Pyrex Journal of Microbiology and Biotechnology Research

Vol 2(2) pp.9-14 August, 2016

http://www.pyrexjournals.org/pjmbr
ISSN: 2985-8836

Full Length Research Paper

A Preliminary record of the entomopathogenic fungus *beauveria bassiana* (Balsamo) viullemin as endophyte in Egypt

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Accepted 18th April, 2016

Abstract

The present study was conducted to survey and isolate entomopathogenic fungi as endophyte from wheat and tomato plants in Assiut, Upper Egypt. Two species of agricultural economic plants (wheat and tomato) were freshly collected two times / month. These plants were wheat and tomato. Plants with no visible symptoms of disease were carefully selected after physical examination. In the present study 30 samples (6 from wheat and 24 from tomato) were surveyed for entophytic fungi. Leaves, shoots, and roots of the two plants were examined. Thirty-three fungal species which belong to 17 genera were isolated as endophytes of the two plants. *Beauveria bassiana* was successfully isolated from the leaves of tomato (*Solanum lycopersicum* L.). Moreover, the pathogenicity of this isolate was assayed as endophyte on tomato and wheat plants. Also, the isolate of *B. bassiana* was assessed against the greater wax moth, *Galleria mellonella* L.

Key words: Entomopathogenic fungi, endophyte, tomato, wheat plants

INTRODUCTION

The term endophyte was coined by the German scientist Heinrich Anton De Bary (1884), and is used to define fungi or bacteria occurring inside plant tissues without causing any apparent symptoms in the host (Wilson, 1995). Endophytes are microorganisms that live within plants for at least a part of their life cycle without causing any visible manifestation of disease (Bacon and White, 2000). Fungal endophytes, fungi that live internally and asymptomatically with plant tissues, are increasingly recognized as important contributors to the phenotype of of host plant (Redman *et al.,* 2002 and Cheplick and Faeth 2009). Fungal endophytes have been detected in hundreds of plants, including many important agricultural commodities such as wheat (Larran *et al.,* 2002a&b).

Several roles have been ascribed to fungal endophytes, including providing protection against herbivorous insects (Breen, 1994 and Clement *et al.,* 1994). Beauveria bassiana is one of the entomopathogenic fungi commonly used as a biocontrol agent for pests of crops. This fungus can be isolated from soil, plant materials as endophytes or insect cadavers. The aim of the present research was to isolate, identify indigenous entomopathogenic fungus as endophyte from wheat and tomato plants and assayed on some plants and some insects and this is for the first time in Egypt.

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MATERIALS AND METHODS

The present study was conducted to survey and isolate entomopathogenic fungi as endophyte from wheat and tomato plants at Assiut. Field samples were collected from the experimental farm of Assiut University throughout the period from January to December 2014. Two species of agricultural economic plants (wheat and tomato) were freshly collected two times / month. These plants were wheat and tomato. Plants with no visible symptoms of disease were carefully selected after physical examination.

Isolation of endophytic fungi

Samples of leaves, stems, and roots were rinsed gently in running water to remove dust and debris. Isolation of endophytic fungi was done according to the method described by (Arnold *et al.,* 2001). Surface sterilization of samples was done by stepwise washing with 70% ethanol for 3 min, sodium hypochlorite solution (4-6%) for 3 min and 70% ethanol for 30 sec., followed by two rinses in sterile distilled water, then they were thoroughly dried between sterilized filter paper (Filip *et al.,* 2003).

The edges were cut by sterilized scalpel to remove dead tissue due to chemical the disinfection application, and samples were cut into about 1 cm2 pieces. Four pieces were placed in 9 cm Petri-dishes containing Potato dextrose agar (PDA) and Sabouraud dextrose agar media. All Petri-dishes were incubated at 25±2ºC for 10-15 days. Periodically the colonies were examined and each colony that emerged from segments was transferred to antibiotic-free potato dextrose agar medium (PDA) to aid identification. Endophytic isolates were identified on the basis of culture characteristics, and spores.

