Biological control of *Bemisia tabaci* gennadius by using entomopathogenic fungi *Aschersonia aleyrodis*

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**Abstract.** *Bemisia tabaci* is the main pest of many food crops, plantations, and horticulture. This pest plays an important role as a vector of various types of viruses. This research was aim to study the efficacy of biological control of *B. tabaci* on soybean by using entomopathogenic fungi *Aschersonia aleyrodis*. The experiment was conducted in the screen house and laboratory of biopesticide, Indonesian Legumes and Tuber Crops Research Institute in 2018. The experiment consists of; the evaluation of LD\(_{50}\) and LT\(_{50}\) of *A. aleyrodis* fungi on nymph and adult stage, pathogenicity of *A. aleyrodis* on various stages of *B. tabaci*, the impact of *A. aleyrodis* fungi infection to *B. tabaci* life cycle, and persistence of *A. aleyrodis* on soybean. The result showed that LD\(_{50}\) of *A. aleyrodis* for controlling *B. tabaci* was used conidia density 10\(^{6}\)/mL with LT\(_{50}\) three days after inoculation. *A. aleyrodis* strain Aa-J18 obtained from *B. tabaci* was very pathogenic because it can kill nymph and adult of *B. tabaci* with the mortality up to 99%. Application of *A. aleyrodis* causing a decrease in fecundity of *B. tabaci* up to 83.84%, thwart egg hatching up to 96.78%, delayed egg hatching period up to three days, and shorten the period of a female adult up to 82.92%. Conidia suspension of *A. aleyrodis* that applied on the soybean surface in the afternoon was able to survive until the fifth week. Therefore, *A. aleyrodis* strain Aa-J18 has the opportunity to be used as a biological agent for controlling *B. tabaci* on soybean and potentially be used as an alternative to replacing chemical insecticide.

1. Introduction

Whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) is one of the important pests of soybean in Indonesia and all over the world. Apart from being a pest, it also plays as a vector of various important viruses namely geminivirus, ipomovirus, torradovirus, and carlavirus that infected agricultural pest [1-3]. *B. tabaci* is a polyphagous insect, attacking food crops, plantation crops, and horticultural plants [4,5]. Therefore, the population development of *B. tabaci* infield was very fast and very abundant year by year especially in the dry season on June-August [6,7].

Besides as a virus vector, *B. tabaci* also produces secretions namely honeydew which serves as a medium to grow black sooty mold. In the dry season, sooty mold grows fast covering the entire surface of the leaves that have honeydew. Therefore, leaves unable to carry out the complete photosynthesis because it was inhibited by sooty mold. Leaf surface covered by sooty mold resulting in dry and fall early, causing high yield losses. Soybean yield loss due to *B. tabaci* attack was varied from 50-80% [8]. This pest also causes crop failure because it acts as a virus vector [9-11].

Various control efforts have been carried out to suppress the development of *B. tabaci* such as planting tolerant varieties, technical culture, trap crops, crop rotation, and intensive use of chemical pesticides [12-15]. However, the population of *B. tabaci* increased continuously in the field because...
most of the insecticide formulations have been broken by this insect. Hereafter, \textit{B. tabaci} formed a new strain in the field [16,17]. The application of chemical insecticide has shortened the life cycle of \textit{B. tabaci} from egg to adult, which was only 12 days, especially in the dry season. Therefore, the appropriate and effective technology innovation for controlling \textit{B. tabaci} should be found to minimize yield loss.

The biological control by using predator, parasitoid, and entomopathogenic fungi has been reported to be able to suppress the \textit{B. tabaci} population and to minimize the pest resistance as well as resurgence [18-21]. \textit{Aschersonia aleyrodis} (Webber) is one of the entomopathogenic fungi in which able to infect the various type of insects that classified in the Homoptera order, especially the family of Diaspididae and Aleyrodidae [22,23]. The advantage of this fungus as compared with others was the time span required to kill the nymph of \textit{B. tabaci} is very short, only three days after application (DAA). Moreover, the conidium of \textit{A. aleyrodis} was able to survive in the leaf surface up to 30 DAA [22]. This phenomenon was marked by the mortality of adult \textit{B. tabaci} up to 90% at 30 DAA. Therefore, the objective of this study was to determine the biological control of \textit{B. tabaci} on soybean by using entomopathogenic fungi, \textit{A. aleyrodis}.

