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Isolation, Characterization, Identification, and Preliminary Pathogenicity Test of Entomopathogenic Fungi Against Whitefly (*Bemesia tabaci*)

Rechelle B. Peningeo*, Judith G. Lawilao, and Asuncion L. Nagpala

Department of Plant Pathology, College of Agriculture, Benguet State University
**Corresponding author email address: peningeorechelle@gmail.com*

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Abstract

Whitefly can transmit many plant viruses to vulnerable vegetable crops such as cabbage, white potato, and chayote. It has been reported to develop resistance to a wide range of insecticides. This study was conducted to isolate entomopathogenic fungi (EPF) from insect cadavers, identify and characterize theisolated EPF through cultural and morphological characterizations and conduct a preliminary pathogenicity test of at least one of the identified EPF on whitefly. Samples of EPF from the BSU Pomology were initially identified and characterized as *Aschersonia placenta, Aschersonia goldiana*, and *Hypocrella epiphylla*. *A. placenta* was selected for pathogenicity test to evaluate against whitefly (*B. tabaci*). Three trials were conducted using conidial concentrations of $1x10^6$, $1x10^7$, $1x10^8$, and $1x10^9$ conidia/ml. The assessed efficacy rate of *A. placenta* on *B. tabaci* indicated that $1x10^9$ conidia/ml gave the highest mortality rate of 7.6% at 7 days and 11.2% at 14 days post-inoculation. The observed highest mortality rate was 11.2% which is less than the standard efficacy rate. However, the capability of *A. placenta* to infect nymphs of *B. tabaci* indicates its potential as a biological control agent against whitefly. Further assessment using field trial to confirm the results, and experiments on the other identified *Aschersonia* species, and the use of supplements for mass production are recommended.

Introduction

Whiteflies (*Bemesia tabaci*) which feed on the sap of plant tissues causing yellowing, necrosis, and death of leaves (Batta, 2003), have become an important pest worldwide. According to Jones (2003), due to the emergence of the B biotype and the rapid expansion of geographic distribution and host range of whiteflies, it has received importance as a pest and vector of different viral diseases of food, fiber, and ornamental plants since the early 1980s. *Bemesia tabaci* belongs to the B biotype, which is considered one of the most invasive species with a broad host range of plants (Sani et al., 2020).

B. tabaci transmits more than 200 plant viruses, most of which belong to the genera Begomovirus,

Carlavirus, Crinivirus, Ipomovirus, and Torradovirus. Some of the most vulnerable crops to these viruses are cassava, cotton, cowpea, cucurbits, crucifers, eggplants, tobacco, tomato, potato, soybean, sweet potato, okra, lettuce, pea, bean, pepper, poinsettia, and chrysanthemum (Sani et al., 2020). Of all the viruses transmitted by *B. tabaci*, begomoviruses are the leading cause of yield losses in crops, ranging from 20 to 100% and losses worth millions of dollars (Sani et al., 2020). Major crops in Benguet, such as cabbage with 78%, white potato with 83%, and chayote with 62% share of the country's total production (Philippine Statistics Authority [PSA], 2021), are highly susceptible to whitefly infestation. In addition to outdoor crops, *B. tabaci* is also a serious pest in protected environments where they survive during the winter in temperate climates (Jones, 2003).

Whiteflies have been reported to develop resistance to a wide range of insecticides, including conventional and novel ones. Conventional insecticides include organophosphates, carbamates, and pyrethroids, while novel insecticides are neonicotinoids and insect growth regulators. Insecticide resistance in whiteflies lowers the control efficacy of commonly used insecticides and accelerates the need for new insecticide chemistries (Yao et al., 2017).

Currently, synthetic insecticides are commonly used to control insect pests. However, the use of synthetic insecticide is hazardous both to human health and the environment. It is also toxic to beneficial insects and microorganisms and to non-target plants. This resistance was attributed to the continuous use of insecticide against this insect pest. Nowadays, the production of safe agricultural products for human consumption has become a global concern. In the Philippines, the government is putting effort into promoting and implementing organic agriculture and good agricultural practices (GAP). These production systems sustain the health of soils, ecosystems, and people by relying on ecological processes, biodiversity, and cycles adapted to local conditions rather than the use of inputs with adverse effects. In these farming systems, integrating biocontrol agents and natural biopesticides such as the entomopathogenic fungi (EPF) in crop protection will help lessen the risk due to insecticides.

Entomopathogenic fungi are beneficial microorganisms that have the ability to parasitize and kill insects. The mechanism of most EPF starts by attaching their spores or conidia to the exoskeleton of the insect. After penetrating the cuticle using cuticle-hydrolyzing enzymes such as lipase, proteases, and chitinases (El Husseini, 2019), the spores or conidia will undergo germination in the epidermis and enter the body of the insect. Internal proliferation for the spores/conidia will take place, resulting in toxicosis and starvation, which will cause the insect's death (Singh et al., 2017). After the fungi kill its host, it will vegetate outside the insect cadavers' body and produce more spores, increasing the risk of other arthropods being infested (Khaleil et al., 2016). Evaluating other fungi with potential EPF, aside from the reported EPF, can help us reduce the utilization of synthetic crop protection products and prevent the increasing population of resistant insect pests.

