January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Yusmani Prayogo, Marida Santi Yudha Ika Bayu, Sri Wahyuni Indiati, et al.

Morphological Characters and Efficacy of Thirteen Entomopathogenic Fungi of *Aschersonia aleyrodis* Webber Isolates on Whitefly (*Bemisia tabaci* Gennadius)

Yusmani Prayogo[a)], Marida Santi Yudha Ika Bayu, Sri Wahyuni Indiati and Made Jana Mejaya

Indonesian Legumes and Tuber Crops Research Institute, IAARD,
Jln. Raya Kendalpayak Km. 8, Malang 65101, East Java, Indonesia

[a)]Corresponding author: yusmani.prayogo@yahoo.com

Abstract. In the intercropping system between of soybean and citrus plant was known as a triggers of the explosion of whitefly (*Bemisia tabaci*) and citrus whitefly (*Dialeurodes citri*) population. These two types of insect pests are in the same order and family which known as an important as vectors for various types of viruses. This study was aimed to identify the morphological characters and efficacy of entomopathogenic fungi that infect *B. tabaci* and *D. citr*i. Insect cadavers colonized by fungal mycelium were taken from three centers of soybean production in East Java Province (Banyuwangi, Jember, and Pasuruan Regencies). Each insect cadaver from the field was sterilized using 0.5% chlorox solution and then incubated in potato dextrose agar medium. The growing colonies were identified based on morphological characters and then tested by Koch's postulates against *B. tabaci*. The results of the identification showed that there were *Aschersonia aleyrodis* Webber fungi obtained with white colony color which then turned into light brown. The size of conidiophores was 39–40 × 2.1–2.3 µm, conidia 10–11 × 2.0 µm, optimum temperature 27–30°C, toxic to egg, nymph, and imago, especially isolates from Banyuwangi Regency with mortality reaching 89% within 3–4 days after application. The finding of *A. aleyrodis* fungal isolates is a new innovation in biological control because both *B. tabaci* and *D. citri* are tolerant to synthetic pesticides due to biotype changes.

INTRODUCTION

Intercropping patterns between soybean, citrus, and dragon fruit are often found in Banyuwangi and Jember Regencies, while intercropping between soybean with pumpkins is found in Pasuruan Regency. The three regencies are the largest center of soybean production in East Java Province, which are often endemic and reported the outbreak of Hemiptera, especially the family Aleyrodidae. The intercropping between of soybean and citrus plant triggers an explosion of whitefly (*Bemisia tabaci* Gennadius) (Hemiptera: Aleyrodidae) and citrus whitefly (*Dialeurodes citri* Ashmead). The soybean intercropping system with pumpkin also triggered the explosion of *B. tabaci* because both types of plants were the main hosts of whiteflies. These two types of insect pests are in the same order and family that function as vectors for various types of viruses.

Entomopathogenic fungi from the genus *Aschersonia* were found to infect and kill whitefly which attacks soybean plants and other food crops. It was also reported that *Aschersonia* fungi were also found and could cause epizootics in various host and locations, especially colonizing citrus whitefly in citrus plantations. According to the report [1], this fungus generally develops in tropical areas on plantations with high humidity. However, several other researchers reported that *Aschersonia* fungi could also grow and develop in low humidity conditions around 50% [2, 3].

It was reported that the genus *Aschersonia* was widespread in Indonesia, China, India, Thailand, Malaysia, Cameroon, New Guinea, Vietnam, Ghana, and Philippines [4, 5]. However, Faria and Wraight [6] and Charnley and Collins [7] reported that *Aschersonia* species that develop in tropical areas, especially in citrus crops, were

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A. aleyrodis and *A. placenta*. In Bali, starting in 2014, exploration was carried out on citrus plantations, which found many fungi that infected and colonized the insect pest *D. citri* and the results of exploration were indicated by the genus *Aschersonia* [8, 9]. Furthermore, [10] cadavers of *B. tabaci* and *D. citri* insects that died from fungal colonization in soybean, citrus, and dragon fruit plantations in Banyuwangi Regency were collected and incubated. The results of the identification and Koch's postulate test indicated that the fungus isolate obtained was *A. aleyrodis*.

