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EXISTENCE OF *BEAUVERIA BASSIANA* (BALSAMO) VULLEMIN AS ENDOPHYTE ON ONION PLANTS AND ITS PATHOGENICITY (*IN VITRO*) AGAINST ONION THRIPS, *THRIPS TABACI* LIND. (THYSANOPTERA: THIRPDAE)

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Abstract

Beauveria bassiana (Balsamo) was successfully isolated from the leaves of onion plants, *Allium cepa* Lind. cultivated in the Experimental Farm of Assiut University. Morphological characters of the fungus were described. Moreover, the pathogenicity of this isolate was assayed (dipping technique) against onion thrips, nympal and adult stages (***In vitro***). Six concentrations ($13x10^4$, $29x10^4$, $42x10^4$, $64x10^4$, $86x10^4$ and $104x10^4$ conidia / ml) were used. Our results demonstrated that mortality increased as the conidial concentrations increased. It seems that the concentration of 104×10^4 conidia / ml is an effective to get sufficient mortality for the nymphal stage (92.50%) and adult stage (88.46%). In general, results refer that *B. bassiana* isolated from onion leaves at the concentration of $104x10^4$ spores/ml is effective to get about 90% mortality for the onion thrips regardless of the pest stage. Also, virulence of *B. bassiana* against onion thrips (nymphal and adult stages) elucidated that the fungus was more effective on nymphal than adult stage as indicated by low value of LC_{50} and LC_{95}. The present results reflect the potential role that can be played by *B. bassiana* endophyte in the biological control of thrips infesting onion plants under field conditions.

Key words: *Beauveria bassiana,* endophyte, onion plant, onion thrips

INTRODUCTION

Onion (Allium cepa Lind.) belonging to the Family Liliaceae is a major horticultural cash crop in Egypt. Onion thrips, Thrips tabaci Lindeman (Thysanoptera: Thripidae), is a cosmopolitan species found in onion-growing regions world-wide (Lewis, 1997, Macintyre-Allen et al., 2005 and Gill et al. 2015). It is one of the key pests of onion crop cultivated in Assiut (Abou-Elhagag and Ezzel-Din 2002 and Mahmoud 2008). Heavy infestations may reduce yield and the marketability of onion bulbs (Mo et al., 2009 and Boateng et al. 2014). Various control tactics such as insecticide application are not sufficient to acheive complete control indicating that a new control tactic is needed to keep the thrips's population under the threshold of economic damage without an environmental negative impact produced (Zereabruk 2017). In the course to seek an effective tactic control we come across that an interesting issue is the presence of fungal endophytes. The term of endophyte was coined by the German scientist, Heinrich Anton De Bary in 1884 and used to define fungi and bacteria occurring inside plant tissues without causing any apparent symptoms on the host (Sasan and

Bidochka 2012). Endophytic fungi, which live within host plant tissues without causing any visible symptoms of infection, are important mutual benefit organisms that mediate plant-herbivore interactions. Fungal entomopathogens are important regulators of insect populations (Parsa et al. 2013).

The present study aimed to isolate and identify of B. bassiana as an endophyte from healthy onion plants and also to investigate its effects (In vitro) against the onion thrips (nymphal and adult stages).

MATERIALS AND METHODS

1-Isolation of B. bassiana as endophyte colonized onion plants

An endophytic fungus, *B. bassiana* used in the present study was isolated from onion plants cultivated at the Experimental Farm of Assiut University. Field samples (twelve samples) of onion plants were collected throughout the period from January to March during 2016 and 2017 onion growing seasons. Onion plants were freshly collected two times / month. Plants with no visible symptoms of disease were carefully selected after physical examination.

