PATHOGENICITY OF ENTOMOPATHOGENIC FUNGI *Lecanicillium lecanii* AND *Beauveria bassiana* AGAINST *Pseudococcus jackbeardsleyi* (PSEUDOCOCCIDAE) INFECTING RAMBUTAN

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ABSTRACT

Pseudococcus jackbeardsleyi is an important pest that attacks rambutan fruit (*Nephelium lappaceum* L.). The *P. jackbeardsleyi* can cause severe damage in rambutan because it is able to survive in postharvest commodities. Therefore, is a need to control this pest to avoid losses. The species of *P. jackbeardsleyi* can be controlled by either chemical means using insecticides or biological means using entomopathogenic fungi. Between the two control measures, the use of entomopathogenic fungi is an environmental friendly control measure and causes no harm to human health. The purpose of this study was to examine the pathogenicity of entomopathogenic fungi *Lecanicillium lecanii* and *Beauveria bassiana* against *P. jackbeardsleyi* (Pseudococcidae) on rambutan. The application method was by spraying *P. jackbeardsleyi* with fungi conidia suspension. The data obtained was analysed using the Statistical Analysis System (SAS) program. The results showed that *L. lecanii* + soap was the most effective cause of mortality for *P. jackbeardsleyi*, then *L. lecanii*, and *B. bassiana* + soap, while the lowest was *B. bassiana*. Based on this study, the use of entomopathogenic fungi is effective against *P. jackbeardsleyi* in rambutan.

Keywords: Pathogenicity, *Lecanicillium lecanii*, *Beauveria bassiana*, *Pseudococcus jackbeardsleyi*, rambutan.

ABSTRAK

Pseudococcus jackbeardsleyi merupakan perosak penting yang menyerang buah rambutan (*Nephelium lappaceum* L.). Spesies *P. jackbeardsleyi* ini mampu menyebabkan kerosakan serius pada buah rambutan kerana spesies ini dapat bertahan pada peringkat komoditi lepas tuai. Oleh itu, spesies ini perlu harus dikawal agar tidak menyebabkan kerugian. Kawalan *P. jackbeardsleyi* dapat dilakukan secara kimia dengan menggunakan inseksitid ataupun dengan kawalan secara biologi menggunakan kulat entomopatogenik. Di antara kedua kawalan tersebut, penggunaan kulat entomopatogenik merupakan kawalan yang mesra alam dan tidak memberikan kesan

negatif terhadap kesihatan manusia. Kajian ini dijalankan adalah bertujuan untuk menguji kesan kepatogenan kulat entomopatogenik *Lecanicillium lecanii* dan *Beauveria bassiana* terhadap *P. jackbeardsleyi* pada rambuatan. Kaedah yang digunakan dalam kajian ini adalah secara semburan *P. jackbeardsleyi* dengan campuran konidia. Data yang diperoleh dianalisis menggunakan perisian *Statistical Analysis System* (SAS). Hasil kajian menunjukkan bahawa campuran *L. lecanii* + sabun paling efektif menyebabkan kesan mortaliti *P. jackbeardsleyi*, kemudian *L. lecanii* dan *B. bassiana* + sabun, sementara yang terendah adalah *B. bassiana*. Berdasarkan kajian ini, penggunaan fungi entomopatogenik adalah efektif terhadap kutu putih *P. jackbeardsleyi* pada rambutan.

Kata kunci: Patogenitas, *Lecanicillium lecanii, Beauveria bassiana*, *Pseudococcus jackbeardsleyi*, rambutan.

INTRODUCTION

Rambutan (*Nephelium lappaceum* L.) is a tropical fruit plant grown in the South East Asian region mainly in Indonesia. The fruit is of a great nutritious value to human health when consumed fresh or as processed fruit. In Indonesia, rambutan is one of the exported fruits and therefore serves a great economic value to the country. The exports of rambutan from Indonesia occupy the second largest position after Thailand (Silitonga 2000). Indonesia's rambutan exports were 47.1 tonnes in 2012 and increased to 66.8 tonnes in 2015 (Indonesia Ministry of Agriculture 2015).

One of the main problems faced by farmers in rambutan cultivation is the insect pests. The presence of insect pests in rambutan affects quality of the fruit and also reduce amount of fruits harvested by farmers. Some important insect pests that attack the rambutan plants including mealybug, *P. jackbeardsleyi* (Hemiptera: Pseudococcidae), *Parasa lepida* (Lepidoptera: Limacodidae), *Adoxophyes privatana* (Lepidoptera: Tortiricidae), *Conopomorpha cramerella* (Lepidoptera: Limacodidae), and *Adoxophyes privatana* (Lepidoptera: Tortiricidae) (Sobir & Martini 2014).

