

The effect of conidia density and application frequency of the entomopathogenic fungus *Aschersonia aleyrodis* Webber in controlling silverleaf whitefly (*Bemisia tabaci* Gennadius) on tomato (*Solanum lycopersicum* Linnaeus)

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Abstract: One of several important pests that attack tomato plants is the silverleaf whitefly (*Bemisia tabaci*) (Hemiptera: Aleyrodidae). An eco-friendly method to control *B. tabaci* utilizes the entomopathogenic fungus, namely *Aschersonia aleyrodis*. This study aimed to determine the effect of *A. aleyrodis* conidia density and the frequency of its application to control silverleaf whitefly (*B. tabaci*) pest on tomato plants under screen house conditions. This study used a randomized completely block design (RCBD) to test ten combination treatments. Each treatment was repeated three times. The results showed that application of *A. aleyrodis* at conidia densities of 10^6 conidia/mL, 10^7 conidia/mL, and 10^8 conidia/mL with an application frequency of up to once every three weeks was still effective in controlling *B. tabaci* populations on tomato plants. The highest population suppression rates for *B. tabaci* (90.6%), tomato yield (1 009 g/plant and 16 fruits/plant), percentage of mycosis (96.6%), and percentage of mummification (97.3%) were found to occur at a conidia density of 10^8 conidia/mL with the application frequency of once a week.

Keywords: population suppression; mycosis; mummification; biological control

Tomato is one of the horticultural commodities that are popular and widely cultivated worldwide. This popularity may be related to tomato-rich nutritional content, such as protein, fat, minerals, carbohydrates, and vitamins (Mubarak et al. 2023; Rahmat et al. 2023). In 2021, tomato production in Indonesia reached 1 114 399 t (BPS 2023). However, the average

tomato production in Indonesia is much lower compared to other countries, such as China, India, and Turkey, which amounted to 67 636 724 t, 21 181 000 t, and 13 095 258 t, respectively (FAOSTAT 2023).

Several problems, including pest infestations, can cause low tomato production, especially in Indonesia. One of the important pests that attack tomato

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plants is the silverleaf whitefly (*Bemisia tabaci*). *B. tabaci* (Hemiptera: Aleyrodidae) is a polyphagous insect that has the ability to infest a wide range of host plants (Frohlich et al. 1999). Additionally, Naranjo et al. (2003) reported that *B. tabaci* exhibits notable migration and reproductive activity levels. *B. tabaci* has the potential to cause both direct and indirect damage. Direct damage occurs when the whitefly punctures the plant tissues with its stylets, developing chlorotic spots (Perring et al. 2018; Sudarjat et al. 2019, 2020) to *B. tabaci* is its role as a disease vector of several pathogenic viruses, including the genus of Begomovirus. One of the pathogenic viruses in the genus of Begomovirus is the *tomato yellow leaf curl virus* (TYLCV) species (Narendra et al. 2017).

Biological control is natural pest management that deliberately utilizes natural enemies to control pest populations (Baker et al. 2020). Biological control agents are organisms used to control pest populations on cultivated plants (Stenberg et al. 2021). Entomopathogenic fungi are one of the biological control agents used to control *B. tabaci*. Entomopathogenic fungi can cause disease to insect pests, leading to the death of infected pests. Several advantages of using entomopathogenic fungi as biocontrol are the high production capacity, the short life cycle of entomopathogenic fungi, and the potential to form conidia resistant to environmental conditions (Shahid et al. 2012).

Aschersonia aleyrodis is one of the entomopathogenic fungi with good pest control prospects. *A. aleyrodis* is an entomopathogenic fungus that is used to control whitefly (Aleyrodidae) and scales (Coccidae) pests (Wei et al. 2016). Entomopathogenic fungi's spore density greatly affects insect host infection's success, mycosis, mummification, and LC50 values (Lethal Concentration 50). *A. aleyrodis* with a spore density concentration of 1.6×10^{11} conidia/mL, can control *Coccus pseudomagnoliarium* up to 95%. Kurnia et al. (2011) also reported that the weekly application of *A. aleyrodis* with a density of 10^8 conidia/mL can suppress the nymph population of *B. tabaci* by 90.93% on hydroponic tomato plants.

