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PATHOGENICITY COMPARATIVE OF SOME EGYPTIAN ISOLATES AND COMMERCIAL INDIANS COMPOUNDS OF ENTOMOPATHOGENIC FUNGI AGAINST SOME INSECT PESTS

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Abstract

The greater wax moth, *Galleria mellonella* Linnaeus, is a ubiquitous pest of the honeybee, *Apis mellifera* Linnaeus, and *Apis cerana* Fabricius. Also, grains are attacked by numerous noxious insect species during their storage like, *Tribolium confusum*. Several crops attacked with many insects and caused serious damage like, *Spodoptera littoralis* and *Aphis craccivora.* Laboratory experiments were done to measure the pathogenicity of three commercial compounds from entomopathogenic fungi, Bio Magic, Bio Power, and Bio Catch and two Egyptian isolates, *Metarhizium anisopliae* and *Beauveria bassiana* against the larvae of *G. mellonella*, *S. littoralis*, *T. confusum* and nymphs of *A. craccivora*. Three concentrations were used from all compounds, 1 x 10^7, 1 x 10^8 and 1 x 10^9 spores/ ml. at 25±2º C and 70± 5 R.H. *M. anisopliae* and *B. bassiana* were more pathogenicity than that Bio Power, Bio Magic, and Bio Catch against the larvae of *G. mellonella* and *S. littoralis*. *M. anisopliae* was the highest pathogenicity against the larvae of *T. confusum* then, Bio Magic, Bio Power, and Catch**.** Bio Catch was the highest pathogenicity against the nymphs of *A. craccivora* then *M. anisopliae* and *B. bassiana*, Bio Power, and Bio Magic. These results confirmed that *M. anisopliae* and *B. bassiana* isolates are the highest pathogenicity than commercial compound against all tested Insects.

***Keywords*:** Pathogenicity, Entomopathogenic Fungi, *G. mellonella*, *S. littoralis, T. confusum, A. craccivora*

Introduction

The greater wax moth, *Galleria mellonella* Linnaeus, is a ubiquitous pest of the honeybee, *Apis mellifera* Linnaeus, and *Apis cerana* Fabricius. The damage caused by *G. mellonella* larvae is severe in tropical and sub-tropical regions, and is believed to be one of the contributing factors to the decline in both feral and wild honeybee populations. This pest has received more attention as a model organism for toxicological investigations involving entomopathogenic organisms than as a honeybee pest, with more focus on proven (demonstrated) control measures, Harding, *et al*., 2013, Ramarao, *et al*., 2012 and Ellis, *et al*., 2013. However, with renewed interests in honeybee health and the moth's increasingly recognized economic role globally, especially in Africa and Asia, Pirk *et al*., 2015 and Chantawannakul *et al*., 2016. Grains are attacked by numerous noxious insect species during their storage. The losses caused by these insects are estimated to be up to 20% or more in developing countries, Neethirajan *et al*., 2007 and Phillips and Throne, 2010. The Biological control of stored-grain insects with natural enemies and pathogens, Thompson and Brandenburg, 2006, Paula, *et al*., 2011, Sabbour and Abd-El-Aziz, 2010, Wakefield, *et al*., 2010 & 2013, and Abdel-Raheem, *et al*., 2015. The cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera, Noctuidae) is one of the most destructive phytophagous pests because it can attack numerous economically important crops in either protected or open fields in the Mediterranean region and the Middle East, Hatem *et al*., 2009; Azab *et al.* 2001; Ahmad, 1988; Blackford *et al*., 1997; Champion *et al*., 1997 and Salama *et al*., 1990. Entomopathogenic fungi have been shown to successfully control a large variety of lepidopteran pests, Quesada-Moraga *et al*., 2013, Schulte *et al*., 2009; Devi *et al*., 2005; Vänninen and Hokkanen, 1997; Zaki and Abdel-Raheem, 2010; Abdel-Raheem *et al*., 2016 and Mohamed Abdel-Raheem, 2018. The cowpea aphid, *Aphis craccivora* Koch is one of the most common and well-known insect pests throughout the world, Zaki and Abdel-Raheem, 2010, Minks and Harrewijn, 1987, Blackman and Eastop, 2006 and Ismail and Abdel-Raheem, 2010. Aphids are important piercing-sucking insects that during feeding cause significant loss of a plant's phloem sap, which is essential for plant growth, Dixon, 1998. Indirectly, cowpea aphid also disturbs the photosynthesis process by the presence of fungus on the leaves that is supported by the aphids' honeydew secretion, Giuseppe *et al*., 2011 and Klingler *et al*., 2001. Plant damage increases because of the aphids' role as vectors for numerous plant viruses, Smitha 2007, such as faba bean necrotic yellow virus, broad bean yellow mosaic virus, and bean leaf roll virus, Aldryhim and Khalil, 1993 and Weigand, *et al*., 1991. Many of entomopathogenic fungi are a great potential for the

