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BIOLOGY OF RED SCALE INSECT, *Aonidiella aurantii* AND ITS BIOLOGICAL CONTROL USING *Beauveria bassiana* AND *Metarhizium anisopliae*

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ABSTRACT

In many regions, including Indonesia, red-scale insect, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) is regarded as a major citrus pest. To reduce the negative impact of pests on citrus production, innovative techniques based on entomopathogenic fungi (EPF) might be an effective alternative for controlling *A. aurantii*. This work aimed to investigate the biology of *A. aurantii*, find the most effective phase for applying EPF, and test the pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* toward *A. aurantii*. Single application and combining these two EPFs at 10^6 and 10^7 conidia mL^{-1} were assayed. It was found that EPFs should be applied to the first instar of *A. aurantii* before the wax layer develops to improve its penetration capacity. As a result, EPF concentration influences the mortality rate of *A. aurantii*. *M. anisopliae* (52.0 and 95.0%) caused more mortality in *A. aurantii* compared to *B. bassiana* (60.0 and 72.5%) 10^6 and 10^7 conidia mL^{-1}, respectively. The pathogenicity of *M. anisopliae* itself is even equivalent to the pathogenicity caused by their consortium. This research concluded that, under laboratory study, *A. aurantii* was effectively controlled by *B. bassiana* and *M. anisopliae*.

Keywords: Citrus, consortium, entomopathogenic fungi, pathogenicity

ABSTRAK

Di banyak kawasan di dunia, termasuk Indonesia, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) dianggap sebagai perosak utama sitrus. Untuk mengurangkan kesan negatif perosak terhadap pengeluaran sitrus, teknik inovatif berasaskan kulat entomopatogenik merupakan alternatif yang berkesan untuk mengawal *A. aurantii*. Tujuan kajian ini dijalankan adalah bagi mengkaji biologi *A. aurantii* untuk mencari fasa yang paling berkesan untuk menggunakan kulat dan menguji kepatogenan *Beauveria bassiana* dan *Metarhizium anisopliae* terhadap *A. aurantii*. Kaedah yang digunakan dalam kajian ini adalah secara semburan tunggal dan gabungan kedua jenis kulat pada kepekatan 10^6 dan 10^7 konidia mL^{-1}. Kajian ini mendapati bahawa kulat perlu digunakan pada instar pertama *A. aurantii*. Kepekatan kulat mempengaruhi kadar kematian *A. aurantii*. Kepekatan *M. anisopliae* pada 10^6 dan 10^7 konidia mL^{-1} menyebabkan lebih banyak kematian *A. aurantii* (52.0 dan 95.0%) daripada *B. bassiana* (60.0 dan 72.5%). Kepatogenan *M. anisopliae* sendiri adalah setara dengan kepatogenan yang disebabkan oleh konsortiumnya. Kajian ini menyimpulkan bahawa *B. bassiana* dan *M. anisopliae* berkesan bagi mengawal jangkitan *A. aurantii*.

Kata kunci: Kulat entomopatogenik, konsortium, kepatogenan, sitrus

INTRODUCTION

Red scale insect, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) is a commonly found pest in various horticultural production areas (Badary & Abd-Rabou 2010; Belguendouz-Benkhelfa et al. 2013; Mo 2018; Wicaksono & Endarto 2018). Particularly, this species is considered a serious pest of citrus in many parts of the world (Badary & Abd-Rabou 2010; El Hassane et al. 2020). In Indonesia, red scale pest has been reported to infest several citrus varieties (Efendi 2009; Syafitri et al. 2017). Defoliation, twig die-back, and finally the death of the tree can result from severe red-scale infestations. Bark longitudinal cracking can also happen when significant infestations occur on tree trunks and branches. Severe infestations can also result in fruit drop in addition to pockmarking (Grout 2012). This will result in economic losses due to a decrease in citrus productivity, as Wicaksono & Endarto (2019) reported that citrus production in Indonesia experienced a decline (1.49%) due to pest attacks.

