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RESEARCH PAPER

Effect of media types on the growth of insect pathogenic fungi (*Aschersonia placenta*)

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ABSTRACT

Bali has favorable conditions for the production and expansion of citrus plantations. However, citrus cultivation in Bali often faces challenges, including reduced yield caused by pest infestations and pathogenic diseases. One significant pest affecting citrus plants is the whitefly. A potential method for controlling whiteflies is the use of natural enemies. The entomopathogenic fungus *Aschersonia placenta* is one of natural enemy that can effectively manage whitefly infestations. However, the succesful utilization of *A. placenta* requires a specialized approach, particularly in selecting an appropriate growth medium. This study aimed to investigate the impact of different growth media on the development of the entomopathogenic fungus *A. placenta.* The research began with field sampling, followed by the isolation and morphological identification of *A. placenta* to obtain a pure culture. Subsequently, media tests were conducted to evaluate fungal growth. Nine treatments were implemented to examine the impact of different media: (1) Water Agar (WA), (2) Potato Dextrose Agar (PDA), (3) Potato Sucrose Agar (PSA), (4) Water Agar + Weaver Ant Eggs Flour (WA-WAEF), (5) Potato Dextrose Agar + Weaver Ant Eggs Flour (PDA -WAEF), (6) Potato Sucrose Agar + Weaver Ant Eggs Flour (PSA-WAEF), (7) Water Agar + Cricket Flour (WA-CF), (8) Potato Dextrose Agar + Cricket Flour (PDA-CF), and (9) Potato Sucrose Agar + Cricket Flour (PSA-CF). The results demonstrated that incorporating cricket insect flour (Gryllidae: Orthoptera) or weaver ant egg flour (*Oecophylla smaragdina*) into potato sucrose media provided the most optimal growth environment for *A. placenta*.

Key words: Citrus whitefly, entomopathogenic fungi, media effect.

INTRODUCTION

Bali possesses favorable conditions for cultivating citrus plants. Based on data from Central Bureau of Statistics of Bali Province (Central Bureau of Statistics, 2020), citrus production in Bali amounted to 2.401.064 tons in 2018. However, major challenges still exist in citrus production in Bali, such as reduced crop yields due to pest infestations and disease outbreaks. One of the pests affecting citrus plants is the whitefly (Sujithra et al., 2019; Merthaningsih et al., 2020; Rodas-Martínez et al., 2023).

The citrus whitefly causes damage by extracting sap from the phloem tissue, which functions as the conduit for nutrient transport within plants. A high population of citrus whiteflies can lead to infestation symptoms, including leaf yellowing, drying, and ultimately, leaf abscission (Flint, 2002). In addition to causing direct harm to citrus plants, the citrus whitefly also plays a role in the development of mold fungi, which thrive on its excretions. Sooty mold can proliferate and coat the surface of citrus plant leaves, interfering with physiological processes and diminishing fruit quality (Rodas-Martínez et al., 2023).

Controlling whiteflies is essential due to their considerable impact on citrus plants. A promising and sustainable approach is the use of entomopathogenic fungi (Liu et al., 2006; Sudiarta et al., 2019; Sudiarta et al., 2024). Meekes et al. (2002) documented the infection of whiteflies by *Aschersonia* sp. fungus in both the nymph and imago stages. *Aschersonia* sp. has been utilized to control whitefly pests in greenhouse environments in multiple countries, including Florida, Bulgaria, China, Japan, Russia, Spain, and Chile. In the Netherlands, *Aschersonia* sp. has been commercially produced and marketed as a biopesticide (Sikder et al., 2019; Sani et al., 2020).

Aschersonia sp. was first identified in Bangli and Gianyar Regencies, Bali, Indonesia, in 2014. In 2018, it was discovered in Gobleg Sukasada (Buleleng Regency), a citrus-producing region. Additionally, in 2018, *Aschersonia* was also reported for the first time in Pancasari Sukasada (Buleleng Regency) on mulberry plants (Suputra et al., 2019). Based on morphological and molecular identification, the *Aschersonia* sp. found in Bali has been identified is *A. placenta* (Sudiarta et

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al., 2019).

Aschersonia placenta has potential as a biocontrol agent against whitefly pest. According to Suputra et al. (2019), the percentage of whitefly infections caused by *A. placenta* reached 78%, demonstrating its potential as a biopesticide. However, cultivating the fungus on synthetic substrates presents significant challenges and requires considerable time. Hence, selecting a suitable medium to enhance the proliferation of the *A. placenta* is crucial.

