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Research Article
Effectiveness of Two Entomopathogenic Fungi Sources Toward Some Sucking Insects and Their Predators on Okra Crop

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Abstract

Background and objective: Mycopathogenic pesticides played a vital role in insect population dynamics making it the earliest insect pests control agents. Use of myco-insecticides in pest management particularly in vegetable production as okra was considered the promising goal to control insects attack plant under field condition. **Materials and Methods:** The experiments were tested 2 entomopathogenic fungi (namely: *Beauveria bassiana* and *Metarhizium anisopliae*) from different sources toward sucking pests which attack okra crop, under laboratory and field conditions. **Results:** Two tested entomopathogenic fungi with different sources caused relatively the same impact on *Aphids gossypii*. The high concentration 1×10^8 caused the best mortality percentage especially after 7 days of treatment for both tested fungi under laboratory conditions. While under field conditions tested materials were elicited different impact on *Aphis gossypii* (Gloner) and leafhopper *Emposca decipiens*. *M. anisopliae* (S1) caused the best impact on population of aphids extend to 10 days. On other view, all the tested materials were recorded the impressive effect on *E. decipiens* reach to approximately more than 90%. **Conclusion:** Finally, the population of predators (F/Coccinellidae) decreased after treatment by *B. bassiana* or *M. anisopliae* (S1 or S2) due to reduction in prey individuals.

Key words: *Beauveria bassiana*, *Metarhizium anisopliae*, sources, okra, aphids, leafhopper, predator

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Naturally, there were many factors had ability to restrict and regulate the insect population, the most widespread and common one were insect diseases. The fungi were potentially the most promising biological control agents due to their wide host range. Mycopathogenic pesticides play a vital role in insect population dynamics making it the earliest insect pests control agents. Over 900 different species of fungi have been reported to infect insects many of them offer great potential for pest management. For instance, *Metarhizium anisopliae* (Metchnikoff) Sorokin can infect as many as 200 different insects[1,2]. The pathogenicity of fungi towards insect has been mainly attributed to the various hydrolytic enzymes, such as chitinases, proteases, lipases etc. Entomopathogenic fungi are component of the natural agents that affect insect population and when host densities are high, they have been known to produce rapid and spectacular epizootics, Mallikarjun[3].

Okra (Bhendi) *Abelmoschus esculentus* (L.) Moench is flowering plant belongs to family mallow which valued for its eaten green seed pods so it considered the most important vegetable grown throughout the tropics and warmer parts of temperate zone. The crop production of okra was mainly used as food source for humans or as fodder for animals. One of the most important constraints in production of okra is insect pests. There are more than 72 species of pests had been recorded on crop[2,4] among which the sucking pest complex consisting of aphids (*Aphis gossypii.* Gloner), leaf hopper (*Emposca decipiens* (Paoli), whitefly (*Bemisia tabacii.* Green) are major pest and causes 17.46% loss in okra, Ghosh *et al.*[5]. Aphids and leafhoppers were attacked the okra crop in early stage and feed on the plant sap, make them weak and reduce the yield. Krishnaiah[6] mentioned that infestation of okra crop by leafhopper caused reduction to field yield reached to 40-56%.

Okra being vegetable crop that has to be harvested at regular interval, it is critical to evaluate safer alternatives likes mycopathogens which have no toxic residues and hence are the best suited for vegetables like okra, cabbage, etc. which is used fresh vegetable for consumption, Anitha[7]. Around the world the most producers was used myco-insecticides to pest management particularly in vegetable production, Abaajeh[8].

The present study was targeted to test efficacy of 2 entomopathogenic fungi from different sources toward major okra sucking pests and their associated predators.

MATERIAL AND METHODS

The experiments were carried out under laboratory and field condition at National Research Center, Egypt during summer season 2018.

Source of fungus culture

There are 2 sources of tested fungi: First one, isolates of *Beauveria bassiana* (Balsamo) and *Metarhizium anisopliae* (Metchnikoff) Sorokin were obtained from Assiut University, Mycological Center Faculty of Science. The isolates were cultured on Sabouraud[9] dextrose yeast agar (SDYA) medium g L^{-1}, containing 40 g glucose, 20 g peptone, 20 g agar, 2 g Yeast extract and 1000 mL of distilled water in flasks. These flasks were autoclaved at 21°C for 15-20 min.