Samples of leaves were rinsed gently with running water to remove dust and debris. Isolation of endophytic fungi was done according to the method described by (Arnold and Lutzoni 2007). Surface sterilization of samples was carried out 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 with sterilized distilled water. Then they were thoroughly dried between sterilized filter papers (Filip *et al*., 2003). The edges were cut by sterilized scalpel to remove dead tissues due to chemical disinfection application, and samples were cut into about 1 cm^2 pieces. Four pieces were placed in 9 cm Petri-dishes containing Potato dextrose agar (PDA) media (ten replicates each sample). All Petri-dishes were incubated at 25±2ºC for 10-15 days. Periodically the colonies were examined and each colony 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 and morphological characteristics.

2- Identification of fungi

The fungus was identified at Assiut Mycological Lab., Botany and Microbiology Department, Faculty of Science according to Domsch *et al*. (1980), Moubasher (1993) and Humber (1997).

3- Inoculums preparation

The endophytic *B. bassiana* strain was streaked on PDA media. The Petri dishes were incubated at 25±2ºC and after two weeks, conidia were gently scraped from Petri dishes using sterile scalpel blade. The conidia were collected and suspended in 25 ml sterilized distilled water (SDW) containing 0.1% Tween 80. To avoid uneven distribution of the conidia, the homogenous conidial suspension was forcefully vortexes for 5 min and used as stock suspension. The conidial concentration was determined using an improved Neubauer Homocytometer (Marien feld, Germany). Concentrations were prepared using micropipette.

4-Bioassay against onion thrips (nymphal and adult stages)

Dipping assay was used to compare the pathogenicity of *B. bassiana* isolated from onion plants as endophyte against nymphal and adult stages of the onion thrips (***In vitro***). Six concentrations of *B. bassiana* were used ($13x10^4$, $29x10^4$, $42x10^4$, $64x10^4$, $86x10^4$ and $104x10^4$ conidia / ml). Leaves containing thrips were placed in conidial suspensions of *B. bassiana* and immersed for 5 seconds. The treatments were replicated four times. Sterilized distilled water and Tween 80 (0.1%) were used as control. After treatments, the insects groups were placed in a sterilized moist chamber consisting of a Petri-dish lined with wet filter paper. After 3, 5, and 7 days the plates were inspected to observe mortality. Percent mortality was calculated. The values of LC_{50}, LC_{95} and slopes were calculated by computerized Probit analysis program (Finny 1971).

RESULTS AND DISCUSSION

Owing to survey studies through onion growing seasons of 2016 and 2017, onion thrips, *Thrips tabaci* Lind. was found infesting onion plants. Nymphs and adults of this pest were commonly intermixed together at the same location on the onion plants. Available literatures in Egypt confirmed our findings and showed that this pest species is infesting onion plants causing severe damage (Mahmoud 2008).

1-B. bassiana *as endophyte isolated from onion*

According to entomopathogenic fungi as endophyte, results indicated that *B. bassiana* (Family: Moniliaceae, Order Moniliales) was identified as endophyte in onion leaves. Plates (1 - 4) show *B. bassiana* grown in the culture media (SDA), by scan electron microscopy (SEM), transmission electron micrograph and those grown on insect body (nymph and adult). The culture characteristics of *B. bassiana* were, colonies growing slowly, wooly; at first white but later often are 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 sub-globose to ellipsoidal or cylindrical venter and a filiform, zigzag- shaped rachis, denticulate rhachis arising by sympodial elongation. Conidia as described by Domsch *et al.*, 1980 and Moubasher, 1993 were hyaline; globose or ellipsoidal with a rounded or slightly pointed base.

Endophytes are microbes which colonize living internal tissues of plants (Stone *et al.*, 2004, Arnold and Lutzoni 2007 and Sanchez *et al.*, 2007). Entomopathogenic fungi gain more and more interest in biocontrol and protection of plants. Many species of entomopathogenic fungi in various genera are known and some of them show promising effects as bio-control agents (BCA). The entomopathogenic fungus *B. bassiana* has the ability to colonize various cultivated plants endophytically (Bing and Lewis, 1991; Akello *et al*. 2008, Ownley *et al*. 2008 and Gurulingappa *et al*. 2010). Many plant used endophytic fungi as a tool of indirect defense against herbivores. Also, the fungus provides benefits to the plant such as increasing water or nutrient uptake. Once associated, the fungi alter nutrient content of the plant and enhance or begin production of secondary metabolites (Schulez *et al.* 2002 and Schardl *et al.* 2004). Several researchers identified and surveyed these fungi species as endophytes from several plants worldwide and is considered to be the best candidates for the biological control of herbivorous insects (Evans *et al.* 2003 and Arnold *et al.* 2003).