2. Methods

The research was conducted in the laboratory of biopesticide dan screen house of Indonesian Legumes and Tuber Crops Research Institute (ILETRI) in June – September 2018. This research was arranged by using randomized complete design, four treatments with five replicates. The treatments were; (1) evaluation of LD$_{50}$ and LT$_{50}$ of \textit{A. aleyrodis} on each stage of \textit{B. tabaci}, (2) evaluation of the pathogenicity of \textit{A. aleyrodis} on each stage of \textit{B. tabaci}, (3) impact of the \textit{A. aleyrodis} infection on life cycle of each stage of \textit{B. tabaci}, and (4) evaluation of \textit{A. aleyrodis} conidia persistence on soybean.

2.1. Mass rearing of \textit{A. aleyrodis} isolates

\textit{Aleyrodis} isolate (Aa-J18) was obtained from \textit{B. tabaci} that colonized by fungus mycelium from soybean plant in Jember, East Java 2018. This fungus already tests by postulate Koch on an adult of \textit{B. tabaci} under laboratory scale with the mortality up to 98% at seven days after inoculation (DAI). Fungus isolates then were grown on potato dextrose agar (PDA) in a glass Petri dish and were transferred in place with a temperature of 26 °C and 80% humidity. At 21 DAI, the fungus colony in each dish was mixed with 10 ml sterile water then the colony surface is scraped using a soft brush. Conidia suspension was filtered by using soft fiber, then the conidia density was counted using hemocytometer up to 10$^6$/mL$^{-1}$. Conidia suspension was added with adhesive 2 mL/L then be whipped before it is applied to test insect followed method developed by li et.al [24] and Zhang et.al [25].

2.2. Mass rearing of test insect

\textit{B. tabaci} was obtained from soybean in Kendalpayak Research Station, Malang, East Java, then maintained on soybean plant in the screen house of ILETRI. Mass rearing of test insects was carried out continuously until obtaining F2 generation that consists of eggs, first instar nymph, second instar, third instar, fourth instar, and adult, according to the amount required.

2.3. Evaluation of LD$_{50}$ and LT$_{50}$ of \textit{A. aleyrodis} on each stage of \textit{B. tabaci}

Trifoliate leaf was inserted into glass Petri dish (d=14 cm), at the bottom was coated with wet filter paper, the petiole was covered by wet cotton to keep the leaf fresh up to few weeks. Various stage of \textit{B. tabaci} that obtained from the laboratory was categorized based on eggs stage, first instar nymph, second instar, third instar, fourth instar, and adult, then was infested to leaf in glass Petri dish. Conidia suspension of 10$^6$, 10$^7$, and 10$^8$/mL$^{-1}$ were used to evaluate LD$_{50}$ and LT$_{50}$. 2 ml conidia suspension was applied on test insect and the mortality was recorded in order to determine LD$_{50}$ and LT$_{50}$ that refer to Qiu et.al [26]. Dead insects were incubated in glass Petri dish coated filter paper with humidity above 90%. Incubated insect bodies were observed using a binocular microscope to ensure that test insects were died due to infection of \textit{A. aleyrodis}. Colonization of \textit{A. aleyrodis} is characterized by the presence of white and orange mycelium on the surface of \textit{B. tabaci} bodies [27].
2.4. Evaluation of the pathogenicity of A. aleyrodis on various stage of B. tabaci

B. tabaci was infested on soybean at 35 DAP. Soybean plant was covered by white fiber in order to keep the insect test. Egg, first to fourth-instar of the nymph, and adult of B. tabaci were treated with A. aleyrodis. The number of 100 individuals from each stage was infested to each plant with five replicates. Test insect was sprayed with \(10^7/\text{mL}^1\) A. aleyrodis conidia suspension, 500 L/ha or 2 mL/plant. The application was done in the afternoon in order to protect A. aleyrodis conidia suspension from ultraviolet exposure. Dead insects due to infected and colonized by A. aleyrodis were counted up to seven days after application.