The study aimed: to isolate entomopathogenic fungi from parasitized insect cadavers collected from the pomology area of Benguet State University (BSU); identify and characterize the isolated entomopathogenic fungi from insect cadavers; and conduct a preliminary pathogenicity test of at least one identified EPF on *B. tabaci*.

Methodology

Collection of *Aschersonia* spp.

Aschersonia spp. was collected from citrus trees at the Pomology area of BSU, wherein two species were observed to have parasitized citrus blackflies, and the other one parasitized scale insects. Parasitized citrus black fly nymphs were observed underneath the citrus leaves, while parasitized scale insects were seen on the twigs and on the back of citrus leaves. Citrus fly nymphs and scale insects infected with the fungus *Aschersonia* spp. were contained in a clean plastic container and were brought to the Plant Pathology laboratory for isolation. The classification of insects parasitized by *Aschersonia* species were determined based on the characteristics observed by Liu and Hodge (2005); Liu et al. (2006), which were frequently used as a taxonomical reference on the reported studies about *Aschersonia* species. The collection site was only at BSU due to the COVID-19 pandemic restrictions limiting movement from one place to another.

Isolation, Characterization, and Identification of the Collected *Aschersonia* spp.

Modifying the methodology of Homrahud et al. (2016), Sudiarta et al. (2019), and Sikder et al. (2019), the stroma that contains the conidia were scraped from the body surface of the collected citrus fly cadavers and scale insects and then crushed in 10 ml sterile water in a test tube. The fungal suspension was poured into a Petri plate containing Potato Dextrose Agar (PDA). The medium (PDA) was tilted until the excess of the fungal suspension was removed, then it was incubated for 14 days. The laboratory incubation conditions were 25±2°C and 75±5% relative humidity (RH) with alternate light and dark exposure as recommended by Sikder et al. (2019).

The cultural characteristics (color, surface texture, shape of the stroma and conidia) and morphological characteristics (presence of hyphae, size of spores, and conidia) of the fungi were directly observed and recorded from both fresh and cultured (fungal growth in PDA) specimens using a compound and stereo microscope. The identification of fungal species was carried out using the taxonomical classification and description of Liu and Hodge (2005); Liu et al. (2006).

Evaluation of Isolated Entomopathogenic Fungi Collection of Host Insect for Entomopathogenicity Test

The host insect used to determine the pathogenicity of the entomopathogenic fungus was the whitefly (*Bemesia tabaci*). Whitefly colonies containing eggs and 1st instar nymphs or crawlers were collected from the vicinity of the BSU main campus. The leaves containing whitefly colonies were soaked in cold, sterile distilled water for a few seconds, and it was set aside for entomopathogenicity trials. *B. tabaci* was selected as the host in this study because of its availability and capacity to infect commercial crops in the Cordillera region. It was also noted that the natural host of the selected EPF (*Aschersonia placenta*), the citrus blackfly (*Aleurocanthus woglumi*), and the selected host on the in-vitro experiment, the silver whitefly (*Bemesia tabaci*), belong to the same family Aleyrodidae.

The first reported species under the genus Aschersonia is the Aschersonia aleyrodis which was used successfully in controlling insect pests in North America and was also an effective biological control agent of citrus whitefly in Florida in the early 1900s. Both *A. aleyrodis* and *A. placenta* have similar morphological characteristics, and according to Wang et al. (2013), having similar morphological characteristics could have great potential in controlling *Bemesia tabaci*. Zhang et al. (2016) reported that *A. aleyrodis* is pathogenic in the nymphal stage of *Bemesia tabaci*. Thus, *Aschersonia placenta*, which was found on the citrus blackfly (host), was chosen as the EPF and was used on the preliminary entomopathogenicity test on *Bemesia tabaci*.

Preparation of Conidial Suspensions of *Aschersonia placenta*

Pure culture of *A. placenta* was suspended in 10ml sterilized distilled water (SDW) with 0.05% Tween 80 and vortexed for one minute to produce a homogenous suspension. The conidial suspensions were diluted and standardized to make concentrations of $1x10^6$, $1x10^7$, $1x10^8$, and $1x10^9$ conidia/mL and were used as inocula in the experiment. The haemocytometer was used to determine conidial count per concentration (Homrahud et al., 2016).

Entomopathogenicity Test of *Aschersonia placenta* Against *Bemesia tabaci*

The entomopathogenicity trial to test the efficacy of *A. placenta* against *B. tabaci* was carried out following a completely randomized design with five treatments. Each treatment had five replicates with four samples each. The inocula used in the experiments contained $1x10^6$ (T2), $1x10^7$ (T3), $1x10^8$ (T4), and $1x10^9$ (T5) conidia/ml. These inocula were transferred to a spray bottle, and it was sprayed on the citrus leaves measuring (3 to 3.5cm in length) and enclosed in a sterilized plastic container. The crawlers or the first instar stage from the previously collected whitefly colonies were picked and transferred in the sterilized plastic containers containing the treated citrus leaves with the aid of a stereomicroscope. It was incubated in an incubation box having a temperature of 24°C-28°C. Five crawlers were introduced per sample, so each treatment has 100 crawlers, which means one trial has 500 nymphs/crawlers. A total of 1500 nymphs/crawlers were used in the three trials conducted. The untreated or control treatment (T1) was prepared in the same manner using SDW and 0.05% Tween 80.