Several techniques have been developed to control *A. aurantii* (Grafton-Cardwell et al. 2021; Pekas 2010; Vacas et al. 2009; Walker et al. 1991; Wicaksono & Endarto 2018). Recently, biological control techniques, including the use of predators, parasitoids, and entomopathogenic microorganisms, have become popular because they are relatively easy, cheap, and safe for humans and the environment (Badary & Abd-Rabou 2010; Dao et al. 2017; El Hassane et al. 2020; Murdoch et al. 2006; Pekas 2010; Permata 2009). Several studies have reported the efficacy of entomopathogen fungi (EPF) against *A. aurantii*, such as *Aspergilllus flavus* and *Fusarium oxysporum* (El Hassane et al. 2020). Other important citrus pests have also been reported to be controlled by EPF. *Beauveria bassiana* effectively controls thrips *Scirtothrips citri* Moulton (Thysanoptera: Thripidae), psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), and red mite *Panonychus citri* McGregor (Acari: Tetranichydae) (Shi & Feng 2006; Ullah et al. 2018; Zahn et al. 2013). In addition, other EPF such *Metarhizium anisopliae* have also been reported to be effective in controlling various species of major pests on rice and tea, peach fruit fly *Bactrocera zonata* Saunders (Diptera: Tephritidae), and *D. citri* on citrus (El-Gendy et al. 2022; Kaushik & Dutta 2016; Lezama-Gutiérrez et al. 2012; Peng et al. 2021). Although these two EPFs have been widely utilized to control a variety of pests, no

studies demonstrate the effectiveness of *B. bassiana* and *M. anisopliae* in controlling *A. aurantii*.

Generally, the biocontrol efficacy of EPF is supported by the susceptible host. Its application at the prone development stage of pests will increase the effectiveness of biocontrol (Mascarin & Jaronski 2016; Peng et al. 2021; Sharma et al. 2020; Walker et al. 1991). Previously, El-Gendy et al. (2022) found that *M. anisopliae* and *B. bassiana* had significant lethal effects at the end of the pupal stage of *B. zonata*, while the pre-nymph male and adult male of *A. aurantii* were more vulnerable to EPF compare to their female counterparts (El Hassane et al. 2020). Thus, this research was conducted to study the biology of *A. aurantii,* to determine the effective phase in applying EPF, and to assay the pathogenicity of *B. bassiana* and *M. anisopliae* against *A. aurantii*.

MATERIALS AND METHODS

Entomopatogenic Fungi Preparation

Entomopathogenic fungi isolate *B. bassiana* and *M. anisopliae* used are from the collection of Integrated Laboratory, Balai Pengujian Standar Instrumen Tanaman Jeruk dan Buah Subtropika (BPSI Jestro), Ministry of Agriculture, Republic of Indonesia. Fungal isolates were first rejuvenated in potato dextrose agar (PDA) media and incubated for 14 days at 26°C. A total of 50 ml of sterile distilled water was put into a Petri dish containing fungal colonies and stirred gently to separate fungal conidia from the PDA. The separated fungal conidia were put into an Erlenmeyer with the addition of 20 µl of Tween 80, homogenized with a rotary shaker for 30 minutes, then diluted to the desired concentration (10^6 and 10^7 conidia mL^{-1}). All of these stages were carried out in a sterile manner.

Rearing of *Aonidiella aurantii* and Its Biological Study

Rearing of *A.aurantii* and its biological study were done in the Entomology Laboratory, BPSI Jestro. Red scale collected from lemon plants in the citrus orchard of BPSI Jestro. The rearing of *A. aurantii* was carried out in a Petri dish containing young lemon leaves (*Citrus limon*) placed on a sponge and covered with tissue at the edges. The tissue is used to create a boundary area because the crawler is still actively moving. Red-scale biological observations were carried out to determine which pest phases were susceptible to the biocontrol treatment. To study the biological development of *A. aurantii*, 40 crawlers that emerged from the imago were collected and put in a new media (each Petri consists of one crawler). The development of the crawler was observed daily until the end of its developmental stage (44 days).

Pathogenicity Assay of Entomopathogenic Fungi

Application of EPF was carried out on one-day-old crawlers. Six treatments of EPF in 40 repetitions were used in this study; *B. bassiana* 10^6 conidia mL^{-1}, *B. bassiana* 10^7 conidia mL^{-1}, *M. anisopliae* 10^6 conidia mL^{-1}, *M. anisopliae* 10^7 conidia mL^{-1}, a combination of *B. bassiana* and *M. anisopliae* 10^6 conidia mL^{-1}, and their combination at 10^7 conidia mL^{-1}. Each Petri consists of 3 crawlers. Spraying of EPF suspension was carried out at a distance of 5 cm. Treated pest was then kept at 30°C and the mortality of *A. aurantii* was observed every day until 12 days after treatment. Data were analyzed by the analysis of variance and further analyzed with the Tukey HSD test at 5% significance level.

RESULTS AND DISCUSSION

Biology of *Aonidiella aurantii*

Aonidiella aurantii is referred to as a crawler when it separates from its female imago. Crawlers are small, round, squishy, yellowish in color, and have short legs and a razor-sharp mouth that allows them to suck food fluids (Figure 1a). According to Grout (2012), crawlers are difficult to spot in the orchard without a magnifying glass, but when they settle, they become incapable of walking, and their bodies start to develop a soft, white covering that resembles scales.