Pseudococcus sp. (Hemiptera: Pseudococcidae) is the commonest amongst all the insect pests that attack rambutan. The pest is widely spread throughout America, Africa, and Asian countries including Taiwan, India, Indonesia, Malaysia, Philippines, Thailand, Singapore, Sri Lanka, and Vietnam (Anura et al. 2012; Ben-Dov et al. 2006; Mani et al. 2013; N'Guessan et al. 2014). The mealybug *P. jackbeardsleyi* is passed to the market commodities by passive dispersal (Williams 2004). It is a big obtastacle to the export of fruits because it can survive in postharvest commodities. This is because the mealybug has a layer of white wax like flour all over the body. The mealybug is polyphagic and has several alternative host plants including *Annona* spp. (Magnoliales: Annonaceae), tomato, *Lycopersicon* spp. (Solanales: Solanaceae), banana, *Musa* spp. (Zingiberales: Musaceae), rambutan, *Nephelium lappaceum* L. (Sapindales: Sapindaceae), durian, *Durio zibethinus* Murray (Malvales: Bombacaceae,) pineapple, *Ananas comosus* (L.) (Poales: Bromeliaceae) and rose mallows, *Hibiscus* spp. (Malvales: Malvaceae) (Ben-Dov et al. 2006).

The mealybug is included in the New Zealand government quarantine list of fruits shipped from Indonesia (Biosecurity Act 2014). To overcome the effects of mealybug pest on plant fruits in Indonesia, farmers have always used chemical control measure which involves the use of pesticides such as Imidacloprid. According to Sartiami et al. (2009), Imidacloprid can reduce the population of mealybug on papaya by 40% after four applications, and 60% if the

application of Imidacloprid is combined with soapy water. Other chemical used to control mealybug long time ago was methyl bromide (Hansen et al. 2000). Although several chemicals have been used to control mealybug, little success has been achieved because the mealybugs have a thick waxy layer that is secreted to cover their body, hence resisting the effect from the chemical substances.

Besides the ineffectiveness of chemicals in control mealybug, the application of synthetic pesticides also has a negative impact on the environment and human health, either hence or so we need for an alternative control that is both environmental friendly and cause no harm to human health. One of the alternative measures is the use of entomopathogenic fungi (Ginting et al. 2020). The use of entomopathogenic fungi has several advantages compared to other biological agents, for example they are able to penetrate the body of insects and epizootic in nature. FitzGerald (2014) explains that isolates of *Metarhizium anisopliae* and *B. bassiana* have the potential to be developed as biological control agents to control mealybugs (*Planococcus citri*) and thrips (*Scirtothrips aurantii*) in citrus plants.

The virulence of *B. bassiana* is higher than *M. anisopliae* and *L. lecanii* at the concentration of $5x10^7$ conidia/ml, and can cause 98% mortality of *P. ficus* (Signoret) (Hemiptera: Pseudococcidae) (Mohamed 2016). *Beauveria bassiana* caused mortality of *P. marginatus* Williams and Granara de Willink faster than *M. anisopliae* and *V. lecanii*, due to faster penetration and colonization rates. Panyasiri et al. (2007) reported the correlation between the concentration of *M. anisopliae* and the percentage mortality of mealybug, *Pseudococcus cryptus* (Hempel). However, the information on pathogenicity of entomopathogenic fungi *L. lecanii* and *B. bassiana* against mealybug in rambutan has not been reported. Based on this description, this study aims to examine the pathogenicity of entomopathogenic fungi *L. lecanii* and *B. bassiana* against mealybug *P. jackbeardsleyi* in rambutan.

METRIALS AND METHODS

Study Site

This study was conducted from February until November 2019. The mealybugs, *P. jackbeardsleyi* were sampled from rambutan trees at Timur Indah, Bengkulu City, Indonesia and brought back to the Plant Protection Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Bengkulu for identification process and bioassay.

Species Identification

The *P. jackbeardsleyi* species was identified using species key (Williams & Granara de Willink 1992) before they were analysed for bioassay using entomopathogenic fungi.