The ability of entomopathogenic fungi to infect their host insects is influenced by the nutritional content of the media, including insect-base media (Sa'idah & Asri, 2019; Sari & Khobir, 2019). Supplementing the media with a protein source can enhance the germination capacity of entomopathogenic fungi (Sa'idah & Asri, 2019; Sari & Khobir, 2019). The growth needs of entomopathogenic fungi can be met by adding substrates containing protein and chitin, such as insects, to the media (Sari & Khobir, 2019; Sari et al., 2023). Some suitable insects included crickets and weaver ant eggs. Previous studies have shown that media composition plays a crucial role in fungal sporulation, viability, and overall growth performance (Fitriana et al., 2018).

Therefore, it is necessary to investigate the impact of different medium types on the growth of the insect-pathogenic fungus *A. placenta*. This research aimed to determined the most suitable medium for fungal growth to facilitate its propagation.

MATERIALS AND METHODS

Isolation and Morphological Identification of the Insect-Pathogenic Fungus *Aschersonia placenta.* *Aschersonia* sp. was isolated from fresh field samples at the Plant Disease Science Laboratory, Agroecotechnology Study Program, Faculty of Agriculture, Udayana University. A modified version of the method from Liu et al. (2006) was employed (Rasjman et al., 2022; Wangi et al., 2023). Morphological identification was conducted on the pure culture of *Aschersonia* sp. to confirm its classification. Observations of PSA medium, colony color, and conidia shape were carried out following the procedure outlined by Liu et al. (2006).

Testing the Effect of Media on the Growth of the *Aschersonia placenta.* The test was carried out by pouring 10 mL of the treatment medium into a 9 cm petri dish and allowing it to cool. *A. placenta* was inoculated in the center of the medium. The experiment involved nine different media treatments, each repeated four times. Each replication consisted of a single culture in a petri dish. Table 1 presents the nine experimental treatments.

All ingredients, including cricket flour and weaver ant eggs flour, were purchased from an animal feed store in Denpasar. Cricket flour and weaver ant eggs flour were used because they contain nutrients suitable for insect-pathogenic fungi, similar to those found in the natural environment. Additionally, these ingredients are readily available in Indonesia.

According to Wang et al. (2005), crickets contain 58.3% protein and 8.7% chitin. Weaver ant eggs (*Oecophylla smaragdina*) content 24.1% crude protein, 4.6% crude fiber, and 42.2% crude fat (Putranto et al., 2021).

Observation. The measured variables included macroscopic fungal characteristics, microscopic features, colony area of *Aschersonia placenta*, conidia density, and conidia size in each treatment.

Colony Area. Colony area measurements were conducted from day 4 to day 21 following inoculation. Each day, colony growth was documented by tracing its area onto transparent paper and then transferring it onto millimeter-marked paper. The colony area was quantified using millimeter grid paper.

Condia Density. Conidia density was calculated by dissolving fungal colonies in 10 mL of distilled water, followed by homogenization. From the homogenized solution, 1 mL was taken, diluted with 9 mL of distilled water, and then homogenized again. This process was repeated six times. Calculations were performed using a hemocytometer using a haemocytometer, following the formula of Gabriel & Riyatno (1989):

$$C = \frac{t}{n \times 0.25} \times 10^6$$

C = Spore density (conidia/mL);
t = Number of total spores in each sample observed;
n = Number of samples observed (5 big square × 16 small square;
0.25 = The correction factor for using small-scale square on a haemocytometer;
10^6 = Constanta.