We found that up to eight weeks, female *A. aurantii* can spawn 100–150 crawlers with an average of 2-3 crawlers per day. The crawler will leave the female's body by moving with its legs to find a suitable place to take nutrients from the host it is attached to. After receiving sustenance and a location to settle, the crawler will change shape, turn brownish, cease moving, and emit a very thin layer of white wax around its body within 4 to 7 hours (Figure 1b). Since the wax layer will get bigger as time passes, *A. aurantii* is best treated in the stage just before the wax layer becomes thicker and the scale body starts to harden.

The effectiveness of biocontrol can be affected by selecting the right pest stage when applying EPF. Therefore, to enhance the fungus's capacity to penetrate the insect's body, entomopathogens should be applied to the first instar of *A. aurantii* before the wax coating thickens. According to Grout (2012), the first instar nymphs are referred to as "white caps", and they get their name from the hard, reddish-orange covering that develops later on. The first instar lasts 4-7 days, while the imago longevity lasts 33-52 days, depending on host plants and temperatures (Badary & Abd-Rabou 2010). Compared to orange trees, lemon trees had higher *A. aurantii* abundance (Belguendouz-Benkhelfa et al. 2013), thus we used *C. limon* as the host of this pest in this study.

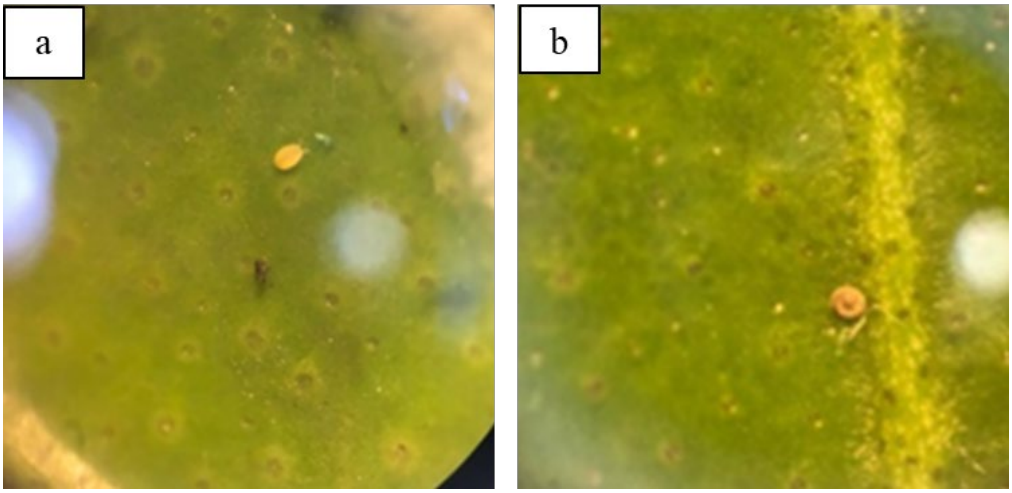

Figure 1. The first instar of *Aonidiella aurantii*; a: soft yellow tiny crawler immediately after leaving its imago's body, b: within 7 hours, the crawler turns brownish and emits a very thin layer of white wax around its body.

Here, this study documented the activity of *A. aurantii* for up to 44 days. The female imago has an orange-red color, which is easily removed (Figure 2). Before reaching the adult stage, females have another immobile nymphal instar. The mature female is easily visible with a diameter of 1 to 2 mm, especially on fruit and green growth. Males go through immobile pre-

pupal and pupal stages, which culminate in winged adults after the first instar stage. Except on sticky traps baited with synthetic female sex pheromones, winged male scales are rarely seen in orchards. The wings of the male red scale are greyish-brown in color. Their antennas are long and thread-like, with a dark stripe running across their backs (Grout 2012). This insect penetrates deeply into plant tissue with its mouthparts to extract sap from parenchyma cells. Citrus foliage, twigs, branches, and fruit are all severely poisoned by saliva injected by this pest (Badary & Abd-Rabou 2010). As shown in Figure 2, a distinctive yellow patch appears on the leaves and around each female scale. Extensive red-scale infestations can cause fruit loss, die-back of the twigs and leaves, and eventually, the death of the tree (Grout 2012).