management of sucking pests, Rabindra and Ramanujam, 2007. The confused flour beetle, *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae), is the most important pests of stored products, Aitken, 1975 and Hill, 1990. It is able to attack whole wheat grains, Aitken, 1975 and Daniels, 1956. It can develop in a considerably wide temperature and moisture range, and is particularly tolerant to low humidities, Aitken, 1975 and Howe, 1960. Like other species, it is now resistant to several conventional insecticides, Abdel-Raheem, 2005, Champ and Dyte, 1976, Zettler, 1991, Arthur and Zettler, 1992 and Wool and Front, 2003.

Materials and Methods

This study was carried out in the Lab. of Pests & plant Protection Department, Agricultural and Biological Research Division, National Research Centre, Egypt.

Entomopathogenic Fungi

Egyptian Isolates: *M. anisopliae* and *B. bassiana* isolated by Abdel-Raheem, 2005 were isolated from *Scrobipalpa ocellatella* and *Cassida vittata.*

Commercial Indians Compounds: This Compounds were Provided from Company of Saiefe Gaarah at Cairo, Egypt, Bio Magic, *M. anisopliae,* Bio Power*, B. bassiana* and Bio Catch, *V. lecanii*.

Preparing of the Concentrations: Three concentrations were used (C_1) 1 x 10^7, (C_2) 1 x 10^8 and (C_3) 1 x 10^9 spores/ ml. and add 0.5 % Tween 80. The spores were counted in the suspension using a haemocytometer (Swastik Scientific Company, India) blood cell counting chambers (Hirscmann 0.1 mm x 0.0025 mm^2). A haemocytometer is essentially a microscope slide bearing a small well of known depth. The base of which is marked with squares of known dimensions. During use the well is covered with a special coverslip (usually 0.4 mm thick).

Mass Rearing:

Greater wax moth, *Galleria mellonella*:

Adults of *G. mellonella* released in plastic jars (10 X 20 cm) for mating and comprised folded sheets for the deposition and collection of eggs. The hatched larvae were reared on a semi-natural diet according to Metwally, 2013 these jars were incubated under the previously conditions till larvae.

The larvae of *Spodoptera littoralis* and nymphs of *Aphis craccivora*:

The larvae of *S. littoralis* and nymphs of *A. craccivora* Strains used in the present studies were taken from laboratory of Department of Plant Protection, Faculty of Agriculture, Al-Azhar Uni., Egypt.

The larvae of *T. confusum*:

The larvae of *T. confusum* individuals were taken from a culture kept on wheat flour plus 5% brewers yeast (by weight) at 28 ±2 ºC and 65±5 % R.H. and continuous darkness. Untreated, clean, infestation-free soft wheat and flour were used for experimentation. The moisture content of the wheat, as determined by a Dickey–John moisture meter (Dickey–John Multigrain CAC II, Dickey–John Co, USA), ranged between 10.7% and 11.0%. In order to measure the moisture content of the flour, quantities of flour (500 g each) were placed in an oven at 130 ° C for 90 min (Brabender III, Brabender Co, USA), and were reweighed at the end of the procedure.

Laboratory Inoculation

Larvae of *G. mellonella*, *S. littoralis*, *T. confusum*, and nymphs of *A. craccivora* were treated with three concentrations ((C_1) 1 x 10^7, (C_2) 1 x 10^8 and (C_3) 1 x 10^9) spores/ ml.) and put in Petri-dishes and incubated in 25±2°C and 75 ±5 % RH. (Five larvae or nymphs / replicate) were used in all treatments. The Entomopathogenic fungi were sprayed using a manual sprayer in a suspension containing (C_1) 1 x 10^7, (C_2) 1 x 10^8 and (C_3) 1 x 10^9 spores/ ml. While sterilized water was sprayed to the leaves disks as blank control. The mortality of larvae and nymphs were observed daily. Mortality data was corrected with that in control by using the Abbott's formula, Abbott, 1925.

