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Efficacy of certain entomopathogenic fungi for controlling the potato tuber moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae) under field conditions in Beheira Governorate, Egypt

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Abstract

The Field study was conducted in Barsiq village, Abu Homs city, Beheira Governorate during two successive seasons, summer seasons 2021 and 2022. The main purpose of this study was to evaluate the efficacy of two entomopathogenic `fungi, *Beauveria bassiana* and *Metarhizium anisopliae* three concentrations $1x10^6$, $1x10^9$ and $1x10^{12}$ spores/ml were used. Also, three commercial bioinsecticides [Biosiana 2.5% WP, Biometa 2.5% WP and Ememactin benzoat 5.7% WG]. The insecticide Methomyl 90% SP, for comparison under field conditions on the population of potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) which is considered the main insect pest that attacks potato *Solanum tuberosum* L. The obtained results showed the efficiency of tested control methods to protect potato foliage from PTM infestation, the highest value was recorded with Methomyl insecticide, followed by E. benzoat, combining E. benzoat with Biosiana, E. benzoat with Biometa, Biosiana, Biometa respectively. While the entomopathogenic fungi *B. bassiana* 1×10^{12}, 1×10^9, 1×10^6 and *M. anisopliae* 1×10^{12}, 1×10^9, 1×10^6, respectively. The relation between treatments and potato yield indicated that the highest increase of the potato yield was recorded with Methomyl insecticide, followed by, E. benzoat, combining E. benzoat with Biosiana, E. benzoat with Biometa, Biosiana and, Biometa respectively, Biosiana, Biometa, *B. bassiana* and *M. anisopliae* three concentrations (1×10^{12}, 1×10^9, 1×10^6) spores/ml.

Introduction

The potato *Solanum tuberosum,* Family Solanaceae is currently the fourth most common food crop in the world in terms of production ranked after rice, wheat, and corn. It plays a relevant role in global nutrition and in the sustainable development goals of the United Nations related to zero hunger and feed security (Ahmadu *et al.,* 2021). Potato *S. tuberosum* is also considered as one of the most important agricultural products that plays a major role as a high-value crop for human health and the food industry (Germchi *et al.,* 2011).

In Egypt, potato is considered as a major energy - rich crop and it is consumed for its high food contents, and it is also required for exportation, representing one of the most important sources of national income (Shoeb and Mostafa, 2004). Potato plant *S. tuberosum* was considered a major export crop in Egypt (Mary *et al*., 1999). Potato plant *S. tuberosum* belongs to the Solanaceae family attacked by the potato tuber moth (PTM) *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae). *P. operculella* is an important and prevalent potato pest in 90 countries in Africa, Asia and Central and South America with tropical and subtropical climates (Lacey *et al*., 2010). *P. operculella* is considered an important economic pest because its larvae cause severe damage to crops especially potatoes, tomatoes, eggplants and many Solanaceae plants (Rondon and Gao, 2018). The potato tuber worm *P. operculella*, is considered to be one of the most destructive pests of the potato *S. tuberosum* crop. This wider niche ability makes this insect difficult to manage and control (Rondon *et al*., 2006). The continuous use of pesticides promotes pesticide resistance in *P. operculella* and better management strategies are needed (Yuan *et al*., 2018). The potato tuber moth *P. operculella* is considered one from the most serious pest of potatoes in Egypt as well as many other countries. It causes severe damage to potato tubers in the field and the storage. Its main hosts are potato, tomato, and pepper (Chandel *et al*., 2020).

The use of chemical insecticides to control potato tuber moth is associated with many problems of creating more resistant strains of the insect, in addition to the treated tubers become invalid for human consumption due to the persistence of pesticide residues in the tubers (Adhikari *et al*., 2022).

This resulted in attempts to develop an integrated control program for potato tuber moth in the field and storage. The present contribution is an attempt to assess different components of IPM (Integrated Pest Management) in controlling the potato tuber moth (Alyokhin *et al*., 2022). Entomopathogenic fungi are one of IPM components to be evaluated, not only due to it is safe alternatives to synthetic insecticides but also compatible with synthetic and botanical pesticides (Bamisile *et al*., 2021).

Additionally, they are insect-specific pathogens. Entomopathogenic fungi such as *Beauveria bassiana* (Bals.) Vuill. belong to the class of Hyphomycetes, are naturally occurring environmentally safe organisms, have a broad spectrum of hosts and, can be mass-produced easily, rapidly and economically (Rumbos and Athanassiou, 2017). This fungus produces aerial conidia, single cell blastospores by germination when they come into contact with the upper skin layer of insects and penetrate directly into the bodies of their hosts from the upper skin (Kim *et al*., 2010). The fungus proliferates rapidly in the body by producing toxins. When a fungus kills its host, it evolves outward by covering the insect with a white layer of mildew. This mildew produces millions of new infective spores that are released into the environment (Irigaray *et al*., 2003).