The efficiency of surface sterilization was confirmed by the following method. Another sterilized plant segments were placed in conical flask containing sterile distilled water, the suspension was shaken by a mechanical shaker for 10 minutes at 150 rpm. One ml of this dilution was transferred into one of five replicates of sterilized Petri-dishes and 12-15 ml of PDA agar medium cooled to just above the solidifying temperature were added to each dish. The dishes were rotated by hand for good dispersion of dilution. The absence of growth of any fungi on the all five Petri-dishes confirmed that the surface sterilization procedure was effective (Schulz et al., 1993).

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Identification of isolated fungi

Identification of fungal species was carried out according to **(**Domsch *et al*., 1980, Moubasher, 1993 and Humber, 1997).

Inoculums preparation

The endophytic *B. bassiana* strain was streaked on PDA media. The Petri dishes were incubated at 25±2ºC in the laboratory. After two weeks, conidia were gently scraped from uncontaminated Petri dishes using sterile scalpel blade. The conidia of 10 Petri dishes cultures of *B. bassiana* were collected and suspended in 25 ml sterilized distilled water (SDW) containing 0.01% Tween 80. To avoid uneven distribution of the conidia, the conidial suspension was forcefully vortexed for 5 min, this homogenous conidial suspension served as stock suspension. The conidial concentration was determined using an improved Neubauer homocytometer (Marien feld, Germany). Concentration of 10^7 conidia / ml was prepared using micropipette and SDW containing 0.01% Tween 80.

Bioassay on insects

Larvae of the 4^{th} instar were placed in conidial suspensions of *B. bassiana*. Submersion for 5 seconds

Table 1. List of fungal species recoded as endophytes from wheat and tomato plantations at Assiut.

Host plant	Genus/ Species	Frequency
	Penicillium	
	P. chrysogenum Thom	*
	P. brevicompactum Dierckx	**
	P. oxalicum Currie and Thom	*
	P. roqueforti Charles Thom in 1906	*
	P. steokii K. M. Zalessky 1927	*
	Aspergillu*s*	
	A. niger Van Tieghem	***
	A. flavus Link	***
	A. candidus Link, H. F. 1809	**
	Cladosporium	
	C. shaerospermum Penzig 1882	*
	C. cladosporioides (Fresenius) de Vries 1952	**
	C. herbarum (Presoon) Link	*
	Alternaria	
Wheat	*A. alternate* (Fries) Keissler 1912	**
	A. tenuissima (Kunze) Wiltshire 1933	*
	Fusarium	
	F. solani (Mart) Appel & Wollenw	*
	F. oxysporum Schlecht. Emend.Snyd. & Hans	*
	Cochliobolus	
	C. hawaiiensis Alcorn	*
	C. spicifera Nelson	*
	Ulocladium	
	U. botrytis Preuss 1851	*
	Nigrospora	
	N. sphaerica (Saccardo) Mason 1927	*
	Trichothecium	
	T. roseum (Pres.) Link 1809	*
	Rhizoctonia	
	R. solan Kuhn, J.G. 1858	*

was used for ten larvae per replicate. The treatment was replicated four times. SDW with 0.1% Tween 80 was used as control. After treatment, the insects group was placed in a sterilized moist chamber consisting of a Petri-dish lined with wet filter paper.

Bioassay on plants

Wheat plant was cultivated in eight pots of 1 kg capacity in the greenhouse. Once plant reach its first true leaf stage (after 15 days of planting), the endophyte, *B. bassiana* conidial suspension of 10^7 was applied to the plant foliage using a manual atomizer. SDW containing 0.01% Tween 80 was used to spray control. The pots were watered till the soil capacity with sterile distilled water one day before inoculation. After 3, 5, and 7 days of inoculation, plant samples were taken and superficially sterilized to detect the endophytic *B. bassiana*. For examination, the plant was uprooted and washed carefully, the shoot and root length were recorded. The plant specimens were selected in random order. Two stem, two true leaves, and two pieces of root, 1 cm^2-long each, were cut and processed for endophyte detection. Surfaces of plant pieces were sterilized as above. Samples were rinsed three times by immersion in SDW and let it dry in sterile filter paper. Then, dissected, the discs' outer edges were discarded, where endophytes might have been eliminated due to contact with disinfectants (Parsa *et al*., 2013). The plant pieces were plated in Petri dishes with PDA media supplemented with the proper antibiotic e.g. tetracycline, at 2 mg/L each to avoid bacterial growth. The plates were sealed well with Para film, and incubated in the dark at 25±2°C. The plates were inspected every day to observe any fungal growth. *Beauveria bassiana* can be identified by characteristic white dense mycelia becoming cream to pale yellow at the edge. When in doubt, mount the specimen in a drop of water and examine under a

Table 1. Continues.