A previous study by Islam et al. (2021) reported that the pathogenicity level of entomopathogenic fungi can be affected by environmental factors, i.e., some environmental conditions may limit the growth of biocontrol fungi. The existence of environmental limiting factors for the success of entomopathogenic infection on insect pests requires repeated application of biocontrol. To increase the effectiveness of using

A. leyrodis fungi as biological control agents, the present study aimed to elucidate the effect of spore density and application frequency of *A. aleyrodis* against *B. tabaci* in tomato cultivation

MATERIAL AND METHODS

Research Preparation. This research was conducted in the Screen House, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor District, Sumedang Regency. The experimental location is at an altitude of ± 752 meters above sea level. Entomopathogenic fungi were propagated at the Laboratory of Pesticides and Environmental Toxicology, Faculty of Agriculture, Universitas Padjadjaran.

***B. tabaci* mass rearing.** *B. tabaci* mass rearing was conducted following the method developed by Farina et al. (2022) with some modifications. The process of mass rearing of *B. tabaci* involved the gathering of adult whiteflies directly from the field. Eggplants (*Solanum melongena* L.) that were three weeks old since planting and had been prepared in advance were introduced into an enclosure, a cube-shaped cage measuring 2 m $\times$ 0.5 m, to serve as a nutritional source for *B. tabaci*. These eggplants were then infested with *B. tabaci* imago. The cage was then covered with gauze.

Preparation of tomato plants. Tomato plants were cultivated following the commonly used cultivation method for growing tomatoes in a screen house. The tomato variety used in this experiment was Servo F1. The seedbed of tomato plants was prepared by thoroughly mixing the soil with cow dung fertilizer in a ratio of 1:1. Transplanting was done when tomato seedlings had 3–5 true leaves or reached the age of three weeks old after germinating. The seedling was then transferred from the seed tray to polybags of 30 cm $\times$ 30 cm, which had been prepared in advance. Polybags contained 2 kg growing media in the form of a good mixing of soil, compost, and husk charcoal in a ratio of 1:1:1. Each polybag was then arranged according to the order of given treatment, with a planting distance of about 50 cm between polybags. Each tomato plant was given a cage covered with gauze of 0.4 m $\times$ 0.44 m $\times$ 1.5 m (l $\times$ w $\times$ h) to avoid other pest infestations, except silverleaf whitefly. Maintenance of tomato plants was carried out by watering once to two times a day in the morning or evening. Application of fertilization in the form of NPK 16:16:16 was carried out five days after

transplanting with a dose of 5 g/plant. Additionally, manual weeding was also carried out to control the weed growth.

Propagation of *A. aleyrodis*. The propagation of *A. aleyrodis* was conducted following the method developed by Mathulwe et al. (2022) with some modifications. *A. aleyrodis* was cultivated on potato dextrose agar (PDA) media under laminar air flow conditions, followed by a 21-day incubation period at room temperature. Following the PDA cultivation, the *A. aleyrodis* fungi were inoculated on cracked corn media. The cracked corn media was prepared by soaking one kilogram of cracked corn in distilled water, thoroughly cleaned and drained. Subsequently, it was steamed using a boiler until the corn was one-third cooked (± 15 minutes). After steaming, the cracked steamed corn was allowed to stand at room temperature, then wrapped with a heat-resistant plastic bag weighing 100 g/plastic. Then the cracked corn media was sterilized inside the autoclave. Pure cultures of *A. aleyrodis* were inoculated into sterilized corn media by inoculating one plug (5 mm) pure culture of *A. aleyrodis* and then incubated at 25–30 °C. *A. aleyrodis* would grow in cracked corn media after a one-week incubation period.