Second source were obtained as wettable powder produced by the Plant Protection Research Institute, Bio-pesticide Production Unit, Dokki-Giza, Egypt. The names of commercial bioagents were Bioranza® WP 10% (*Metarhizium anisopliae* Sorok.) Active ingredients 10%, Inert Ingredient 90%. It is formulated as a Wettable Powder with count of 1×10^8 spore mL^{-1} and Biovar® WP 10% (*Beauveria bassiana Balsamo*) active ingredients 10%, Inert Ingredient 90%. It is formulated as a Wettable Powder with count of 1×10^8 spore mL^{-1}.

Preparation tested bioagent: The 2 fungal sources were prepared as solution as follows:

- **Source 1 (S1):** Fungal cultures were grown on Sabouraud[9] dextrose yeast agar (SDYA) medium g L^{-1} and incubated at 25±2°C in darkness for 14 days. Conidial suspensions were prepared by scraping cultures with a sterile objective glass and transferred to 10 mL of sterile water containing 0.05% Tween 80 in a laminar flow chamber. The conidia were harvested by scraping the surface of the culture with inoculation needle. The mixture was stirred for 10 min the hyphal debris was removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined by direct count using Haemocytometer. Serial dilutions were prepared in distilled water containing 0.1% Tween-80 and preserved at 5°C until used
- **Source 2 (S2):** To prepare stock solution (1×10^8) spore mL^{-1} from commercial bioagent was weighted 1 g of powder and dissolved at 90 mL sterile water. From stock solution (1×10^8) spore mL^{-1} take 10 mL and dissolved at 90 mL of sterile water to prepare concentration (1×10^7) spore mL^{-1}. From concentration (1×10^7) spore mL^{-1} take 10 mL and dissolved at 90 mL sterile water to prepare concentration (1×10^6) spore mL^{-1}

A volume of 1 mL of the adjustable concentrations 1×10^6, 1×10^7 and 1×10^8 viable conidia (From S1or/and S2) was directly spray into target insect pests. Three replicates/treatment were made.

Pathogenic bioassay: The experiments were involved laboratory and field treatments as follows:

- **Under laboratory condition:** Fresh healthy okra leaves were gathered from special farm and clean them well by wet cotton. After that it was placed in Petri-dish (20 cm.) which was provided by wet cotton to act as substrate for keeping okra leaves fresh till end the test. A viviparous female of *Aphis gossypii* was collected from okra plant farm to be made artificial infestation (20 individual/ leave) was provided at each Petri-dish. Then 1 mL of the prepared tested materials was sprayed directly on infested leaves. Examination the experiments were carried out after 3, 5 and 7 days to calculate percentage of mortality. Each test was replicated 20 times
- **Under field condition:** The present study was carried out during summer growing seasons of 2018 at special farm in Motamedia region Giza governorate in which cultivated Baldy strain of Okra

An area of about 700 m^2 (4 Karats) was divided into 4 equal plots. Each plot with ridges (3 replicates) of 5 m length and 60 cm apart, all normal cultural practices of land preparation, thinning, irrigation, mechanical weed control were followed out and kept free from any insecticidal application. After month of plantation before treatment, Samples were picked randomly from 3 levels of the plant (10 leaves of okra plants from each replicate making a sum of 30 leaves for each treatment). After that spraying tested fungus was carried out by using handling sprayer (to cover one karat was sprayed 10 L from prepared fungi solution concentration). The random samples were picked up at interval time after spraying. Each one was kept in tight close paper bag and transferred to the laboratory to estimate the different piercing sucking pests (aphids and leafhopper) attacked the leaf surface and their associated predators by the aid of the Stereo-binocular microscope.

