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Effect of entomopathogenic fungi on *Phenacoccus solenopsis* and *Icerya aegyptiaca* in Egypt

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Abstract

Background: Cotton Mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) is a major cotton pest in several countries. It is highly invasive, cryptic and polyphagous pest inflicting severe crop damage in economically important crops worldwide. *Icerya aegyptiaca* (Douglas, 1890) (Hemiptera: Coccoidea: Monophlebidae) is commonly known as Egyptian mealybug.

Materials: Pre adult, Ovipositor female and Adult female of *Phenacoccus solenopsis* and *Icerya aegyptiaca* were treated with the spores of *B. bassiana* and *M. anisopliae* using the concentrations of $1x10^2$, $1x10^3$, and $1x10^4$ spores / ml.

Results: The percent mortality of Pre adult of *Phenacoccus solenopsis* was 86.04 % at 1 X 10^4 spores/ ml. of *Beauveria bassiana* after 5th days of infection and 81.39 % mortality at-the same concentration of *Metarhizium anisopliae* with. The ovipositor female of *P. solenopsis* was 95.5 % at 1 X 10^4 spores/ ml. of *B. bassiana* after 5th days of infection and 97.9 % the same concentration of, *M. anisopliae*. The adult female of *P. solenopsis* was 95.0 %, at 1 X 10^4 spores/ ml. of *B. bassiana* after 5th days of infection and 100 % at the same concentration of *M. anisopliae*. The Pre adult of *Icerya aegyptiaca* was 60.0 % after 5th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but after 5th days of infection with, *M. anisopliae* was 52.0 % with the same concentration. The ovipositor female of *I. aegyptiaca* was 43.0 % after 5th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but after 5th days of infection with, *M. anisopliae* was 34.0 % with the same concentration. The adult female of *I. aegyptiaca* was 50.0 % after 5th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but after 5th days of infection with, *M. anisopliae* was 75.0 % with the same concentration.

Keywords: virulence, entomopathogenic fungi, *Phenacoccus solenopsis*, *Icerya aegyptiaca*

Introduction

Phenacoccus solenopsis was discovered in 1898 by Tinsley (1898a) in New Mexico, USA. This mealybug was later reported to have spread to Arizona, California, Colorado, Mississippi, Washington D.C., and Texas, USA [1]. This insect has the ability to spread rapidly to un infested areas by natural carriers such as the wind, rain and water-ways, on farm equipment, and by clinging to clothing and animals. International trade plays a major role in the spread of this pest to new regions of the world. It was discovered to be infesting ornamentals in Nigeria [2]. This insect sucks cotton sap, injects toxins and secretes "honeydew" stimulating the development of black sooty moulds which adversely affect photosynthesis [3, 5]. *Phenacoccus* is one of the largest genera in family Pseudococcidae or mealybugs [6] as it contains about 180 species. Several *Phenacoccus* species are known to be important plant pests and potentially (if not actually) invasive (e.g. *P. aceris*, *P. madeirensis* Green, *P. manihoti* Matile-Ferrero and *P. solani* Ferris). Entomopathogenic fungi were able to decrease the infestation by *Fiorina fiornii* on date palm trees. Percent mortality of nymphs was 79.6 % after 7th days, at the concentration (3.2 X 10^4 spores/ ml.) of Biover but after 7th days of infection of adults was 71.63 % with the same concentration. While percent mortality of nymphs was 83.7 % after 21st days, at the concentration (3.2 X 10^4 spores/ ml.) of Biover but after 21st days of infection of adults was 83.28 % with the same concentration [7]. *Metarhizium anisopliae* was the most virulence fungi than *Beauveria bassiana* and *Virticillium lecanii* against black scale insect, *Saissetia oleae* [8]. *Icerya aegyptiaca* (Douglas, 1890) (Hemiptera: Coccoidea: Monophlebidae) is commonly known as Egyptian mealybug. *I. aegyptiaca*, when abundant, causes defoliation and in some cases dieback of the branches and the entire plant [9]. It egests honeydew which induces sooty blotch that covers leaf surface and, make fruits unmarketable [10]. However, in Japan, *I. aegyptiaca* is found to egest little to no honeydew and, this monophlebid species is rarely associated with sooty mould, [10]. In Kiribati and some other Micronesian atolls, the greatest impact of *I. aegyptiaca* is on the breadfruit tree (*Artocarpus altilis*) with crop loss as high as 50% or more [11]. In Chahbahar, Iran, *I. aegyptica* caused considerable damage on mango (*Mangifera indica*) and tropical fruits. In China, *I. aegyptiaca* was listed as one of the dangerous garden pests [10]. *Icerya aegyptiaca* has been recorded as a serious pest of citrus, fig and shade trees in Egypt, although it is largely controlled by natural enemies. It is also recorded as a pest of commercial rose production in greenhouses in Egypt [12]. It is a pest of breadfruit, avocado, banana, citrus, and ornamentals in the South Pacific, of annona, jackfruit, sapote (*Pouteria sapota*), mulberry and guava in India, and breadfruit in the Maldives Islands [13]. Entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* are ubiquitous soil borne organisms that are pathogenic to various arthropod pests, such as Sugar-beet Tortoise beetle, *Cassida vittata* Vill, *Scrobipalpa ocellatella* Boh, *Bemisia tabaci*, [14, 25].