Conidia Length. Conidia length was measured by

Table 1. Types of media treatment and their preparation

Symbols	Type of treatment/media	Preparation (All media were sterilized by autoclaving at a temperature of 121 ºC and a pressure of 2 atm for 15 min (Terrones-Fernandez et al., 2024)
T1	Media Water Agar (WA)	WA media was prepared following standard procedures (Maia & Yano-Melo, 2001) by boiling 1000 mL of distilled water; after boiling, 15 g of agar was added.
T2	Water agar media + Cricket flour (WA-CF)	Boil 1000 mL of distilled water and then add 20 g of cricket flour and 15 g of agar.
T3	Water agar media + Weaver Ant Eggs flour (WA-WAEF)	Boil 1000 mL of distilled water and then add 20 g of Weaver Ant Eggs flour and 15 g of agar.
T4	Potato Dextrose Agar (PDA) media	PDA media was made with 200 g potatoes, 10 g dextrose, and 15 g agar, boiled using 1000 mL distilled water.
T5	Potato Dextrose Agar + Cricket Flour (PDA-CF) media	PDA-CF media was made with 200 g potatoes, 10 kg dextrose, 15 g agar, and 20 g cricket flour, boiled using 1000 mL distilled water.
T6	Potato Dextrose Agar Media + Weaver Ant Eggs Flour (PDA-WAEF)	PDA-WAEF media was made with 200 g potatoes, 10 g dextrose, 15 g agar, and 20 g Weaver Ant Eggs flour, boiled using 1000 mL distilled water.
T7	Potato Sucrose Agar (PSA) Media	PSA media was made with 200 g potatoes, 10 g sugar, and 15 g agar, boiled in 1000 mL distilled water.
T8	Potato Sucrose Agar Media + Weaver Ant Eggs Flour (PSA-WAEF)	PSA-WAEF media is made with 200 g potatoes, 10 g granulated sugar, 15 g agar, and 20 g Weaver Ant Eggs flour, 1000 mL distilled water.
T9	Potato Sucrose Agar + Cricket Flour (PSA-CF)	PSA-CF media is made with 200 g potatoes, 10 g granulated sugar, 15 g agar, 20 g cricket flour, and 1000 mL distilled water.

dissolving the fungal colony in 10 mL of distilled water and diluting it six times. A total of 1 mL the solution was taken and placed onto the hemocytometer. The conidia length was measured using the hemocytometer, with measurements taken from 10 fungal conidia per treatment medium.

Data Analysis. The statistical analysis in this study aimed to determine the effect of different media types on the growth of the *Aschersonia placenta*. The acquired data were analyzed using single-factor analysis of variance (ANOVA) at a significance level of 5%.

RESULTS AND DISCUSSION

Specimen Collection and Identification. Specimens of *Aschersonia placenta* were collected from whitefly insects infested with *A. placenta* on citrus plants in Kerta Village, Payangan District, Gianyar Regency. In this study, a single infected whitefly was utilized. To verify the identity of *Aschersonia*, the specimen was compared with a fungal strain from the laboratory collection that had been previously identified both morphologically and molecularly.

The fungus is characterized by the distinctive shape and color of its sexual stroma, which facilitates its identification. The sexual stroma of *A. placenta* typically exhibits a flat shape with a slightly convex section, containing a mass of orange to yellowish-orange fungal conidia (Figure 1A). Fungal colonies are found on the underside of citrus plant leaves, in accordance with the findings reported by Sudiarta et al. (2019).

The identification of *A. placenta* is carried out to confirm that the fungal samples collected in the field belonged to *A. placenta*. The method of Liu et al. (2006) was used for fungal identification. Cultivation of *A. placenta* on PSA media resulted in the formation of white fungal colonies with a mass of yellowish-orange conidia. The conidial mass became visible on the 21st day of incubation at 23 ºC (Figure 1B). The presence of conidia is a critical characteristic for the successful isolation of *A. placenta*.

Microscopic examination confirmed that the

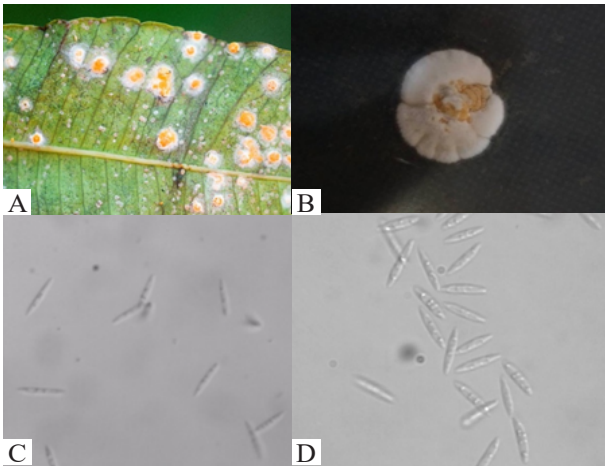

Figure 1. Identification of *Aschersonia placenta* in whiteflies. A. Orange to yellowish-orange fungal stroma collected from Kerta Village; B. *A. placenta* on PSA media 21 days after inoculation; C. Conidia of *A. placenta* obtained from the stroma; D. Conidia of *A. placenta* from PSA media culture.

conidial form of *A. placenta* was consistent between the conidia obtained from the fungal stroma in the field (Figure 1C) and those cultivated on PSA media (Figure 1D). This demonstrates that the *Aschersonia* fungus grown on the PSA medium is indeed *A. placenta*. The conidia observed in both the PSA medium and field samples exhibited a fusoid morphology, as depicted in Figure 1C and 1D. This findings align with the documented characteristics of *A. placenta* described by Liu et al. (2006) and Sudiarta et al. (2019).