Entomopathogenic fungi such as *B. bassiana* offer promise in the microbial control of certain economic crop pests (Jaronski, 2010). This fungus has been scrutinized worldwide as a microbial control agent of many insect pests (Hoffman *et al*., 1993). More than 30 entomopathogenic fungi have been tested as biological control preparations for different insect pests (Ferron *et al*., 1976). The ubiquitous fungus *B. bassiana* (Balsamo) Vuillemin causes common diseases associated with dead moribund insects in nature (McCoy *et al*., 1988). The advantages of using natural products such as *B. bassiana* are numerous, including being safe for the environment when compared to chemical insecticides, relatively easy to handle and safer for handlers. However, *B. bassiana* depends on favorable environmental conditions during application and they are heavily humidity-dependable (Shah and Pell, 2003). They found that the utilization of a combination of Emamectin benzoate (Absolute) and methomyl (Jito) with *B. bassiana* is feasible for the control of polyphagous insects, S. littralis especially the second larval instar (Ibrahim *et al*., 2017). There is great potential for the use of *B. bassiana* in biological control, because it can be cost-effective to locally mass-produce. Moreover, many strains are also already commercially available. *B. bassiana* has been mass-produced on different solid substrates, including steamed rice (El-Wakeil *et al*., 2020), Also the ubiquitous fungi, *Metarhizium anisopliae* (Metsch.) Sorokin (Ascomycota: Hypocreales) is an important agent for biological control of several insect pests (Bamisile *et al*., 2021).

The entomopathogenic filamentous fungus *M. anisopliaeis* popular as a pathogen of arthropods applied in biological control strategies against numerous serious insect pests (Sharma and Sharma, 2021). *M. anisopliae* (Metsch) Sorok (Ascomycota: Clavicipitaceae) usually multiply in the form of blastospores or hyphae structures in their host, similar to yeasticidal and spread to the body cavity with hemolymph fluid movement in the insect body. The spores of these fungi penetrate into the insect body by contact and kill the host (Driver *et al*., 2000). It produces mycotoxins and destruxins, a group of secondary metabolites, which are considered as an important new generation of insecticides (Mannino *et al*., 2021). It is a recognized pathogen of more than 200 insect species, including several major pests (Roberts and Hajek, 1992). The potential of *M. anisopliae* (Metsch.) as a cost-effective management agent for subterranean insect pests has been demonstrated (Peng *et al*., 2022).

The objective of this study is to evaluate the efficacy of entomopathogenic fungi as one of IPM components for controlling potato tuber moth (PTM), *P. operculella*.

Materials and methods

The main purpose of the present study is to evaluate the efficacy of two entomopathogenic fungi, *B. bassiana* and *M. anisopliae* three concentrations1x10^6, 1x10^9 and 1x10^{12} spores/ml. In addition to three commercial bioinsecticides [Biosiana 2.5% WP (*B. bassiana* 1x10^8 CFU's/gm), Biometa 2.5% WP (*M. anisopliae* 1x10^8 CFU's/gm) and Ememactin benzoat 5.7% WG (ALASKA)]. The insecticide Methomyl 90% SP (KREMAX), for comparison under field and storage on the population of potato tuber moth (PTM) *P. operculella* which is considered the main insect pest that attacks potato *S. tuberosum* crop (Spunta variety).

1. Field studies:

The Field study was carried out in Barsiq village, Abu Homs city, Beheira Governorate during two successive seasons: summer seasons 2021 and 2022 to study the effect of treatment on the population reduction of potato tuber moth larvae on potato leaves (Spunta variety) under field conditions during summer seasons

2021 and 2022. Three concentrations of *B. bassiana* and *M. anisopliae* $1x10^6$, $1x10^9$ and $1x10^{12}$ spores/ml were used in the fungus, also Commercial vehicles bioinsecticide (Biosiana 2.5% WP, Biometa 2.5% WP and E. benzoat 5.7% WG (ALASKA) and addition to mixing bioinsecticides and comparing them with Methomyl 90% SP (KREMAX) as standard insecticide during the two seasons. Each treatment was divided into four pieces 52 (Replicates). In the design of a whole random block showed population reduction due to treatments, the population reduction was calculated according to the insect number in treated blokes in comparison to insect number in control plots (Untreated check): (Henderson and Tilton equation, 1955).