Statistical analysis: The percent reduction of infestation was statistically calculated according to the equation of Henderson and Tilton[10]:

$$\text{Mortality (\%)} = \frac{1\text{-Ta}\times\text{Cb}}{\text{Tb}\times\text{Ca}}100$$

Where:

Ta = Post treatment insect counts
Cb = Untreated insect count before treatment
Tb = Pretreatment counts
Ca = Untreated insect count after treatment

RESULTS

Effect of tested entomopathogenic fungi under laboratory condition: The data at Table 1 indicated that 2 entomopathogenic fungi with different sources caused relatively the same impact on *A. gossypii* but the best one was *B. bassiana* (S1) was recorded 100% mortality at 1×10^8 conc. As usual, the high concentration $1x10^8$ caused the best mortality percentage especially after 7 days of treatment for both tested fungi which approximately $\geq$98.7%, respectively. The symptoms of aphids mortality was cleared in Fig. 1.

Effect of tested entomopathogenic fungi under field conditions

on *Aphids gossypii*: Table 2 described that *M. anisopliae* (S1) caused the best impact on population of aphids extend their effect to 10 days that were recorded reduction reach to 68.4 and 54.6% after 5 and 10 day of treatment, respectively.

It was followed by *B. bassiana* (S2) which was recorded reduction in population of aphids reach to 53 and 48.2% after 5 and 10 days of treatment, respectively. While the remaining treatments had ability to overcome aphids for 5 days and after that their effect restricted.

On *Emposca decipiens*: All the tested materials were recorded the impressive effect on *E. decipiens* which was extend to 10 days after treatments and caused reduction in number of target insect reach to approximately more than 90% as clear at Table 3.

On predators (F/Coccinellidae): Figure 2 cleared that the population of predators decreased after treatment by *B. bassiana* or *M. anisopliae* (S1 or S2). From the investigation the treated leaves were observed no dead among individuals of predators so the reduction in predator individuals might be attributed to reduction their prey which pushes them to migrate to other place.

DISCUSSION

From the obtained results showed that the high concentration 1×10^8 of both tested entom-pathogenic fungus sources were elicited impressive effects toward aphids that after 7 days of treatment which reached to $\geq$98.7% mortality under laboratory test. While *M. anisopliae* (S1) and *B. bassiana* (S2) were showed the best effect toward reduction

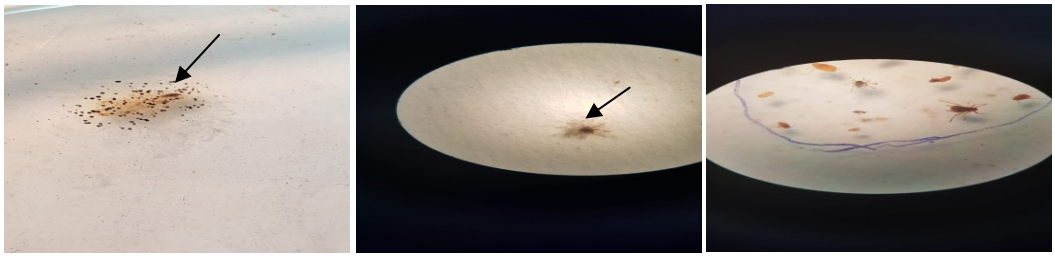

Fig. 1: Morality symptom of aphids after treatment by pathogenic fungus
Arrow show growth of fungus on the outer surface of aphids

Table 1: Effect of different concentration of 2 entomopathogenic fungi on *Aphid gossypii* mortality under laboratory conditions

		Mortality (%) (Mean±SE)			
Tested materials	Concentration	3 days	5 days	7 days	Statistical analysis
Metarizium anisopliae (S1)	10^6	37.1±1.0	66.3±5.70	85.7±2.33	$LSD_{0.05}$ = 3.61
	10^7	48.7±6.2	73.8±8.70	93.2±2.31	
	10^8	62.1±3.8	92.1±1.69	98.7±0.90	
Metarizium anisopliae (S2)	10^6	38.6±10.6	73.4±1.82	81.9±3.33	$LSD_{0.01}$ = 5.14
	10^7	60.7±2.61	84.2±4.22	94.5±1.29	
	10^8	69.4±3.4	94.5±2.46	98.3±1.04	
Beauveria bassiana (S1)	10^6	41.4±9.2	89.4±2.20	45.3±0.96	$LSD_{0.05}$ = 12.53
	10^7	49.7±5.6	72.3±6.90	94.5±18.6	
	10^8	57.2±2.4	87.8±5.19	100.0±0.0	
Beauveria bassiana (S2)	10^6	28.5±1.8	70.3±1.65	74.8±1.45	$LSD_{0.01}$ = 17.87
	10^7	49.1±4.5	81.4±2.77	91.8±2.90	
	10^8	78.6±7.9	94.1±1.97	99.1±0.86	

