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Original article

Cultivation of *Aschersonia placenta* Berkeley and Broom and its efficacy for controlling *Parlatoria ziziphi* (Lucas) (Hemiptera: Diaspididae)

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ABSTRACT

The entomopathogenic fungus genus *Aschersonia* (Deuteromycotina: Hyphomycetes) is host specific to some aleyrodids and scale insects. In search of the Thai endemic species, fungus samples were isolated from cadavers of citrus whiteflies (*Aleyrodes tabaci* Gennadius) found in citrus orchards in Trat province, Thailand. After morphological analysis and scanning electron microscopic examination, it was identified as *Aschersonia placenta* Berkeley and Broom. Seven synthetic media, namely: potato dextrose agar (PDA), PDA with pasteurized milk (Foremost®) (PDA + M), Sabouraud dextrose agar with yeast extract (SDAY), SDA with pasteurized milk (Foremost®) (SDA + M), corn meal agar (CMA), water agar with juice of eight vegetable species (V8®) (WA + V8) and WA were explored as appropriate media for fungal cultivation. SDAY and SDA + M gave the best colony radial growth, producing 2.04 ± 0.13 cm and 2.09 ± 0.10 cm in 21 d, respectively. However, based on the ability of *A. placenta* to produce conidia, PDA and SDAY which produced 2.59×10^8 conidia/mL and 2.69×10^8 conidia/mL, respectively, were considered as the most suitable media for this fungal species. The efficiency assessment of *A. placenta* for controlling black parlatoria (*Parlatoria ziziphi* (Lucas)), indicated that a conidial suspension at 1×10^9 conidia/mL gave 23.73% and 27.42% mortality at 14 and 21 d post inoculation, respectively.

Introduction

The application of entomopathogenic microorganisms for the control of insect pests is one of the alternative tactics to reduce pesticide use as fungal spores of some biological control agents have been regarded as potential alternatives to agrochemicals because they can often be easily produced and may be adapted to survive unfavorable conditions (Qiu et al., 2013).

The utilization of the fungal genus *Aschersonia* for control of insect pests has a long history and has been studied extensively in other countries. *Aschersonia aleyrodis* was the first fungal species used for the control of insect pests in North America with the successful control of citrus whiteflies in Florida being achieved in the early 1900 s through the introduction of *A. aleyrodis* into a citrus orchard to seed epizootic disease in the whitefly population (Burger, 1921; Liu et al., 2006). *Aschersonia* spp. are not hazardous to humans and some species can be potential biological control agents against insect pests (Qiu et al., 2013).

Aschersonia placenta Berkeley and Broom belongs to the Ascomycota, Deuteromycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Clavicipitaceae. This fungus has been recognized as an important biological control agent able to cause spectacular epizootic disease in whiteflies (Aleyrodidae) and scale insects (Coccidae) in the tropics and subtropics (Evans and Hywel-Jones, 1990; Fransen, 1990; Zhu et al., 2008). *A. placenta* has morphological characteristics similar to *A. aleyrodis* which means it could have great potential to be used to control whiteflies (Wang et al., 2013).

Black parlatoria (*Parlatoria ziziphi* (Lucas) [Hemiptera: Diaspididae]) is an important pest of citrus in many countries such as Brazil, China, Egypt, Iran, Italy, France, Libya, Nigeria, Puerto Rico, Taiwan, and Tunisia (Hanene, 2011). It was noted that in some countries the scale insect may not be considered as a pest, but a population occasionally causes problems in localized areas (Hanene, 2011). In Thailand, to date *P. ziziphi* populations have reached outbreak levels only in certain areas, but in the future, *P. ziziphi* may spread and cause problems in citrus orchards. Hence, prevention by gaining some basic information is necessary. The destruction of plants by *P. ziziphi* was described by Hanene (2011) as follows. The insect absorbed sap on the leaves of pomelo

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which caused the fruit color to become yellow—a state known as "chlorosis". The immature fruits, which were infested by this insect species, had a hard pulp, stopped development and later dropped from the tree. The infestation on mature fruits had no effect on the pulp but reduced market prices.

In Thailand, most research studies on entomopathogenic fungi have been done on *Metarhizium anisopliae* and *Beauveria bassiana* (Thaochan et al., 2011; Pankamjon et al., 2012; Petlamul and Prasertsan, 2012). However, for biological control with entomopathogenic microorganisms, legal restrictions of the application of exotic species should be considered. To avoid these complications, searching for promising, endemic fungal species is the solution. In addition, media components, which significantly affect the sporulation of the collected endemic species have to be explored. Study on the pathogenicity of the species on some insect pests is a general practice for basic biological control before field application. More minute information on the physiology of the collected endemic species is required for its application in biological control.