Fungal Growth on Different Media. The size of fungal colonies was recorded in each culture medium for 21 days following inoculation. Fungal growth began on the 4th day after inoculation on water agar media. Rapid growth was observed from the 5th day onward, reaching its peak on the 21st, with an average colony area of 90.50 mm^2.

On all other media, except PDA-CF, growth began on day 5th. On PDA media, fungal growth continued until the 21st day, reaching 533.50 mm^2. On PSA media, growth appeared on the 5th day and peaked on the 21st day, reaching 624.50 mm^2. WA-WAEF media supported continuous growth until the 21th day, with a peak colony area of 507.00 mm^2.

On PDA-WAEF media, growth initiated on the 5th day and peaked on the 21st day at 487.75 mm^2. On PSA-WAEF media, growth also began on the 5th day and peaked on the 21st day, reaching 590.75 mm^2. On WA-CF media, growth was observed from the 5th day, with a significant increased on the 19th day before peaking at 531.25 mm^2 on the 21st day.

PDA-CF media exhibited the earliest growth, beginning on the 4th and peaking on the 21st day with colony area of 385.75 mm^2. On PSA-CF media, growth started on the 5th day reaching a peak of 645.75 mm^2 on the 21st day, making it the most effective medium for *A. placenta* growth.

The graph of average colony area growth indicates that PSA medium supplemented with cricket flour (PSA-CF) has the most favorable effect. This is evident from the consistently larger fungal colony sizes observed daily (Table 2).

Fungal Colony Growth and Statistical Analysis. The fungal colony area in WA media reached 90.50 mm^2 ± 11.12%. Statistical analysis revealed that the colony area in WA media differed significantly from that in other media. The colony area on PDA media was 385.75 mm^2 ± 71.39, which was also significantly different from other media. The colony area on WA-WAEF media was 531.25 mm^2 ± 27.83, a value statistically similar to those observed on PDA-WAEF, WA-CF, and PDA-CF media. The colony area on PSA media was 590.75 mm^2 ± 4.99, which was not statistically different from the fungal colony size observed on PSA-WAEF and PSA-CF media. Data on the colony area is presented in Table 2.

The statistical data in Table 2 indicate that the incorporation of cricket flour or weaver ant egg flour into WA media (WA-CF) significantly enhanced fungal colony growth compared to WA media alone. However, fungal development remained suboptimal, with notably thin growth, as shown in Figure 2. The colony areas in PDA, PDA-CF, and PDA-WAEF media were not significantly different from those in WA-CF and WA-WAEF media. This suggest that *A. placenta* can thrive without potato and dextrose supplementation. The increased proliferation of *A. placenta* colonies in WA media with supplemented with cricket flour and weaver

Table 2. The effect of media on colony area, conidia density, and conidia length of *Aschersonia placenta* fungus on day 21

No.	Treatment	Observation variables		
		Colony size (mm^2)	Conidia density ($\times 10^3$ conidia/mL)	Conidia length (µm)
1.	WA	90.50 ± 11.12 d	0.10 e	3.52 f
2.	PDA	385.75 ± 71.39 c	1.05 c	3.95 d
3.	PSA	590.75 ± 99.20 a	5.55 b	4.25 c
4.	WA-WAEF	531.25 ± 27.83 b	0.30 d	3.80 e
5.	PDA-WAEF	487.75 ± 124.07 b	22.95 a	4.38 b
6.	PSA-WAEF	624.50 ± 68.95 a	21.50 a	4.70 a
7.	WA-CF	533.50 ± 81.10 b	0.35 d	3.84 e
8.	PDA-CF	507.00 ± 22.64 b	22.30 a	4.98 a
9.	PSA-CF	645.75 ± 4.99 a	25.90 a	5.03 a