Table 2: Effect of some entomopathogenic fungi against *Aphid gossypii* on Okra under field condition

	Number of apterous aphids/10 leaflets ±SE					
Tested materials	Before treatment	After 5 days	Reduction (%)	After 10 days	Reduction (%)	Statistical analysis
Metarizium anisopliae (S1)	85.6±24.8	23.3±8.1	68.4	31.0±8.6	54.6	$LSD_{0.05}$ = 20.5
Metarizium anisopliae (S2)	72.6±17.4	37.0±5.2	41.0	52.0±3.1	10.2	$LSD_{0.01}$= 29.7
Beauveria bassiana (S1)	69.0±7.8	26.3±3.5	56.0	45.3±8.4	18.0	$LSD_{0.05}$ = 32.59
Beauveria bassiana (S2)	62.0±27.6	25.3±4.6	53.0	25.6±1.8	48.2	$LSD_{0.01}$ = 46.9
Control	86.0±9.5	74.0±7.2	--	68.6±19.2	--	

Table 3: Effect of some entomopathogenic fungi against *Empoasca decipiens* on Okra under field condition

	Number of apterous aphids/10 leaflets ±SE					
Tested materials	Before treatment	After 5 days	Reduction (%)	After 10 days	Reduction (%)	Statistical analysis
Metarizium anisopliae (S1)	11.7±2.02	2.0±2.0	87.6	1.66±1.7	90.6	$LSD_{0.05}$ = 10.01
Metarizium anisopliae (S2)	24.0±5.8	4.3±1.9	87.2	0.00±0.0	100.0	$LSD_{0.01}$ = 14.5
Beauveria bassiana (S1)	18.0±1.7	0.0±0.0	100.0	0.60±0.7	97.8	$LSD_{0.05}$ = 15.75
Beauveria bassiana (S2)	44.3±11.4	3.7±0.3	94.0	0.00±0.0	100.0	$LSD_{0.01}$ = 22.66
Control	20.0±5.13	27.6±1.3	--	30.30±4.4	--	

population of aphids because their effect extended to 10 days and caused reduction reached to more than 45% under field test. On other side, leafhopper, *E. decipiens* were affected by all tested materials and caused mortality percent (more than 90%) extended to 10 day. The predators' family coccinellidae were reduced due to reduction in population of preferring prey.

Most of entomopathogenic fungi infect the host through the cuticle. The posses of pathogenesis being with adhesion of fungal spore on cuticle followed by germination, penetration and development fungal inside the host and leading to the death of the host. Fungal usually cause insect mortality by nutrition deficiency, destruction the tissue and releasing their toxin[11,12].