2.5. Effect of A. aleyrodis application against B. tabaci life cycle

Each stage of B. tabaci was infested to soybean at 21 DAP which was previously inserted into a plastic mylar (t: 30 cm; d: 10 cm). The test insect was sprayed with \(10^9/\text{mL}^1\) conidia density of A. aleyrodis suspension. Life cycle change in each stage of B. tabaci was observed.

2.6. Persistence of conidia A. aleyrodis on soybean leaf

Soybean plant at 35 DAP was put in the open space exposed to sunlight than was sprayed by conidia suspension of A. aleyrodis with a density of \(10^9/\text{mL}^1\). The application time were; (1) 07.00 am; (2) 10.00am; (3) 13.00pm; and (4) 15.00pm. Each treatment repeated five times. Persistence of A. aleyrodis was known from conidia growth attached to soybean leaf. Soybean leaf (1 g) was transferred into a mortar, added with 10 ml fresh water then mesh up. One ml leaf liquid formed was taken, then put into a Petri dish and added with 10 ml of PDA. Petri dishes were shaken clockwise slowly in order to mix grows media with leaf liquid homogeneously, then incubated for three days at room temperature (26 °C). The number of conidia that formed to be colonies on PDA was used to measure the conidia density and LT50 was required to measure the effectiveness and toxicity of entomopathogenic fungi that will be used as biological agents for pest management. The result showed that LD30 of A. aleyrodis on conidia density of \(10^7/\text{mL}^1\) has been able to kill various stages of B. tabaci (Tabel 1). LD50 of A. aleyrodis to kill the nymph of B. tabaci was different from LD 50 to kill the adult, but it was not significantly different. The higher the insect stage requires a higher conidia density. Meanwhile, LT50 of A. aleyrodis for nymph stage was 3.50-3.75 days and LT50 for an adult was 4.50 days. This result indicated that A. aleyrodis isolates used was more virulent because LD50 only at 6-7 x \(10^9/\text{mL}^1\) conidia density and LT50 only take 3.5 - 4.50 days.

This result was different from LD50 of isolates Aa005 of A. aleyrodis for killing B. tabaci in which required higher conidia density, \(10^7/\text{mL}^1\) and LT50 4-6 days [30]. This condition is caused by various factors including; the origin of fungal isolates, the nutritional quality of media growth used to produce conidia, application time, and host insect stage [31-33]. Isolates of A. aleyrodis used by Zhang et.al [30] were obtained from Dialeurodes citri (Ashmead) on citrus, however, this research used isolates of A. aleyrodis from B. tabaci. It is suspected that the difference in the origin of A. aleyrodis isolates used is one of the factors that significantly influence LD50 and LT50. This was also reported by Qiu et.al [26], LD50 of A. aleyrodis isolates obtained from B. tabaci was only use \(10^9/\text{mL}^1\) conidia density and within three days were able to kill B. tabaci up to 100%.

3. Results and discussion

Evaluation of LD50 and LT50 was required to measure the effectiveness and toxicity of entomopathogenic fungi that will be used as biological agents for pest management. The result showed that LD50 of A. aleyrodis on conidia density of \(10^9/\text{mL}^1\) has been able to kill various stages of B. tabaci (Tabel 1). LD50 of A. aleyrodis to kill the nymph of B. tabaci was different from LD 50 to kill the adult, but it was not significantly different. The higher the insect stage requires a higher conidia density. Meanwhile, LT50 of A. aleyrodis for nymph stage was 3.50-3.75 days and LT50 for an adult was 4.50 days. This result indicated that A. aleyrodis isolates used was more virulent because LD50 only at 6-7 x \(10^9/\text{mL}^1\) conidia density and LT50 only take 3.5 - 4.50 days.

3.1. Pathogenicity of A. aleyrodis on various stage of B. tabaci

The results showed that A. aleyrodis was very pathogenic for controlling B. tabaci because B. tabaci was dead within 48 hours after inoculation (HAI). Meanwhile, colonization of white fungus of A. aleyrodis mycelium on the body of insects was seen from 72 HAI, then the mycelium developed to cover the entire surface of the insect’s body (Figure 1). The pathogenicity of fungi was known from the mortality of each stage of B. tabaci, which ranges from 88-98% within three to four days after
inoculation (Figure 2). The highest mortality of \textit{B. tabaci} occurred in nymph instar 1, instar 2 and instar 3 (98%), while the mortality in instar 4 was 95%. However, the lowest mortality (88.15%) occurred in the adult stage.