The aims of this study were threefold: firstly to search for Aschersonia sp. as a biological control agent to control hemipteran insect pests, particularly aleyrodids and coccids; secondly to investigate the most suitable synthetic medium that accelerated the growth and sporulation of the fungus for laboratory cultivation; and finally to evaluate the laboratory infection of the fungus for the control of the black parlatoria (*P. Ziziphi*).

Materials and methods

Collecting the fungus Aschersonia sp.

Insect cadavers covering the stroma of entomopathogenic fungi in the genus *Aschersonia*, following species descriptions by Liu et al. (2006), were collected from citrus orchards in Khao Saming district in Trat province (12°21′12″N, 102°26′6″E″), in eastern Thailand in September 2011. The insect samples were kept in sterile vials and placed in an ice box containing an ice pack for transportation to the National Biological Control Research Center, Central Regional Center (NBCRC, CRC) Laboratory, Kasetsart University for isolation and identification of both the fungal and insect species.

Morphological characteristics

Isolation of the fungus, Aschersonia sp.

Isolation of the collected *Aschersonia* sp. was modified from Liu et al. (2006). Stromata from the body surface of the collected whitefly cadavers were scraped and then crushed in sterile water. The fungal suspension was cross streaked on potato dextrose agar (PDA; Difco; Becton Dickinson; Franklin Lakes, NJ, USA) medium and incubated for 14 d. The laboratory incubation conditions were 25 ± 2 °C and 75 ± 5% relative humidity (RH) with 24 h of light. All single colonies of the fungus were collected and subcultured on PDA medium to obtain pure cultures.

Macromorphological analysis

The macromorphological characteristics of *Aschersonia* sp. were observed as the field collection was being carried out. The colony size, shape and color of the mycelia and conidial masses, the presence or absence of the hypothallus and the texture of the stroma surface were recorded for species identification according to descriptions by Liu et al. (2006) and Wang et al. (2013).

Scanning electron microscopic study

The morphological characteristics of the isolated fungus were observed using a scanning electron microscope (SEM) at the Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), Kasetsart University, Bangkok, Thailand. Preparation of the materials for the study was as follows. Stromata with arising conidiophores and conidia were cut, fixed in 5% (v/v) glutaraldehyde at 4 °C for 2 h and then in 2% (v/v) osmium tetroxide (OsO_4) for 2 h on ice. The chemically fixed specimens were then subjected to dehydration through an ethanol series: 30%, 50%, 70%, 90% and 100%, each for 10 min. The samples were dried in a critical-point drier (HCP-2; Hitachi; Koki Co Ltd.; Tokyo, Japan) and then gold coated for 10 min in FINE COAT Ion Sputter (IB-2; Eiko Engineering; Ibaraki, Japan). The specimens were finally examined under an SEM (JSM-5600 LV; JEOL Ltd.; Tokyo, Japan) operated at 10–15 kV.

Identification of the fungus and the infected insect cadaver

Identification of the collected *Aschersonia* isolate according to the macromorphological and SEM observations was accomplished based on Samson et al. (1988), Humber (1992), Liu et al. (2006) and Luansa-ard et al. (2007). All species of the infected insect were confirmed by Associate Professor Kosol Charernsom, Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.

Appropriate synthetic media for cultivation of Aschersonia placenta isolate Asp001

Seven synthetic media, namely: PDA, PDA + M (180:1 mL of PDA: pasteurized milk) (Foremost®), SDAY (Sabouraud dextrose agar (Difco) with yeast extract (Difco)), SDA + M (180:1 mL of SDA: pasteurized milk) (Foremost®)), CMA (corn meal agar (Difco)), WA (water agar) + V8 (180:1 mL of WA (Difco):juice of eight vegetable species (V8®); tomatoes, carrots, celery, beets, parsley, lettuce, watercress and spinach) and WA were assigned as treatments and laid out in a completely randomized design with five replications. Isolated *A. placenta* was cultured on PDA medium in a 9 cm-diameter Petri dish. A cork borer (0.6 cm in diameter) was used to drill a piece of jelly at the edge of the colony. The jelly was then placed on the Petri dishes containing separately each of the tested media. The Petri dishes were incubated at 25 ± 2 °C and 75 ± 5% RH with 24 h of light.

To assess the growth of *A. placenta* cultivated on different media, two parameters were taken into account—the radius of the fungal colony and the number of produced conidia. The radial growth was measured on days 7, 14 and 21 post incubation and the conidia were counted at day 21 post incubation. One mL of 0.1% Tween 80 in sterile water was dropped onto the culture plate and a stainless spatula was used to scrape stromata of the fungus into the water. The suspension was vortexed to make a homogeneous fungal suspension. Finally, a spore count was made using a hemacytometer.