Statistical Analysis

The per cent corrected cumulative mortality of each fungus was subjected to ANOVA test and the means were separated by Duncan's Multiple Range Test (DMRT).

Results

The % mortality of larvae of *G. mellonella*:

Table 1 showed the % mortality of larvae of *G. mellonella* was 15.0 and 20.3 % after 3rd days, at the concentration (C_3) 1 x 10^9 spores/ ml. from *M. anisopliae* and *B. bassiana*. Also, % mortality of larvae of *G. mellonella* was 12.5, 15.6 and 3.2% after 3rd days, at the concentration (C_3) from Bio Magic*,* Bio Power and Bio Catch, respectively. All the entomopathogenic fungi in concentration (C_3) produced high mortality ranging from 50.0 to 100 %, after 8th days of infection. *B. bassiana* reached to 100 % mortality after 6th days of infection. *B. bassiana* was the highest pathogenicity then, *M. anisopliae*, Bio Power*,* Bio Magic and Bio Catch, 100, 100, 100, 85.0 and 50.0 % mortality, respectively.

The % mortality of larvae of *S. littoralis*

Table 2: Showed the % mortality of larvae of *S. littoralis* was 20.0 and 13.0 % after 3rd days, at the concentration ((C_3) 1 x 10^9 spores/ ml.), *M. anisopliae* and *B. bassiana*.

Also, % mortality of larvae of *S. littoralis* was 10.2, 14.8 and 3.2% after 3rd days, at the concentration (C_3) from Bio Magic, Bio Power and Bio Catch, respectively. All the entomopathogenic fungi in concentration ((C_3) produced high mortality ranging from 35.0 to 100%, after 8th days of infection. *M. anisopliae* was the highest pathogenicity then Bio Power then, *Beauveria bassiana*, Bio Magic and Bio Catch, 100, 90.0, 70.7, 50.0 and 35.0 % mortality, respectively.

The % mortality of larvae of *Tribolium confusum*:

Table 3: Showed that % mortality of larvae of *T. confusum* was 25.0 and 17.9 % after 3rd days, at the concentration ((C_3) 1 x 10^9 spores/ ml.) from *Metarhizium anisopliae* and *Beauveria bassiana*. Also, % mortality of larvae of *T. confusum* was 12.0, 12.0 and 11.0% after 3rd days, at the concentration (C_3) from Bio Magic, Bio Power and Bio Catch, respectively. All the entomopathogenic fungi in concentration (C_3) produced high mortality ranging from 40.0 to 100 %, after 8th days of infection. *Metarhizium anisopliae* was the highest pathogenicity then Bio Magic, *Beauveria bassiana*, Bio Power, and Bio Catch, 100, 100, 100, 90.0 and 60.0 % mortality, respectively. *Metarhizium anisopliae* was the highest pathogenicity against the larvae of *T. confusum* then, Bio Magic, *Beauveria bassiana*, Bio Power and Bio Catch.

The % mortality of nymphs of *A. craccivora*

Table 4: Showed that % mortality of nymphs was 10.0, 8.0 % after 3rd days, at the concentration ((C_3) 1 x 10^9 spores/ ml.), *M. anisopliae* and *B. bassiana*. Also, % mortality of nymphs of *A. craccivora* was 6.2, 8.2, 5.1, 4.8 and 13.3 % after 3rd days, at the concentration (C_3) from Bio Magic, Bio Power, and Bio Catch, respectively. All the entomopathogenic fungi in concentration (C_3) produced high mortality ranging from 75.0 to 100 %, after 8th days of infection.

Bio Catch was the highest pathogenicity then *M. anisopliae*, *B. bassiana*, Bio Power and Bio Magic, 100, 100, 90.0, 84.4, and 75.0 % mortality, respectively.