$$\textbf{Corrected \% = (1 -} \frac{\textbf{n in Co before treatment * n in T after treatment}}{\textbf{n in Co after treatment * n in T before treatment}} \textbf{)} \times \textbf{100}$$

Where: n = Insect population, T = treated, Co = control

The area of each plot was 21 m² (3x7 m²); so, that the area of all treatments was 1092 m² cultivated by potato (Spunta variety). The untreated plot was subjected to natural infestation. The potato Spunta variety was selected and planted at the first day of March of 2021 and 2022 seasons, respectively. The potato variety was obtained from the International Potato (HZPC) Holland, Abu Homs city, Beheira Governorate, Egypt. The regular seeding practices were applied by sowing in 25 cm apart in rows (80 cm wide) with the soil ridged up to bury the tuber seeds at a depth 20 cm below soil surface. Irrigation intervals and fertilization were conducted as usual. Two sprays were applied for all treatments, the first spray was applied at 81 days after sowing and the second spray was applied at 15 days after the first spray during 2021 and 2022 seasons.

2. Preparation of *Beauveria bassiana* and *Metarhizium anisopliae* culture:

The fungus *B. bassiana* and *M. anisopliae* were grown on artificial media in the laboratory, Bioinsecticide Production Unit at the Plant Protection Research Institute - Agricultural Research Center, Dokki, Giza, Egypt. The materials used for preparing the selective medium were composed of 20g dextrose, 10g peptone, 18g agar-agar and 1000 ml of distilled water. These components were mixed thoroughly and dissolved in the water in a conical flask. The prepared medium was sterilized in an autoclave at 120°C with a pressure of 15 lb for 15 min. When the medium was cooled down to 60°C, 0.6g streptomycin, 0.05g tetracycline, and 0.05g cyclohexamide dissolved in 20ml sterilized distilled water was poured into the conical flask that contained the medium and thoroughly mixed. The sterilized liquid medium was then poured into sterilized petri plates (l16°C for 4 hrs. in the oven) and kept solidifying for streaking fungus culture. The culture was incubated at 24°C and 75% RH. to induce growth and sporulation of fungus in an aseptic condition in the laboratory. After 16 days, the conidia were harvested by scrapping off the contents of each Petri dish using a sterile metal spatula. The culture was stored at a cool temperature at 4°C.

3. Preparation of *Beauveria bassiana* and *Metarhizium anisopliae* concentrations:

For the preparation of *B. bassiana* and *M. anisopliae* concentrations, the conidia scrapped off from the Petri dish were mixed homogenously in water together with 2 drops of Tween 20 (0.l%, dispersing agent). Conidia in the stock solution was quantified by counting under a microscope using the hemocytometer (Thoma) counting chamber and the stock solution was adjusted to $1x10^7$ conidia/ml by adding water. Five bioassays were conducted, for each bioassay a new stock solution (No. I, III-V) was prepared except of bioassay II, for which purpose the stock solution No. I was stored over a month period at ambient temperature. Since exact adjustment to $1x10^7$ conidia/ml was not always possible, the quantity (titer) assessed through counting (average of three counts) of conidia was taken as the most exact estimate of the highest concentration used in each bioassay. The stock suspension "Ma-stock" was further diluted using different dilution factors to obtain different concentration levels $1x10^6$, $1x10^9$, $1x10^{12}$ spores/ml.

4. Insect pathogens and Insecticides Used:

4.1. Biosiana 2.5% WP: Isolated, developed, propagated and prepared the required concentration in the Bioinsecticide Production Unit at the Plant Protection Research Institute – A.R.C., Dokki, Giza, Egypt.

Formulation Type: Wettable Powder and dust.

Active ingredient: (*B. bassiana*)

Recommended rate: 250 gm./100 L. water

Concentrations used: $1x10^8$ CFU's/gm.

4.2. Biometa 2.5% WP: Isolated, developed, propagated and prepared the required concentration in the Bioinsecticide Production Unit at the Plant Protection Research Institute – A. R. C., Dokki, Giza, Egypt.

Formulation Type: Wettable Powder and dust.

Active ingredient: (*M. anisopliae*)

Recommended rate: 250 gm./100 liter water

Concentrations used: $1x10^8$ CFU's/gm.

4.3. Ememactin benzoat 5.7% WG (ALASKA): Prepared the required concentration in the Jingbo Agrochemicals Technology Co., Ltd., China, and packaging Zein Group for Agricultural Development Egypt.

Formulation Type: Water dispersible granules.

Active ingredient: Emamectin Benzoate

Recommended rate: 60 gm./fed.

Concentrations used: 5.7% WG.

