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Physiological concepts and bioassays of *Beauveria bassiana* and *Paecilomyces lilacinus* attacking the corn leaf aphid *Rhopalosiphum maidis* (Hemiptera:Aphididae) in Assiut, Egypt

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Abstract

Enzymatic activity and pathogenicity of *Beauveria bassiana* and *Paecilomyces lilacinus* against the corn leaf aphid *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) were studied in the laboratory. The results showed that two isolates of *B. bassiana* and one isolate of *P. lilacinus* were found effective at all concentrations on *R. maidis*, but the uppermost concentration (2×10^7 conidia/ml) of *P. lilacinus* provided maximum mortality (100%). While *B. bassiana* isolate No. (1) and *B. bassiana* isolate No. (2) provide mortality rates of 85% and 95% at their highest concentrations of 1.8×10^7 and 1.30×10^7, respectively. All isolates revealed enzymatic activity such as lipase, protease, and chitinase, but *P. lilacinus* showed the highest activity. *B. bassiana* (2) isolate was more aggressive than other isolates, as indicated by the low value of LC_{50} compared with other isolates.

Introduction

Aphids are one of the most detrimental pests to agricultural productivity. By removing carbs and amino acids from plant phloem, they directly harm plants, and they also indirectly harm plants by dispersing different viruses (Dedryver *et al*., 2010 and Kim *et al*., 2013). The corn leaf aphid, or *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae), is a global pest of maize (Corn) and other allied crops. This pest can infest every portion of the corn plant above ground, severely reducing production (Bitsadze *et al*., 2017). According to the majority of researchers, the most popular way to control aphids is to use chemical pesticides; nevertheless, this approach is ineffective since aphids quickly proliferate and readily develop resistance to chemical insecticides. Additionally, the excessive use of pesticides has led to the degradation of the environment and negative impacts on human and other organisms' health (Dedryver *et al*., 2010). Global agriculture is becoming more dependent on ecologically responsible pest management as a result of the growing volume of commercial crop production worldwide. Biocontrol methods can be used to control plant pests in place of chemicals. Entomopathogens, for example, are microbes that control insects. Microorganisms that cause insect pests are mostly entomopathogenic fungi. Unlike bacteria and viruses, which must be consumed to cause disease, fungi

usually infect insects by penetrating their cuticles directly and then multiplying in the hemocoel (McCoy *et al*., 2019; Lacey *et al*., 2001; Kılıç and Yıldırım (2008), and Leger *et al*. (2011). EPF estimates that pathogenic fungi are responsible for about 60% of insect diseases (De Faria and Wraight, 2007). Aphids can be infected by nearly thirty commercial mycoinsecticides, and several species commonly induce naturally occurring epizootics in aphid populations (Goettel *et al*., 2005 and Kim *et al*., 2013). Although the majority of aphid-pathogenic fungi belong to the Entomophthorales (Zygomycota) order, many species in the Hypocreales (Ascomycota) order, including *Beauveria, Verticillium*, and *Paecilomyces*, are also known as aphid pathogens. According to Lotfy *et al.* (2021), *P. lilicanus*, an entomopathogenic fungus, is a promising biological control agent that can replace chemical management for sucking pests. According to the majority of researchers, *B. bassiana* and *M. anisopliae* can be used as microbial control agents for aphids, which are found throughout the world and have a broad host range. These species are also easily isolated from soil and insects (Meyling *et al*., 2006 and Freed *et al*., 2011a and b). The purpose of this work was to ascertain the pathogenicity of two isolates of *B. bassiana* and one isolate of *P. lilacinus*, which were obtained from infected aphids, against the corn leaf aphid *R. maidis*, in a laboratory setting, as well as their enzymatic activity.

Materials and methods

1. Mycopathogens associated with aphids infesting sorghum plants:

Hyphal tips from external growth mycoses cadavers were taken by the tiny fine tip of a sterile isolation needle and inoculated on appropriate Potato dextrose agar (PDA) or Sabouraud Dextrose Yeast Agar (SDYA) media in Petri dishes (9cm diam. X 1.5cm) at 25ºC. Incubated dishes were inspected daily to observe the fungal growth that was purified and used to confirm the disease cycle, then stored on slants of PDA and SDYA artificial media at 4ºC until they were used in subsequent experiments.