Materials and methods

Fungi culture

Entomopathogenic Fungi were grown and maintained on Potato dextrose agar (250 g potatoes, 20 g agar and 1000 ml distilled water. The media was autoclaved at 120 ºc for 20 minutes, and poured in Petri- dishes (9 cm diameter x 1.5 cm). The fungal isolates were re-cultured every 14-30 days and isolates were kept at 4 ºC. To restore the virulence of the isolates they were passed through their natural host or through the wax moth larvae, *Galleria mellonella*.

Rearing of test insect

In the present study the insects *Phenacoccus solenopsis* was collected from potatoes and *Icerya aegyptiaca* from *Ficus virens* trees and transferred to the laboratory of Pests Plant Protection Department, National Research Centre to be infected with entomopathogenic fungi.

Laboratory application

Fungal inoculate

Spores of fungal isolates harvested by rinsing with sterilized 0.5 % Tween 80 from 14day old culture (PDA) media grown at 25±2º C for *B. bassiana* and *M. anisopliae*. The suspensions were filtered through cheese cloth reduce mycelium clumping. The spore was counted in the suspension using haemocytometer (Hirscmann 0.1 mm x 0.0025 mm^2). The concentrations were used $1x10^2(c_1)$, $1x10^3(c_2)$, and $1x10^4(c_3)$ spores / ml.

Treatment of Pre adult, Ovipositor Female and Adult female of *Phenacoccus solenopsis* and *Icerya aegyptiaca*:

Pre adult, ovipositor female and adult female of *Phenacoccus solenopsis* and *Icerya aegyptiaca* were treated with the spores of *B. bassiana* and *M. anisopliae* using the concentrations of $1x10^2$, $1x10^3$, and $1x10^4$ spores / ml. each treatment contained 4 replicates and each replicate contained twenty insects transferred in group in a plastic boxes, placed on a wetted filter paper. After dipping the insects in the suspension, the insects were transmitted on parts of Potatoes and *Ficus virens* leaves to feed. Each treatment was incubated at 25 ± 2 ºC and 85 ± 5 % R.H. and observed daily and the control were treated with distilled water only.

Statistical analysis

Mortality data was corrected with that in control by using [26].