Host plant	Genus/ Species	Frequency
Tomato	*Penicillium*	
	P. brevicompactum Diercks	**
	P. chrysogenum Thom	**
	A. oryzae (Ahlburg) Chohn	*
	A. terreus Thom	*
	Cladosporium	
	C. shaerospermum Penzig 1882	**
	C. herbarum (Presoon) Link	**
	Alternaria	
	A. chlamydospora Mouchacca 1973	*
	A. tenuissima (Kunze) Wiltshir 1933	*
	A. alternate (Fries) Keissler 1921	**
	Fusarium	
	F. oxysporum Schlecht.Emend.Snyd & Hans	**
	Cochiliobolus	
	Cochiobolus hawaiiensis Alcorn	*
	+Beauveria	
	***Beauveria bassiana* (Bals.) Vuill**	*****

+ First record in Egypt as endophyte.
***** = Scarce ******= Moderately
*******= Abundant ********= More abundant

microscope, looking for globose conidia and zigzag-shaped conidiophores, characteristic of the species. The mounted slide was examined microscopically for characteristic *B. bassiana* features (globose conidia and zigzag-shaped conidiophores) (Humber, 1997).

RESULTS AND DISCUSSION

Survey of endophyte entomopathogenic fungi

The survey studies extended through the period from January to December 2014. Wheat and tomato plants were used to survey fungal endophytes. Thirty-three fungal species were identified as endophytes from wheat and tomato crops, 21 and 13 species, respectively (Table, 1). *Beauveria bassiana* was identified as endophyte was isolated also from leaves of tomato plants.

Description of the species

According to measurements of this species According to Domsch *et al.* (1980) and Moubasher (2010) (Figure. 1) shows colonies growing slowly, wooly; at first white but later often becoming yellow to slightly pinkish. Conidiogenous cells arising either singly; in whorls from vegetative hyphae; or more commonly in clusters from swollen stalk cells. Conidiogenous cells differentiated into a subglobose to ellipsoidal or cylindrical venter and a filiform, zigzag- shaped rachis, denticulate rhachis arising by sympodial elongation. Conidia hyaline; globose or ellipsoidal with a rounded or slightly pointed base.

Conidiogenous apparatus forming dense clusters of swollen stalk cells, which consist of a subglobose to flask-shaped venter 3-6×2.5-3.5µm, and zig-zag shaped. Conidiogenous cell length 8.7µm; conidiogenous cell width 2.9µm; hyphae width 2-3µm; and conidia subglobose 2-4µm diameter.