2-Bioassay of B. bassiana against nymphal and adult stages

The efficiency of *B. bassiana* isolated from onion plants as endophyte was assayed (***In vitro***) against nymphal and adult stages of the onion thrips, *Thrips tabaci* Lind. Six concentrations were prepared and tested. Data presented in Table (1) show the percentages of mortality among tested concentrations after 5-days. Relationships between probits equivalent to percentage of mortality and log concentrations (conidia / ml) of *B. bassiana* used are presented in Figure (1). The calculated LC_{50} and LC_{95} values are given in Table (4).

2.1-Nymphal stage

Data in Table 1 reveal that concentrations of $13x10^4$, $29x10^4$, $42x10^4$, $64x10^4$, $86x10^4$ and $104x10^4$ conidia / ml were able to kill 33.33%, 50.00%, 64.86%, 70.51%, 87.50%, and 92.50% of nymphal stage, respectively. By contrast, mortalities of nymphs in the control treatment was acheived by 1.02%. Mortality increased as the conidia concentration increased. It seems that the *B. bassiana* as endophyte at the concentration of 104 x 10^4 conidia / ml is effective to get sufficient mortality (92.50%) for the nymphal stage of onion thrips. Pathogencity of *B. bassiana* against the nymphal stage of *T. tabaci* was summarized in Table (4). The LC_{50} and LC_{90} were calculated as $1.23x10^5$ and $7.02x10^6$, respectively.

2.2- Adult stage

Adult stage of the onion thrips infesting onion plants was treated with different concentrations of *B. bassiana* (conidia/ml). Mortality rates among treated individuals of onion thrips showed a dose-mortality trend as presented in Table 2. Data exhibited that the different concentrations resulted in considerable mortalities of the treated adults. By contrast, mortality in the control treatment was acheived by 4.65%. Recorded mortality values among the tested concentrations reached 88.46, 75.76, 78.95, 73.68, 53.13 and 28.57% for the concentrations of $104x10^4$, $86x10^4$, $64x10^4$, $42x10^4$, $29x10^4$ and $13x10^4$ conidia / ml, respectively.

The calculated LC_{50}, LC_{95} and slope values were observed in Table (4). The LC_{50} and LC_{95} values of the adult stage were $1.42x10^5$ and $8.96x10^6$ conidia/ml, respectively.

2.3- Regardless of T. tabaci stages

Data shown in Table (3) demonstrate the relative pathogencity of *B. bassiana* isolated from onion leaves as endophyte against onion thrips. Concentrations of $13x10^4$, $29x10^4$, $42x10^4$, $64x10^4$, $86x10^4$ and $104x10^4$ conidia / ml were able to kill 30.77%, 51.92%, 69.33%, 73.27%, 85.48% and 90.22%, respectively. In contrast, mortality in the control treatment was acheived by 2.11%. Mortality increased as the conidia concentration increased. It seems that the *B. bassiana* isolate as endophyte at the concentration of 104 x 10^4 conidia / ml is an effective to get 90.22% mortality for the onion thrips regardless of the pest stage.

Virulence of *B. bassiana* against the onion thrips was summarized in Table (4). The LC_{50} and LC_{95} were calculated as $5.43x10^6$ and $1.34x10^5$ (slope = 0.93±0.05). Thus *B. bassiana* was more effective against nymphal stage than adult stage of the onion thrips as indicated by low value of LC_{50} (Table 4).