Calculation of conidia density of *A. aleyrodis*. Pure culture of *A. aleyrodis* grown on corn media for three to four weeks weighed as much as 10 g, then mixed with 100 mL aquadest and 0.1% Tween 80 (SmartLab, Indonesia). The mixture was then vigorously shaken at a speed of 500 rpm for 10 min. Conidia density was determined using a haemocytometer under a microscope. Approximately one drop of the *A. aleyrodis* conidia suspension was placed onto the haemocytometer counting chamber, which was then covered with a glass cover slip. Then, the number of conidia contained in the counting box on five view fields with 40 times magnification was counted by hand counter. The counting for each field was done two times. Conidia densities of 10^8 conidia/mL, 10^7 conidia/mL, and 10^6 conidia/mL were obtained by using the following formula (Fuentes 2016):

$$\text{Conidia density (conidia/mL)} = \frac{\text{Average conidia} \times \text{Dilution factor}}{\text{Square volume}} \quad (1)$$

Infestation *B. tabaci* on tomato plants and application of *A. aleyrodis*. Before applying *A. aleyrodis*, post-transplanted tomato seedlings (two weeks after transplanting) were infested with *B. tabaci* imago that had been propagated and main-

tained. *B. tabaci* was infested into the tomato plant with 20 individuals per plant using an insect aspirator. The application of *A. aleyrodis* fungal suspension to *B. tabaci* was carried out two weeks after *B. tabaci* infestation with three levels of conidia densities, namely (10^6 , 10^7 and 10^8 conidia/mL), and various application frequencies as follows: A – spore density 10^6 conidia/mL with the frequency of application weekly, B – spore density 10^7 conidia/mL with the frequency of application weekly, C – spore density 10^8 conidia/mL with the frequency of application weekly, D – spore density 10^6 conidia/mL with the frequency of application fortnightly, E – spore density 10^7 conidia/mL with the frequency of application fortnightly, F – spore density 10^8 conidia/mL with the frequency of application fortnightly, G – spore density 10^6 conidia/mL with the frequency of application triweekly, H – spore density 10^7 conidia/mL with the frequency of application triweekly, I – spore density 10^8 conidia/mL with the frequency of application triweekly, J – control (without *A. aleyrodis* treatment).

Application of *A. aleyrodis* fungus was carried out in the afternoon with a spray volume of 9–21 mL/plant with a spray target on the upper and lower leaf surfaces.

Population density analysis, mycosis, and mummification. The population density of *B. tabaci* was carried out two weeks after *B. tabaci* infestation and before the application of *A. aleyrodis* fungi to tomato plants. Observations were made on the population of *B. tabaci* in the nymph phase, which was observed weekly at intervals of once every week. *B. tabaci* population density was calculated by sampling ten leaves per plant. Mycosis observations were carried out every day until mycelia grows, and then the percentage is calculated with the following formula by Pramono and Purnomo (2019) as follows:

$$\text{Mycosis percentage} = \frac{\text{Number of mycosis insect}}{\text{Number of dead insect}} \times 100 \quad (2)$$

As for the percentage of mummification, it could be calculated by the following formula by Pramono and Purnomo (2019) as follows:

$$\text{Mummification percentage} = \frac{\text{Number of mummification insect}}{\text{Number of mycosis insect}} \times 100 \quad (3)$$

Analysis of tomato plant growth and yield. Tomato fruit was ready to be harvested when the fruit's skin colour changed from green to yellowish. Harvesting was carried out when tomato plants reached the

age of three months. The yield of tomatoes is measured by weighing only good-quality tomatoes. Plant growth measurement was measured in the form of plant height seven days after planting by measuring the height of the plant from the soil surface up to the highest active growing point of tomato plants.

Statistical data analysis. The Kolmogorov-Smirnov was used for testing the normalities of data distributions. For the statistical data analysis, data were subjected to a one-factor analysis of variance (ANOVA) followed by the Scott-Knott at $P < 0.05$ for comparisons among the investigated data. All statistical analyses were performed using SPSS statistical software (version 23.0).

RESULTS

Population Density of *B. tabaci*. The statistical analysis revealed that various treatments of *A. aleyrodis* fungal conidia density and its application frequency could suppress *B. tabaci* population density (Table 1). In the first week after the application of *A. aleyrodis*, whitefly population density on tomato

plants showed no significant difference between treatments, where the whitefly population density was evenly distributed on all plants. Also, in the second to fourth observations, the application of *A. aleyrodis* on various levels of conidia density and application frequency still could not suppress the population density of *B. tabaci*. Based on the observation in the second week after, there was an increase in the number of *B. tabaci* nymphs in each treatment. It was likely that not all conidia of applied entomopathogenic fungi could reach the target, so there was still some *B. tabaci* to grow and breed actively (Table 1).