2. Cultivation:

For cultivating and preserving fungi, potato dextrose agar was utilized (250 grams of potatoes, 25 grams dextrose, 20 grams agar, and 1000 ml distilled water) or Sabouraud Dextrose Yeast Agar (10 grams peptone, 40 grams dextrose, 15 grams agar, 1 gram yeast extract, and 1000 ml distilled water). Petri dishes measuring, (9cm diameter x 1.5cm) were used to transfer the media after autoclaving for 20 minutes at 121 ºC. Approximately ten to fourteen days after incubation, fungi were left to grow in the dark. Keeping the fungal isolates at 4 ºC and re-culturing them every 14 to 30 days were the main cultivation procedures.

3. Identification:

To make sure the same fungus was maintained, insect cadavers were placed on slides stained with lactophenol cotton blue, inspected under a microscope, and then cultivated in PDA or SDYA media. The fungi were then incubated for 14 days at 25 ºC. Regular inspections were conducted on each isolate. A color atlas of pathogenic fungi was utilized to distinguish between the various fungal species (Domsch *et al*., 1980; Moubasher, 1993 and Key of Humber (1989), Mycology online (https://www.mycology.adelaide.edu.au) and Assiut University Mycological Center (AUMC).

4. Morphological characters of the identified fungi species:

4.1. *Paecilomyces lilacinus* (Plate 1):

Conidiophores primarily arose from submerged mycelium conidiogenous structures consisting of verticillate branches with whorls of 2 to 4 phialides ellipsoidal to fusiform conidia walled was smooth and hayaline the aphids fungus produced a white mycelial felt from which a large number of conidiophores, typically several white to yellow synnemata rose in a dense

powdery layer from all parts of the insect body colonies on SADY the conidiophores grew fairly quickly reaching a diameter of 4-6 cm in 14 days at 25 ºC.

4.2. *Beauveria bassiana* (Plate 2):

Probosci were used to bind the cadavers to the substrate; white mycelial development covered the cadavers, and later on, the conidia became powdery; they were almost globose and were carried alone on conidigenous cells with flask bases.

5. Enzymatic activity:

The present investigation employed two species of entomopathogenic fungus, specifically *B. bassiana* (Balsamo) Vuillemin and *P. lilacinus*. The following tests were performed on one isolate of *P. lilacinus* and two isolates of *B. bassiana*.

5.1. Lipase:

The medium outlined by Ulman and Blasins (1974) was used to evaluate the isolates.

- Its composition is as follows (g/L): MgSO4.7H2O, 0.2; Tween 80, 10 ml; CaCl2.2H2O, 0.2, peptone 10, and agar, 15.
- . For 15 minutes, the medium was autoclaved at 121°C to sanitize it.
- The Tween 80 was added to the sterile, chilled basal medium after being autoclaved separately.
- A 15-ml test tube that was vertically oriented was filled with the medium (10 ml/tube).
- Three days later, if there were any contaminated tubes, they were injected with discs of the fungal colony with a diameter of 0.5 cm.
- For fourteen days, the tubes were incubated at 25°C, and the mean of three replicates was used to record the results.

5.2. Protease:

It was examined using a casein hydrolysis medium (Paterson and Bridge, 1994), which contains the following components (g/L):

- 15% skim milk, 25 ml; glucose, 10 and agar, 15; CaCl2.2H2O, 0.1; KH2PO4, 1.0; KCL, 0.5; MgSO4.7H2O, 0.2; distilled water to one liter.
- After thoroughly mixing the medium to create a miscible medium solution, 10 milliliters of the medium were placed in each test tube and stored vertically.
- After three days, discs of the fungus colony with a diameter of 0.5 cm were added to each tube.
- For 14 days, the tubes were incubated at 25°C, and the mean of three replicates was used to record the results.

5.3. Chitinase:

A modified version of the medium used by Sherief *et al.* (1991) was employed. The media had the following composition (g/L):

- Agar, 15, MgSO4.7H2O, 0.5, FeSO4.7H2O, 0.01; KH2PO4, 1.0; and KCL, 0.5.
- Plates were infected with spore suspension of each isolate using a bacterial needle loop to identify the clear zone.
- For 14 days, the cultures were incubated at 25°C, and the outcomes were noted.

6. Bioassay:

6.1. Rearing of test insects:

The experimental colonies (Apterous form) of the corn leaf aphids were collected from severely infested sorghum plants grown at the Experimental Farm of Assiut University (Faculty of Agriculture, Plant Protection Department), Assiut governorate. Culture was maintained under laboratory conditions on sorghum plants for 6 months before being used in the bioassay experiments.

6.2. Preparation of conidial suspension:

A combination of conidia and hyphal debris was harvested from a 7-day-old culture of each isolate using a sterile blade. Sterile distilled water with 0.5 Tween 80 was used to suspend the mixture. Cheesecloth was used as a filter to lessen mycelium clumping in the

suspensions. The spores in the suspensions were counted with a hemocytometer. Each isolate was created using a different set of live conidial suspensions.