The entomopathogenic fungus *A. aleyrodis* is a widely distributed species infecting whitefly insect pests. Since the early 1900s, there were many reports stating that this fungus has been developed as a biological agent in various developed countries [6, 11, 12]. The further research indicated that the genus *Aschersonia* apart from being used as a pest control agent of *B. tabaci* can also suppress the development of rice blast disease caused by *Magnaporthe grisea* [13, 14]. This study was aimed to identify the morphological characters and efficacy of entomopathogenic fungi that infect *B. tabaci* and *D. citri*. Insect cadavers colonized by fungal mycelium were taken from three centers of soybean production in East Java Province.

MATERIALS AND METHODS

Description of the Study Area

The research was conducted in three centers of soybean production in East Java Province which are generally endemic to whitefly and in the laboratory of the Indonesian Legumes and Tuber Crops Research Institute. The research was carried out from 2019 to 2020 in dry season II (DSII) in order to obtain a population of both types of whitefly insects in the field in abundant conditions reaching their peak. Therefore, it was easy to find healthy and dead insects infected with entomopathogenic fungi. Insect cadaver of *B. tabaci* colonized by fungal mycelium were taken from three centers of soybean production in East Java, namely Banyuwangi, Jember, and Pasuruan Regencies. The cadaver of *B. tabaci* and *D. citri* were obtained from soybean plants intercropped with citrus and dragon fruit. Meanwhile, from intercropping of soybean and pumpkins in Pasuruan Regency were obtained only *B. tabaci* cadaver.

Exploration of Dead Insects Infected by the Fungus

The cadaver of *B. tabaci* and *D. citri* colonized with mycelium from the field were put into a plastic vial, then stored in a box with ice cubes to retain moisture. Each fungal isolate from insect cadaver obtained from each location was given an identity according to the type of plant and place of collection. The results of the exploration of all isolates from the field are then taken to the laboratory for preparation according to the Koch's postulate procedure which refers to the method of Faria and Wraight [6].

Isolation, Identification, and Efficacy of the Fungi

Insect cadaver from the field which was then taken to the laboratory then immersed in 0.5% chlorox solution for 3 min. After that, each isolate was put in sterile water for up to 1 min, then dried using sterile filter paper. Each isolate was grown on potato dextrose agar (PDA) in a laminar flow cabinet for 24 h in the dark then incubated for 96 h under irradiated conditions [15]. The fungal mycelium growing from each isolate was then observed using an Olympus Microscope CX31 which refers to the method of Faria and Wraight [6]. Each isolate was tested for its efficacy on egg, nymph, and imago of *B. tabaci* in a Petri dish. Then, from each isolate the fungus that grew was matched with the morphological characters of the fungus *Aschersonia* sp. [12, 16].

RESULTS AND DISCUSSIONS

Identification of Entomopathogenic Fungi

The results of isolation step in PDA media showed that the growth of all fungal mycelium starting 3 days after isolation (DAI) with visible shape of white colonies (Fig. 1), then with increasing age the colony turned to light brown (Fig. 2) after the age of 14 DAI (Table 1). Out of the thirteen fungal isolates obtained from three regencies in

East Java Province, none of them showed a difference in colony color, even though they were isolated from two different types of insects, whitefly (*B. tabaci*) and citrus whitefly (*D. citri*).