| \textit{B. tabaci} stages | \text{LD}_{50} \text{ (conidia/mL)} | \text{LT}_{50} \text{ (days)} |
|---------------------------|----------------------------------|------------------|
| Instar 1                  | $6.85 \times 10^6$ a             | 3.50 a           |
| Instar 2                  | $6.85 \times 10^6$ a             | 3.50 a           |
| Instar 3                  | $7.88 \times 10^6$ a             | 3.75 a           |
| Instar 4                  | $7.97 \times 10^6$ a             | 3.75 a           |
| Imago                     | $8.61 \times 10^6$ a             | 4.50 b           |

In this study, \textit{A. aleyrodis} was more pathogenic because the mortality of \textit{B. tabaci} is higher than the mortality of \textit{A. aleyrodis} reported by Meekes et.al [23], which is only 70%. The results of the study conducted by Zhang et.al [25] showed that the pathogenicity of \textit{A. aleyrodis} isolate Aa005 was lower than the results from this study because of \textit{A. aleyrodis} only able to kill \textit{B. tabaci} 80%. Therefore, isolates of \textit{A. aleyrodis} from this study has great prospects to be used as a potential biological agent for controlling \textit{B. tabaci}. This phenomenon was characterized by the number of insects that die quite a lot due to the infection of \textit{A. aleyrodis}, besides that the colonization of fungi in the body of \textit{B. tabaci} grows relatively quickly. Insect carcasses colonized by \textit{A. aleyrodis} fungi created abundant conidia and these conidia are potential to be sources of inoculums that serve as the transmission of pathogens to cause epizootics. The more conidia formed from colonization in insect carcasses, the more effective the biological agent for causing epizootics and for suppressing the development of pest populations in the field [34,35].

3.2. Impact of \textit{A. aleyrodis} infection to fecundity and hatchability of \textit{B. tabaci}

The results showed that the application of \textit{A. aleyrodis} conidia could have a significant effect on fecundity, hatchability, hatching period, a lifetime of \textit{B. tabaci} female. \textit{B. tabaci} female infected with \textit{A. aleyrodis} was only able to produce 15.20 eggs/female, while a healthy female was able to produce eggs up to 98.10 eggs/female (Table 2). \textit{A. aleyrodis} was ovicidal because it is able to thwart egg hatch up to 96.80%, only 3.20 eggs capable to hatch from totally 100 eggs. The impact of \textit{A. aleyrodis} infection can be seen from the hatching period of \textit{B. tabaci} eggs which is up to three days late (9.80 days) when compared to normal eggs (6.70 days). Moreover, \textit{A. aleyrodis} infection caused the life of a female to be limited to only 3.50 days, while the lifetime of a healthy female was 20.50 days.

Huang et.al [36] stated that entomopathogenic fungal infections can shorten the lifetime of \textit{B. tabaci} adult up to 9 days when compared to healthy adult which is up to 16.80 days. This condition is caused by the entire blood cavity of the insect (hemocoel) and hemolymph contaminated by a toxin produced by the fungus. At that time, there was an increase in blood pH which caused the disruption of the nervous system of the insect, then the insect stopped their activity and finally died [37,38]. Huang et.al [36] and Kaur et.al [39] reported that entomopathogenic fungal infection was able to shorten the lifetime of female \textit{B. tabaci} and also reduce the fecundity and the number of eggs produced by the female. Moreover, the influence of entomopathogenic fungal infections can thwart the egg hatch so that it is very effective for suppressing the pest population and their development [32,40]. Another study stated that the \textit{Lecanicillium lecanii} fungus was able to thwart the egg hatching of \textit{Riptortus linearis} and delayed hatching period, the nymphs formed were also abnormal or malformed and finally died [41]. Torrado-Leon et.al [42] confirmed that entomopathogenic fungal infections such as \textit{B. bassiana} were not only able to reduce the fecundity but also caused the malformation of nymphs so that \textit{B. tabaci} insects cannot alive because they die prematurely. The results of this study informed that the infection of \textit{A. aleyrodis} has a negative impact on fecundity, the number of eggs hatched, and a lifetime of the
female so that the fungus can suppress the development of the *B. tabaci* population. Therefore, *A. aleyrodis* has great prospects as a biological agent for controlling *B. tabaci*.