Discussion

M. anisopliae and *B. bassiana* were more virulent than Bio Power, Bio Magic, and Bio Catch against *G. mellonella* larvae according to, Abdel-Raheem *et al*., 2016; Saleh *et al*., 2016 and Abdel-Raheem and Lamya Ahmed Al-Keridis, 2017. Also, the pathogenicity of *M. anisopliae* and *B. bassiana* were more virulent than Bio Magic, Bio Power and Bio Catch towards *S. littoralis* larvae. *Metarhizium anisopliae* was the highest pathogenicity against the larvae of *T. confusum* then, Bio Magic, *Beauveria bassiana*, Bio Power and Bio Catch. *M. anisopliae* was the highest pathogenicity then Bio Power, Bio Magic and Bio Catch according to Resquín-Romero, 2016 and Sahab and Sabbour, 2011. But, the pathogenicity of Bio Catch and *M. anisopliae* was the highest pathogenicity then *B. bassiana*, Bio Magic and Bio Power, towards *A. craccivora* nymphs. Bio Catch was the highest pathogenicity then *M. anisopliae* and *B. bassiana,* Bio Power and Bio Magic according to Zaki and Abdel-Raheem, 2010, Abdel-Raheem *et al*., 2009, Sabry, *et al*., 2011 and Abdel-Rahman and Abdel-Raheem, 2018, mentioned that the reason of different pathogenicity between the fungus and other due to the fraction exhibiting antimicrobial activity of some polar compounds ranging between 1000 and 1500 Dalton in the extraction of fungi.

Conclusion

M. anisopliae and *B. bassiana* were more virulent than Bio Power, Bio Magic and Bio Catch against *G. mellonella* larvae. *M. anisopliae* and *B. bassiana* were more virulent than Bio Magic, Bio Power and Bio Catch towards *S. littoralis* larvae. *M. anisopliae* was the highest pathogenicity against the larvae of *T. confusum* then, Bio Magic, *B. bassiana*, Bio Power and Bio Catch. Bio Catch and *M. anisopliae* was the highest pathogenicity against the nymphs of *A. craccivora* then *B. bassiana*, Bio Magic and Bio Power.

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Table 1: % Mortality of larvae of *G. mellonella* at different time after infection with entomopathogenic fungi at 25±2º C and 70± 5 R.H.

Entomopathogenic Fungi	Concentrations (Spores / ml.)	Corrected mortality % day						
		2nd	3rd	4th	5th	6th	7th	8th
M. anisopliae	C_1	0.0	7.0	23.3	39.3	56.0	75.0	88.0
	C_2	0.0	10.0	24.8	45.0	58	80.0	90.0
	C_3	0.0	15.0	30.0	50.0	78.8	85.0	100
B. bassiana	C_1	0.0	12.0	35.0	40.8	75.2	83.3	100
	C_2	0.0	17.2	37.3	47.3	88.8	100	100
	C_3	0.0	20.3	40.4	75.9	95.7	100	100
Bio Magic	C_1	0.0	6.2	18.9	32.2	46.8	48.0	65.2
	C_2	0.0	8.9	22.6	35.2	49.9	55.3	72.8
	C_3	0.0	12.5	27.5	39.8	69.8	73.0	85.0
Bio Power	C_1	0.0	8.2	23.5	34.6	58.9	76.2	84.0
	C_2	0.0	10.9	24.0	39.3	73.5	83.6	84.9
	C_3	0.0	15.6	29.7	66.4	85.2	94.2	100
Bio Catch	C_1	0.0	1.0	4.2	10.0	33.3	34.2	40.4
	C_2	0.0	2.0	5.6	11.2	33.0	34.7	45.0
	C_3	0.0	3.2	8.2	13.2	39.0	42.4	50.0

(C_1) 1 x 10^7, (C_2) 1 x 10^8 and (C_3) 1 x 10^9 spores/ ml.

Table 2 : % Mortality of larvae of *S. littoralis* at different time after infection with entomopathogenic fungi at 25±2 ºC and 70± 5 R.H.