In the fifth to ninth observations, all treatments tested were able to suppress the population density of *B. tabaci*. In the fifth and sixth observations, the suppression of the *A. aleyrodis* on the population of *B. tabaci* was significantly higher compared to controls. All tested treatments decreased the population density of *B. tabaci*, while in the control treatment, the population density of *B. tabaci* remained increased. This is due to no *A. aleyrodis* spore suspension applied; therefore, the *B. tabaci* were able to grow and to breed actively. Finally,

Table 1. Effect of conidia density and application frequency of *Aschersonia aleyrodis* fungi on population density of silverleaf whitefly (*Bemisia tabaci*) nymph

Treatment (conidia density, frequency application)	Population density of <i>Bemisia tabaci</i> pests at times of observation (weekly)								
	1	2	3	4	5	6	7	8	9
A (10^6 conidia/mL, weekly)	281.3 ^a	282.7 ^a	274.3 ^a	256.3 ^a	233.3 ^b	199.7 ^b	169.0 ^c	116.0 ^c	73.0 ^c
B (10^7 conidia/mL, weekly)	286.0 ^a	280.3 ^a	262.7 ^a	241.0 ^a	214.7 ^b	183.0 ^b	146.3 ^c	101.7 ^c	57.0 ^c
C (10^8 conidia/mL, weekly)	314.3 ^a	314.0 ^a	278.3 ^a	252.0 ^a	220.7 ^b	179.3 ^b	139.0 ^c	78.3 ^c	29.0 ^c
D (10^6 conidia/mL, weekly)	270.0 ^a	279.0 ^a	268.0 ^a	248.7 ^a	242.0 ^b	203.0 ^b	189.7 ^c	128.3 ^c	103.3 ^b
E (10^7 conidia/mL, fortnightly)	322.3 ^a	326.3 ^a	324.3 ^a	303.3 ^a	286.0 ^b	238.0 ^b	223.7 ^b	165.0 ^b	130.0 ^b
F (10^8 conidia/mL, fortnightly)	295.3 ^a	293.3 ^a	293.7 ^a	276.0 ^a	268.0 ^b	222.3 ^b	210.3 ^b	144.0 ^b	92.3 ^c
G (10^6 conidia/mL, fortnightly)	247.3 ^a	253.7 ^a	258.7 ^a	265.0 ^a	236.3 ^b	230.3 ^b	231.3 ^b	184.0 ^b	149.0 ^b
H (10^7 conidia/mL, triweekly)	287.0 ^a	292.7 ^a	288.0 ^a	288.7 ^a	269.7 ^b	260.3 ^b	249.0 ^b	198.7 ^b	176.7 ^b
I (10^8 conidia/mL, triweekly)	271.7 ^a	263.0 ^a	260.7 ^a	256.0 ^a	237.7 ^b	229.3 ^b	216.3 ^b	149.3 ^b	120.3 ^b
J (Control)	238.7 ^a	295.3 ^a	324.3 ^a	353.0 ^a	374.0 ^a	398.0 ^a	423.7 ^a	455.7 ^a	486.3 ^a

The mean followed by the same letter in the same column did not differ significantly based on the Scott-Knott at 5%

the population density of *B. tabaci* was fairly high, reaching 486.3 individuals (Table 1).

Significant variation in the percentage of suppression of population density of *B. tabaci* was also found in response to conidia density and application frequency of *A. aleyrodis* (Table 2). The higher the application frequency of *A. aleyrodis*, the higher the suppression of *B. tabaci* population density, leading to the lower population density of *B. tabaci*. The highest suppression (90.6%) was achieved in treatment C (conidia density 10^8 conidia/mL applied weekly). In comparison, the lowest suppression (39.9%) of *B. tabaci* population was obtained in treatment G (conidia density 10^6 conidia/mL applied triweekly). The shorter the frequency of application, the more effective the application of *A. aleyrodis*. However, the effect of *A. aleyrodis* application on the population of *B. tabaci* was only noticed after some time as the fungus required time to infect the insect, unlike the chemical insecticide application in which the effect can be seen immediately.