The mortality rate was assessed and recordedafter 3, 7, and 14 days post-application of the *A. placenta*. Nymphs observed using a stereomicroscope were considered infected when they turned opaque white, orange, or brown, depending on the fungal isolate used (Homrahud et al., 2016). Only nymphs that died due to Aschersonia placenta infection were considered in the mortality calculation.

Statistical Analysis

The number of dead nymphs per replicate after 3, 7, and 14 days were counted, recorded, and subjected to Abbot's formula for mortality rate (1925) to compute the percentage mortality of Bemesia tabaci. Means were subjected to the analysis of variance (ANOVA), and the comparison of means was determined by using Duncan's multiple range test (DMRT) at P≤0.05.

Results and Discussion

Collection of *Aschersonia* spp.

The collected fungi (Figure 1) showed stroma that covers the insect cadavers, similar to the appearance of the genus *Aschersonia* described by Liu et al. (2005 & 2006). The collected *Aschersonia* spp. totally covered the nymph of the citrus fly and scale insects, which made the insect stage identification indistinguishable. Three different *Aschersonia* spp. were collected from the Pomology area of BSU. Two species were from citrus blackfly, and the other species was from a scale insect that is also from citrus. Of the two *Aschersonia* spp. collected from citrus blackfly (Figure 1 A-D), one has a light yellow to orange convex stroma with a smooth surface and embedded conidiomata when viewed under the stereomicroscope (Figure 1D). The other one with a bigger stroma (Figure 1 A-B) has yellowish to orange conidial masses contained on a scattered conidiomata.

On the other hand, the stroma observed on the *Aschersonia* found on the scale insects (Figure 1 E-F) has a convex hemiglobose shape with smooth brown to gray color. No apparent conidiomata were observed under the stereomicroscope. Based on the descriptions and based on the taxonomical identification for *Aschersonia* spp. by Liu and Hodge (2005); Liu et al. (2006), the three collected fungal isolates were initially identified as *Aschersonia placenta*, *Aschersonia goldiana*, and *Hypocrella epiphylla* (Anamorph: *Aschersonia cubensis*) (Figure 1 and

Figure 1

(A) Aschersonia placenta Attached on Leaves (B) Stromata with Orange Conidiomata of Aschersonia placenta in 30x Magnification (C) Aschersonia goldiana with its Host (H: citrus blackfly) (D) Yellow Stromata of Aschersonia goldiana in 30x Magnification (E) Hypocrella epiphylla (F) Brown stromata of Hypocrella epiphylla under 30x Magnification

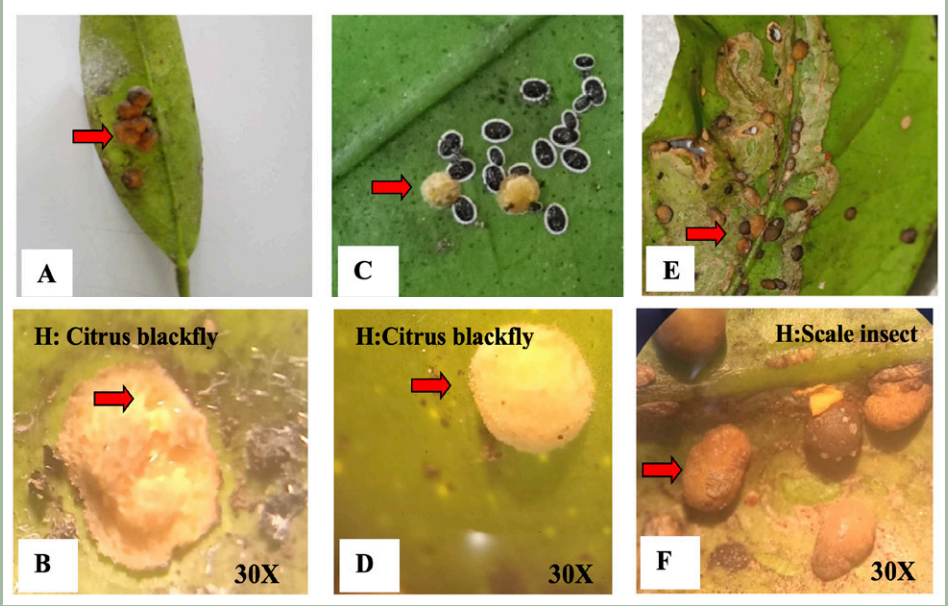


Table 1

Characteristics of Fresh Specimen of Aschersonia placenta

Aschersonia spp.	Cultural and Morphological Characteristics	Reference
Aschersonia placenta	Yellow to orange color stromata	Liu et al., 2006
	Yellow Fusoid conidia (15.5 µm)	Liu et al., 2006
	Presence of paraphyses	Liu et al., 2006
	Conidiogenenous cells arising from hyphae	Liu et al., 2006
	Flask-shaped Perithecium	Liu et al., 2006
	Cylindrical asci and cylindrical with rounded ends ascospores	Liu et al., 2006