6.3. Pathogenic test:

To compare the virulence of two isolates of *B. bassiana* and one isolate of *P. lilacinus* against the corn leaf aphid *R. maidis.* Three replicates of four conidial concentrations were used in the bioassay of each fungal isolate plus a control. Aphids to be treated were sorted into 48 batches, each containing ten aphids to a leaf in an assay cell. Each assay cell consisted of a 60 mm glass Petri dish containing a 5 cm length of sorghum leaf portion from a greenhouse-grown plant 2-3 weeks old, the bottom of the cell contained a sterile piece of cotton soaked with sterile water and covered with sterile filter paper. The assay cells were maintained at 25 ºC. The moist cotton maintains the humidity within each cell at or near saturation. The treated aphids were observed daily for 7 days to observe and record the number of dead aphids and the date.

Results and discussion

1. Morphological characters of the identified fungi species:

1.1. *Paecilomyces lilacinus* (Plate 1):

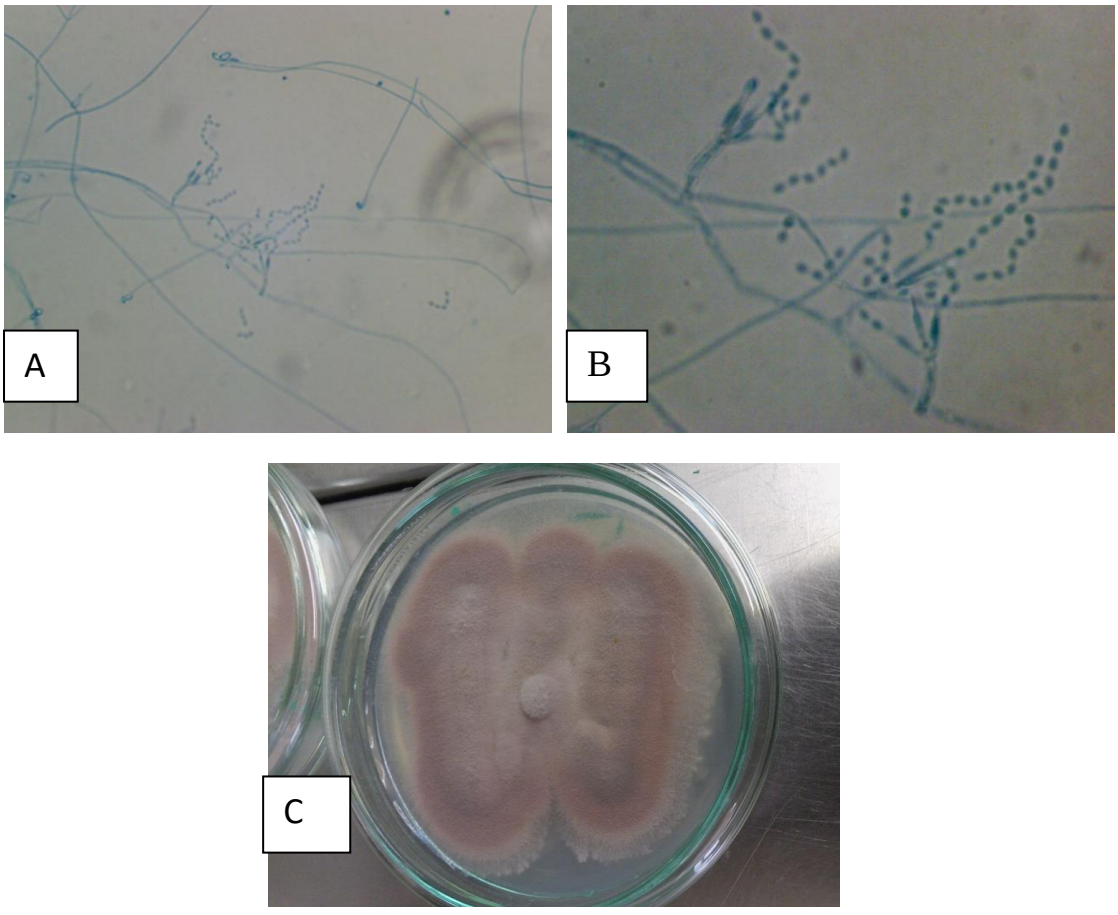

Plate (1): *Paecilomyces lilacinus*: (A): Series of conidia on phailides x 40; (B): Series of conidia on phailides x 100 and (C); culture.