Table 2. Suppression of *Bemisia tabaci* population density at several weeks

Treatment (conidia density, frequency of application)	Suppression of <i>Bemisia tabaci</i> population density (%)		
	7 th week	8 th week	9 th week
A (10^6 conidia/mL, weekly)	40.1 ^b	59.0 ^c	74.2 ^b
B (10^7 conidia/mL, weekly)	48.8 ^a	64.5 ^b	80.1 ^b
C (10^8 conidia/mL, weekly)	55.7 ^a	74.8 ^a	90.6 ^a
D (10^6 conidia/mL, fortnightly)	29.6 ^c	52.4 ^d	61.5 ^d
E (10^7 conidia/mL, fortnightly)	29.7 ^c	48.8 ^d	60.6 ^d
F (10^8 conidia/mL, fortnightly)	28.6 ^c	51.1 ^d	68.6 ^c
G (10^6 conidia/mL, triweekly)	6.6 ^e	25.6 ^e	39.9 ^e
H (10^7 conidia/mL, weekly)	14.5 ^d	32.5 ^e	40.5 ^e
I (10^8 conidia/mL, triweekly)	20.1 ^d	45.2 ^d	55.2 ^d
J (Control)	—	—	—

The mean followed by the same letter in the same column did not differ significantly based on the Scott-Knott at 5%

Furthermore, the ability of the fungus to give a better controlling effect was also determined by the conidia density. The higher the conidia density, the greater the suppression of the population density of *B. tabaci*. The more often *A. aleyrodis* is applied to *B. tabaci*, the better the suppression effect.

Mycosis. Application of *A. aleyrodis* fungus to *B. tabaci* on tomato plants leads to the infection of *B. tabaci* by *A. aleyrodis*. Based on statistical data analysis showed that the various treatments of *A. aleyrodis* conidia density and application frequency significantly affect the percentage of mycosis of *B. tabaci* (Table 3).

During the initial through the last observation, all *A. aleyrodis* treatments exhibited mycosis in the tested *B. tabaci*, and this had a significantly greater impact compared to the control treatment. Among these treatments, determining the best one was challenging during the first three initial observations as all treatments, except for control, yielded similar significant results. However, from the fourth to the eighth observations, the most effective treatment was identified as treatment C (conidia density 10^8 conidia/mL applied weekly) with a percentage of mycosis of 96.6%. This outcome is believed to be influenced by both the spore density and frequency of application. The higher the spore density, the more conidia are attached to the insect's body, thereby accelerating the infection of *B. tabaci* by *A. aleyrodis*.

Mummification. *B. tabaci* is considered to be in a mummified state when approximately 90% of its body is covered by the mycelia of the entomopathogenic fungi (Pramono & Pramono 2019). When infected by *A. aleyrodis*, the body of *B. tabaci* undergoes a transformation marked by the development of white mycelia; the body then becomes hard, which resembles a mummy. The average percentage of mummification for each treatment is listed in Table 4, and it showed a significant result in response to different conidia densities and application frequencies of *A. aleyrodis*.

The result demonstrated that all treatments can induce mummification except control. However, no significant difference between the treatments was found up to the first four observations. Therefore, it is difficult to determine the best treatment with the significantly highest percentage of mummification among all *A. aleyrodis* treatments in the first to fourth weeks of observation. However, from the fifth and final observations, the triweekly application of

Table 3. Effect of conidia density and application frequency of *Aschersonia aleyrodis* fungi on the percentage of mycosis at several observation times