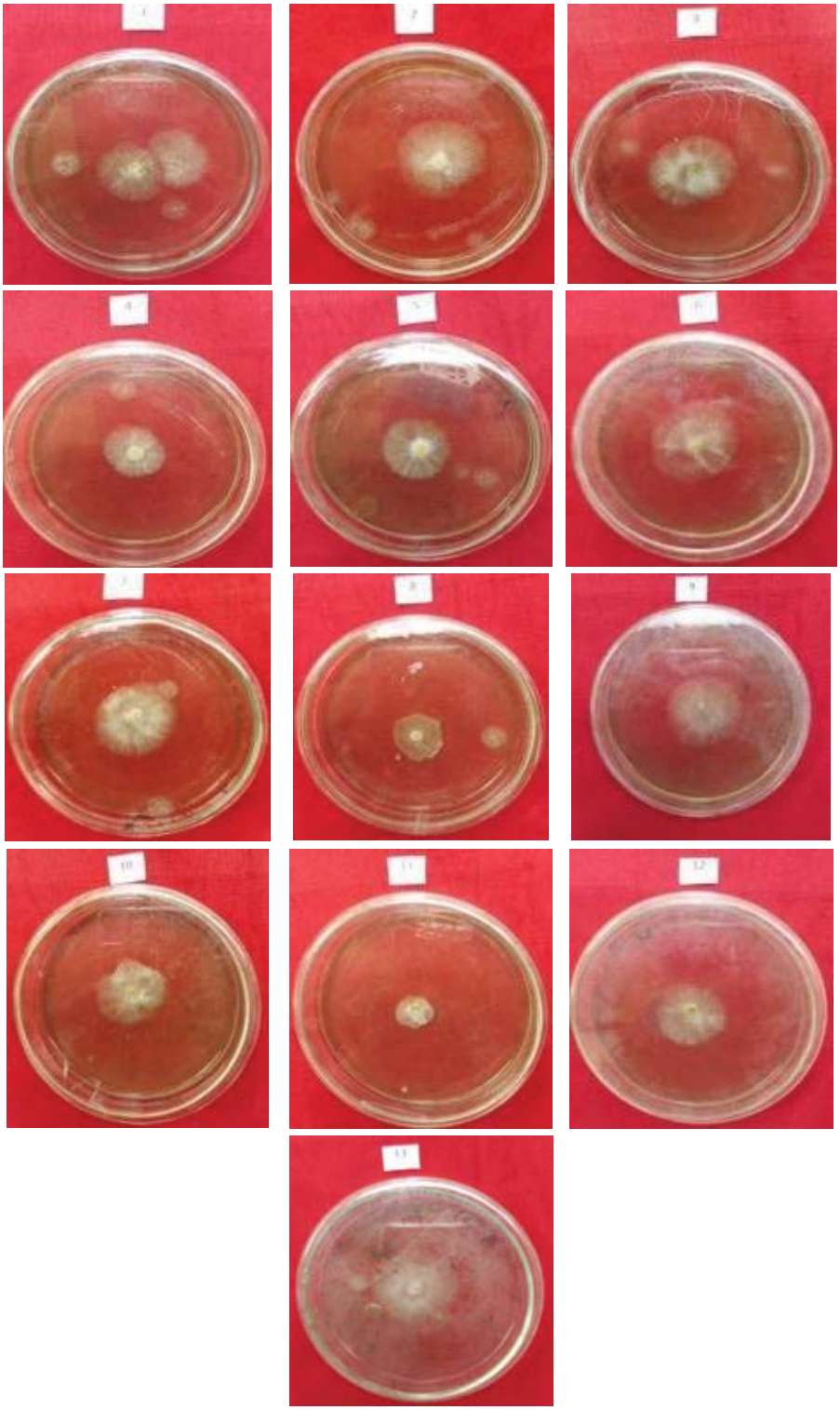

FIGURE 1. Colony shape and color of thirteen *Aschersonia aleyrodis* fungi isolates (3-day-old) obtained from three centers of soybean production in East Java Provinces.

(a) **(b)** **(c)**

FIGURE 2. Changes of colony color of *Aschersonia aleyrodis* on PDA media became light brown after 14 DAI. (a) Jember isolate no. 3. (b) Banyuwangi isolate no. 7. (c) Pasuruan isolate no. 13.

The results of the identification of fungal isolates that have been obtained based on colony color, conidiophores shape and size, conidial shape and size, as well as the results of reisolating the isolates from the tested insect cadavers, the fungal isolates colonizing the insects *B. tabaci* and *D. citri* is *A. aleyrodis* [16–18]. The results of this identification support the observations of previous researchers which indicated that the fungus *A. aleyrodis* has a specific host, the group of insect pests from the order Hemiptera, especially the family Aleyrodidae [6, 17, 19, 20].