1.2. *Beauveria bassiana* (Plate 2):

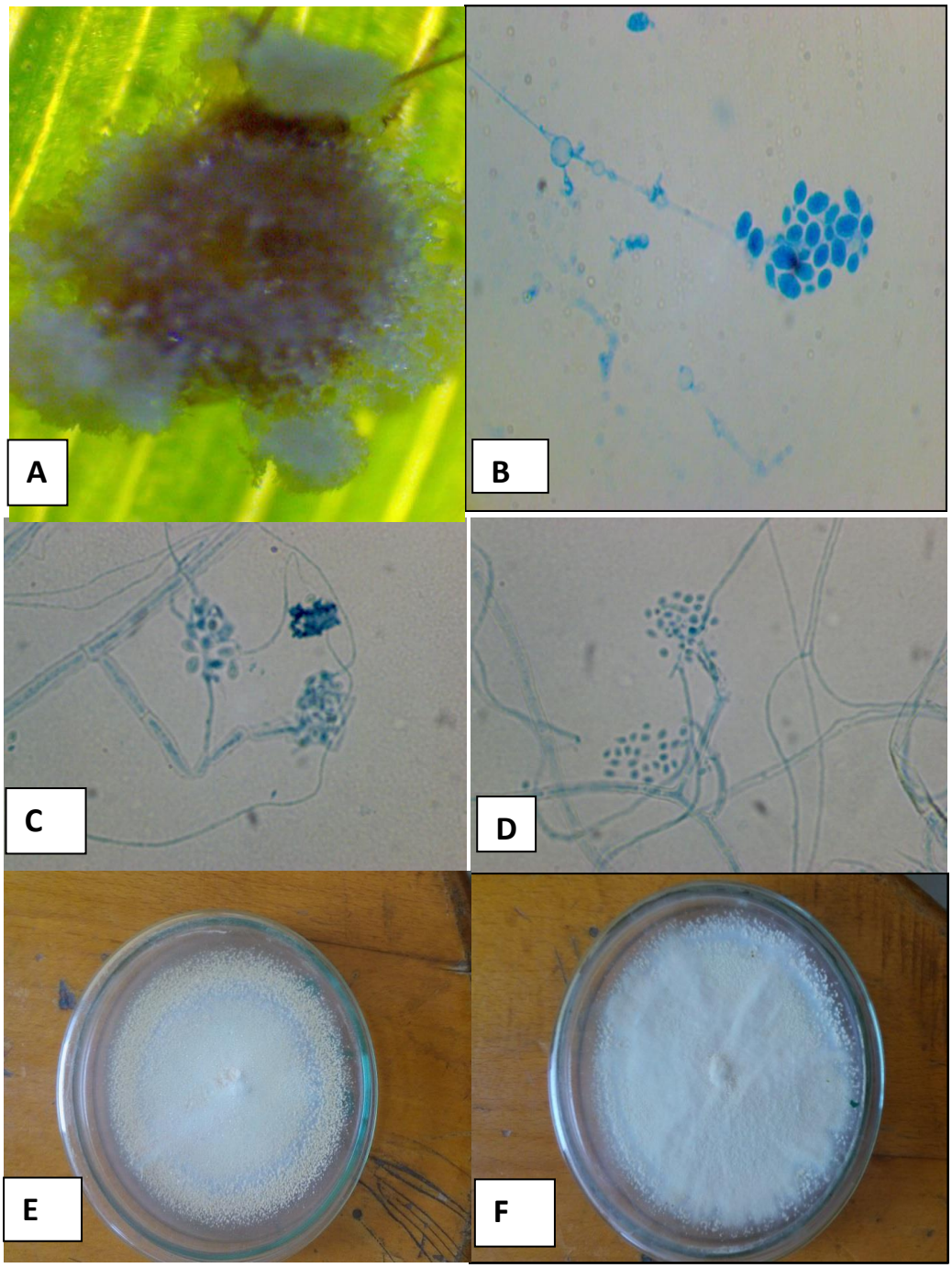

Plate (2): (A): An infected aphid with *Beauveria bassiana*; (B, C. and D): Show conidia and conidiogenous cells of *Beauveria bassiana*, (E): Isolate No. (1) and (F) Isolate No. (2).

2. Enzymatic activity of *Beauveria bassiana* and *Paecilomyces lilacinus* isolates:

Data in Table (1) show the enzymatic activity of two isolates of *B. bassiana* and one isolate of *P. lilacinus* isolated from cereal aphid cadavers directly collected from wheat plants Assiut.

2.1. Lipase :

Data indicates that all tested isolates were able to produce lipolytic

enzymes, but the isolation of *P. lilacinus* was the most active.