Table 1). *Aschersonia placenta* and *Aschersonia goldiana* were collected from the cadaver of citrus blackfly nymph, while *Hypocrella epiphylla* was collected from the cadaver of scale insect. *Aschersonia placenta* (Figure 1 A-B) has the most noticeable stromata when it comes to volume, followed by *Aschersonia goldiana* (Figure1C-D) and *Hypocrella epiphylla* (Figure E-F).

Morphological and Cultural Characteristics of *Aschersonia* spp. *Aschersonia placenta*

Table 1 and Figure 2 show the cultural and morphological characteristics of *A. placenta* determined through a microscopic examination conducted from fresh specimens and the fungal growth in PDA. From the fresh specimen, A. *placenta* has yellow conidia that are fusoid (Figure 2B), multicellular with thickened wall at ends (15.5 µm), and hymenium showing long paraphyses (Figure 2D). A cross-section of its stroma showed an embedded flask-shaped perithecium (Figure 2A). Orange conidiomata (Figure 1B) were seen, which appear as simple depressions and are irregularly arranged. Asci (Figure 2F) are cylindrical with rounded, and some has square ends and ascospores produced were cylindrical. Conidiogenous cells (Figure 2E) arising individually from branched hyphae were also observed from the fresh specimen. Paraphyses which arise from the hymenium were seen under the compound microscope.

Pure culture of *A. placenta* in PDA (Figure 3 and Table 2) showed a colony that has a relatively rapid growth measuring 37.5mm in diameter in less than three weeks at 25°C. Colonies produced were white-colored and had irregular forms with condensed surfaces and undulated margins (Figure 3A). A microscopic examination from the colony growth displayed a verticillate branching of conidiophores producing ovoid conidiogenous cells (Figure 3B). Conidia are fusoid measuring 12.6µm (Figure 3C). Microscopes produced from the isolate measured 6.5µm x 2.6µm and had cylindrical with rounded ends, and some were ovoid (Figure 3D).

Aschersonia goldiana

Table 3 and Figure 4 present the morphological characteristics of *Aschersonia goldiana*. Fresh mount of *A. goldiana* (Figure 4) had a flask-shaped perithecium (Figure 4B) located at the center of the stroma. Paraphyses were absent, but fusoid conidia, measuring 9.5µm (Figure 4C), and asci, both containing cylindrical ascospores, were observed from the cross-sections of stroma using high power objective (400x magnification) but were not clearly seen in the picture (Figure 4D).

Pure culture of *Aschersonia goldiana* (Figure 5 and Table 4) was observed to have mycelia wit a white circular form and convex elevation measuring 23.5mm (Figure 5A). Hyaline fusoid conidia measuring 10.2µm (Figure 5B) were produced, and single branched conidiophores (Figure 5C) were evident under the microscope.

Hypocrella epiphylla

Figure 6 shows and Tables 5 and 6 describes the cultural and morphological characteristics of *H. epiphylla*. Sections of the stroma from the fresh mount of *H. epiphylla* showed obovoid-shaped perithecium (Figure 6A) embedded and well-separated (Figure 6A). No paraphyses were

Figure 2

Fresh Specimen of Aschersonia placenta Observed Under the Microscope at 400x Magnification (A) Flask-shaped Perithecium (B) Yellow fusoid conidia (15.5 µm) (C) Multicellular fusoid conidia (15.5µm) (D) Paraphyses (E) Conidiogenous Cell Arising from Branched Hyphae (F) Cylindrical asci