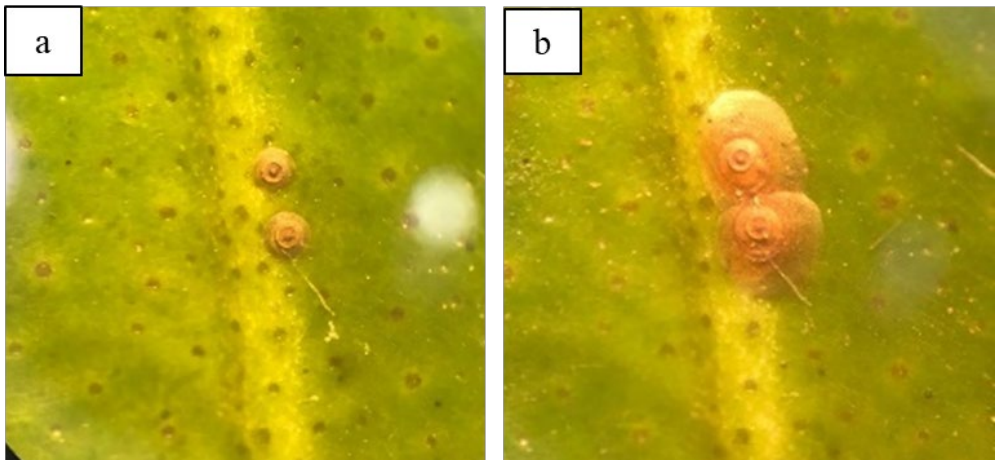

Figure 2. Yellow spots appear on the leaves and around the female scale; a: day 5, b: day 20.

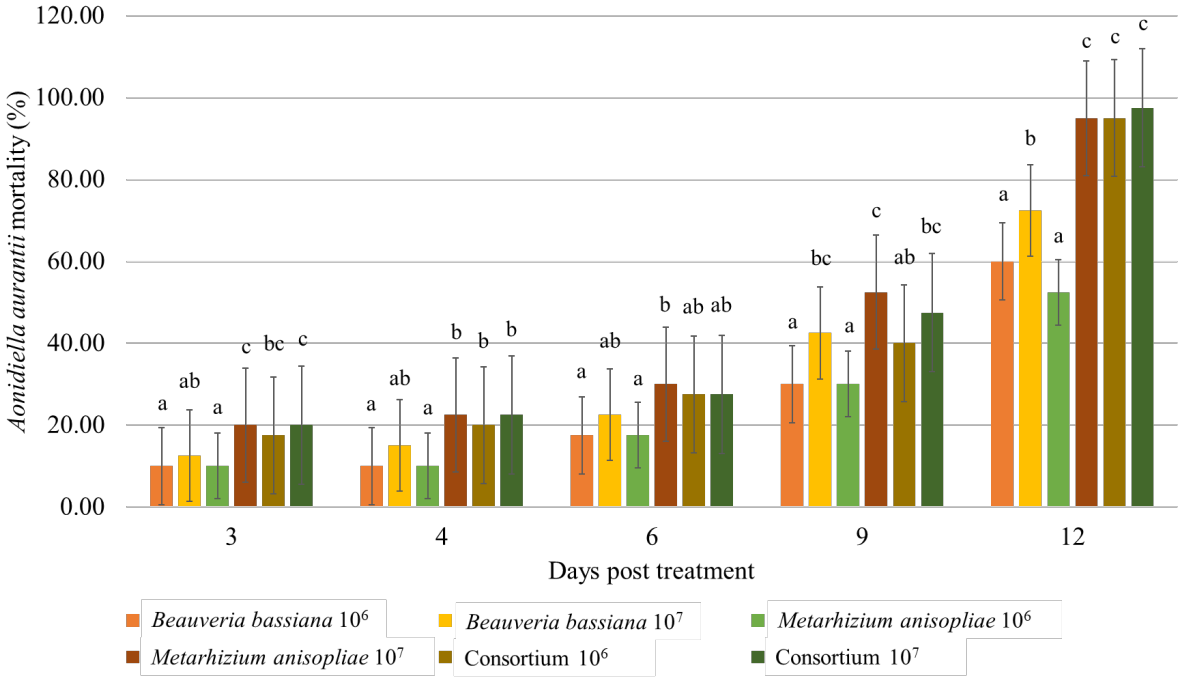

Figure 3. Mean % Mortality of *Aonidiella aurantii* toward the application of *Beauveria bassiana* and *Metarhizium anisopliae*. Different letters above the bars indicate significant differences among the treatments according to the Tukey HSD test ($P<0.05$).

Pathogenicity Test of *Beauveria bassiana* and *Metarhizium anisopliae* Against *Aonidiella aurantii*

Result presented in Figure 3 showed that, in general, higher concentrations of EPF were directly proportional to the mortality of *A. aurantii*. Entomopathogenic effects of *B. bassiana* and *M. anisopliae* began to appear on day 3 after treatment. However, up to six days post-treatment, the mortality of *A. aurantii* did not alter according to variations in *B. bassiana* concentrations. The effect of *B. bassiana* concentration was only visible on day 12; *B. bassiana* 10^6 and 10^7 conidia mL^{-1} caused 60.0 and 72.5% mortality, respectively. Similarly, the laboratory assay conducted by El-Gendy et al. (2022) resulted in 63.9% mortality of *B. zonata* due to the application of *B. bassiana*. We found that in the same concentration, *B. bassiana* is least effective in controlling *A. aurantii* compared to *M. anisopliae*. Accordingly, *B. bassiana* was found to be least effective in controlling *Helopeltis antonii* and *Pseudococcus jackbeardsleyi* compared to *Lecanicillium lecanii* (Anggarawati et al. 2017; Ginting et al. 2020).