The results of microscopic observations on the size of the conidiophores, ranged from 39–40 × 2.1–2.3 µm. Fungal isolates obtained from the cadavers of *B. tabaci* or *D. citri* did not show any differences in conidiophores size even though they were found in different intercropping systems. The largest size of conidiophores was found in fungal isolates obtained from soybean + citrus cultivation from Jember Regency or soybean + pumpkin in Pasuruan Regency. This condition might be due to the fact that the two regencies are close to each other, even though the fungi were isolated from different insects. In addition, the two insects are in the same family. Meanwhile, the fungi which were isolated from Banyuwangi Regency had a smaller conidiophore size compared to the size of the conidiophores from Jember or Pasuruan Regency, although it was not significantly different. The conidia size of the fungal isolates obtained from Banyuwangi Regency appears to be longer when compared to the isolates obtained from Jember or Pasuruan Regency. According to the report by Faria and Wraight [6], the size of *A. aleyrodis* conidiophores was up to 40 × 2–3 µm, while the size of conidia ranged from 10–12 × 2.0 µm, although the difference between conidiophore and conidia was not so significant.

Testing of temperatures was only started from 25–30°C, this was due to the results of previous studies conducted by Saka *et al*. [14] which indicated that *A. aleyrodis* isolates preferred temperatures of 20°C, although there were also isolates that were tolerant to temperatures of 30°C [15]. Therefore, the temperature test was conditioned to the temperature which common in Indonesia so that the isolates obtained were also tolerant up to 30°C. The temperature suitability study showed that all the isolates obtained were more tolerant to high temperature, ranging from 27–30°C (Table 1). This condition indicates that all isolates have the same opportunity to be developed as biological agents, especially *B. tabaci* and *D. citri* in Indonesia, where population development of both types of pests occurs during the dry season. Thus, farmers or users will not experience losses due to the explosion of the two types of pests. In addition, farmers will no longer depend on the efficacy of synthetic pesticides whose prices are increasingly unreachable. In addition, there are various negative impacts caused by synthetic pesticides such as pollution to the environment and water sources as well as exposure to pesticides on crop products.

The time required for the fungal isolate to kill the tested insects was around 3.25–4.50 days after application (DAA). The results showed that all *A. aleyrodis* isolates obtained from Banyuwangi Regency only needed the shortest time to kill the *B. tabaci* test insects, namely 3.25–3.50 DAA, with a faster mycelium colonization rate (Table 2). The time of death of the tested insects was relatively longer required for the fungal isolates obtained from Jember Regency when compared to isolates from Banyuwangi Regency. Meanwhile, the fungal isolates obtained from Pasuruan Regency reached 4.00–4.50 DAA. This condition is thought to have the effect of differences in the microclimate of the isolates. Therefore, the fungal isolates required adjustment in the process of infection against the tested insects. Fungal isolates from Pasuruan Regency were obtained from soybean + pumpkin plants which had a relatively higher temperature when compared to fungal isolates obtained from soybean + citrus + dragon fruit intercropping plants in Banyuwangi Regency.

TABLE 1. Morphological characters of thirteen *Aschersonia aleyrodis* fungi isolates from three centers of soybean production in East Java Province.

No.	Origin of *A. aleyrodis* isolates	Insect	Colony color (DAI)		Conidiophore (µm)	Conidia (µm)	Temperature (°C)	Medium
			4	14				
1	Soybean + citrus (Kaliwates, Jember)	*B. tabaci*	White	Light brown	40 × 2–3	10.0 × 2.0	27.5	PDA
2	Citrus (Umbulsari, Jember)	*D. citri*	White	Light brown	40 × 2–3	10.0 × 2.0	28.0	PDA
3	Soybean + citrus (Umbulsari, Jember)	*D. citri*	White	Light brown	40 × 2–3	10.0 × 2.0	27.0	PDA
4	Soybean + citrus (Kencong, Jember)	*B. tabaci*	White	Light brown	40 × 2–3	10.0 × 2.0	27.5	PDA
5	Soybean (Ambulu, Jember)	*B. tabaci*	White	Light brown	39 × 2.1	10.0 × 2.0	28.0	PDA
6	Soybean + citrus (Tegaldlimo, Banyuwangi)	*D. citri*	White	Light brown	39 × 2.1	11.0 × 2.0	29.5	PDA
7	Soybean + citrus + dragon fruit (Tengaldlimo, Banyuwangi)	*B. tabaci*	White	Light brown	39 × 2.1	11.0 × 2.0	29.5	PDA
8	Soybean + citrus + dragon fruit (Jajag, Banyuwangi)	*B. tabaci*	White	Light brown	39 × 2.1	11.0 × 2.0	29.5	PDA
9	Soybean + citrus (Genteng, Banyuwangi)	*D. citri*	White	Light brown	39 × 2.1	11.0 × 2.0	30.0	PDA
10	Soybean + citrus (Gambiran, Banyuwangi)	*D. citri*	White	Light brown	39 × 2.1	11.0 × 2.0	30.0	PDA
11	Soybean + citrus (Tapan, Banyuwangi)	*B. tabaci*	White	Light brown	39 × 2.1	11.0 × 2.0	30.0	PDA
12	Soybean + pumpkin (Kejayan, Pasuruan)	*B. tabaci*	White	Light brown	40 × 2.1	10.0 × 2.0	29.5	PDA
13	Soybean + pumpkin (Wonorejo, Pasuruan)	*B. tabaci*	White	Light brown	40 × 2.1	10.0 × 2.0	29.5	PDA