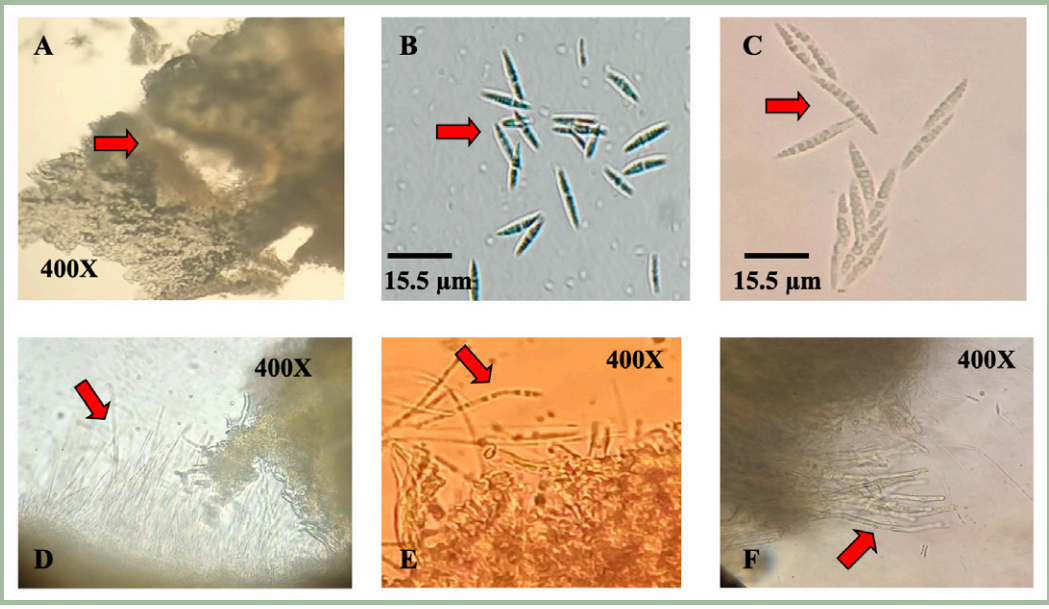


Table 2

Characteristics of Aschersonia placenta on PDA

Aschersonia spp.	**Cultural and Morphological Characteristics**	**Reference**
Aschersonia placenta	White mycelia colony; relatively rapid growth (37.5 mm)	Liu et al., 2006
	Verticillate branching of conidiophores producing ovoid conidiogenous cells	Liu et al., 2006
	Production of fusoid (12.6 µm) and ovoid part spores (6.5 µm x 2.6 µm)	Liu et al., 2006

observed. Conidia produced are hyaline, ovoid, and acute ends measuring 9.5 µm (Figure 6C). It has shorter cylindrical end asci and ascospores (Figure 6B) that are filiform in shape.

Cultures of *H. epiphylla* showed an elevated irregular white filamentous convex colony measuring 25mm, as shown in Figure 6D and Table 6. Conidia produced are hyaline, unicellular, ovoid, and others have acute ends measuring 5.2µm x 2.6µm (Figure 6E). Conidiophores observed were penicillate with several branching (Figure 6F).

Preliminary Entomopathogenicity of *Aschersonia placenta* for the Control of *Bemesia tabaci*

The mortality rate of *Bemesia tabaci* was recorded at 3, 7, and 14 days after inoculation of different conidial concentrations. No fungal infections and mortality were incurred by the whitefly nymphs 3 days after the application of *A. placenta*. At 7 days after application of *A. placenta*, infection by the fungus was manifested by the presence of white fringes of hyphae (Figure 7) from the marginal area of the nymphs' body as observed under the stereomicroscope (30x

magnification). The presence of such structure is an indication of an early infection, according to Homrahud et al. (2016). At 14 days after inoculation of *A. placenta*, fungal sporulation is evident on infected nymphs. White mycelia were visible on top and the side of the nymph's body (Figure 7C). Microscopic examination of the infected nymphs showed cylindrical and ovoid conidia.

Table 7 presents the effects of the different fungal concentrations of *A. placenta* on the percentage mortality rate of *B. tabaci* for the three trials. There is no mortality incurred by the 1st instar nymphs or crawlers at 3 days after the application of different conidial concentrations of EPF in all three trials conducted. The percentage of mortalities recorded on day 7 showed a similar trend to that on day 14, according to the increased fungal concentration. In Trial 1, the highest mortality rate was observed at a conidial concentration of $1x10^9$ with 7.6% and 11.2% and significantly differed in all treatments at 7 and 14 days after application of the EPF, significantly differing from all other treatments.

The concentration in Trial 2 that has incurred the highest mortality rate is the 1×10^9 (T5) with

Figure 3

Aschersonia placenta on PDA (A) Mycelia (37.5 mm) (B) Verticillate Branching of Conidiophores Producing Ovoid Conidiogenous Cells (C) Fusoid conidia Measuring 12. 6µm (D) Ovoid Part Spores (6.5µm x 2.6µm); B–D (400x Magnification)