Evaluation of the efficiency of the fungus for the control of Parlatoria ziziphi (Lucas)

Rearing of P. ziziphi

Infested leaves with *P. ziziphi* were collected from pomelo trees growing at the NBCRC. Small pieces of leaves containing colonies of *P. ziziphi* were cut and then placed on leaves of pomelo seedlings which had been planted in small plastic pots (15 cm deep and 21 cm in diameter). The pots were placed in small plastic cages (20 × 15 × 12 cm) and left for 1–3 d in a growth chamber in darkness at 25 ± 2 °C and 75± 5% RH for settlement of crawlers (Hanene, 2011). Successfully established colonies of *P. ziziphi* were used in further experiments.

Preparation of conidial suspensions

Pure fungal culture was suspended in 0.1% Tween 80 and vortexed for 1 min to produce a homogenous suspension. The conidial

suspension was then diluted with sterile, distilled water to make concentrations of 1×10^6 conidia/mL, 1×10^7 conidia/mL, 1×10^8 conidia/mL and 1×10^9 conidia/mL to be used as inocula in the experiment. The hemacytometer was used to provide a conidial count.

Experimental design

A completely randomized design with five treatments (four concentrations of the fungus and the control) and five replications was employed for the experiment. The experimental procedure was as follows. A piece of pomelo leaf washed with sterile water was placed in a Petri dish to provide a food source for the tested insects that had been transferred onto plates and then the plates were sprayed with 1 mL of each concentration of the fungal inoculum using aqueous 0.1% Tween 80 for the control. The laboratory conditions during the experiment were 25 ± 2 °C and 75 ± 5% RH. Accumulative mortality rates were recorded daily for 21 d after inoculation using microscopic examination. A nymph infected with the fungus *A. placenta* was considered dead when there was a white fringe around the insect's body or it had turned white or orange, depending on the growth progress of the fungal isolate, as described by Wang et al. (2013). Only nymphs that had died due to *A. placenta* infection were counted in the mortality calculation.

Statistical analysis

To assess the radial colony growth and the number of conidia produced and the differences in insect mortality among concentrations, the usual assumptions of analysis variance (that is, variance normality and homogeneity of variance) were tested. The square-root transformation method described by Gomez and Gomez (1984) was applied to the insect mortality data. Tukey's honestly significant difference test was used for multiple mean comparisons.

Results and discussions

Collecting the fungus, Aschersonia sp.

The collected fungi displayed stroma on the insect cadavers with an appearance described as the genus *Aschersonia* (Liu et al., 2006). The stroma was convex with white mycelia which changed to yellow and orange with time, and covered the dead insects' bodies on the ventral side of the leaf. The stroma itself was orange, yellow or light yellow in color and some exhibited a shape similar to a fried egg with the conidial mass on the top and center of the colony (Fig. 1A). The infected insect was identified as *Aleyrodes citri* Gennadius (Hemiptera: Aleyrodidae) as shown in Fig. 1B.

Morphological characteristics

Colony appearance on potato dextrose agar medium

When cultured on PDA medium, the isolated fungus, *Aschersonia* sp. (Fig. 2), produce many small mycelia which afterward became tightly packed into a hard and compressed colony that grew into the medium. The colony had a convex and spherical surface with a smooth edge. Mycelia were irregularly large, white in color and soft bodied. The colony color on the medium ranged from light orange to orange-red and sometimes white and yellow. At the center of the colony, there was a conidial mass with a light yellow to orange color. When examined under a microscope, a small ostiole in the middle of the stroma was found, which indicated the exit point of the conidia. The conidia were fusoid with a length of 12.22 ± 0.26 μm.

Macromorphological analysis

The colony of the fungus was composed of a group of short mycelia that were woven together into a mass called the “stroma” (shaped like a cushion and soft and white, yellow and orange in color) covering the dead body of *A. tabaci*. An orange or yellow and light yellow conidial mass, shaped like a drop of water, was formed near the top and middle of the stroma which could be visually observed (Fig. 1A). When the stroma was cut transversely, there

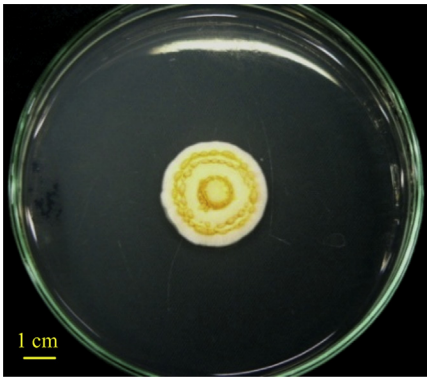

Fig. 2. Colony of field-collected *Aschersonia* sp. on potato dextrose agar medium on day 21. Stromata are variable in shape usually circular, white to yellow white, with a conidial surface that is orange in color and the conidial mass is white or light yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