2.1. Protease ;

Proteolytic activity (Caseinolysis) was positive in all cultures, and the best activity was detected in the culture of *P. lilacinus*.

2.2. Chitinase:

Table (1) shows that the tested isolates gave positive activity (Growth and clear zone). However, the best isolate of chitinase activity was *P. lilacinus*.

Table (1): Enzymatic activity (lipase, protease, and chitinase) of *Beauveria bassiana* and *Paecilomyces lilacinus* isolated from cereal aphid cadavers directly collected from wheat plants in Assiut.

Isolate	Lipase	Protease	Chitinase
	Turbidity depth (mm)	Clear zone depth (mm)	Growth + clear zone
***Baeuveria bassiana* (1)**	9.33±0.58b	16.33±1.53b	5.33±0.29b
***Baeuveria bassiana* (2)**	9.00±0.00b	15.00±0.00b	5.33±0.58b
Paecilomyces lilacinus	15.00±5.00a	38.33±2.89a	8.50±0.00a

Means followed by the same letters vertically are not significantly different at 0.05 level of probability.

3. Pathogenicity of *Beauveria bassiana* and *Paecilomyces lilacinus* against the corn leaf aphid *Rhopalosiphum maidis*:

In this study, one isolate of *P. lilacinus* and two isolates of *B. bassiana* were tested against *R. maidis* infesting wheat plants at Assiut. Data in Table (2) show the percentages of mortality in relation to fungal isolate concentration.

3.1. *Paecilomyces lilacinus*

Apterous forms of *R. maidis* were inoculated with different concentrations of *P. lilacinus* conidia (2×10^7, 2×10^6, $2\times10^{5,}$ and $2\times10^{4)}$. All the experimented concentrations resulted in mortality percentages of 100, 80, 65, and 45%, respectively (Table 2).

3.2. *Beauveria bassiana* (1)

Data in Table (2) reveal that concentrations of 1.80×10^7, 1.80×10^6, 1.80×10^5, and 1.80×10^4 conidia/ml were able to kill 85, 65, 60, and 55%, respectively.

3.3. *Beauveria bassiana* (2)

Results in the same table show that all concentrations of *B. bassiana* (2) spore suspensions are able to kill the corn leaf aphid in various mortality percentages ranging from 65 to 95%. Generally, the rise of the conidial concentration induced a regular increase in mortality percentage. By contrast, mortality in the control treatment was recorded at 2.5%.

Table (2): Effect of *Paecilomyces lilacinus* (One isolate) and *Beauveria bassiana* (Two isolates) against the corn leaf aphid *Rhopalosiphum maidis*.

Fungi species	Concentrations	No. Insect	Mortality (%)
***Baeuveria bassiana* (1)**	1.80×10^7	20	17(85)
	1.80×10^6	20	13(65)
	1.80×10^5	20	12(60)
	1.80×10^4	20	11(55)
***Baeuveria bassiana* (2)**	1.30×10^7	20	19(95)
	1.30×10^6	20	16(80)
	1.30×10^5	20	14(70)
	1.30×10^4	20	13(65)
Paecilomyces lilacinus	2×10^7	20	20 (100)
	2×10^6	20	16 (80)
	2×10^5	20	13 (65)
	2×10^4	20	9 (45)
Control	……	40	1(2.5)

The degree of virulence of the three isolates against *R. maidis* is presented in Table (3). The LC_{50}were calculated as 1.35×10^5 , 4.45×10^3 and 5.52×10^2 (slope = 063±0.03, $0.28\pm1.92\times10^2\times$and $0.37\pm2.38\times10^2$)for isolates of *P. lilacinus* , *B. bassiana* (1) and *B. bassiana* (2) respectively. *B. bassiana* (2) isolate was more aggressive than other isolates as indicated by low the value of LC_{50} compared with other isolates as given in Table (3).

Table (3): Results of LC_{50} and LC_{95} *Paecilomyces lilacinus* (One isolate) and *Beauveria bassiana* (Two isolates) against the corn leaf aphid *Rhopalosiphum maidis*.

Fungi	Regression	Slope ±SE	LC_{50}	LC_{95}
Beauveria bassiana (1)	Y=-1.51+0.28×	$0.28\pm1.92\times10^2\times$	4.45×10^3	5.06×10^6
Beauveria bassiana (2)	Y=-1.69+0.33×	$0.37\pm2.38\times10^2\times$	5.52×10^2	4.88×10^6
Paecilomyces lilacinus	Y=-3.92+0.68×	0.63±0.03	1.35×10^5	1.46×10^6

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