Rather than its single application, *B. bassiana* showed better efficacy when combined with *M. anisopliae*. Prior studies revealed that *B. bassiana is* compatible with the wide spectrum of botanical/chemical pesticides and natural enemies in controlling pests and plant diseases (Bayu et al. 2021; Shi & Feng 2006). A combination of *B. bassiana* and entomopathogenic nematode *Heterorhabditis bacteriophora* significantly controlled *Rhynchophorus ferrugineus* (Wakil et al. 2017), while its combination with nucleopolyhedrovirus effectively controlled *Spodoptera frugiperida* (Gómez-Valderrama et al. 2022). Correspondingly, compared to its single application, the combination of *B. bassiana* and soap, and *B. bassiana* and pyridaben showed better efficacy against *P. jackbeardsleyi* and *P. citri*, respectively (Ginting et al. 2020; Shi & Feng 2006). These findings are supported by a previous study by Upamanya et al. (2020) that reported compatibility between *B. bassiana* and *M. anisopliae*, their combination even effectively reduced damping off disease incidence on brinjal.

Environmental biotic parameters like host age, susceptibility, and behavior, as well as abiotic factors like humidity, temperature, and solar radiation, all inherently impact efficacy (Mascarin & Jaronski 2016). According to Bayu et al. (2021), the earlier instars of nymphs such *Bemissia tabaci, Nezara viridula, Riptortus linearis*, and *Spodoptera litura* larvae are more sensitive to EPF than the later instars. This is because matured instars' integument surfaces are generally covered in a thick coating of wax, making it difficult for conidia to penetrate and infect. Likewise, in this investigation, EPF was given to the first instar of *A. aurantii* before the wax coating hardened. The concentration of EPF and their mode of action also influenced their ability to control pests. *B. bassiana* has the potential to eradicate all stages of insects on a variety of nuisance plants, including ovicidal ability, which can stop egg hatching, hence preventing the spread of pest populations and avoiding pest outbreaks. Their efficacy is also influenced by the production of toxins that can disrupt the nervous system and kill the host (Bayu et al. 2021; Keswani et al. 2013). Additionally, *B. bassiana*'s ability to inhibit soil-borne disease growth due to its endophytic nature is one of its benefits (Bayu et al. 2021).

In other insect pests species, symptoms of EPF infection can be seen in the growth of EPF hyphae from inside the insect's organs that emerge through the legs, antennae, and mouthparts (Bayu et al. 2021; Mascarin & Jaronski 2016), however, symptoms of EPF infection in *A. aurantii* are difficult to observe because they are small, transparent yellow, and actively moving. The initial instar of *A. aurantii*, which should last no more than 7 days, can

be halted by the application of *B. bassiana* and *M. anisopliae*. On the third day, the mortality of *A. aurantii* due to the EPF infection was noticed by brownish-yellow crawlers that did not move.

Since day three post-treatment, *M. anisopliae* consistently shows better biocontrol efficacy than *B. bassiana* and a consortium of these EPF. A single application of *M. anisopliae* 10^7 conidia mL^{-1} did not even differ from the combination of the two EPF in 10^6 and 10^7 conidia mL^{-1}, which caused *A. aurantii* mortality by 95.0, 95.0, and 97.5% at day 12, respectively. Similarly, El-Gendy et al. (2022) also reported that *M. anisopliae* was more virulent than *B. bassiana* in controlling peach fruit flies. In addition, Lezama-Gutiérrez et al. (2012) reported the effectiveness of this species in decreasing *D. citri* density in lime.

Over the last few decades, natural and biological control of cultivated plant pests and diseases has received increased attention to minimize agricultural production's reliance on chemical products. Using live organisms has proven to be an effective and long-term pest management strategy. These include the most well-known strains from the genera *Beauveria* and *Metarhizium* (Bamisile et al. 2021; Deka et al. 2021). They are the two principal EPF for biocontrol, primarily because of their widespread, global dispersion and simple mass production utilizing artificial media (Apriyanto et al. 2021; Mascarin & Jaronski 2016).