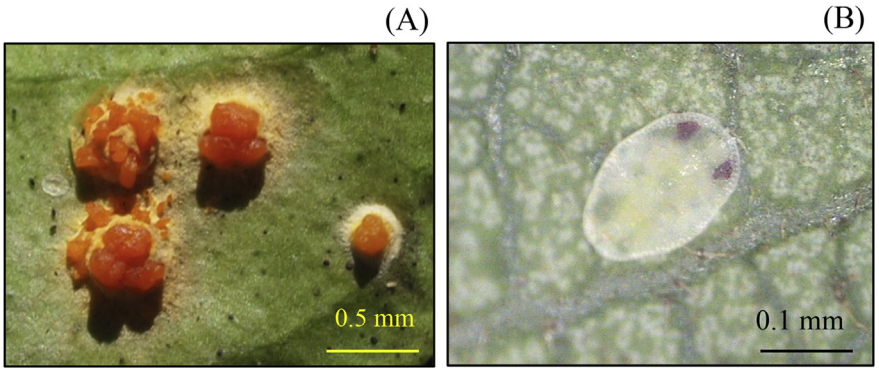

Fig. 1. Morphology of (A) stromata of the collected fungus, *Aschersonia* sp. with the conidial mass in the center, covering the cadaver of citrus whiteflies, *Aleyrodes citri* Gennadius (Hemiptera: Aleyrodidae) that were found on the ventral citrus leaf (scale bar = 0.5 mm); (B) immature citrus whitefly (*A. citri*) (scale bar = 0.1 mm).

was a group of flask-shaped perithecia (Fig. 3A), and fusoid conidia inside the stroma (Fig. 3B). It was possible that this *Aschersonia* sp. was collected in the period of anamorph of *Hypocrella raciborskii* (Liu et al., 2006).

Morphological analysis using scanning electron microscopy

SEM observation revealed that the stroma of the isolate *Aschersonia* sp. was a pack of tightly woven fungal mycelia (Fig. 4A). Mycelia were irregular in size, partial and branching (Fig. 4B). At the top and middle of the colony, a conidial mass was observed. When the conidia dried out, they did not drop off from the colony (Fig. 4C). The conidia were of the fusoid type (Fig. 4D), which confirmed the micromorphological observation.

The macromorphological and SEM studies revealed that the collected *Aschersonia* specimen was *A. placenta*. This identification mainly followed Liu et al. (2006) and other reports (Samson et al., 1988; Humber, 1992; Luansa-ard et al., 2007). This *A. placenta* sample was designated as *A. placenta* isolate Asp001 and deposited in the NBCRC-CRC, Thailand.

Appropriate synthetic media for cultivation of Aschersonia placenta isolate Asp001

Culture media influenced the mycelial growth and sporulation. Observation on days 7, 14 and 21 of the fungus *A. placenta* isolate Asp001 showed colony growth on the seven tested media as shown in Fig. 5. On all media, the fungus grew as a single colony with white mycelia, being pulvinate and spherical and producing viscous, conidial masses. Conidia were similar in shape and size to those derived from natural specimens. Fungal colonies on PDA, PDA + M,

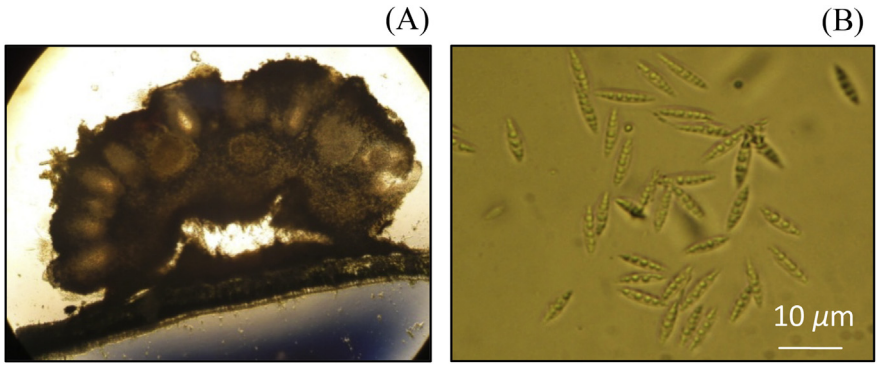

Fig. 3. Morphology of citrus whiteflies infected with *Aschersonia* sp., *Aleyrodes citri* Gennadius (Hemiptera: Aleyrodidae): (A) cross section of a stroma and perithecia 10×; (B) fusoid conidia 40×.

