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Efficacy of bio-pesticide *Lecanicillium lecanii* against soybean-sucking bugs *Riptortus linearis* during field application

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Abstract. *Mulyati Y, Zubaidah S, Prayogo Y. 2023. Efficacy of bio-pesticide* Lecanicillium lecanii *against soybean-sucking bugs* Riptortus linearis *during field application. Biodiversitas 24: 4829-4836.* This study aimed to determine the effect of application time and surfactants on the efficacy of *Lecanicillium lecanii* strain Probolinggo in controlling the soybean pod-sucking bug *Riptortus linearis* in a field test. The study was conducted in a randomized block design consisting of two treatments, namely time of application (7 am, 10 am, 1 pm, and 4 pm) and type of surfactant (alkyl glycerol phthalate and alkylaryl polyglycol ether). Application time at 4 pm showed 93% highest mortality of *R. linearis* than the other treatments, with the lowest number of punctures (86 punctures) and the highest soybean dry weight (11.38 g), which were not significantly different from the application time of 10 am. The efficacy of *L. lecanii* increased by 74.3% after adding alkylaryl polyglycol ether. The efficacy of *L. lecanii* with alkyl glycerol phthalate was not significantly different from the control. In the parameters of the number of seed punctures and dry weight of soybeans, the surfactant treatment gave similar results to the control. The results showed that in the field test, the highest efficacy of *L. lecanii* against *R. linearis* occurred at 4 pm with alkil aril polyglycol ether surfactant.

Keywords: Application time, entomopathogenic fungi, *Lecanicillium lecanii*, *Riptortus linearis*, soybean, surfactant

INTRODUCTION

Soybean is one of the global primary crops, aside from wheat, paddy, and corn (Tantawizal 2021). Globally, soybean consumption accelerates annually (Najafabadi et al. 2022; Parrini et al. 2023), including in Indonesia (Baroh et al. 2022). However, crop failures have resulted in insufficient domestic demand for soybeans. The majority of soybean crop failures in Indonesia are due to pest attacks, including pests that destroy seeds, destroy leaves, and damage pods (Sari and Suharsono 2011). In Indonesia, crop failure is mainly caused by soybean pod-sucking pest, *Riptortus linearis*. This pest has a wide range in soybean production centers in Indonesia, including Java, Lampung, Sumatra and Kalimantan. The *R. linearis* attack causes shriveled seeds, flat seeds and pods fall. Damage due to *R. linearis* attack reaches 80-100% (Li et al. 2021).

The current pest control mainly focuses on integrated pest control such as using bio-pesticide containing active entomopathogenic fungi (Dinkwar and Ashwini 2022; Trela and Szpyrka 2022; Ferreira and Soares 2023). Therefore, an investigation on the potential entomopathogenic fungi has been carried out to control *R. linearis*. A previous study reported the highest potential from *Lecanicillium lecanii* in controlling *R. lienaris* in comparison to *Beauveria bassiana* (Riningrum et al. 2020) and *Metarhizium* (Astuti et al. 2020).

However, despite its potential to regulate the pest, the application of *L. lecanii* still does not meet expectations related to the stress of abiotic factors in the field, especially temperature and sunlight (Kaiser et al. 2018; Mantzoukas et al. 2020; Alhadidi 2023; Ferreira and Soares 2023). Exposure to temperatures outside the optimum range of fungi can inhibit conidial germination (Wu et al. 2020), reduce conidia viability (Coucéiro et al. 2021), and slow penetration into the host (Wu et al. 2020). Meanwhile, sun exposure affects the virulence of fungus (Mulyati et al. 2015; Khan et al. 2021), causes DNA damage (Mulyati et al. 2015; Wong et al. 2019), death of fungal propagules (Fernández-Bravo et al. 2017) and inhibits the growth of fungus (Couceiro et al. 2021; Subramaniam et al. 2021). Based on these facts, it can be assumed that temperature and UV radiation are the main limiting factors for the efficacy of fungi when applied in the field. Therefore, using bio-pesticides with active ingredients, *L. lecanii* in the field must consider the proper application time. To date, there has been no research on the application time of bio-pesticides made from active entomopathogenic fungi on a field scale.

Implementing fungus with surfactants is one solution to maintain its efficacy during application in the field (Dinkwar and Ashwini 2022; Ferreira and Soares 2023). These additives act as the UV protectant for conidia (Fernandes et al. 2015; Wu et al. 2020), allowing e conidia for longer survivability (Kaiser et al. 2018). Several surfactants have been investigated for use along with *L.*

lecanii bio-pesticides, such as glycerol, tween-20, tween-80, carboxy methyl cellulose, chitin-enriched groundnut oil, and several other surfactants (Nithya and Rani 2019; Ritika et al. 2019). None of the available studies examined the potential surfactant with active alkylaryl polyglycol ether or alkyl glycerol phthalate for supporting the *L. lecanii* bio-pesticide efficacy. In addition to being low-cost, these two surfactants are commonly used by farmers to control the *R. linearis*. The aim of this study was to investigate the efficacy of *L. lecanii* against *R. linearis* in a field trial.