The biocontrol efficacy of *M. anisopliae* is supported by its ability to penetrate the host's body. The infection process can be broken down into the following steps: (1) conidia adherence to the host cuticle; (2) conidia germination and development; (3) appressorium production; (4) cuticle penetration; (5) colonization of hemolymph; and (6) extrusion and sporulation (Brunner-Mendoza et al. 2019; Chintkuntlawar et al. 2015; Devi 2018; Peng et al. 2022). Additionally, *M. anisopliae* produces a variety of toxins that are crucial in lowering host immune defense, harming the muscular system and Malpighian tubule, limiting host excretion, and impairing feeding and mobility (Devi 2018; Peng et al. 2022). Because of its low impact on the environment, safety for people and other mammals, and simplicity of mass production, *M. anisopliae* has been the subject of intensive research (Peng et al. 2021; Peng et al. 2022). According to reports, *M. anisopliae* can infect a wide range of arachnid and insect hosts, including agricultural pests and human disease vectors (Kaushik & Dutta 2016; Peng et al. 2021; Peng et al. 2022).

CONCLUSION

This study concluded that the prone stage of *A. aurantii* toward the application of EPF is their first instar. The mortality of *A. aurantii* commenced on the third day after application. As the EPF concentration grew, so did the mortality rate. In the same concentration level (10^7 conidia mL^{-1}), *M. anisopliae* caused higher mortality (95.0%) than *B. bassiana* (72.5%). In other words, the biocontrol efficacy of *B. bassiana* can be increased by combining it with *M. anisopliae*. However, the efficacy of their consortia (caused 97.5% mortality) is equivalent to the pathogenicity of *M. anisopliae* alone. We conclude that both *B. bassiana* and *M. anisopliae* had significant effects on *A. aurantii* control. This knowledge may be useful in integrated pest management and organic agriculture systems.

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AUTHORS DECLARATIONS

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Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
No ethical issue is required for this research.

Data Availability Statement
The data are also available in the undergraduate thesis of Faiqotul Jannah (2022).

Authors' Contributions
The authors state that Anang Triwiratno (AT), Susi Wuryantini (SW), Sudarminto Setyo Yuwono (SSY), Faiqotul Jannah (FJ), and Arrohmatus Syafaqoh Li'aini (ASL) are the primary contributors to this paper, while Agus Sugiyatno (AS), Joko Purnomo (JP), Cinta Badia Ginting (CBG), and Imro'ah Ikarini (II) serve as co-contributors. AT arranged the research concept and reviewed the manuscript. SW conducted the laboratory work and composed the manuscript, SSY reviewed the manuscript, FJ conducted the laboratory work and analyzed the data, ASL composed and evaluated the manuscript, AS and JP arranged the research concept, CBG reviewed the manuscript, and II analyzed the data.

REFERENCES

Anggarawati, S.H., Santoso, T. & Anwar, R. 2017. The use of entomopathogenic fungi *Beauveria bassiana* (Balsamo)Vuillemin and *Lecanicillium lecanii* (Zimm) Zare & Gams) for controlling *Helopeltis antonii* Sign (Hemiptera: Miridae). *Jurnal Silvikultur Tropika* 08(3): 197–202.

Apriyanto, D., Bustamam, H. & Zarkani, A. 2021. Effect of *Beauveria bassiana* against coffee bean borer, *Hyphothenemus hampei* (Ferrari) in small scale field trials. *Serangga* 26(3): 69–88.

Badary, H. & Abd-Rabou, S. 2010. Biological studies of the California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) under different host plants and temperatures with an annotated list of natural enemies of this pest in Egypt. *Egyptian Academic Journal of Biological* Science 3(1): 235–242.

Bamisile, B.S., Akutse, K.S., Siddiqui, J.A. & Xu, Y. 2021. Model application of entomopathogenic fungi as alternatives to chemical pesticides: prospects, challenges, and insights for next-generation sustainable agriculture. *Frontiers in Plant Science* 12: 741804.

Bayu, M.S.Y.I., Prayogo, Y. & Indiati, S.W. 2021. *Beauveria bassiana*: The eco-friendly and effective biopesticide to control pest and plant diseases. *Buletin Palawija* 19(1): 41–63.

Belguendouz-Benkhelfa, R., Biche, M., Adda, R. & Allal-Benfekih, L. 2013. Bio-ecology of a citrus pest (*Aonidiella aurantii* Maskell) (Hemiptera, Diaspididae): Spatiotemporal relationship with its host plants *Citrus limon* and *C. sinensis* in Algiers region. *American-Eurasian Journal of Sustainable Agriculture* 7(1): 14–20.

Brunner-Mendoza, C., Reyes-Montes, M.D.R., Moonjely, S., Bidochka, M.J. & Toriello, C. 2019. A review on the genus *Metarhizium* as an entomopathogenic microbial biocontrol agent with emphasis on its use and utility in Mexico. *Biocontrol Science and Technology* 29(1): 83-102.