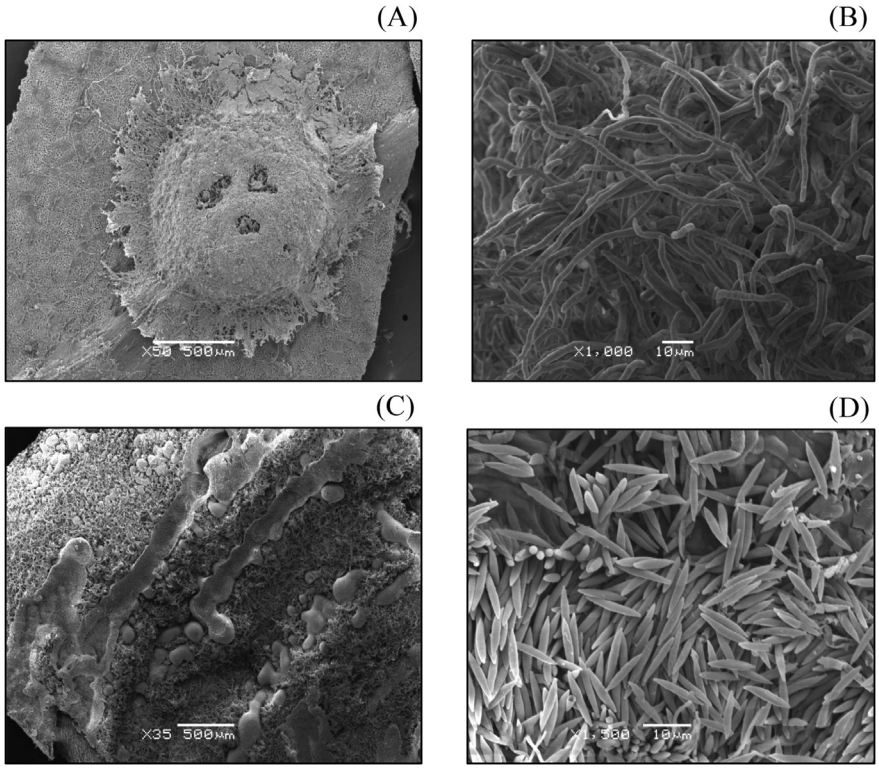

Fig. 4. Scanning electron micrographs of the collected fungus, *Aschersonia* sp.: (A) stroma composed of mycelia and the conidial mass (50×) (scale bar = 500 μm); (B) intertwined and woven mycelia (1000×) (scale bar = 10 μm); (C) conidia produced in center of stroma appear as slimy mass (35×) (scale bar = 500 μm); (D) fusoid conidia (1500×) (scale bar = 10 μm).