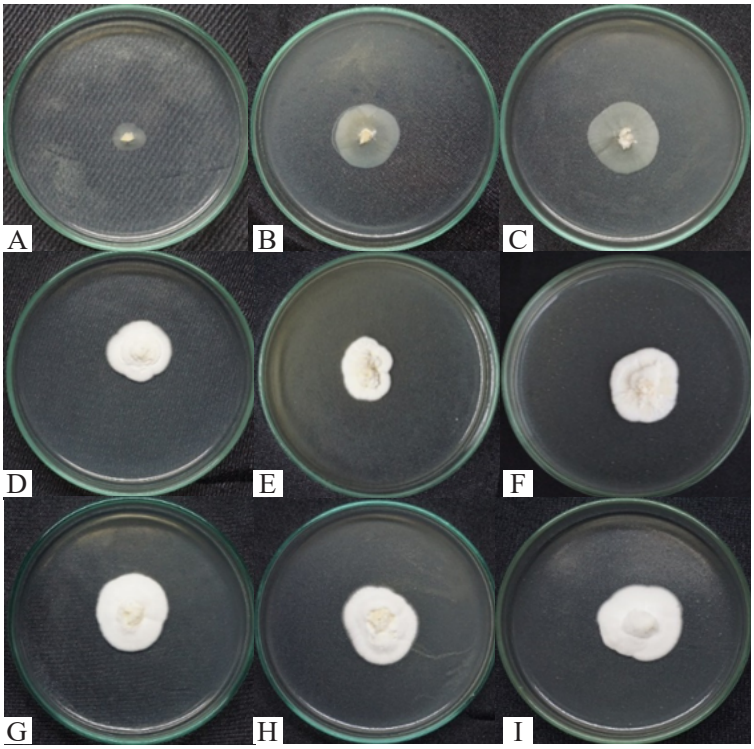

Figure 2. Colony area of *Aschersonia placenta* fungus on day 21 after inoculation. A. WA Media; B. WA-WAEF media; C. WA-CF Media; D. PDA media; E. PDA-WAEF media; F. PDA-CF media; G. PSA media; H. PSA-WAEF media; I. PSA-CF media.

ant eggs flour suggest that the nutritional composition of these facilitated fungal growth.

According to Sari & Khobir (2019), both protein and chitin enhance the germination capacity of entomopathogenic fungi. Wang et al. (2005) reported that crickets contain 58.3% protein and 8.7% chitin. Additionally, the eggs of weaver ants (*Oecophylla smaragdina*) contain 24.1% crude protein, 4.6% crude fiber, and 42.2% crude fat (Putranto et al., 2021). The nutritional composition of culture media plays a significant role in fungal colony growth. Elevated levels of carbohydrates and proteins facilitate conidial germination and promote fungal growth (Sa'idah & Asri, 2019; Sari & Khobir, 2019; Sari et al., 2023).

Conidial Density Across Media. At a 10^3 dilution, the lowest *A. placenta* conidia density was observed in WA media. Similarly, PDA media had density of 1.05×10^3 conidia/mL, which was not significantly difference from the other media. The conidia density on WA-WAEF media was 0.30×10^3 conidia/mL, which was not significantly different from WA-CF media ($0.35 \times$

10^3 conidia/mL) but was significantly different from other media. PSA media had a density of 5.55 × 10^3 conidia/mL and was significantly different from other treatments. The analysis revelead that PSA-CF, PDA-WAEF, PDA-CF, and PSA-WAEF media were not significantly different from each other. However, the highest conidial density was found in PSA-CF (25.90 × 10^3 conidia/mL), followed by PDA-WAEF (22.95 × 10^3 conidia/mL), PDA-CF (22.30 × 10^3 conidia/mL), and PSA-WAEF (21.50 × 10^3 conidia/mL) (Figure 3).

The increased fungal density observed in PSA and PDA media supplemented with cricket flour or weaver ant egg flour suggests that these media provide adequate nutrition for *A. placenta*. In addition to supplying sugar as a carbon source, the addition of cricket flour enhances the media's protein, lipid, carbohydrate, and nucleic acid content, all of which contribute to fungal cell structure (Sari et al., 2023).

Impact of Media on Conidia Size. The media had a significant effect on conidia size. In WA treatment, conidia measured 3.52 µm in length, which was significantly different from the other treatments. Similarly, conidia in PDA media had a length of 3.95 µm, showing a significant difference compared to the other media. Conidia in WA-WAEF and WA-CF media measured 3.80 µm and 3.84 µm, respectively, and were not significantly different from each other.

PDA-WAEF media yielded conidia measuring 4.38 µm, significantly different from other media. Observations showed that PSA-CF was not significantly different from PDA-CF and PSA-WAEF media. This indicates that the combination of these three media produces optimal results. Combining cricket flour with PSA or PDA increase conidial size, while weaver ant egg flour enhance colony length when combined with PSA. The impact of cricket flour was particularly evidence in the differences between PSA and PSA-CF. Adding 20 g of cricket flour to 1 L of PSA increase colony length, as shown in Figure 4.