Identification of Entomopathogenic Fungi

The entomopathogenic fungus used was a collection of the Plant Protection Laboratory, Department of Plant Protection, Faculty of Agriculture, Bengkulu University. Morphological identification of the fungi was carried out using Humber (1997). The optical microscope was used to make observations on the morphological features of the fungi colonies grown on Potato Dextrose Agar (PDA) media in petri dishes. The colony features observed include colony's color, shape, texture and edge shape. Other observations made include the forms of conidia and the presence of hyphae.

Preparation of the *B. bassiana* and *L. lecanii*

Fungi isolates of *B. bassiana* and *L. lecanii* were used for insect bioassay. The isolates were prepared from the entomopathogenic fungi grown on petri dishes with Potato Dextrose Agar (PDA) (potato 200g, dextrose 20g, agar 15g, chloramphenicol 0.5g, and aquadest 1L) at 21ºC. After 3 weeks, the fungi conidia were harvested, by adding 10 ml of sterilized aquades to the fungi colonies to form a suspension. Then 0.1% Triton X-100 was added to the suspension as an adhesive and grading agent. Its density was calculated using hemocytometer.

Pathogenicity of *B. bassiana* and *L. lecanii* against Nymph of *P. jackbeardsleyi*

Second instar of *P. jackbeardsleyi* nymphs were used for bioassay. A total of 5 nymphs of *P. jackbeardsleyi* were placed in a plastic container with a diameter of 6.5 cm and height of 4.5 cm and fed with rambutan fruit. Concentration of entomopathogenic fungi use for bioassay is shown in Table 1 (*B. bassiana* (Bb): Bb 10^6, Bb 10^7, Bb 10^8 (conidia/ml), Bb 10^6 + Soap 0.2%, Bb 10^7 + Soap 0.2%, Bb 10^8 + Soap 0.2%; *Lecanicillium lecanii* (LL): LL10^6, LL10^7, LL10^8 (conidia/ml), LL 10^6 + Soap 0.2%, LL 10^7 + Soap 0.2%, LL 10^8 + Soap 0.2%, soap 0.2%, and control (sterile water)). The soap used in the test was Benzalkonium Chloride. Each treatment was repeated three times. Fungal application was carried out by spraying nymphs with a conidia suspension of 10 ml (Tefera & Pringle 2003). Larval mortalities were recorded every day for five days after application.

Data Analysis

Data obtained were analyzed using the SAS program version 6.2. Duncan's Multiple Range Test at the 5% significance level was performed if there were differences between treatments. Determination of lethal concentration (LC); LC_{50}, LC_{75}, and lethal time (LT); LT_{50}, LT_{75} values was carried out by Probit Analysis (Finney 1971).

RESULTS

Mortality of *P. jackbeardsleyi* Nymphs after Treatments

All treatments caused mortality of *P. jackbeardsleyi* nymphs under laboratory conditions. The highest cumulative mortality rate of *P. jackbeardsleyi* nymphs recorded was 93% and 100% at concentrations of 10^7 and 10^8 (conidia/ml), respectively for entomopathogenic fungi treatments; *B. bassiana*, *B. bassiana* + soap, and *L. lecanii* + soap, and *L. lecanii* (Table 1).

Table 1 Cumulative mortality of *P. jackbeardsleyi* for five days after treatment with various concentrations of entomopathogenic fungi *B. basianna* and *L. lecanii*, and fungi mixture with soap.

Treatment	% Mortality (±SD)
Control (sterile water)	0±0.00a
Soap	13±0.67a
Bb 10^6 (conidia/ml)	53±0.58b
Bb 10^7 (conidia/ml)	73±1.15b
Bb 10^8 (conidia/ml)	93±0.58d
Bb 10^6 (conidia/ml) + Soap	80±1.00c
Bb 10^7 (conidia/ml) + Soap	87±0.58c
Bb 10^8 (conidia/ml) + Soap	100±0.00e
LL 10^6 (conidia/ml)	67±0.58b
LL 10^7 (conidia/ml)	73±0.58b

LL 10^8 (conidia/ml)	93±0.58d
LL 10^6 (conidia/ml) + Soap	80±1.00c
LL 10^7 (conidia/ml) + Soap	93±0.58d
LL10^8 (conidia/ml) + Soap	100±0.00e

*The number followed by the same letter in the same column is not significantly different according to Duncan's test at 5% significance level. Bb: *B. basianna*, LL: *L. lecanii*.
*Soap: Benzalkonium chloride 0.2%