Treatment	Percentage of mycosis (%) at several observation times							
	1	2	3	4	5	6	7	8
A (10 ⁶ conidia/mL, weekly)	63.3 ^a	82.8 ^a	80.5 ^a	80.0 ^b	83.8 ^b	85.6 ^a	87.9 ^b	87.5 ^b
B (10 ⁷ conidia/mL, weekly)	62.0 ^a	83.0 ^a	82.6 ^a	82.3 ^b	87.2 ^a	85.5 ^a	90.9 ^a	89.3 ^b
C (10 ⁸ conidia/mL, weekly)	79.2 ^a	87.5 ^a	91.2 ^a	93.5 ^a	93.7 ^a	91.9 ^a	95.9 ^a	96.6 ^a
D (10 ⁶ conidia/mL, fortnightly)	63.9 ^a	73.6 ^a	81.6 ^a	59.9 ^c	76.9 ^b	67.4 ^b	79.3 ^c	74.4 ^d
E (10 ⁷ conidia/mL, fortnightly)	64.4 ^a	65.8 ^a	82.7 ^a	65.7 ^c	80.7 ^b	67.2 ^b	86.8 ^b	77.4 ^c
F (10 ⁸ conidia/mL, fortnightly)	78.0 ^a	87.3 ^a	87.9 ^a	72.9 ^c	89.5 ^a	67.3 ^b	92.9 ^a	81.9 ^c
G (10 ⁶ conidia/mL, triweekly)	73.9 ^a	66.0 ^a	74.6 ^a	76.5 ^b	52.2 ^d	58.9 ^b	74.8 ^d	70.3 ^d
H (10 ⁶ conidia/mL, triweekly)	69.7 ^a	80.6 ^a	70.0 ^a	79.1 ^b	65.0 ^c	59.4 ^b	82.9 ^c	74.7 ^d
I (10 ⁸ conidia/mL, triweekly)	74.4 ^a	72.2 ^a	67.1 ^a	89.2 ^a	71.1 ^c	63.1 ^b	91.6 ^a	78.7 ^c
J (Control)	0.0 ^b	0.0 ^b	0.0 ^b	0.0 ^d	0.0 ^e	0.0 ^c	0.0 ^e	0.0 ^e

The mean followed by the same letter in the same column did not differ significantly based on the Scott-Knott at 5%

A. aleyrodis at a concentration of 10⁸ conidia/mL gave the best percentage of mummification. The mummification time begins on the 4th to 10th day after the fungus application until the insect's body is filled with mycelia and begins about two days after the occurrence of mycosis in insects. During the last observation, it becomes evident that the maximum mummification rate was achieved in treatment C (10⁸ conidia/mL with weekly application), reaching an impressive 97.3%

Tomato fruit yield. Tomato fruit yield was measured by involving two variables, i.e., fruit weight and number of fruits. Tomato harvesting was carried out every four days, as much as five harvesting times, at 11 to 13 weeks after planting. Based on

Table 5, there were significant differences in fruit yields as the result of the application of conidia density and application frequency of *A. aleyrodis* fungi. The statistical data analysis showed that the best treatment was obtained from the plant that was applied weekly with a conidia density of 10⁸ conidia/mL (C) with a fruit weight of 1 009 g/plants and the number of fruits per plant was 16 fruit that was significantly higher than other treatments and control (Table 5). The increasing yield of tomato fruit after the treatment of *A. aleyrodis* fungi has a positive correlation with low silverleaf whitefly population density (Table 1), percentage of mycosis (Table 3), and percentage of mummification (Table 4).

Table 4. Effect of conidia density and application frequency of *Aschersonia aleyrodis* fungi on the percentage of mummification at several observation times

Treatment (Conidia density, frequency of application)	Mummification Percentage (%) at time of observation							
	1	2	3	4	5	6	7	8
A (10 ⁶ conidia/mL, weekly)	80.6 ^a	63.2 ^a	69.7 ^a	80.1 ^a	82.4 ^a	88.8 ^a	87.9 ^b	88.2 ^b
B (10 ⁷ conidia/mL, weekly)	84.7 ^a	81.9 ^a	78.4 ^a	82.7 ^a	80.8 ^a	85.4 ^a	91.0 ^b	90.2 ^b
C (10 ⁸ conidia/mL, weekly)	82.2 ^a	92.5 ^a	90.3 ^a	90.1 ^a	89.4 ^a	93.3 ^a	97.1 ^a	97.3 ^a
D (10 ⁶ conidia/mL, fortnightly)	75.0 ^a	78.8 ^a	68.5 ^a	70.0 ^a	69.6 ^b	65.9 ^b	69.0 ^e	76.9 ^c
E (10 ⁷ conidia/mL, fortnightly)	71.2 ^a	73.3 ^a	69.4 ^a	70.6 ^a	85.7 ^a	80.2 ^a	80.8 ^c	83.6 ^b
F (10 ⁸ conidia/mL, fortnightly)	84.2 ^a	79.9 ^a	80.6 ^a	71.5 ^a	91.7 ^a	78.1 ^b	87.5 ^b	87.8 ^b
G (10 ⁶ conidia/mL, triweekly)	63.9 ^a	58.9 ^a	63.9 ^a	64.6 ^a	63.9 ^b	75.0 ^b	76.9 ^d	71.5 ^c
H (10 ⁷ conidia/mL, triweekly)	72.7 ^a	72.2 ^a	75.6 ^a	82.2 ^a	84.9 ^a	70.3 ^b	80.8 ^c	74.6 ^c
I (10 ⁸ conidia/mL, triweekly)	85.6 ^a	72.2 ^a	78.3 ^a	80.2 ^a	71.5 ^b	74.5 ^b	86.6 ^b	83.1 ^b
J (Control)	0.0 ^b	0.0 ^b	0.0 ^b	0.0 ^b	0.0 ^c	0.0 ^c	0.0 ^e	0.0 ^e