Entomopathogenic Fungi	Concentrations (Spores / ml.)	Corrected mortality % day						
		2nd	3rd	4th	5th	6th	7th	8th
M. anisopliae	C_1	0.0	9.0	20.0	35.0	55.0	75.0	85.0
	C_2	0.0	11.0	24.0	45.2	60.9	79.2	100
	C_3	0.0	20.0	45.0	75.2	80.0	100	100
B. bassiana	C_1	0.0	8.0	17.0	35.0	65.2	70.0	70.0
	C_2	0.0	10.5	20.2	56.0	56.5	73.3	80.0
	C_3	0.0	13.0	35.0	75.0	80.5	87.0	90.0
Bio Magic	C_1	0.0	4.2	14.0	30.0	35.2	40.0	42.2
	C_2	0.0	6.3	15.2	31.0	39.0	43.3	45.0
	C_3	0.0	10.2	18.4	33.9	42.2	45.9	50.0
Bio Power	C_1	0.0	6.2	17.0	30.0	44.6	55.0	60.0
	C_2	0.0	10.9	20.4	33.0	49.9	64.3	66.0
	C_3	0.0	14.8	25.8	40.9	52.2	67.0	70.7
Bio Catch	C_1	0.0	1.0	3.7	8.8	19.3	27.7	30.0
	C_2	0.0	2.2	4.9	8.9	23.0	32.2	33.0
	C_3	0.0	3.2	7.7	10.2	25.0	35.0	35.0

(C_1) 1 x 10^7, (C_2) 1 x 10^8 and (C_3) 1 x 10^9 spores/ ml.

Table 3 : % Mortality of larvae of *Tribolium confusum* at different time after infection with entomopathogenic fungi at 25±2 ° C and 70± 5 R.H.

Entomopathogenic Fungi	Concentrations (Spores / ml.)	Corrected mortality % day						
		2nd	3rd	4th	5th	6th	7th	8th
M. anisopliae	C_1	0.0	10.5	30.0	75.0	78.5	85.5	100
	C_2	0.0	14.4	36.2	77.2	85.4	100	100
	C_3	0.0	25.0	60.0	85.0	100	100	100
B. bassiana	C_1	0.0	8.5	25.0	55.0	69.5	75.5	85.0
	C_2	0.0	11.7	33.0	75.2	76.4	86.5	100
	C_3	0.0	17.9	39.0	80.0	95.2	100	100
Bio Magic	C_1	0.0	10.0	27.0	70.0	77.5	84.0	95.0
	C_2	0.0	11.0	35.0	75.5	80.0	87.0	100
	C_3	0.0	12.0	55.0	83.0	95.0	100	100
Bio Power	C_1	0.0	9.0	19.0	35.5	55.0	65.0	70.0
	C_2	0.0	10.0	21.3	40.0	60.0	75.0	80.0
	C_3	0.0	12.0	25.3	45.8	75.2	80.0	90.0
Bio Catch	C_1	0.0	8.0	25.0	27.0	33.0	35.0	45.0
	C_2	0.0	9.0	30.0	30.0	35.0	45.0	50.0
	C_3	0.0	11.0	35.0	40.0	45.0	59.0	60.0

(C_1) 1 x 10^7, (C_2) 1 x 10^8 and (C_3) 1 x 10^9 spores/ ml.

Table 4: % Mortality of nymphs of *A. craccivora* at different time after infection with entomopathogenic fungi at 25±2 ° C and 70± 5 R.H.

Entomopathogenic Fungi	Concentrations (Spores / ml.)	Corrected mortality % day						
		2nd	3rd	4th	5th	6th	7th	8th
M. anisopliae	C_1	0.0	5.0	12.0	25.0	45.0	65.0	75.0
	C_2	0.0	6.0	15.0	35.2	50.9	69.9	85.0
	C_3	0.0	10.0	25.0	45.2	67.0	86.0	90.0
B. bassiana	C_1	0.0	4.0	17.0	35.0	55.5	60.0	65.0
	C_2	0.0	6.5	20.2	52.0	56.5	70.0	72.0
	C_3	0.0	8.0	25.0	55.0	65.5	77.0	80.0
Bio Magic	C_1	0.0	2.0	7.5	14.2	40.9	41.2	45.4
	C_2	0.0	3.1	9.8	16.6	47.5	55.3	63.0
	C_3	0.0	6.2	12.8	18.3	68.2	73.0	75.0
Bio Power	C_1	0.0	2.9	4.3	15.2	43.6	58.3	69.3
	C_2	0.0	5.2	12.5	20.4	52.2	70.0	75.0
	C_3	0.0	8.2	18.1	32.3	70.7	83.0	84.4
Bio Catch	C_1	0.0	7.7	20.3	35.2	56.4	75.0	83.2
	C_2	0.0	10.2	22.0	36.9	69.8	84.0	84.2
	C_3	0.0	13.3	25.2	49.8	79.8	90.9	100

(C_1) 1 x 10^7, (C_2) 1 x 10^8 and (C_3) 1 x 10^9 spores/ ml.

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