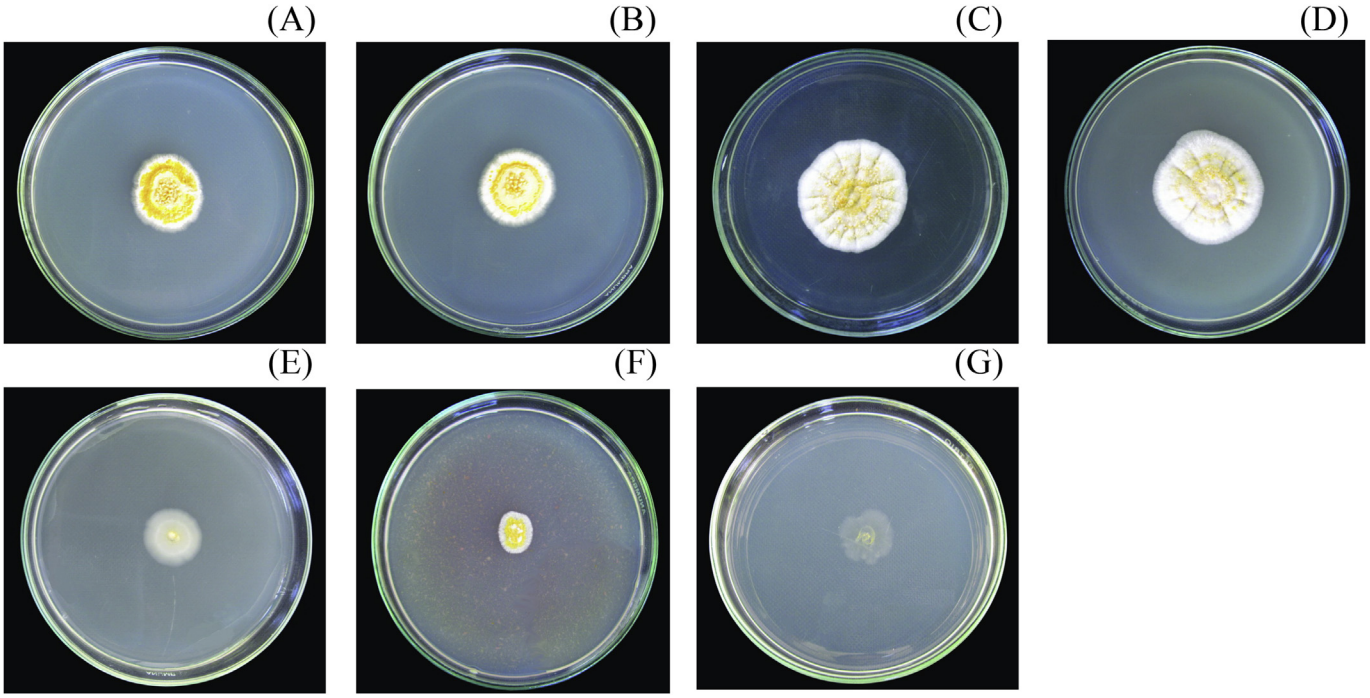

Fig. 5. Appearance of colonies of the fungus, *Aschersonia placenta* isolate Asp001 cultivated on different synthetic media at day 21 post incubation at 25 ± 2 °C, 75 ± 5% RH under 24 h of light: (A) PDA (Potato Dextrose Agar); (b) PDA + M (180: 1 ml of PDA: pasteurized milk); (C) SDAY (Sabouraud dextrose agar with yeast extract); (D) SDA + M; (E) corn meal agar; (F) WA + V8 (180: 1 ml of Water Agar (WA): juice of 8 vegetable species; (G) WA (scale bar = 1 cm).

CMA and WA + V8 had a spherical surface with a smooth edge and conidial masses were light yellow in color (Fig. 5A, B, 5E and 5F) but colonies on SDA + M and SDAY had a condensed surface and the conidial masses were pale yellow in color (Fig. 5C, D). On the WA medium, no conidial masses were observed (Fig. 5G).

The media SDA + M and SDAY were ranked first and second, respectively, as suitable media for fungal growth with radial growth of 0.99–2.09 cm and 0.90–2.04 cm, respectively, and were not significantly different ($p > 0.05$). Significant differences on radial growth were observed between these two media and the others ($p < 0.05$). On the WA + V8 medium, the fungus showed no growth from day 14–21. Interestingly, the fungus showed a growth increase on the WA medium with radial growth of 0.54–1.14 cm (Table 1).

The numbers of conidia/mL produced by *A. placenta* isolate Asp001 when cultured on synthetic media, PDA, PDA + M, SDAY, SDA + M, CMA and WA + V8 were 2.59×10^8, 1.02×10^8, 2.69×10^8, 2.17×10^8, 1.88×10^6 and 3.22×10^7, respectively, with no conidia observed on the WA medium (Table 1). Although the fungus cultured on SDAY and SDA + M provided the best results based on the colony radius measurement, on SDA + M, the fungus produced only 2.17×10^8 conidia/mL, which was statistically less than that on PDA and SDAY which produced 2.59×10^8 conidia/mL and 2.69×10^8 conidia/mL, respectively. No significant difference ($p > 0.05$) was found in the numbers of conidia produced using the latter two media (Table 1).

The fungus *A. placenta* isolate Asp001 cultured at 25 °C resulted in slow growth and few conidia, which was different from the previous finding of Ibrahim et al. (1993) and may have been due to the interaction of the isolate and the temperature as discussed by Lutthisungneon (1998). Nonetheless, in the present study, PDA and SDAY (Difco) were the appropriate synthetic media for culturing the fungus, *A. placenta*, which supported the results on congenic species reported by Lutthisungneon (1998) and Hywel-Jones (2002). Ibrahim et al. (1993) studied factors affecting the growth of the fungi, *A. placenta* (anamorph) and *H. raciborskii* (telemorph), and found that

Table 1
Mean radial growth (±SD) and number of conidia produced by the fungus, *Aschersonia placenta* isolate Asp001 on seven synthetic media on days 7, 14 and 21 after incubation at 25 ± 2 °C, 75 ± 5% RH and 24 h of light.