The mean followed by the same letter in the same column did not differ significantly based on the Scott-Knott at 5%

Table 5. Effect of conidia density and application frequency of *Aschersonia aleyrodis* fungi on the weight and number of fruits of tomato

Treatment (conidia density, frequency of application)	Tomato yield	
	fruit weight (g)	number of fruits
A (10 ⁶ conidia/mL, weekly)	533.7 ^c	12.3 ^c
B (10 ⁷ conidia/mL, weekly)	705.0 ^b	14.0 ^b
C (10 ⁸ conidia/mL, weekly)	1 009.0 ^a	16.0 ^a
D (10 ⁶ conidia/mL, fortnightly)	401.7 ^e	7.0 ^e
E (10 ⁷ conidia/mL, fortnightly)	456.7 ^d	10.0 ^d
F (10 ⁸ conidia/mL, fortnightly)	549.0 ^c	10.0 ^d
G (10 ⁶ conidia/mL, triweekly)	219.3 ^h	5.0 ^f
H (10 ⁷ conidia/mL, triweekly)	273.3 ^g	4.7 ^f
I (10 ⁸ conidia/mL, triweekly)	353.0 ^f	8.3 ^e
J (Control)	160.7 ⁱ	3.3 ^f

The mean followed by the same letter in the same column did not differ significantly based on the Scott-Knott at 5%

DISCUSSION

B. tabaci is a significant pest within the agricultural sector, especially in Solanaceae plants such as tomatoes. The insect can cause damage directly by eating plant leaves and as vectors of virus plants, leading to reduced crop yields and decreased plant productivity. To overcome these pest attacks, pesticides are generally carried out, but in the long term, they can be detrimental to humans and the environment, such as insecticide resistance, environmental pollution, and effects on non-target organisms (Sani et al. 2020). Natural/biological control using entomopathogenic fungi can be used as an alternative to control *B. tabaci*. Liu et al. (2006) stated that the application of *A. aleyrodis* has been proven effective in parasitizing whiteflies to control *B. tabaci*. Therefore, the fungus is a promising biological control candidate. In this study, we evaluated the effect of conidia density and application frequency of *A. aleyrodis* fungi on controlling *B. tabaci* in tomatoes.

Low spore density could affect the effectiveness of entomopathogenic fungi in infecting insects. This was revealed in the research of Hasnah et al. (2012), who stated that the effectiveness of insect pathogenic fungi in controlling target pests depends on the density of conidia applied. In this study, at the seventh and eighth observations, the population density of *B. tabaci* decreased due to the treatment

of *A. aleyrodis*. Among several tested treatments, the best treatment to suppress the population density of *B. tabaci* was treatment with high conidia density and applied more frequently in this case; they were conidia density 10⁶ conidia/mL, 10⁷ conidia/mL, 10⁸ conidia/mL, which applied weekly and 10⁸ conidia/mL which applied fortnightly.

Kurnia et al. (2011) also stated that the spore density of *A. aleyrodis* is good in suppressing the population of *B. tabaci* at a spore density of 10⁸ conidia/mL. However, the frequency of application is very influential in suppressing the population density of *B. tabaci*. Repeated application of *A. aleyrodis* fungus could support suppressing *B. tabaci* population density.

