the potatoes was significantly decreased in the plots treated with *B. bassiana* and *M. anisopliae.*

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