4.4. Methomyl 90% SP (KREMAX):

Has been made the required concentration in the Shandong Huayang Pesticide Chemical Industry Group Co., Ltd., China, and packaging Misr Agricultural Development Company Egypt.

Formulation Type: Soluble powder.

Active ingredient: Methomyl

Recommended rate: 300 gm./fed.

Concentrations used: 90% SP.

5. Statistical analysis:

The obtained data were statistically analyzed with one-way analysis of variance (ANOVA) ($P<0.05$) according to Snedecor and Cochran (1980), and multiple range test means was analyzed by using method of Duncan (1955).

Results and discussion

1. Field evaluation:

The Field evaluation of the present work is to evaluate the efficacy of two entomopathogenic fungi, *B. bassiana* (Balsamo) and *M. anisopliaem*, three concentrations $1x10^6$, $1x10^9$ and $1x10^{12}$ spores/ml. Also, three commercial bioinsecticide [Biosiana 2.5% WP (*B. bassiana* $1x10^8$ CFU's/gm), Biometa 2.5% WP (*M. anisopliae* $1x10^8$ CFU's/gm) and mixing the fungus with E. benzoat 5.7% WG (ALASKA)]. The insecticide Methomyl 90% SP (KREMAX), for comparison under field on the population of potato tuber moth (PTM), *P. operculella* (Zeller) which is considered the main insect pest attacks potato *S. tuberosum* L. (Spunta variety), were conducted during two successive seasons; summer season 2021 and summer season 2022.

Table (1) shows the percentage of potato tuber moth larval population reduction in the treated fields during 2021 season, which was as follows: the entomopathogenic fungi *B. bassiana* were three concentrations ($1×10^6$, $1×10^9$ and $1×10^{12}$), were the reduction caused (68.05, 72.04 and 72.31%), respectively. The percentage of reduction in the larval population of the *M. anisopliae* fungus using the three concentrations ($1×10^6$, $1×10^9$ and $1×10^{12}$), achieved a value (59.72, 69.38 and 70.61%) respectively. When treated with the biocide Biosiana and Biometa, the results were 76.38% and 74.70%. On the other hand, application of the biopesticide E. benzoat to population reduction by 88.22%, while mixing with Biosiana and Biometa the reduction rate was (87.57% and 86.93%, respectively). The chemical pesticide Methomyle was used for comparing with biological treatments, where the reduction 92.90.

Table (2) shows the percentage of potato tuber moth larval population reduction in treated field during 2021 season, which was as follows: where three concentrations of *B. bassiana* ($1×10^6$, $1×10^9$ and $1×10^{12}$), where the reduction caused (77.56, 82.22 and 83.97%) respectively. While the percentage of reduction in the larval population of the three concentrations of *M. anisopliae* ($1×10^6$, $1×10^9$ and $1×10^{12}$), where it achieved a value (75.96, 79.80 and 80.96%), respectively. When treated with the biocide Biosiana and Biometa, the results were 85.57% and 84.31%. On the other hand, application of the biopesticide E. benzoat to population reduction of 91.29%, while mixing with Biosiana and Biometa the reduction rate was (87.05% and 86.73%) respectively. The chemical pesticide Methomyle was used for comparison with biological treatments, where the reduction 93.76%.

Table (1): Effect of the insecticide applications on the population reduction of *Phthorimaea operculella* larvae on the leaves of potato (Spunta variety) under field conditions during summer season 2021.

Treatments	Population reduction% after the 1st spray	Population reduction% after the 2nd spray	Mean of total reduction %
Beauveria bassiana **$3×10^6$**	61.66de	74.44de	68.05e
Beauveria bassiana **$3×10^9$**	68.05c	76.04d	72.04cde
Beauveria bassiana **$3×10^{12}$**	60.06e	84.02c	72.31cde
Metarhizium anisopliae	48.89f	70.56e	59.72f
Metarhizium anisopliae	68.05c	70.71e	69.38e
Metarhizium anisopliae	59.11e	82.11c	70.61de
Biosiana 2.5% WP	66.66c	86.11c	76.38c
Biometa 2.5% WP	65.39cd	84.02c	74.70cd
Ememactin benzoat + Biosiana 2.5% WP	82.25ab	92.90b	87.57b
Ememactin benzoat + Biometa 2.5% WP	79.67b	94.19b	86.93b
Ememactin benzoat 5.7% WG (ALASKA)	79.82b	96.63ab	88.22b
Methomyle 90% SP (SHANDONG HUAYANG)	85.80a	100.0a	92.90a
P-value	0.000	0.000	0.000

Table (2): Effect of the insecticide applications on the population reduction of *Phthorimaea operculella* larvae in the leaves of potato (Spunta variety) under field conditions during season summer 2022.