Medium	Radial growth *A. placenta* (cm)[a]			Conidial density (conidia/mL)[b]
	7 d[c]	14 d[c]	21 d[c]	21 d[c]
PDA	0.88 ± 0.03b	1.36 ± 0.04c	1.50 ± 0.06b	2.59×10^8a
PDA + M	0.85 ± 0.06b	1.27 ± 0.14b	1.50 ± 0.09b	1.02×10^8c
SDAY	0.90 ± 0.04a	1.54 ± 0.04a	2.04 ± 0.13a	2.69×10^8a
SDA + M	0.99 ± 0.05a	1.59 ± 0.03a	2.09 ± 0.10a	2.17×10^8b
CMA	0.56 ± 0.04d	0.88 ± 0.04d	1.22 ± 0.09c	1.88×10^6e
WA + V8	0.72 ± 0.14c	0.88 ± 0.14d	0.88 ± 0.14d	3.22×10^7d
WA	0.54 ± 0.05d	0.82 ± 0.06d	1.14 ± 0.14c	−4/[d]

[a] Average of five replicates.
[b] Average of five replicates, counted using a hemacytometer.
[c] Means followed with the same lowercase online in the same column do not differ significantly from each other using Tukey's honestly significant difference ($p > 0.05$).
[d] No conidia were observed.

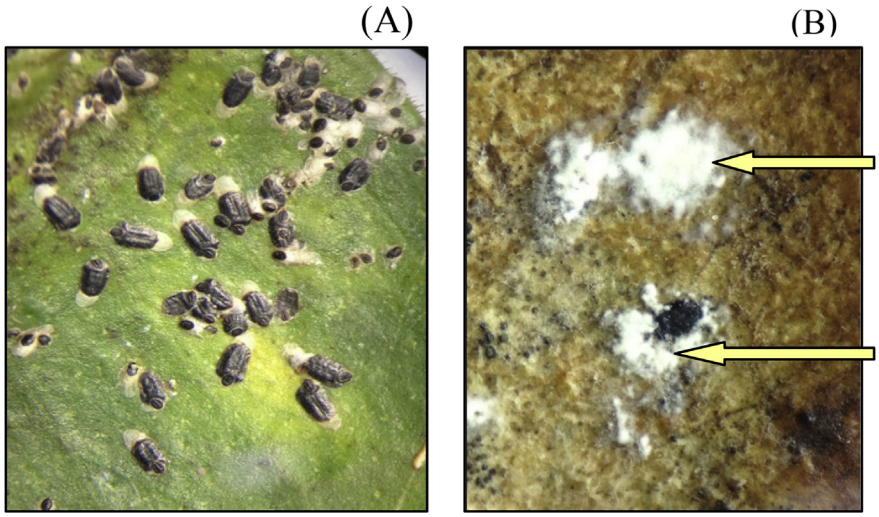

Fig. 6. Morphology of (A) live nymphs of *Parlatoria ziziphi* (Lucas) (Hemiptera: Diaspididae) on pomelo leaf in control treatment; (B) mycelia of *Aschersonia placenta* isolate Asp001 (arrows) covering infected *P. ziziphi* observed at 14 d after spraying conidial suspension.

media mixed with vegetables could create the highest growth. However, the WA + V8 in this present investigation did not provide a significantly lower ($p > 0.05$) density of conidia (3.22×10^7 conidia/mL) than the media composed of either SDAY (2.69×10^8 conidia/mL) or PDA (2.59×10^8 conidia/mL), probably due to differences in the medium texture and the vegetables juice used in this study. They used a semi-solid media mixed with pumpkin or cassava, but the present study employed solid media mixed with eight different species of vegetables other than pumpkin or cassava.

Mycelial growth and sporulation on artificial media are important biological characteristics of entomopathogenic fungi. The basic nutritional requirements can have a profound effect on culture growth, sporulation and morphology in entomopathogenic fungi (Zhu et al., 2008). Qiu et al. (2013) applied the response surface method in a Box-Behnken design to study the interactions of the important components of the media on *A. placenta*. They used a biphasic system in fungal cultivation experiments. In the first phase, liquid media for mycelial production was used, which produced an average of 2.14 ± 0.17 g per 100 mL. In the second phase, a solid PDA media was employed for conidial production, which produced 9.30 ± 0.8 × 10^7 spores per cm^2. The current study obtained 2.69×10^8 conidia/mL when cultured with the fungus *A. placenta* isolate Asp001 on solid SDAY medium for 21 d.