Results

Table (1) showed that percent mortality of pre adult of *Phenacoccus solenopsis* was 86.04 % after 5th days, at (1 X 10^4 spores/ ml.) of *Beauveria bassiana* but after 5th days of infection with, *Metarhizium anisopliae* was 81.39 %. While percent mortality Pre adult was 100 % after 9th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but after 9th days of infection with *M. anisopliae* was 100 %. Percent mortality ranging between 58.13 % to 100 % at the concentration (1 X 10^4 spores/ ml.) of *B. bassiana* but was 55.11% to 100% at the concentration (1 X 10^4 spores/ ml.) of *M. anisopliae*. *B. bassiana* was the most effective on the pre adult of *P. solenopsis* than *M. anisopliae*

Table 1: Percent mortality of Pre adult of *Phenacoccus solenopsis* after infected with *Beauveria bassiana* and *Metarhizium anisopliae* at 25 ± 2 ºC and 85 ± 5 R % R.H.

Days after treatment	Control	*Beauveria bassiana*			*Metarhizium anisopliae*		
		C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	25.58	53.48	58.13	27.9	52.13	55.11
3rd	0.0	53.48	60.46	62.79	52.11	55.13	62.09
4th	0.0	55.81	72.09	76.74	55.09	66.74	69.06
5th	0.0	76.74	83.72	86.04	74.41	80.39	81.39
6th	0.0	79.06	83.72	90.69	78.06	81.04	86.04
7th	0.0	81.39	86.04	93.02	80.39	83.37	93.02
8th	0.0	83.72	88.37	100	80.04	87.69	93.02
9th	0.0	86.04	100	100	83.37	90.0	100

1 X 10 ^{2}(C_1), 1 X 10 ^{3} (C_2) and 1 X 10 ^{4} (C_3) spores/mg

Table (2) showed that percent mortality of ovipositor female of *P. solenopsis* was 95.5 % after 5th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but after 5th days of infection with, *M. anisopliae* was 97.9 %. While percent mortality Ovipositor Female was 100 % after 9th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* and *M. anisopliae*. Percent mortality ranged between 82.5 % to 100 % at the concentration (1 X 10^4 spores/ ml.) of *B. bassiana* but was 85.0 % to 100% at the concentration (1 X 10^4 spores/ ml.) of *M. anisopliae*. *M. anisopliae* was the most effective on ovipositor Female of *P. solenopsis* than *B. bassiana*.

Table 2: Percent mortality of ovipositor female of *Phenacoccus solenopsis* after infected with *Beauveria bassiana* and *Metarhizium anisopliae* at 25 ± 2 ºC and 85 ± 5 % R.H.

Days after treatment	Control	*Beauveria bassiana*			*Metarhizium anisopliae*		
		C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	75.0	80.5	82.5	80.0	87.5	85.0
3rd	0.0	80.0	84.5	92.0	82.5	87.5	93.5
4th	0.0	82.5	86.5	92.5	82.5	90.0	94.5
5th	0.0	82.5	87.5	95.5	87.5	95.0	97.9
6th	0.0	*85.*0	92.5	97.5	*90.*0	92.5	98.5
7th	0.0	87.5	95.0	100	95.0	95.0	100
8th	0.0	92.5	95.0	100	97.5	100	100
9th	0.0	95.0	97.5	100	97.5	100	100

1 X 10 ^{2}(C_1), 1 X 10 ^{3} (C_2) and 1 X 10 ^{4} (C_3) spores/mg

Table (3) showed that percent mortality of adult female of *P. solenopsis* was 95.0 % after 5th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but after 5th days of infection with, *M. anisopliae* was 100 %. While percent mortality of adult female was 100 % after 9th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* and *M. anisopliae*. Percent mortality ranging between 65.0 % to 100 % at (1 X 10^4 spores/ ml.) of *B. bassiana* but was 75.0 % to 100% at the concentration (1 X 10^4 spores/ ml.) of *M. anisopliae*. *M. anisopliae* was the most effective on adult female of *P. solenopsis* than *B. bassiana*.