![Image](image1.png)

**Figure 1.** *B. tabaci* nymph infected and colonized by *A. aleyrodis*.

![Image](image2.png)

**Figure 2.** Mortality of *B. tabaci* stages that infected by *A. aleyrodis* at four days after application.

![Image](image3.png)

**Table 2.** Impact of *A. aleyrodis* infection to fecundity and the number of egg hatch.

| Variable                | *A. aleyrodis* infection | Control  |
|-------------------------|--------------------------|----------|
| Fecundity (egg)         | 15.20 b                  | 94.10 a  |
| Egg hatch (egg)         | 3.20 b                   | 99.50 a  |
| Hatching period (days)  | 9.80 b                   | 6.75 a   |
| Female lifetime (days)  | 3.50 b                   | 20.50 a  |

3.3. Persistence of *A. aleyrodis* conidium on soybean leaf

Persistence of *A. aleyrodis* conidium assessed from the number of conidia grows and creates a colony on PDA. The results of isolation of *A. aleyrodis* showed that *A. aleyrodis* conidia that grew and create colonies were quite a lot to cover the entire surface of the media, especially in the treatment of P4 (Figure 3). The highest *A. aleyrodis* colonies in the first week occurred in P4, reached 151 colonies, while the lowest found in P1, 49 colonies. Moreover, the number of colonies grew in P2 and P3 in the first week were 52 and 76 colonies, respectively.
Persistence of *A. aleyrodis* conidia on soybean leaves decreases with increasing the exposure time in the field, especially in P1, P2 and P3 (Figure 4). The decreased persistence of *A. aleyrodis* conidia in P1, P2, and P3 appeared to be significant after the third weeks, even in the fourth weeks, the conidia were not found. However, the persistence of *A. aleyrodis* conidia in P4 reached 78.65% because there was a decreasing of about 21.35%. Up to fifth weeks, *A. aleyrodis* conidia were survived on the surface of soybean leaves because there were 32.50 conidia grew into colonies.

![Figure 3. The colony of *A. aleyrodis* at P4 (a) and P1 (b) on the first week, and P2 on the second week (c).](image)

The decreasing of *A. aleyrodis* persistence applied in the morning up to noon (07.00-13.00) be suspected because the conidia wall was damaged due to lysis from ultraviolet exposure so the conidia are unable to grow. The study of Rodrigues et.al [43] indicated that the conidia of *Beauveria bassiana* and *Metarhizium anisopliae* which are exposed to ultraviolet light for five minutes can cause conidia to die up to 50%. Braga et.al [44] stated that ultraviolet has a negative impact, which is delaying the conidia germination of *L. lecanii*, even the conidia could not grow due to death.

![Figure 4. *A. aleyrodis* population that grew on PDA after exposed on the surface of soybean leaves for several weeks.](image)

The results of this study provide information that conidia persistence takes place up to five weeks if applied in the afternoon. Therefore, controlling *B. tabaci* using *A. aleyrodis* is recommended to be conducted in the afternoon. This is due to the conidial will last longer and conidia will not experience desiccation due to the wall damage as a consequence of ultra-violet rays. However, the persistence of conidia can withstand the exposure of ultraviolet in the field if adhesive oil is added [45-47]. The
addition of adhesive compounds can increase the humidity in the conidia wall so that it can minimize the impact of ultraviolet rays and the conidia germination is not disturbed [48-50].

4. Conclusion

*A. aleyrodis* is very pathogenic for controlling *B. tabaci* with LD<sub>50</sub> at a conidia density of 10<sup>6</sup>/mL<sup>1</sup> and LT<sub>50</sub> within three days after application. Application of *A. aleyrodis* has a negative impact on fecundity, the number of eggs hatch, hatching period, and shorten female lifetime of *B. tabaci*. Application of *A. aleyrodis* conidia suspension in the morning up to afternoon (07.00am-13.00 pm) caused conidia become unable to survive on the surface of soybean leaves due to the exposure of ultraviolet rays. *A. aleyrodis* has great potential to be used as a biological agent for controlling *B. tabaci* and can be used as an alternative to replace chemical insecticides.

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