Chintkuntlawar, P.S., Pramanik, A., Solanki, R. & Rathod, A. 2015. *Metarhizium anisopliae*: New trend entomopathogenic fungus for management of sucking pests in vegetable crops. *Popular Kheti* 3(1): 98–101.

Dao, H.T., Beattie, G.A.C., Spooner-Hart, R., Riegler, M. & Holford, P. 2017. Primary parasitoids of red scale (*Aonidiella aurantii*) in Australia and a review of their introductions from Asia. *Insect Science* 24(1): 150-168.

Deka, B., Baruah, C. & Babu, A. 2021. Entomopathogenic microorganisms: Their role in insect pest management. *Egyptian Journal of Biological Pest Control* 31: 1-8.

Devi, G. 2018. Nematophagous fungi: *Metarhizium anisopliae*. *International Journal of Environment, Agriculture and Biotechnology* 3(6): 268294.

Efendi, M. 2009. Distribusi hama kutu sisik merah *(Aonidiella aurantii)* pada perkebunan jeruk manis *(Citrus sinensis)* dan jeruk keprok *(Citrus reticulata)*. Undergraduate Thesis.

Jurusan Biologi, Fakultas Sains dan Teknologi, Universitas Islam Negeri Maulana Malik Ibrahim, Malang.

El Hassane, L., AbdelAziz, Z., Ayoub, H., Abdellah, A., aissam, E.F., Hassan, B. & Abdelhamid, E.M. 2020. Biological control of *Aonidiella aurantii* (Hemiptera-Diaspididae) by the entomopathogenic fungi *Aspergilllus flavus* and *Fusarium oxysporum*. *Journal of Entomology* 17(2): 48–57.

El-Gendy, I.R., Zawrah, M.F.M. & El-Banobi, M.I. 2022. Virulence effect of *Metarhizium anisopliae* (Met.) and *Beauveria bassiana* (Bals.) fungi against the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *Egyptian Journal of Biological Pest Control* 32(1): 1–7.

Ginting, S., Djamilah, Pamekas, T., Bustaman, H., Priyatiningsih, Sipriyadi & Wibowo, R.H. 2020. Pathogenicity of entomopathogenic fungi *Lecanicillium lecanii* and *Beauveria bassiana* against *Pseudococcus jackbeardsleyi* (Pseudococcidae) infecting rambutan. *Serangga*, 25(3): 1–11.

Gómez-Valderrama, J., Cuartas-Otálora, P., Espinel-Correal, C., Barrera-Cubillos, G. & Villamizar-Rivero, L. 2022. Fungal and viral entomopathogens as a combined strategy for the biological control of fall armyworm larvae in maize. *CABI Agriculture and Bioscience* 3(1): 24.

Grafton-Cardwell, E.E., Leonard, J.T., Daugherty, M.P. & Headrick, D.H. 2021. Mating disruption of the California red scale, *Aonidiella aurantii* (Hemiptera: Diaspididae) in Central California citrus. *Journal of Economic Entomology* 114(6): 2421–2429.

Grout, T. 2012. Red scale *Aonidiella aurantii* (Maskell). *Citrus Research International (Pty) Ltd.* III(3): 1–10. https://www.citrusres.com/wp-content/uploads/2021/08/Ch-3-11-4-Red-scale-Jan-2012.pdf [23 October 2023].

Kaushik, H. & Dutta, P. 2016. Establishment of *Metarhizium anisopliae*, an entomopathogen as endophyte for biological control in tea. *Research on Crops* 17(2): 375–387.

Keswani, C., Singh, S.P. & Singh, H.B. 2013. Beauveria bassiana: Status, mode of action, applications and safety issues. *Biotech Today* 3(1): 16-20.

Lezama-Gutiérrez, R., Molina-Ochoa, J., Chávez-Flores, O., Ángel-Sahagún, C.A., Skoda, S.R., Reyes-Martínez, G., Barba-Reynoso, M., Rebolledo-Domínguez, O., Ruíz-Aguilar, G.M.L. & Foster, J.E. 2012. Use of the entomopathogenic fungi *Metarhizium anisopliae, Cordyceps bassiana* and *Isaria fumosorosea* to control *Diaphorina citri* (Hemiptera: Psyllidae) in Persian lime under field conditions. *International Journal of Tropical Insect Science* 32(1): 39–44.

Mascarin, G.M. & Jaronski, S.T. 2016. The production and uses of *Beauveria bassiana* as a microbial insecticide. *World Journal of Microbiology and Biotechnology* 32(11): 1–26.