Table 3**:** Percent mortality of adult female of *Phenacoccus solenopsis* after infected with *Beauveria bassiana* and *Metarhizium anisopliae* at 25 ± 2ºC and 85 ± 5 % R.H

Days after treatment	Control	*Beauveria basin*			*Megatherium anisopliae*		
		C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	50.0	50.0	65.0	50.0	60.0	75.0
3rd	0.0	50.0	75.0	75.0	75.0	75.0	100
4th	0.0	50.0	75.0	90.0	75.0	100	100
5th	0.0	70.0	75.0	95.0	100	100	100
6th	0.0	75.0	80.0	100	100	100	100
7th	0.0	80.0	90.0	100	100	100	100
8th	0.0	85.0	100	100	100	100	100
9th	0.0	95.0	100	100	100	100	100

1 X 10^2(C_1), 1 X 10^3 (C_2) and 1 X 10^4 (C_3) spores/mg

Table (4) showed that percent mortality of Pre adult of *Icerya aegyptiaca* was 60.0 % after 5th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but after 5th days of infection with, *M. anisopliae* was 52.0 %. While percent mortality of Pre adult was 88.0 % after 9th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but at *M. anisopliae* was 80.0 % after 9th days from infection. Percent mortality ranging between 16.0 % to 88.0 % at (1 X 10^4 spores/ ml.) of *B. bassiana* but was 20.0 % to 80.0 % at (1 X 10^4 spores/ ml.) of *M. anisopliae*. *M. anisopliae* was the lowest effective on Pre adult of ***I. aegyptiaca*** than *B. bassiana*.

Table 4: Percent mortality of pre adult of *Icerya aegyptiaca* after infected with *Beauveria bassiana* and *Metarhizium anisopliae* at 25 ± 2 ºC and 85 ± 5 % R.H.

Days after treatment	Control	*Beauveria bassiana*			*Metarhizium anisopliae*		
		C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	8.0	8.0	16.0	12.0	12.0	20.0
3rd	0.0	20.0	24.0	32.0	20.0	24.0	36.0
4th	0.0	40.0	42.0	52.0	28.0	28.0	44.0
5th	0.0	52.0	56.0	60.0	32.0	36.0	52.0
6th	0.0	64.0	65.0	68.0	36.0	52.0	60.0
7th	0.0	64.0	68.0	76.0	44.0	64.0	76.0
8th	0.0	68.0	72.0	88.0	48.0	72.0	76.0
9th	0.0	68.0	76.0	88.0	48.0	76.0	80.0

1 X 10^2(C_1), 1 X 10^3 (C_2) and 1 X 10^4 (C_3) spores/mg

Table (5) showed that percent mortality of Ovipositor Female of *I. aegyptiaca* was 43.0 % after 5th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but after 5th days of infection with, *M. anisopliae* was 34.0 %. While percent mortality of ovipositor Female was 60.0 % after 9th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but at *M. anisopliae* was 56.0 % after 9th days from infection. Percent mortality ranging between 8.0 % to 60.0 % at (1 X 10^4 spores/ ml.) of *B. bassiana* but was 14.0 % to 56.0 % at (1 X 10^4 spores/ ml.) of *M. anisopliae*. *M. anisopliae* was the lowest effective on Ovipositor Female, *I. aegyptiaca* than *B. bassiana*.

Table 5: Percent mortality of ovipositor female of *Icerya aegyptiaca* after infected with *Beauveria bassiana* and *Metarhizium anisopliae* at 25 ± 2 ºC and 85 ± 5 % R.H.