Macroscopic and Microscopic Observations. Macroscopic and microscopic observations indicate that sucrose supplementation enhances fungal colony

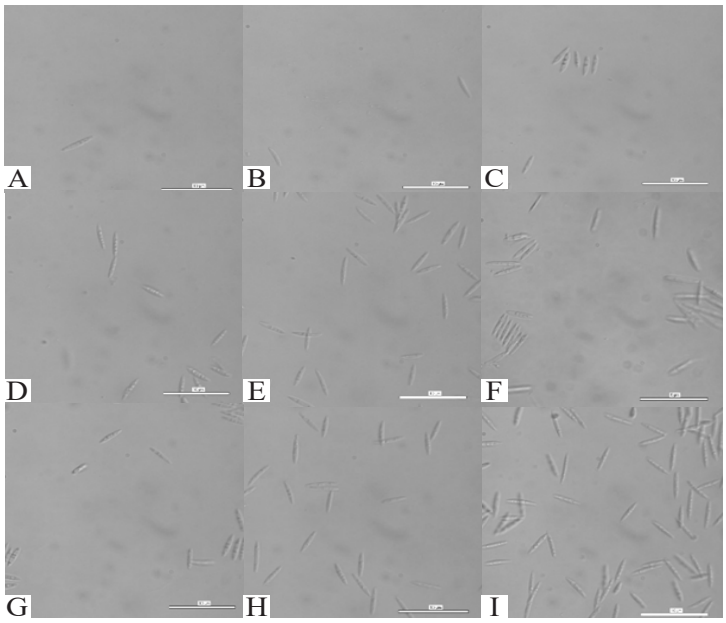

Figure 3. Conidia density on day 21 after inoculation. A. WA media; B. WA-WAEF media; C. WA-CF media; D. PDA media; E. PDA-WAEF media; F. PDA-CF media; G. PSA media; H. PSA-WAEF media; I. PSA-CF media.

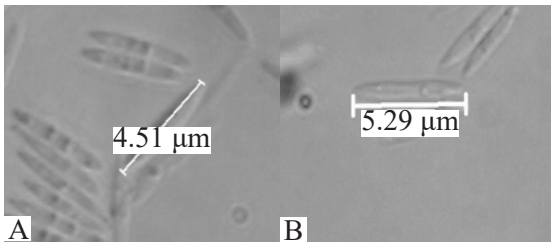

Figure 4. Comparison of conidia length on day 21 after inoculation. A. Colonies on PSA media; B. Colonies on PSA-CF media.

growth. This study demonstrated that incorporating cricket flour and weaver ant eggs flour into the media promotes *A. placenta* growth. The presence of these two insects-derived nutrients, which serve as carbon and nitrogen sources, is essential for fungal development (Sari et al., 2023).

The treatment outcomes in WA-WAEF and WA-CF media showed a significant effect on fungal growth in terms of colony area, conidial density, and colony length compared to WA media alone. The addition of cricket flour or weaver ant eggs flour alone significantly increase fungal growth. Media substrate composition play a critical role in the growth of entomopathogenic fungi. Sowmya et al. (2022) stated that the solid substrate in the medium substantially impacts the mass production of *Metarhizium anisopliae.*

The addition of cricket or weaver ant egg flour in various ratios to the fungal culture media did not yield significantly different results. Both insects enhance *A. placenta* growth*;* however, supplementation with dextrose or sucrose as a carbohydrates source is necessary. Among the tested media, PSA-CF is recommended as it contains the highest carbohydrate content while remaining cost-effective. Granulated sugar is both readily available and affordable, while crickets are inexpensive and easy to source, either from nature or through farming. According to Sari et al. (2023), cricket flour provides essential nutrients that positively influence the growth of *M. majus.*

CONCLUSION

The addition of cricket insect flour (Gryllidae: Orthoptera) or weaver ant egg crumbs (*Oecophylla smaragdina*) to potato sucrose media provides the optimal medium for the growth of the insect-pathogenic fungus *Aschersonia placenta*, in terms of colony area, conidia density, and conidia length.

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AUTHORS' CONTRIBUTIONS

IPS conceptualized and designed the experiment. IPS, IKGSH, KAY, GNASW, and IPWS conducted fields surveys, sampling, and observation of insect pathogen in both fields and laboratory setting. DGWS and LL reviewed the manuscript. All authors contributed to manuscript preparation, read, and approved the final version.

COMPETING INTEREST

The authors declare no relevant financial or non-financial competing interests.

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