Mo, J. 2018. *Development of Phenology Models and A Timing Guide For The Management of Red Scale in Australian Citrus*. Sydney: Hort Innovation Publishing.

Murdoch, W.W., Swarbrick, S.L. & Briggs, C.J. 2006. Biological control: Lessons from a study of California red scale. *Population Ecology* 48: 297-305.

Pekas, A. 2010. Factors affecting the biological control of California red scale *Aonidiella aurantii* (Hemiptera: Diaspididae) by *Aphytis* (Hymenoptera: Aphelinidae) in Eastern Spain citrus: host size, ant activity, and adult parasitoid food sources. Doctoral Thesis. Departamento de Ecosistemas Agroforestales, Universidad Politécnica de Valencia, Valencia.

Peng, G., Xie, J., Guo, R., Keyhani, N.O., Zeng, D., Yang, P. & Xia, Y. 2021. Long-term field evaluation and large-scale application of a *Metarhizium anisopliae* strain for controlling major rice pests. *Journal of Pest Science* 94(3): 969–980.

Peng, Z.Y., Huang, S.T., Chen, J.T., Li, N., Wei, Y., Nawaz, A. & Deng, S.Q. 2022. An update of a green pesticide: *Metarhizium anisopliae*. *All Life 15*(1): 1141–1159.

Permata, P. 2009. Biology aspect and preying ability of *Harmonia axyridis* Pallas (Coleoptera: Coccinelidae) to the scale insect *Aonidiella aurantii Maskell* (Hemiptera: Diaspididae) on citrus plant. Undergraduate Thesis. Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Brawijaya, Malang.

Sharma, A., Srivastava, A., Shukla, A.K., Srivastava, K., Srivastava, A.K. & Saxena, A.K. 2020. Entomopathogenic fungi: A potential source for biological control of insect pests. In Solanki, M. (ed.), *Phytobiomes: Current Insights and Future Vistas*, pp. 225–250. Singapore: Springer Nature Singapore Pte Ltd.

Shi, W.B. & Feng, M.G. 2006. Field efficacy of application of *Beauveria bassiana* formulation and low rate pyridaben for sustainable control of citrus red mite *Panonychus citri* (Acari: Tetranychidae) in orchards. *Biological Control* 39(2): 210–217.

Syafitri, D.D., Fauzana, H. & Salbiah, D. 2017. Abundance of aphids and mealybugs of the Siam citrus plant (*Citrus nobilis* Lour.) in the Kuok Village, Kuok District, Kampar Regency, Riau Province. *Jom Faperta* 4(1): 1–11.

Ullah, M.I., Arshad, M., Abdullah, A., Khalid, S., Iftikhar, Y. & Zahid, S.M.A. 2018. Use of the entomopathogenic fungi *Beauveria bassiana* (Hyphomycetes: Moniliales) and *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) to control *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) under laboratory and semi-field conditions. *Egyptian Journal of Biological Pest Control* 28(1): 1–5.

Upamanya, G.K., Bhattacharyya, A. & Dutta, P. 2020. Consortia of entomo-pathogenic fungi and bio-control agents improve the agro-ecological conditions for brinjal cultivation of Assam. *3 Biotech* 10(10): 1–19.

Vacas, S., Alfaro, C., Navarro-Llopis, V. & Primo, J. 2009. The first account of the mating disruption technique for the control of California red scale, *Aonidiella aurantii* Maskell (Homoptera: Diaspididae) using new biodegradable dispensers. *Bulletin of Entomological Research* 99(4): 415–423.

Wakil, W., Yasin, M. & Shapiro-Ilan, D. 2017. Effects of single and combined applications of entomopathogenic fungi and nematodes against *Rhynchophorus ferrugineus* (Olivier). *Scientific Reports* 7(1): 1–11.

Walker, G.P., Richards, C.B., Jones, W.G. & Aitken, D. 1991. Toxicity of five insecticides used to control California red scale (Homoptera: Diaspididae) against susceptible red scale strains. *Journal of Economic Entomology* 84(1): 17–24.

Wicaksono, R.C. & Endarto, O. 2019. The role of kaolin in thrips pest control in orange fruit. *Jurnal Agronida* 5(1): 7–11.

Wicaksono, R. & Endarto, O. 2018. Efektifitas kapur gamping untuk mengendalikan kutu sisik (*Aonidiella aurantii*) pada tanaman apel. *Prosiding Seminar Nasional VI Hayati* 351–355.

Zahn, D.K., Haviland, D.R., Stanghellini, M.E. & Morse, J.G. 2013. Evaluation of *Beauveria bassiana* for management of citrus *Thrips* (Thysanoptera: Thripidae) in California blueberries. *Journal of Economic Entomology* 106(5): 1986–1995.