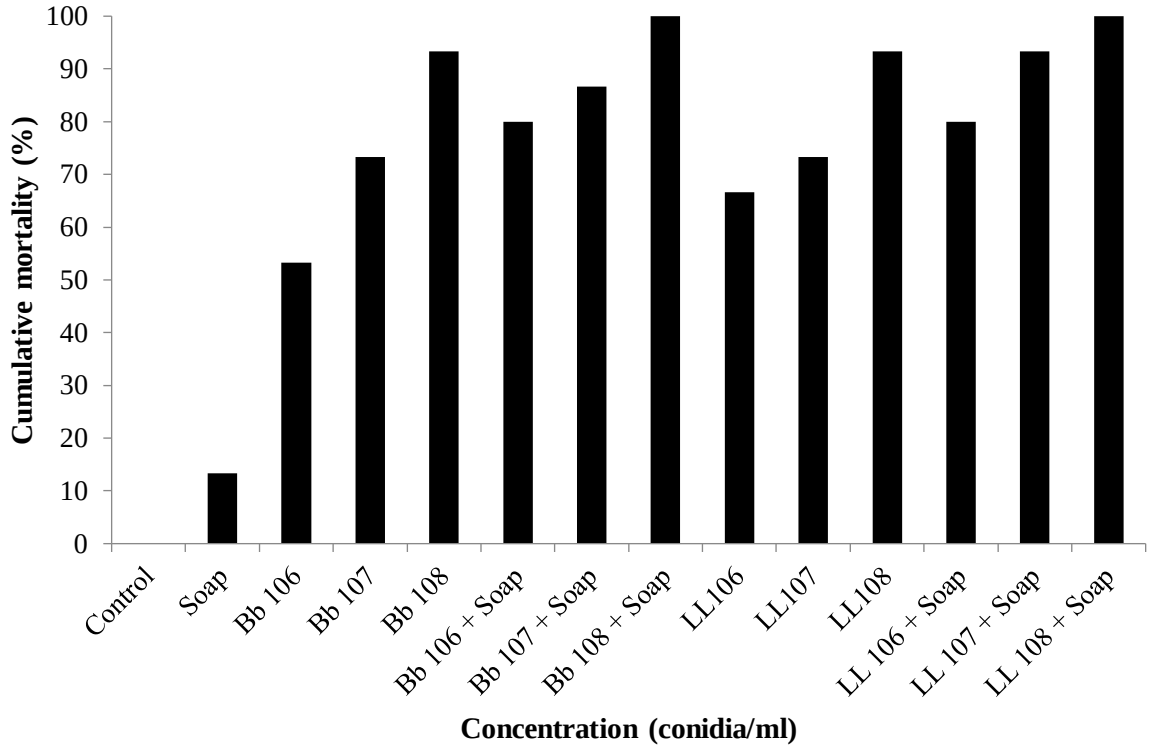

Figure 1 The cumulative mortality of *P. jackbeardsleyi* nymphs against treatment of various with various concentrations of entomopathogenic fungi, *B. basianna* and *L. lecanii*, and fungi mixture with soap

Lethal Concentration LC_{50} and LC_{75} of Treatments

The density of the conidia of fungus was used to determine the mortality of *P. jackbeardsleyi* nymphs. Based on the results of Probit Analysis on the fifth day observation, treatment with the LC_{50} and LC_{75} was found *L. lecanii* + soap, followed by *L. lecani*, *B. bassiana* + soap, *B. bassiana* (Table 2).

Table 2. LC_{50} and LC_{75} values of each treatment of entomopathogenic fungi, *B. basianna*, *L. lecanii*, and fungi mixture with soap

Treatments	**LC_{50} (conidia/ml)**	**LC_{75} (conidia/ml)**
Beauveria bassiana	21.6 x 10^8	2.83 x 10^9
Beauveria bassiana + soap	20.0 x 10^8	2.74 x 10^9
Lecanicillium lecanii	17.2 x 10^8	1.91 x 10^9
Lecanicillium lecanii + soap	16.8 x 10^8	1.87 x 10^9

Lethal Time LT_{50} and LT_{75} of Treatments

The ability of the treatments to cause mortality of *P. jackbeardsleyi* nymphs was shown by LT_{50} and LT_{75} values for each treatment against *P. jackbeardsleyi* nymphs at a density 10^8 (conidia/ml). The results showed that mixture of *B. basianna* and *L. lecanii* with soap as treatment gave the lowest lethal time values, LT_{50} (3.5 days) and LT_{75} (3.9 days). These treatments were followed by *L. lecanii,* and *B. bassiana* (Table 3).