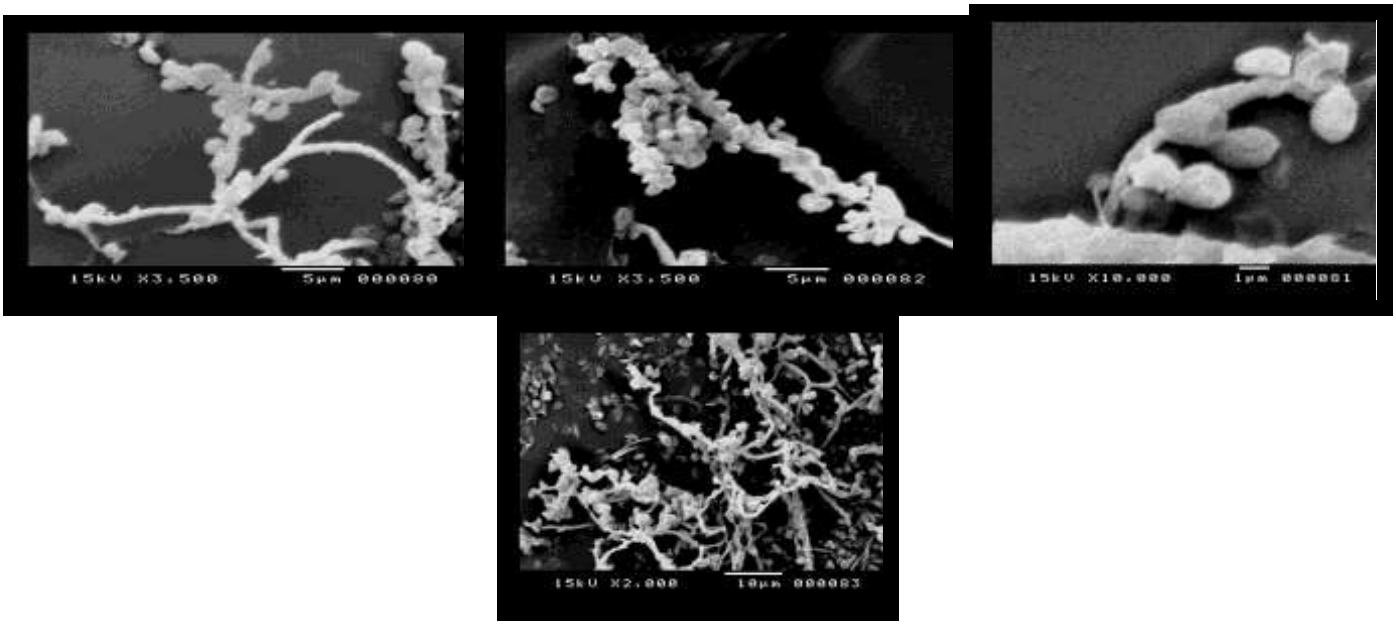
Inoculation test on wheat plant

B. bassiana was able to endophytically-colonize wheat plants in response to the demonstrated inoculation treatments**.** Foliar sprays drenches resulted in endophytic colonization by *B. bassiana* in over 80% of the treated plants (Figure 2). However, the extent of colonization depended on the plant part evaluated. Treatment and control plants were visibly indistinguishable two weeks after inoculations. No differences were detected in their dry weight and in their height.

Pathogenicity of insects

A conidial suspension of the fungus *B. bassiana* isolated from tomato leaves was pathogenic on the fourth instar lavae of the greater wax moth (Lepidoptera: Pyralidae). Figure 3 shows the dead larvae after 7-days old. Pathogensity test of the fungal against showed that this

A)



B)

Figure 1. *B. bassiana* characteristic. A) 7-days-old colony on PDA B) Using different powers of scanning electron microscope.

(A) (B) (c)

Figure 2. Representative results of inoculation treatments on endophytic colonization of wheat plants by *Beauveria bassiana*. **Figure (B):** control plates with no growth. **Figure (A):** *B. bassiana* growing from four plant sections after 7-Days old. **Figure (C):** Endophytic *B. bassiana* conidia and conidiophores as seen under a microscope.

Figure 3. The dead larvae with condense growth of *B. bassiana.*

fungi could be considered the most promising candidate as biocontrol of insect pests. Isolation of fungi as endophyte from wheat and tomato organs collected in Assiut, Upper Egypt revealed the presence of 33 species related to 17 genera of fungi. One species of them (*Beaveria bassiana*) was identified as entomopathogenic isolated from tomato plants. Fungal entomopathogens are important regulators of insect populations with considerable potential as biological control agent. Recently fungal entomopathogens have been shown to occur as endophytes, both naturally and in response to various inoculation methods (Vega *et al*., 2008 and Michael *et al.,* 2012).

The ecological function of endophytic fungal entomopathogens remains largely unknown, but some studies have implicated them in plant growth, herbivore resistance, and disease resistance (Vega *et al*., 2008). *Beauveria bassiana* (Balsamo) Vullemin (Ascomycota: Hypocreales) is the best candidate endophytic fungal entomopathogen and it is available as a commercial mycopesticide. From the foregoing results, it could be mentioned that entomopathogenic fungi as endophyte appeared to be of a significant value in the natural control of some herbivores.

Results of the present study are in general agreement with those previously reported by Dean and Wilding 1973, Dedryver 1983 and Feng *et al*. 1991. Members of Hyphomycetes are excellent candidates for biological control as endophytic fungi (Latge and Papierok 1988). *B. bassiana* as endophyte has recently attracted increasing attention in recent years as plant defense against pests. The results of this work can be useful in planning a successful pest management programs for some herbivore.

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