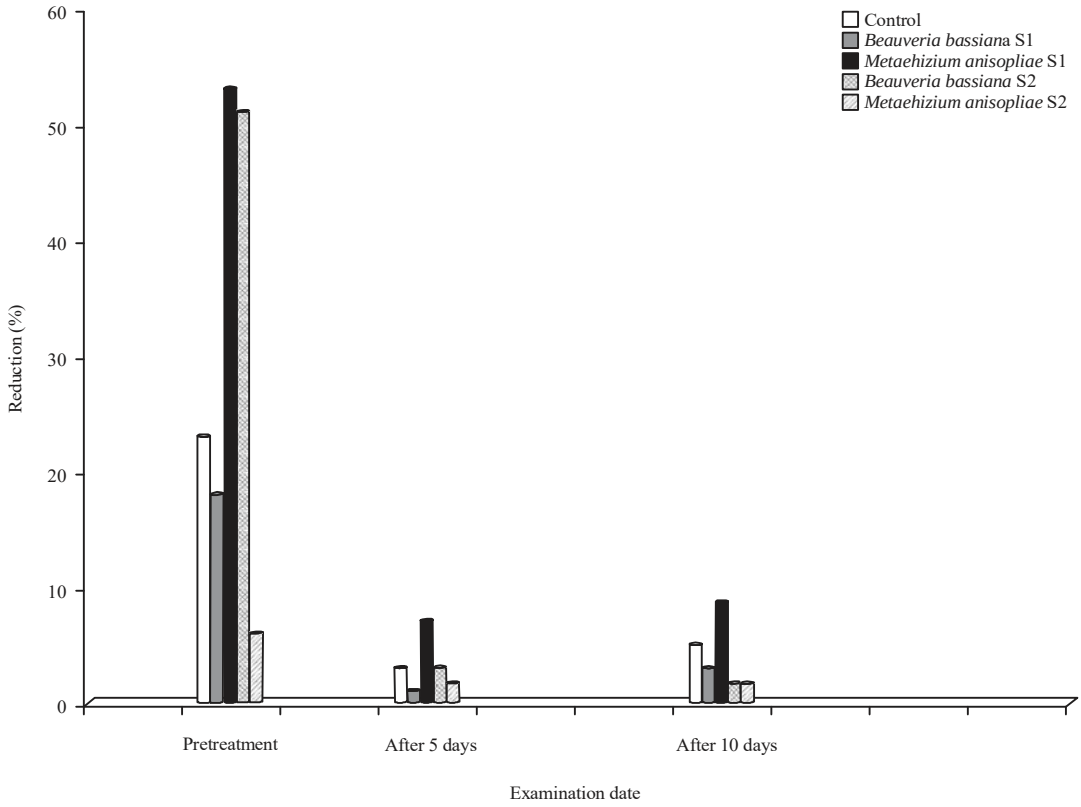

Fig 2: Effect of entomopathogenic fungi on predators of from family Coccinellidae

There are different researchers interested to showed effects of different fungal isolation toward sucking insect pest and agree with the present study as Nirmala *et al.*[13], who tested 12 fungal isolation belong to *B. bassiana*, *M. anisopliae* and *Verticillium lecanii* (Zimmerman) against 3 aphids species and found that, all 12 isolates of the 3 fungi were found to be pathogenic toward three aphids species with different degree under laboratory conditions. Deka *et al.*[14] showed that 2 entomopathogenic fungus (*Metarhizium anisopliae* and *Nomuraea releyi*) when used at 10^8 CFU mL^{-1} were highly virulent against mustard aphid population. Ujjan and Shahzad[15] reported that some isolates of *M. anisopliae* showed 64% virulence against mustard aphid population after 3.8 days while in another study, *M. anisopliae* isolate showed 72% mortality within 3 days Araujo *et al.*[16].

Recently, the largest single microbial control program using fungi on other insect species as the use of *M. Anisopliae* for control of spittlebugs (Cercopidae) in South American sugarcane and pastures, Li *et al.*[17]. The application of *B. bassiana* for the control of the pine moth *Dendrolimus* spp. in China probably represents one of the largest uses of a biocontrol agent over 1 m ha of pine forest Lord[18].

CONCLUSION

Strains of *B. bassiana* and *M. anisopliae*, can be considered the promising materials to be used in biological control program of sucking insect especially *A. gossypii* and leaf hopper in the field level.

The tested entomopathogenic fungi were elicited impact role toward the sucking insects and that can be used at overcome programs of pests.

SIGNIFICANCE STATEMENT

The present study gives excellent indication to researchers around the ability of the local 2 strains of mycopathological fungi to reduce the population of sucking insect for 10 days. Fungal pathogens have certain advantages in pest control programs over other insect pathogens like bacteria and viruses. Mass production techniques of fungi are much simpler, easier and cheaper than those used for Bt and NPVs. Myco-insecticides able to pest management particularly in vegetable production because it was considered the most safety for living organism and environment.

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