Treatments	Population reduction% after the 1st spray	Population reduction% after the 2nd spray	Mean of total reduction %
Beauveria bassiana	69.55i	85.57k	77.56h
Beauveria bassiana	72.82g	91.63g	82.22f
Beauveria bassiana	79.39c	88.55i	83.97e
Metarhizium anisopliae	69.95i	81.97l	75.96i
Metarhizium anisopliae	73.07g	86.53j	79.80g
Metarhizium anisopliae	71.95h	89.98h	80.96g
Biosiana 2.5% WP	78.84d	92.30f	85.57d
Biometa 2.5% WP	74.91f	93.72e	84.31e
Ememactin Benzoat + Biosiana 2.5% WP	77.81e	96.30b	87.05c
Ememactin Benzoat + Biometa 2.5% WP	78.44d	95.02d	86.73cd
Ememactin Benzoat 5.7% WG (ALASKA)	87.17b	95.42c	91.29b
Methomyle 90% SP (SHANDONG HUAYANG)	89.31a	98.21a	93.76a
P-value	0.000	0.000	0.000

Where the highest value was recorded with Methomyl insecticide, followed by E. benzoat + Biosiana, E. benzoat + Biometa, Biosiana, Biometa. While the entomopathogenic fungi *B. bassiana* 1×10^{12}, 1×10^{9}, 1×10^{6} and *M. anisopliae* 1×10^{12}, 1×10^{9}, 1×10^{6} , respectively. There were significant differences at (P = 0.5) between treatments. Data agree with the percentage of moth emergence showed a highly progressive decrease with the increase in the concentrations of *B. bassiana*. Thus, emergence decreased from 96.7% in the control to 10% at 16.5 x 10^{8} conidia/ml. (Hafez *et al*., 1997).

Where, found that the combination of insecticides with *B. bassiana* and *M. anisopliae* showed 1.05-1.24- and 1.19-1.42-fold increase in virulence over the sole treatment, respectively (Dayakar *et al.*, 2000). In another study, Purwar and Sachan (2004) also found similar results with *Lipaphis erysimi*. Thus, the combination of insecticide and entomogenous fungi was more deleterious to the insect than application of insecticides or entomogenous fungi alone.The utilization of combination of E. benzoate (Absolute) and methomyl (Jito) with *B. bassiana* is feasible for the control of polyphagous insect, *S. littralis* especially the second larval instar. (Ibrahim *et al*., 2017).

2.The relation between treatments and potato yield:

The results of potato yield during 2021 and 2022 seasons are listed in Table (3), the data revealed that all tested treatments resulted in increasing the potato yield; there were significant differences at (P = 0.5), between Methomyl, E. Benzoat, E. benzoat + Biosiana, E. benzoat + Biometa, Biosiana, Biometa, *B. bassiana* and *M. anisopliae* three concentrations ($1x10^{6}$, $1x10^{9}$ and $1x10^{12}$) spores/ml and untreated checks.

Table (3): The relation between insecticide used and potato yield (Spunta variety) during 2021 and 2022 seasons.

Treatments	Rate of Application /fed.	Mean (Kg/plot).		Yield increase %.	
		2021	2022	2021	2022
Beauveria bassiana **3×10^6**	250 ml	133.80d	121.03fg	18.21e	05.58g
Beauveria bassiana **3×10^9**	250 ml	130.19d	135.81bcd	15.02h	18.47c
Beauveria bassiana **3×10^{12}**	250 ml	133.12d	137.00bc	17.61ef	19.51c
Metarhizium anisopliae **3×10^6**	250 ml	131.15d	126.54ef	15.87gh	10.38f
Metarhizium anisopliae **3×10^9**	250 ml	134.02d	127.06ef	18.41e	10.84f
Metarhizium anisopliae **3×10^{12}**	250 ml	132.04d	137.12bc	16.66fg	19.62c
Biosiana 2.5% WP	250 g	142.12c	129.41de	25.56d	12.89e
Biometa 2.5% WP	250 g	129.89d	132.81bcde	14.76h	15.86d
Ememactin benzoat + Biosiana 2.5% WP	60 g + 250 g	147.33bc	126.98ef	30.17c	10.77f
Ememactin benzoat + Biometa 2.5% WP	60 g + 250 g	142.21c	129.81cde	25.64d	13.24e
Ememactin benzoat 5.7% WG (ALASKA)	60 g	154.06a	139.18b	36.12a	21.42b
Methomyle 90% SP (SHANDONG HUAYANG)	300 g	149.86ab	152.13a	32.40b	32.71a
Control		**113.18e**	**114.63 g**	**--**	**--**

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