Evaluation of the efficiency of the fungus for the control of Parlatoria ziziphi (Lucas)

The cumulative mortality of black parlatoria (*P. ziziphi*), was calculated at 7, 14 and 21 d after inoculation using different concentrations of the conidial suspension. In the control treatment (sprayed with sterile water; Fig. 6A), no mortality was observed until the end of the experiment (21 d). Seven days after inoculation, no dead insects were observed in any treatment. The cumulative mortalities recorded on days 14 and 21 were significantly different ($p < 0.05$) when compared with the control. The percentage of cumulative mortality on day 14 showed a similar trend to that on day 21 according to the increased fungal concentration. The cumulative mortality of the *P. ziziphi* when applied with 1×10^8 conidia/mL and 1×10^9 conidia/mL suspensions of the fungus was not significantly different ($p > 0.05$) but did differ significantly from treatments of 1×10^6 conidia/mL and 1×10^7 conidia/mL (Table 2). The symptoms of the *P. ziziphi* when infected by *A. placenta* isolate Asp001 is shown in Fig. 6B. Early infection of the fungus on the black parlatoria was usually detected as some white fringes of hyphae extending from the marginal area of the larval body and as pustules at the weak points on the insect dorsum. A few conidia were observed arising directly from the conidiogenous cells. By the end of the infection, mat-like pustules were observed completely covering the larval body and no recognizable remnants of the insects could be observed. Colonies were white at first, and became orange to reddish in color as sporulation began. The stroma consisted of a compact accumulation of mycelia covered by thinner, fertile mycelia bearing conidiogenous structures, which were direct, at first, but later became confluent. On sporulating colonies, conidial masses were readily seen as orange-red slimy droplets on the insect cadavers.

Although *A. placenta* isolate Asp001 gave low control efficiency on *P. ziziphi* with cumulative mortality less than 50% within 21 d, its

Table 2
Mean cumulative mortality (±SD) of *Parlatoria ziziphi* (Lucas) (Hemiptera: Diaspididae) at 7, 14 and 21 d after spraying with conidial suspensions of *Aschersonia placenta* isolate Asp001.

Conidial suspension of *A. placenta* (conidia/mL)	Mortality of *P. ziziphi* infected by *A. placenta* (%)[a]		
	7 d[b]	14 d[b]	21 d[b]
Control	0.0	0.0a	0.0a
1×10^6	0.0	5.74 ± 8.6b	7.71 ± 9.9b
1×10^7	0.0	16.00 ± 17.5c	17.46 ± 17.6c
1×10^8	0.0	21.81 ± 17.2d	23.89 ± 17.8d
1×10^9	0.0	23.73 ± 11.3d	27.42 ± 15.2d

[a] Average of 5 replicates.
[b] Means within a column followed by different lowercase online are significantly different using Tukey's honestly significant difference ($p < 0.05$).

persistence on the leaf surface and tolerance to relative humidity were evident under field conditions. This would be advantageous regarding using this fungal species for insect pest control. Previous research indicated that another *Aschersonia* species (*A. aleyrodis*), which was a promising whitefly control agent, also had these properties (Fransen, 1987; Meekes et al., 2002).

The great variance in the insect mortality in this investigation was probably due to the experimental conditions which were unsuitable for fungal infection to the tested insects. At a higher conidial concentration, the fungus *Aschersonia* spp. produced greater infection in nymphs, pupae and adults of *A. citri* (Uchida, 1970). The effect of the environmental conditions, especially the weather, was revealed by Ponomarenko et al. (1975) who reported that inoculation of isolates of *A. placenta* from Vietnam and China resulted in 90% mortality of citrus whitefly, *Dialeurodes citri* (Ashmead) (Hemiptera: Aleyrodidae) in citrus orchards in Georgia due to appropriate weather conditions.

Attathom (2012) described several restrictions on the use of entomopathogenic fungi to introduce disease infection in insect pests—drought, ultraviolet radiation, temperature, insect characteristics and age and the virulence of the fungus—which could be solved by using a suitable fungal formulation and an effective application technique, for example, spraying conidial suspension of the fungus in an appropriate manner and at a suitable time, as most entomopathogenic fungi produce spores with a solid wall (the chlamydospore), which is resistant to adverse environmental conditions such as temperature or unusual climate conditions or insufficient food for long period of time.

Samples of the fungus *Aschersonia* sp. isolated from naturally infected whitefly (*A. citri*) collected from citrus orchards in Trat province were identified as *Aschersonia placenta* based on macromorphological characteristics and SEM observation. It was designated as *A. placenta* isolate Asp001. In search of suitable synthetic media for the fungal cultivation, seven media were compared. Based on the radial growth of the fungal colony, the medium SDA + M gave the best result followed by SDAY. However, based on the ability of the fungus to produce conidia, either SDAY or PDA was considered the most suitable medium for *A. placenta* Asp001 mass cultivation. An experiment to determine the efficacy of *A. placenta* Asp001 toward black parlatoria (*P. ziziphi*) indicated that the higher the concentration applied, the greater the observed accumulative mortality. On days 14 and 21 post inoculation with 1×10^9 conidia/mL of the fungal conidia suspension the accumulative mortality was 23.73% and 27.42%, respectively.

Conflict of interest

None.

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