Table 3 LC_{50}, LC_{75} values of with various concentrations of entomopathogenic fungi, *B. basianna* and *L. lecanii*, and fungi mixture with soap against *P. jackbeardsleyi* nymphs

Treatments	**LT_{50} (Day)**	**LT_{75} (Day)**
Beauveria bassiana	3.8	4.2
Beauveria bassiana + soap	3.5	3.9
Lecanicillium lecanii	3.6	4.05
Lecanicillium lecanii + soap	3.5	3.9

Growth of white mycelia after mortality of *P. jackbeardsleyi* nymphs

Observation through the microscope shown growth of white mycelia from *P. jackbeardsleyi* nymphs' cadavers (Figure 2).

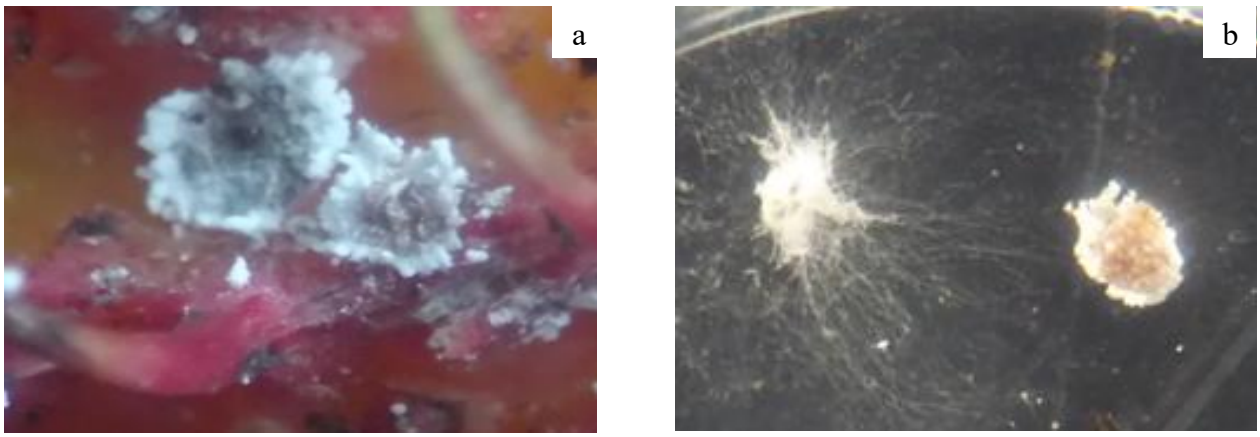

Figure 2. a) White mycelia formed on *P. jackbeardsleyi* nymphs cadavers by a, *B. bassiana*; b), *L. lecanii.*

DISCUSSION

The difference in concentration of treatments cuased the differences in conidia density hence differences in percentage mortality of *P. jackbeardsleyi* nymph. The treatments with high conidia density gave greater opportunities for conidia to penetrate into the body of *P. jackbeardsleyi* nymphs and cause mortality. The highest *P. jackbeardsleyi* nymph mortality was at the conidia density Bb 10^8 + 0.2% soap and LL 10^8 + 0.2% soap which reach 100% mortality. The lowest *P. jackbeardsleyi* nymph mortality was with Bb 10^6 treatment. These results correspond with the findings from a number of studies. Shinde et al. (2010) explains that one of the factors of entomopathogenic fungal infections in insects is the number of inoculums. Inglis et al. (2001) reported the relationship between the virulence of *B. bassiana, M. anisopliae,* and the

concentration of entomopathogenic fungi for insect pests. The higher conidia density was applied, so the chance of conidia contacts greater with pests, that it gives more opportunities to penetrate the pest body. Chavan and Kadam (2009) also reported that mortality of mealybug *M. hirsutus* nymphs was 82.5% with *L. lecanii* application. Furthermore, Jeyarani et al. (2011) reported that *B. bassiana* was effective than *Cladosporium cladosporioides* (Fresenius) against papaya mealybug, *Paracoccus marginatus*. Moreover, *B. bassiana* isolated from Bengkulu caused 76.7%-80.6% mortality on *Hyphotenemus hampei* Ferrari when applied at 10^9 conidia/ml (Apriyanto & Nadrawati 2019). Agustin (2014) reported that *L. lecanii* at a density of 10^8 conidia/ml, was able to cause 67.62% mortality of *O. furnacalis* larvae, while at a density of 10^9 conidia/ml the mortality rate was 71.25%.