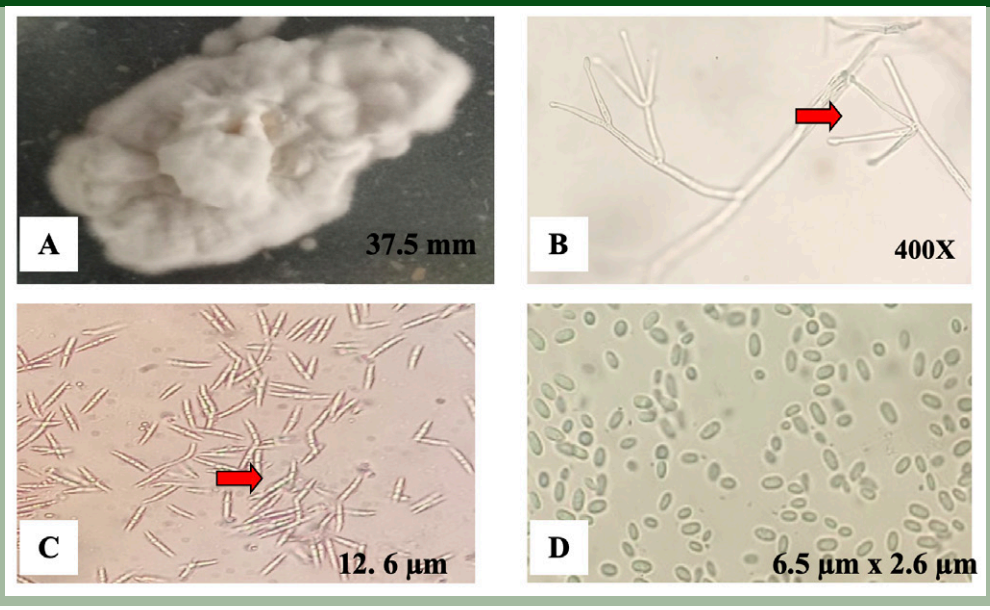


Table 3

Characteristics of Fresh Specimen of Aschersonia goldiana

Aschersonia spp.	**Cultural and Morphological Characteristics**	**Reference**
Aschersonia goldiana	Flask-shaped perithecium	Liu et al., 2006
	Fusoid conidia with thickened wall at ends	Liu et al., 2006
	No presence of paraphyses	Liu et al., 2006
	Yellow stromata	Liu et al., 2006
	Asci with cylindrical ends	Liu et al., 2006

Figure 4

Fresh Specimen of Aschersonia goldiana (A) Yellow Stroma on Citrus Blackfly Nymph (30x Magnification) (B) Cross-section of Stroma with Embedded Empty Flask-shaped Perithecium (C) Fusoid conidia 9.5µm (B-C:400x Magnification) (D) Ascus

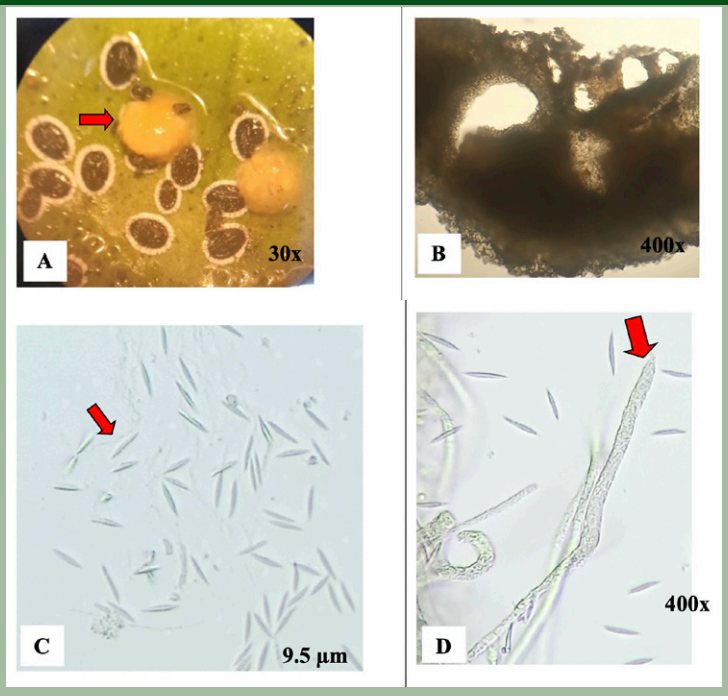


Table 4

Characteristics of Aschersonia goldiana on PDA

Aschersonia spp.	**Cultural and Morphological Characteristics**	**Reference**
Aschersonia placenta	White-colored mycelia with moderate growth (23. 5 mm)	Liu et al., 2006
	Fusoid conidia	Liu et al., 2006
	Single branched conidiophores	Liu et al., 2006

Figure 5

Aschersonia goldiana on PDA (A) White Circular Convex Mycelia (B) Fusoid conidia 10.2µm (400x Magnification) (C) Single-branched Conidiophore

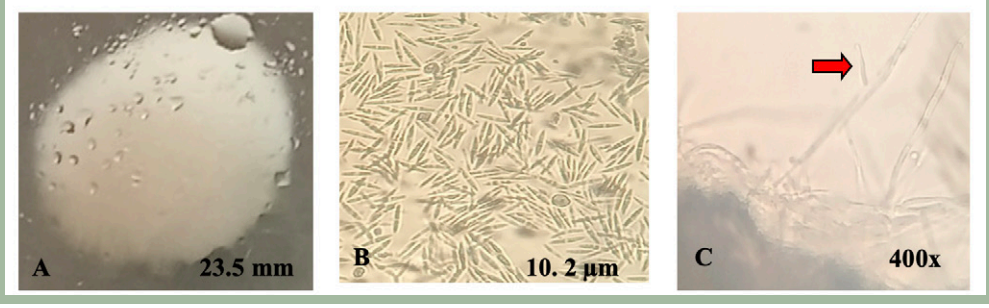


Figure 6

Hypocrella epiphylla (Fresh Specimen Under 400x Magnification: A-C) (A) Obovoid Perithecium (B) Asci and ascospores (C) Conidia Measuring 9.5µm (D) Colony on PDA (E) Ovoid Shaped conidia (5.2µm x 2.6µm in 400x Magnification) (F) Penicillate conidiophores in 400x Magnification