In the present study, *B. bassiana* as an endophytic fungus was tested against nymphal and adult stages of the onion thrips **(*In Vitro*)**. This fungus has been used as an important criterion for selecting some other fungal species in the microbial control of agricultural pests (Lai *et al*., 1982; Zoberi and Grace, 1997; Wells *et al*., 1995; and Almeida *et al*., 1997).

The ability of entomopathogenic fungi to infect the nymphal and adult stages of *T. tabaci* or the potential to control this pest through nymph and / or adult infestation has been poorly studied. The results reported herein indicate that *B. bassiana* as endophyte was more effective against nymphal stage of the onion thrips than adult stage since, the LC_{50} were calculated as $1.23x10^5$ and $1.42x10^5$ for nymphs and adults, respectively. Observations on infected nymph and adult stages indicated that differences in virulence between both stages may be due to the differences in ability to adhere to the surface of infected stage, subsequently facilitates penetration into the infected individual (Couch and Ignoffo 1981, and Samuels *et al*, 2002). El-Sufty *et al*. (1982) evaluated the efficiency of *B. bassiana* against the cotton leaf worm. They found that treating larvae with suspensions of low and middle spore densities (up to 10^8 spores / ml) yielded not more than 16% mortality. The authors interpreted the low susceptibility of *S. littoralis* larvae to *B. bassiana* infection as a result of the physical and chemical structure of the larval cuticle.

In general, *B. bassiana* as an endophyte at the concentration of 104×10^4 conidia / ml is an effective to get 90.22% sufficient mortality for the onion thrips, in addition to its higher effectiveness against nymph than adult as indicated by low value of LC_{50}.

From the aforementioned results it could be concluded that biological control with pathogenic fungi is a promising alternative to chemical control against the agricultural pests all over the world, (Wells *et a*l., 1995; Grace, 1997; and Milner *et al*., 1998).

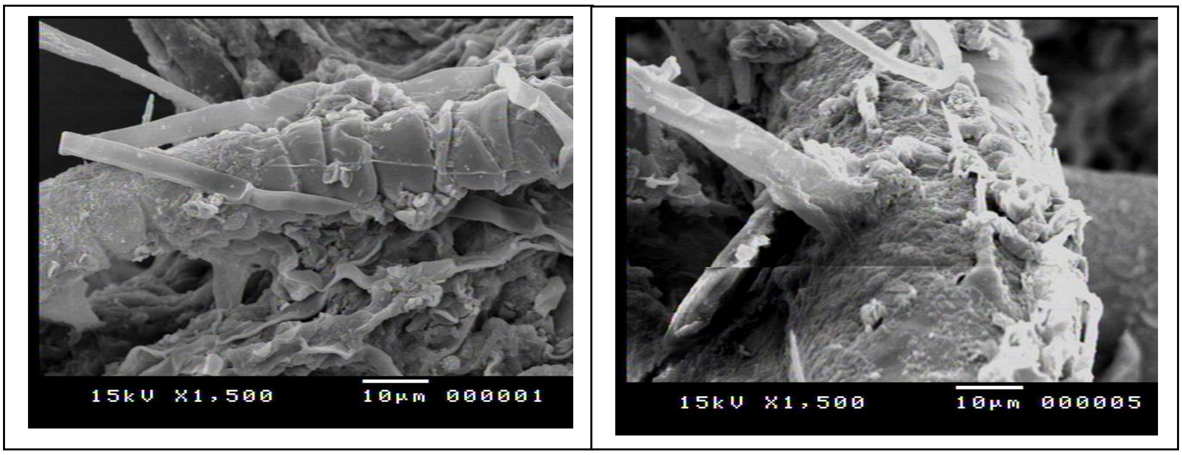

Plate (1): SEM showing *B. bassiana* network forming hyphal mycelium covering dead thrips. Mycelia covered whole surface of thrips and penetrated body wall and legs.