Days after treatment	Control	*Beauveria bassiana*			*Metarhizium anisopliae*		
		C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	4.0	4.0	8.0	8.0	12.0	14.0
3rd	0.0	16.0	16.0	20.0	12.0	28.0	28.0
4th	0.0	24.0	28.0	32.0	16.0	28.0	32.0
5th	0.0	40.0	41.0	43.0	20.0	28.0	34.0
6th	0.0	40.0	42.0	48.0	24.0	32.0	35.0
7th	0.0	44.0	48.0	52.0	24.0	40.0	47.0
8th	0.0	48.0	50.0	56.0	28.0	48.0	50.0
9th	0.0	48.0	48.0	60.0	36.0	52.0	56.0

1 X 10^2(C_1), 1 X 10^3 (C_2) and 1 X 10^4 (C_3) spores/mg

Table (6) showed that percent mortality of adult female of *I. aegyptiaca* was 50.0 % after 5th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but after 5th days of infection with, *M. anisopliae* was 75.0 %. While percent mortality of adult female was 75.0 % after 9th days, at (1 X 10^4 spores/ ml.) of *B. bassiana* but of *M. anisopliae* was 100 % after 9th days from infection. Percent mortality ranging between 25.0 % to 75.0 % at (1 X 10^4 spores/ ml.) of *B. bassiana* but was 25.0 % to 100 % at (1 X 10^4 spores/ ml.) of *M. anisopliae*. *M. anisopliae* was the most effective on adult female, *I. aegyptiaca* than *B. bassiana*.

Table 6: Percent mortality of adult female of *Icerya aegyptiaca* after infected with *Beauveria bassiana* and *Metarhizium anisopliae* at 25 ± 2 ºC and 85 ± 5 % R.H.

Days after treatment	Control	*Beauveria bassiana*			*Metarhizium anisopliae*		
		C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	0.0	0.0	0.0	0.0	25.0	25.0
3rd	0.0	0.0	0.0	25.0	0.0	50.0	50.0
4th	0.0	0.0	25.0	25.0	0.0	75.0	75.0
5th	0.0	25.0	25.0	50.0	25.0	75.0	75.0
6th	0.0	25.0	50.0	50.0	50.0	75.0	75.0
7th	0.0	50.0	50.0	75.0	50.0	75.0	80.0
8th	0.0	50.0	50.0	75.0	50.0	75.0	100
9th	0.0	50.0	50.0	75.0	50.0	75.0	100

1 X 10^2(C_1), 1 X 10^3 (C_2) and 1 X 10^4 (C_3) spores/mg

Discussion

The results according with [17, 18] where used the entomopathogenic fungi against some insect pests attacking peanuts and sugar-beet in Egypt **(***Spodoptera littoralis; Spodoptera exigua; Aphis craccivora; Pegomyia mixta*). Also, according with Mohamed Abdel-Raheem and Amany Elbahrawy [5], Abdel-Raheem *et al* [8]. and Abdel-Raheem [15] where using of some entomopathogenic fungi against the oliver black scale insect, *Saissetia oleae* (Oliver) on olive trees. They found the three entomopathogenic fungi due to reduction in number the insects after being treated with *B. bassiana*, *M. anisopliae* and *V. lecanii* as compared the control and against *Pulvinaria tenuivalvata* (Newstead) Infesting Sugar-cane in Egypt and used against *Phenacoccus solenopsis.* This result also according with Mohamed Abdel-Raheem *et al.* [4], Abdel-Raheem *et al.* [7], García Morales *et al.* [13], Zaki and Abdel-Raheem [17] and Abdel-Raheem *et al.* [18].

Conclusion

B. bassiana was the most effective on the pre adult of *P. solenopsis* than *M. anisopliae*. *M. anisopliae* was the most effective on ovipositor Female of *P. solenopsis* than *B. bassiana*. *M. anisopliae* was the most effective on adult female of *P. solenopsis* than *B. bassiana*. *M. anisopliae* was the lowest effective on pre adult of *I. aegyptiaca* than *B. bassiana*. *M. anisopliae* was the lowest effective on ovipositor female, *I. aegyptiaca* than *B. bassiana*. *M. anisopliae* was the most effective on adult female, *I. aegyptiaca* than *B. bassiana*. so we can use of entomopathogenic fungi as bio control agents against *P. solenopsis* and *I. aegyptiaca*.

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