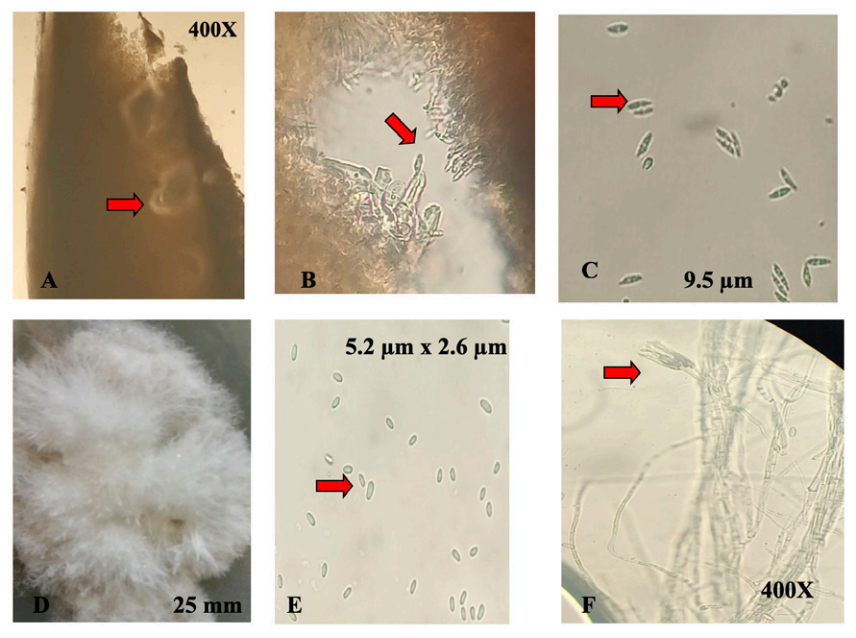


Table 5

Characteristics of Fresh Specimen of Hypocrella epiphylla

Aschersonia spp.	**Cultural and Morphological Characteristics**	**Reference**
Hypocrella epiphylla	Brown to black stroma	Liu et al., 2005
	Obovoid shaped perithecium	
	No presence of paraphyses	Liu et al., 2005
	Fusoid and Ovoid with acute ends conidia	Liu et al., 2005
	Shorter cylindrical asci and ascospores, which are filiform shaped	Liu et al., 2005

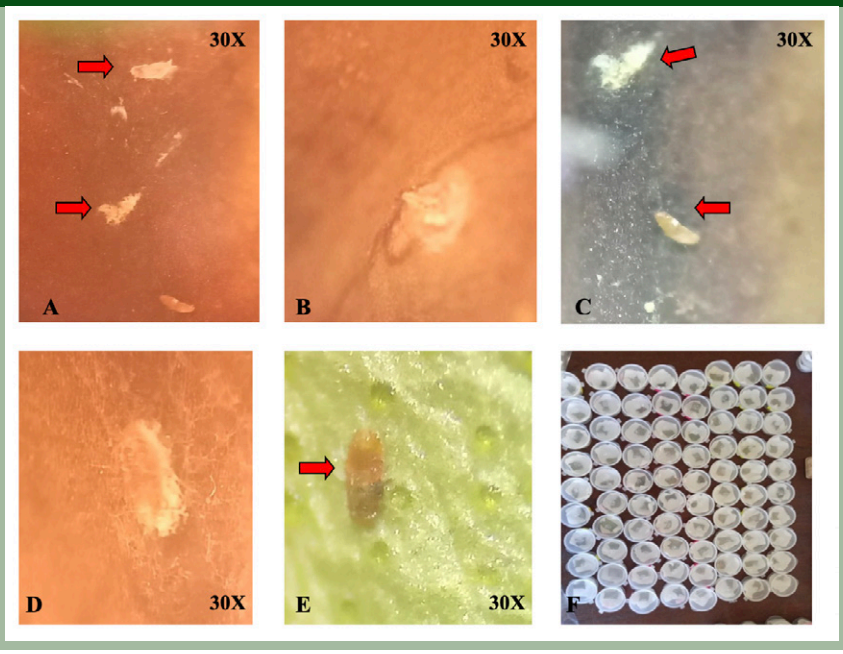
Table 6

Characteristics of Hypocrella epiphylla on PDA

Aschersonia spp.	**Cultural and Morphological Characteristics**	**Reference**
Hypocrella epiphylla	Elevated irregular white convex mycelia (25 mm)	Liu et al., 2005
	Penicillate conidiophores branching more than three times	
	Hyaline ovoid conidia with acute ends (5.2 µm x 2.6 µm)	Liu et al., 2005
	Shorter cylindrical asci and ascospores, which are filiform shaped	Liu et al., 2005

Figure 6

Bemesia tabaci Nymphs Observed on 30x Magnification Under a Stereo Microscope (A-D) Infected Nymphs with White Fungal Sporulation of Aschersonia placenta (C-D) White Mycelia of Aschersonia placenta Observed at 14 Days After Spraying Conidial Suspensions (arrows) (E) Control Treatment - Uninfected Nymph (arrow) (F) Sterilized Plastic Container Enclosing the Treated Citrus Leaves with Nymphs



3.6% and 7% which is significantly different with $1x10^6$ (MR: 2.4% & 3.2%) but comparable with $1x10^8$ (MR: 3.2% & 6.2%) and $1x10^7$ (MR: 2.8 & 5.0%) at 7 and 14 days after application of *A. placenta*. In Trial 3, the mortality rate was significantly higher at $1x10^9$, having 4.2% and 6.8% compared with other treatments except at $1x10^8$ where it is comparable with mortality percentages of 3.8 and 5.4. This observation is consistent at 7 and 14 days after application of inocula.