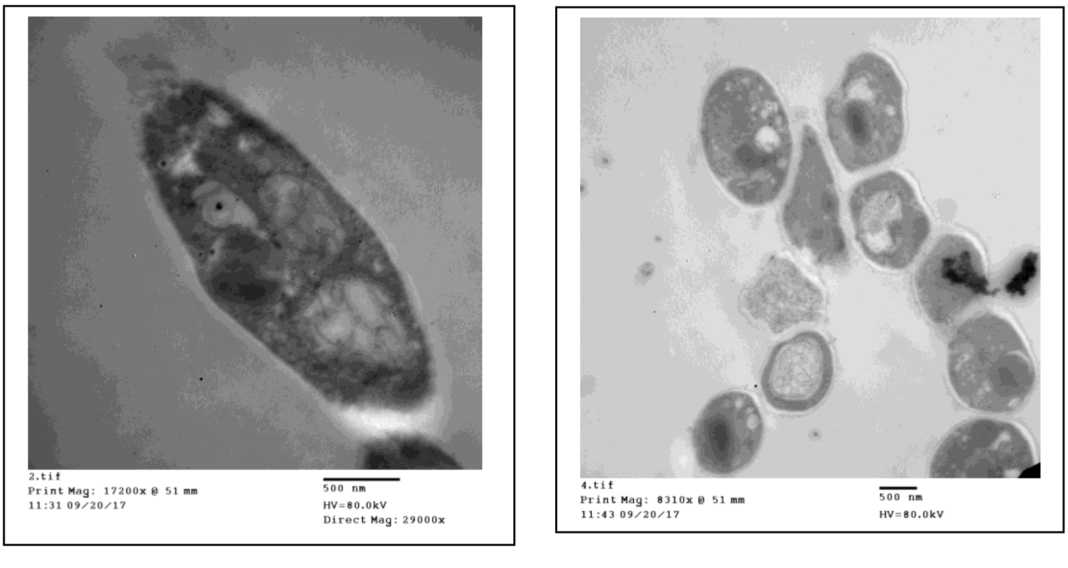

Plate (2): Transmission electron micrograph showing the conidia of *B. bassiana* identified.