DAI = days after isolation, PDA = potato dextrose agar.

The infectivity test of thirteen fungal isolates was able to kill the nymph and imago stages with conidia density above 7.10×10^7/ml (Table 2). However, the fungal isolates obtained from Banyuwangi Regency were able to kill the egg, nymph, and imago phases which only required a lower conidia density of $5–6 \times 10^6$/ml. Eggs are the initial phase of insect pest breeding, so the biological agents capable of killing the egg phase have a better advantage over synthetic pesticides. This condition is due to the biological agents being able to suppress the development of the insect population early, so that the opportunity for population explosion is difficult to occur. Another advantage of the isolates obtained from Banyuwangi Regency was that they were able to infect and colonize the egg phase of *B. tabaci*. Therefore, all the eggs colonized by the fungus were ultimately unable to hatch to form nymphs. The nature of the entomopathogenic fungus which can kill the egg phase is called ovicidal. The advantages of the ovicidal properties of the fungus *A. aleyrodis* were also shown by the results of the study by Zhang *et al*. [1] which it was the egg phase of *B. tabaci*, so that the nymph population formed is increasingly limited. The superiority of ovicidal properties has also been reported by Prayogo [21] of the entomopathogenic fungi *Lecanicillium lecanii* brown stink bug eggs (*Riptortus linearis*), so that the development of the nymph population was limited.

The efficacy test carried out on *B. tabaci* insects at both the nymph and imago stages obtained the test insect mortality ranging from 55–89% (Table 2, Fig. 3). The highest mortality occurred in all fungi isolated from *B. tabaci* and *D. citri* were obtained from Banyuwangi Regency, ranging from 75–89%. Fungal isolates which were obtained from Jember and Pasuruan Regencies caused lower mortality in the tested insects. However, these isolates also had a high enough contribution as biological agents because they were able to kill the tested insects above 50%. The entomopathogenic fungi are a component of biological agents so that the efficacy of these agents is higher when combined with other components of biological control.

TABLE 2. Efficacy of thirteen *Aschersonia aleyrodis* entomopathogenic fungi in the egg, nymph, and imago stages of *Bemisia tabaci*.