The acquired percentage mortality rate during the in-vitro experiment was 11.2% which is less than the standard efficacy rate (50%) according to the Philippine National Standard (2016). However, even if the percentage mortality acquired in the in-vitro experiment was <50%, its persistence in infecting *Bemesia tabaci* nymphs is an indication that it can be a possible biocontrol for *B. tabaci*. According to Evans and Hywel-Jones (1990), Fransen (1990), Zhu et al. (2008) as cited by Homrahud et al. (2016) and Sikder et al. (2019), the genus *Aschersonia* has been recognized as an important biological control agent able to cause spectacular epizootic disease in whiteflies (Family: *Aleyrodidae*) in the tropics and subtropics which concludes the natural parasitism or infection of the *Aschersonia placenta* on *B. tabaci*. *Aschersonia* spp. was described as having an active toxin component that is toxic to insects called "destruxin" which acts as repellant and antifeedant (Goettel et al., 2005).

However, in a study by Qui et al. (2013), their in-vitro experiment showed a lower mortality rate than the field trial they conducted. Their field trial of applying *Aschersonia placenta* against 1st instar of *Bemesia tabaci* revealed an 80-100% mortality rate using their highest treatment $1x10^8$ conidial/ml.

Table 7

Mortality Rate (%) of B. tabaci After 3, 7, and 14 Days Inoculation with Different Fungal Concentrations of A. placenta in Three Trials

Conidial Suspension of *A. placenta* (conidia/ml)	**Mortality Rate**								
	TRIAL 1			**TRIAL 2**			**TRIAL 3**		
	3 days	7 days	14 days	3 days	7 days	14 days	3 days	7 days	14 days
Control	0.0	0.0^{d}	0.0^{d}	0.0	0.0^{c}	0.0^{c}	0.0	0.0^{c}	0.0^{c}
1×10^{6}	0.0	1.8^{c}	4.0^{c}	0.0	2.4^{b}	3.2^{b}	0.0	2.6^{b}	4.0^{b}
1×10^{7}	0.0	2.6^{c}	5.0^{c}	0.0	2.8^{ab}	5.0^{ab}	0.0	2.8^{b}	4.8^{b}
1×10^{8}	0.0	4.0^{b}	7.2^{b}	0.0	3.2^{ab}	6.2^{a}	0.0	3.8^{ab}	5.4^{ab}
1×10^{9}	0.0	7.6^{a}	11.2^{a}	0.0	3.6^{a}	7.0^{a}	0.0	4.2^{a}	6.8^{a}

Data are means of 5 replicates with 4 samples (n=20)
Means in the same column under each observation time followed by the same letter are not significantly different (P≤0.05)

Conclusions

Collected samples of *Aschersonia* spp. from the BSU Pomology citrus field were initially identified as *Aschersonia placenta*, *Aschersonia goldiana*, and *Hypocrella epiphylla* based on the taxonomic identification of morphological and cultural characteristics described by Liu et al. (2005 & 2006). While the observed highest percentage mortality in this in-vitro experiment was only 11.2%, the capability of *Aschersonia placenta* to infect nymphs of *Bemesia tabaci* is an indication that it is a potential biological control agent of whitefly. The entomopathogenicity conducted to test the different rates of fungal suspensions of *Aschersonia placenta* on the mortality rate of *Bemesia tabaci* indicated that as the fungal concentration of *A. placenta* increases, the mortality rate of *Bemesia tabaci* also increases.

Recommendations

The recommended conidial suspension 1x109 of *A. placenta* can be used in the management of *B. tabaci*. It is recommended that conducting a field trial is necessary to confirm the results obtained from this in-vitro experiment and include a standard insecticide as a reference. This further assessment of the performance of *A. placenta* in a natural environment will provide a more conclusive result on the efficacy of *A. placenta* as a potential entomopathogenic fungus against *B. tabaci*. Performing a field trial will also determine conditions such as temperature that are favorable for the *A. placenta* and will aid in improving the percentage mortality of the whitefly. Since *A. placenta* is a slow-growing fungus, the use of supplements on growing media such as millet with the addition of KH_2PO_4 $MgSO_4$ (Qui et al., 2013) for mass production could also be studied. *A. goldiana* and *H. epiphylla* should also be used for entomopathogenicity test to assess their efficacy as entomopathogenic fungi. It is also recommended that the isolated entomopathogenic fungi be subjected to molecular identification to confirm their identity.

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