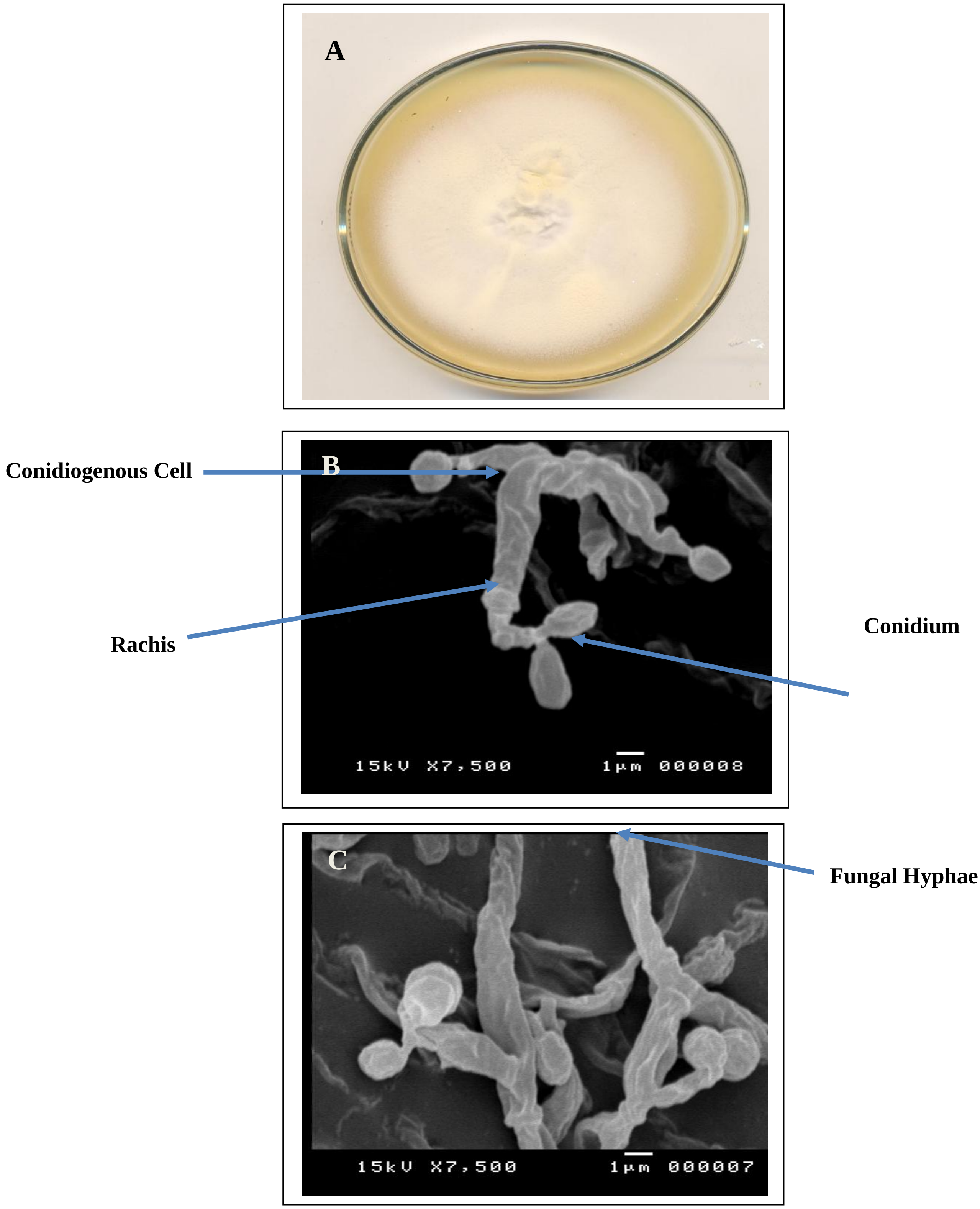

Plate (3): Morphological characteristic of *B. bassiana*:

(A) 7-days old colony on PDA. (B & C) Scanning Electron Micrographs showing fungal hyphae with flask shaped conidiogenous cells and rachis elongated in a long Zig-Zig extension.