The effectiveness of entomopathogenic fungi in infecting insects is influenced by spore density, frequency of application, environmental conditions, and virulence of entomopathogenic fungi. Similar to these findings, Sopialena and Hutajulu (2022) also states that several factors, including environmental conditions, spore viability, and spore count, influence the effectiveness of entomopathogenic fungi in infecting insects. Environmental factors, especially temperature and relative humidity, greatly affect the virulence of entomopathogenic fungi. The optimum temperature for spore growth ranges from 25–30 °C (Wallstad et al. 1970). A favourable relative humidity for spore growth is above 85% (Liu et al. 2006). However, the entomopathogenic fungus *A. aleyrodis* had a high tolerance to low relative humidity conditions (Ingle et al. 2022).

To minimize the failure of *A. aleyrodis* conidia in infecting *B. tabaci*, it is necessary to apply *A. aleyrodis* fungi repeatedly. In early-stage applications, conidia that cannot infect target pests need to be replaced by conidia that are applied at a later stage (Gul et al. 2014). Early symptoms of *B. tabaci* death as the effect of *A. aleyrodis* infection was characterized by a rigid discolouration of the insect's body to become dull. Infected insects become weak and have low sensitivity and low feeding activity, so the insect gradually dies. According to Kumar et al. (2020), the symptoms of entomopathogenic fungi that infect insects are sluggish body movements, reduced appetite, hiding behind leaves, changes in colour on the body of insects to paleness, and even completely difficult to move.

In this study, the death of *B. tabaci* pests infected with entomopathogenic fungi was observed two days after application. This is in accordance with previous reports by Cloyd (2003) that entomopathogenic fungi cause insect symptoms that take about 24–72 hours.

The time of mycosis begins on the 2nd to 3rd days after application of the fungus. Mycosis is a term used for a disease caused by fungi infection. Mycosis triggers physiological changes in insects. Insects suffering from mycosis show several abnormalities, such as convulsions, absence of synchronization, behavioural changes, and paralysis (Mora et al. 2017). However, to give a controlling effect, entomopathogenic fungi require a period of time to infect and kill pests because the conidia attached to the insect's cuticle need to germinate before penetrating the cuticle.

Differences in mummification values as a result of the application of *A. aleyrodis* were only seen at the age of five weeks, where the mummification occurred within four to ten days after application of the *A. aleyrodis*. Abbas (2020) stated that fungal growth occurs in the body of insects, and dead insects harden like mummies. A previous study by Butt et al. (2000) showed insect death was strongly influenced by spore density and virulence of entomopathogenic fungi. The increasing spore density in entomopathogenic fungi and the more conidia attached to the body of insects, the faster the infection process that makes the metabolic system disrupted in the body so that the percentage of mummification becomes high.

After mycosis occurs, the fungus grows through the insect integument, characterized by white mycelium on the external insect body surface (Islam et al. 2021). Infection with entomopathogenic fungi causes death in host insects (Meekes et al. 2000). So, in the second week of observation after application, there were still mummified *B. tabaci* observed. Pramono and Purnomo (2019) reported a direct relationship between spore density and the percentage of mummification. The higher the spore density, the higher the percentage of mummification, and *vice versa*; the lower the spore density, the lower the percentage of mummification occurred. The frequency of application also has substantial effects on the percentage of mummification. Repeated application of *A. aleyrodis* can reduce the likelihood of spore failure to attach to the insect body or germinate. If the application is more frequent, then the percentage of mummification is high; on the contrary, less frequent application is associated with a low percentage of mummification.

CONCLUSION

It can be concluded that the application of *A. aleyrodis* entomopathogenic fungi at conidia densities of

10⁶ conidia/mL, 10⁷ conidia/mL, and 10⁸ conidia/mL with application frequency of once in one, two, and three weeks were still effective in controlling the population of *B. tabaci* on tomato plants. The highest population suppression rate of *B. tabaci* (90.6%), tomato yield (1 009 g/plant and 16 fruits/plant), mycosis percentage (96.6%), and mummification percentage (97.3%) were found at conidia density of 10⁸ conidia/mL with application frequency of once a week.

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