No.	Origin of *A. aleyrodis* isolates	Insect	Density of conidia (per ml)	Infectivity on *B. tabaci*	Mortality (%)	Time of mortality (DAA)
1	Soybean + citrus (Kaliwates, Jember)	*B. tabaci*	7.10×10^7	Nymph and imago	70.50	3.50–3.75
2	Citrus (Umbulsari, Jember)	*D. citri*	7.20×10^7	Nymph and imago	67.00	3.50–3.80
3	Soybean + citrus (Umbulsari, Jember)	*D. citri*	7.25×10^7	Nymph and imago	61.50	3.50–3.75
4	Soybean + citrus (Kencong, Jember)	*B. tabaci*	7.18×10^7	Nymph and imago	70.50	3.50–3.80
5	Soybean (Ambulu, Jember)	*B. tabaci*	7.50×10^7	Nymph and imago	69.80	3.50–3.75
6	Soybean + citrus (Tegaldlimo, Banyuwangi)	*D. citri*	5.25×10^6	Egg, nymph, and imago	85.70	3.25–3.50
7	Soybean + citrus + dragon fruit (Tengaldlimo, Banyuwangi)	*B. tabaci*	6.10×10^6	Egg, nymph, and imago	75.60	3.25–3.50
8	Soybean + citrus + dragon fruit (Jajag, Banyuwangi)	*B. tabaci*	5.12×10^6	Egg, nymph, and imago	82.50	3.25–3.75
9	Soybean + citrus (Genteng, Banyuwangi)	*D. citri*	6.15×10^6	Egg, nymph, and imago	75.50	3.25–3.50
10	Soybean + citrus (Gambiran, Banyuwangi)	*D. citri*	6.25×10^6	Egg, nymph, and imago	81.00	3.25–3.50
11	Soybean + citrus (Tapan, Banyuwangi)	*B. tabaci*	5.30×10^6	Egg, nymph, and imago	89.50	3.25–3.50
12	Soybean + pumpkin (Kejayan, Pasuruan)	*B. tabaci*	7.50×10^7	Nymph and imago	56.60	4.25–4.50
13	Soybean + pumpkin (Wonorejo, Pasuruan)	*B. tabaci*	7.15×10^7	Nymph and imago	55.80	4.00–4.50

DAA = days after application.

(a) **(b)** **(c)**

FIGURE 3. The efficacy test for *Aschersonia aleyrodis* isolates against the nymphs and imago of *Bemisia tabaci* in the laboratory. (a) Jember isolate no. 3. (b) Banyuwangi isolate no. 7. (c) Pasuruan isolate no. 13.

The time required for the fungal isolate to cause mortality on the tested insects was around 3.25–4.50 DAA. The results showed that all *A. aleyrodis* isolates obtained from Banyuwangi Regency needed the shortest time to kill the *B. tabaci* test insects, namely 3.25–3.50 DAA. The time of death for the tested insects was relatively longer than the fungal isolates obtained from Jember Regency, even the isolates from Pasuruan Regency one day longer. This condition might be due to the differences in the microclimate of the isolates obtained. Therefore, the fungal isolates

require adjustment in the process of infection against the tested insects. Fungal isolates from Pasuruan Regency were obtained from soybean and pumpkin intercropping which had relatively high temperatures when compared to fungi isolates obtained from intercropping plants between soybeans + citrus + dragon fruit.

The results of this study indicated that the different types of insects used as the source of the inoculum did not affect the differences in the species obtained. This condition is due to the fact that the two types of insects (*B. tabaci* and *D. citri*) belong to one order (Hemiptera) and one family (Aleyrodidae). Evans and Hywel-Jones [22] stated that the fungus *A. aleyrodis* has a more specific host insect, especially whitefly in various types of food crops and commercial plants, especially citrus plants. Meanwhile, several previous reports stated that *A. placenta* fungi isolates were more specifically found in scale insects from the order Cocoidae apart from whitefly from the order Aleyrodidae [23–25]. Therefore, *A. placenta* has become a biological agent that is growing quite rapidly in various developed countries where the majority of the levels of education and knowledge are higher so that the lifestyle of the people is also better when compared to developing countries [26, 27].

By obtaining the entomopathogenic fungi isolate *A. aleyrodis*, this is a new breakthrough in biological control against the pest *B. tabaci*, which recently reported that the *B. tabaci* pest is tolerant to various types of synthetic pesticides in Indonesia. According to some entomologists, biotype Q formed as a result of biotype B. This biotype change is caused by environmental stress that occurs as the result of excessive application of synthetic pesticides [28–32].

CONCLUSION

Based on the results of this study, it could be concluded as follows: (1) the fungal identification results obtained were *A. aleyrodis* based on the colony color, conidiophores size and shape, as well as conidia shape; (2) the source of inoculum obtained from two types of insects in the same order or family did not affect the genus and species of the isolates obtained; (3) the finding of *A. aleyrodis* fungi isolates is a new innovation in the field of biological control for controlling the pests of *B. tabaci* and *D. citri*, therefore, this technological innovation needs to be promoted immediately to farmers.

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