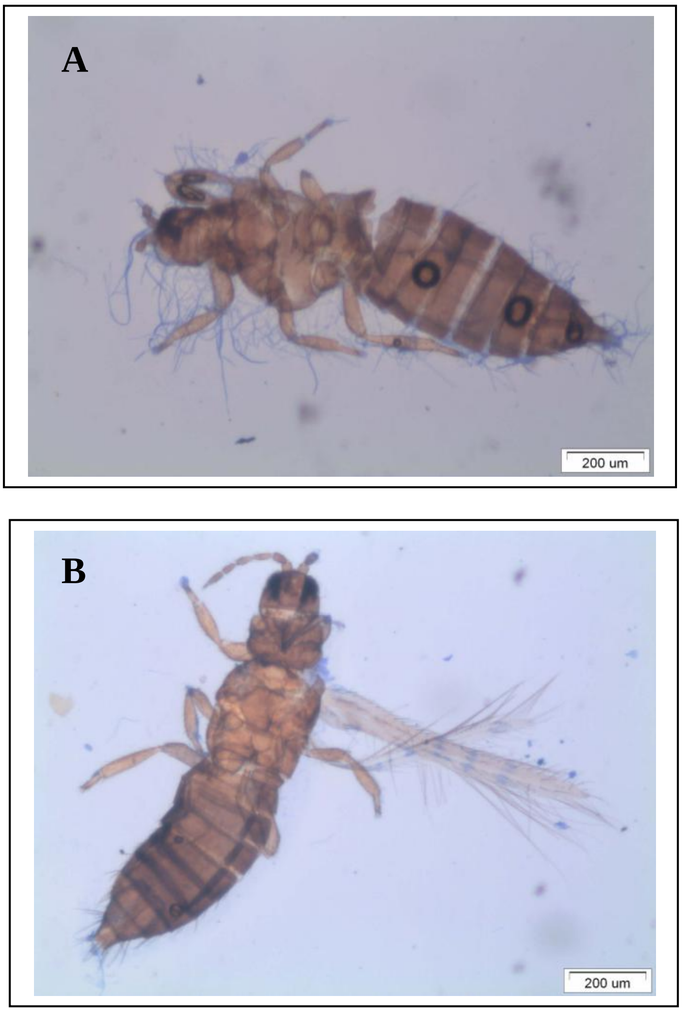

Plate (4): Showing *B. bassiana* hyphae colonize the abdomen of the onion thrips under light microscopy using high power (x 500)

(A) Nymph

(B) Adult

Table (1): Mortality (%) of the onion thrips (nymphal stage) treated with different concentrations of *B. bassiana*.

Concentrations (Conidia / ml)	Total no. of individuals	No. of dead individuals	Mortality (%)
$13x10^4$	40	37	3.33
$29x10^4$	64	56	50.00
$42x10^4$	78	55	64.86
$64x10^4$	37	24	70.51
$86x10^4$	40	20	87.50
$104x10^4$	60	20	92.50
Control (untreated)	98	1	1.02

Table (2): Mortality (%) of the onion thrips (adult stage) treated with different concentrations of *B. bassiana*.

Concentrations (Conidia / ml)	Total no. of individuals	No. of dead individuals	Mortality (%)
$13x10^4$	52	46	28.57
$29x10^4$	66	50	53.13
$42x10^4$	38	30	73.68
$64x10^4$	38	28	78.95
$86x10^4$	64	34	75.76
$104x10^4$	70	20	88.46
Control (untreated)	43	2	4.65

Table (3): Mortality (%) of the onion thrips (nymphal & adult stages) treated with different concentrations of *B. bassiana*.

Concentrations (Conidia / ml)	Total no. of individuals	No. of dead (individuals)	Mortality (%)
$13x10^4$	92	83	30.77
$29x10^4$	124	106	51.92
$42x10^4$	116	85	69.33
$64x10^4$	75	52	73.27
$86x10^4$	104	54	85.48
$104x10^4$	130	40	90.22
Control (untreated)	142	3	2.11

Table (4). Results of LC_{50} and LC_{95} of *B. bassiana* as endophyte against onion thrips (nymph and adult).

Stage	LC_{50}	LC_{95}	Slope ± SE	Regression equations
Nymphal	$1.23x10^5$	$7.02x10^6$	0.94±0.05	Y=-4.77+0.94x
Adult	$1.42x10^5$	$8.96x10^6$	0.91±0.06	Y=-4.71+0.91x
Total	$5.43x10^6$	$1.34x10^5$	0.93±0.05	Y=-5.43x1.02x

Lc_{50} (Lethal concentration 50): is the concentration of *B. bassiana*, which kills 50% of a sample population.
Lc_{95} (Lethal concentration 95): is the concentration of *B. bassiana*, which kills 95% of a sample population.

CONCLUSION

Entomopathogenic fungi gain more and more influence in biocontrol and protection of plants. Many species of entomopathogenic fungi in various genera are known and some of them show promising effects as bio-control agents (BCA).From the aforementioned results it could be concluded that biological control with pathogenic fungi as endophyte is a promising alternative to chemical control against the agricultural pests all over the world. Endophytes are microbes which colonize living internal tissues of plants. *Beuveria bassiana* is a fungal entomopathogen with the ability to colonize onion plants endophytically cultivated in Assiut, Egypt. Plant use of endophytic fungi in defense occurs when endophytic fungi, which live symbiotically with the majority of plants by entering their cells, are utilized as an indirect defense against herbivores.

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