The lowest lethal concentration ($LC_{50, 75}$) was found in the treatment of *L. lecanii* + soap. This was followed by the treatment of *L. lecanii*, *B. bassiana* + soap, *B. bassiana*, and soap. These results showed that the treatment of *L. lecanii* + soap was the most effective against mealy bug. These results correspond with the findings by Bhadani et al. (2017), which showed that *L. lecanii* 2.0 gm/l + profenophos 50 EC 0.025% and *L. lecanii* @ 2.0 gm/l + flonicamid 50 WG 0.0125% was more effective to reduce the population of mealy bugs, *Maconellicoccus hirsutus* in apples both under laboratory and field conditions with mortality of 68.14-72.69%, on the fifth day, than *B. bassiana* 2.0 gm/l + profenophos 50 EC 0.025% @ 0.5ml/l and *B. bassiana* 2.0 gm/l + flonicamid 50 WG 0.0125% @ 0.25 gm/l with mortality of 44.40-51.23%. Leland et al. (2005), explains that, the differences in pathogenicity of different entomopathogenic fungi strains is influenced by the physiological and enzymatic properties of each isolate. The pathogenesis of entomopathogenic fungi depends on the pathogen and host, whereby the cuticle of host affects the infection process, adhesion, germination and differentiation of aspersorium (Vega & Kaya 2012). The lethal concentration also range of a fungus isolate depends on the strain, the host insect, and the application method (Baidoo & Ackuaku 2011).

Entomopathogenic fungi function in several ways during control of insect populations. During the process of infection, the entomopathogenic fungi produce a number of enzymes, such as proteases, chitinases, and lipases that function to degrade the host cuticle and facilitate attachment of the entomopathogenic fungus conidia to the cuticle of the host. The entomopathogenic fungi also produce several types of toxins (e.g. *B. bassiana* produces bassianin, beauvericin, bassianolide, beaverolides and tenellin while *L. lecanii* produces dipcolonic acid, hydroxy carboxylic acid and cyclosporine) which cause an increase in the pH of haemolymph, clotting and stopping the circulation of haemolymph in insect. As a result, the function of the insect's hemolymph and nucleus are interfered, resulting in death of the infected insect due to swelling and hardening (Vey et al. 2001).

The results of the lethal time values showing the time taken by the entompathogenic fungi (*B. bassiana* and *L. lecanii*) treatments to cause mortality of the *P. jackbeardsleyi* nymphs showed that *B. bassiana* + soap and *L. lecanii* + soap treatments had the lowest LT_{50} value of 3.5 days each, followed by *L. lecanii* (3.6 days) and then *B. bassiana* (3.8 days). The LT_{50} value of *B. bassiana* treatment different with findings by Ahmed (2013), which showed that the application of *B. bassiana* strain PDRL1187 against *Phenacoccus solenopsis* has LT_{50} value of 13.4 days, and *B. bassiana* PDRL1147 has LT_{50} 13.9 days.

The lethal time values obtained were related to isolate virulence and host susceptibility in a way that the isolate with higher virulence took less time than the isolate with lower virulence to kill the host (Vega & Kaya 2012). Inglis et al. (2001) explains that the difference in virulence of the isolate is affected by the process of fungus infecting insects starting from attaching of

conidia, germination, penetration, invasion and colonization in haemocells, tissues, and organs of the host. (Vega & Kaya 2012) explains that the effectiveness of entomopathogenic fungi begins from 48 hours post inoculation when the hyphae penetrates the integument in the trachea, epithelium, and epidermal cells. At the 72 hours, the fat tissue is damaged and after 96 hours the mortality of the host occurs.

CONCLUSION

The entomopathogenic fungi treatment of *L. lecanii* + soap is the most effective, followed by *L. lecanii*, *B. bassiana* + soap and *B. bassiana* against *P. jackbeardsleyi*. This is because *L. lecanii* + soap had the lowest LC_{50} and LC_{75} value amongst all the treatments tested. Also, *L. lecanii* + soap and *B. bassiana* + soap treatments had the lowest lethal time value compared to others. Therefore, it can be concluded that entomopathogenic fungi *L. lecanii and B. bassiana* tested are appropriate control alternatives against *P. jackbeardsleyi* in rambutan.

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