MATERIALS AND METHODS

Sample preparation

In this study, *L. lecanii* isolate and *R. linearis* pest were collected from the planting land of the Indonesian Research Center of Tubes and Beans in Muneng, Probolinggo, East Java, Indonesia (Figure 1).

The *L. lecanii* suspension was prepared from one-month *L. lecanii* culture. As much as 10 mL of *L. lecanii* suspension was poured into an erlenmeyer, filled with semi-cooked sterile rice, and set aside for 30 days at room temperature. After 30 days of inoculation, the culture was added to sterile water and shacked until the conidia were released from the rice. The conidial density was adjusted to 3.8 x 10^6/mL using the hemocytometer. Then, 2 mL/L alkyl glycerol phthalates and alkylaryl polyglycol ethers were added to each *L. lecanii* suspension.

Rearing *R. linearis* was carried out in an iron cage (30 cm in diameter and 40 cm in height), then covered with gauze. The pests were fed with long beans that were replaced every two days. The eggs of insects were placed into another cage to examine *R. linearis* growth from eggs, nymphs, and imago. The uniform population of imago was used as a bioassay.

Procedures

Planting of soybean wilis variation in the experimental field

The wilis variety of soybean was planted in polybags (20 cm in diameter and 25 cm in height) with a soil capacity of approximately 5 kg. Each pot contains two plants as experimental units, then maintained (fertilizing, watering, and weeding) according to the recommendations. At the age of 35 days after planting, each pot was given a bamboo stake according to the height of the plant, then covered with gauze.

Infestation of Riptortus linearis *imago*

Ten pairs of imago *R. linearis* were placed into plastic mylar. The *R. linearis* imago was sprayed with *L. lecanii* suspension added with different surfactants, and the control was sprayed with water by 2 mL/unit per treatment. The imago *R. linearis* that was given treatment was placed in a gauze cover following the application period, namely 7 am, 10 am, 1 pm, and 4 pm. The determination of the four treatment times for biopesticide application was based on the evaluation and research suggestions of Bayu and Prayogo (2018) and Faria et al. (2010) that the application of entomopathogenic fungi must pay attention to the favorable application time.

Observation of Riptortus linearis *imago mortality*

The number of died *R. linearis* infected by *L. lecanii* from each treatment was calculated every day until 14 days after treatment (DAT). The fungi efficiency was measured using formula:

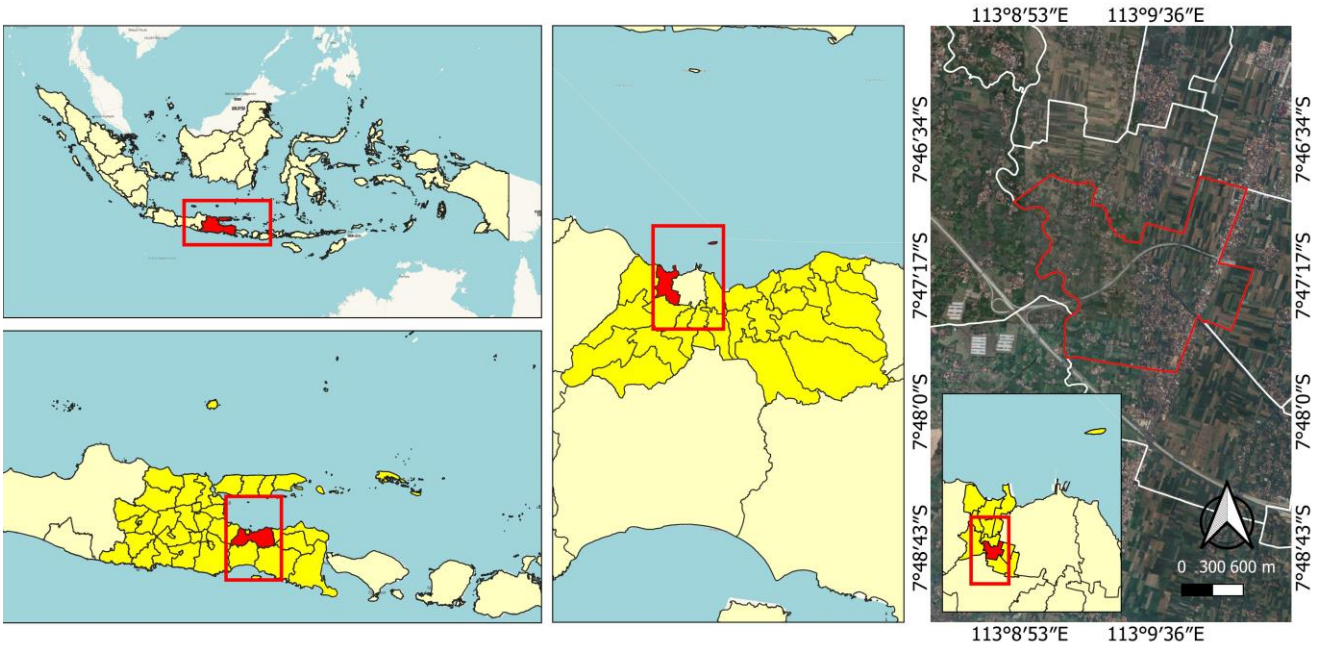

Figure 1. Origins of *Lecanicillium lecanii* isolate strain and *Riptortus linearis* pest from Muneng, Probolinggo District, East Java, Indonesia

$$M = \frac{a}{b} x\ 100\%$$

Where:

M = mortality of tested insect (%)

a = number of died imago *R. linearis* infected by *L. lecanii*

b = the total number of *R. linearis* in the test

The obtained percentage of imago *R. linearis* was transformed into arc sin $\sqrt{x}$, prior to the calculation with variance.

Observation of soybean quality

The parameters for soybean quality examination included bean weight and number of punctures on the bean caused by *R. linearis*. All harvested soybean pods were peeled, dried, and weighed using the digital scale. Meanwhile, the puncture number was measured through observation using a stereo microscope.

Data analysis

The mortality of imago *R. linearis*, the number of pest punctures, and the weight of dried beans were analyzed using SPSS 22.0 software. Before the ANOVA analysis, the arcsine transformation was conducted toward the mortality of imago *R. linearis*. The *R. linearis* imago mortality analysis was conducted every two days, from 2 DAT to 14 DAT. If the statistical analysis results suggested the treatments have effects, then LSD test was conducted (*Least Difference Significance*) with α 0.05 to identify the best treatment.

RESULTS AND DISCUSSION

The analysis of variance results indicates interactions between the application time and the addition of surfactants toward the efficacy of *L. lecanii* biopesticide in regulating the soybean pod-sucking bugs of *R. linearis*. The impacts of application time and surfactant addition were revealed after 12 days of treatment. Within 2 to 10 DAT, no interaction observed between application period and surfactant addition. However, each application period and surfactant addition had an effect on the efficacy of *L. lecanii* fungi in controlling the *R. linearis*.

Effects of application time on *Lecanicillium lecanii* efficacy in controlling *Riptortus linearis*

The statistical analysis showed that the application time affected the efficacy of *L. lecanii* in controlling *R. linearis* in the field. The mortality of imago *R. linearis* in the 4 pm application carries higher significant effects than the other three application times. The difference in mortality of *R. linearis* imago in the 4 pm application was observed starting from 2 DAT and remaining until 14 DAT. Meanwhile, the application at 7 am, 10 am, and 1 pm suggested no significant differences in the mortality of *R. linearis* imago, as summarized in Table 1.

Effects of surfactant addition on *Lecanicillium lecanii* efficacy in regulating *Riptortus linearis* imago

According to the results of statistical analysis, addition of surfactant impacts the efficacy of *L. lecanii* in controlling *R. linearis*, as shown in Table 2. The application of *L. lecanii* with alkylaryl polyglycol ether surfactants (B2) showed the highest efficacy against *R. linearis* compared to other treatments. Meanwhile, adding active alkyl glycerol phthalate surfactant (B1) showed similar results to the control (water). The results of 14 DAT indicated that adding alkylaryl polyglycol ether surfactant enhanced *L. lecanii* biopesticide efficacy to 74.3%, which was higher than that of alkyl glycerol phthalate (61.4%). The effect of interaction between surfactants - application time on the efficacy of *L. lecanii* was observed at 12 DAT and 14 DAT. At 4 pm, biopesticides with active *L. lecanii* + alkylaryl polyglycol ether surfactants showed the highest efficacy in controlling *R. linearis*. Observations at 14 DAT showed that the addition of alkylaryl polyglycol ether surfactants increased the efficacy of *L. lecanii* by 71.93% compared to the control and 57.89% compared to alkyl glycerol phthalate (Figure 2).

Effects of application time and surfactant addition toward the number of punctures *Riptortus linearis* and pod-dried weight

Statistical analysis showed that application time had no significant effect on the number of punctures, but had an effect on soybean dry weight. In the 4 pm application, *R. linearis* made the lowest 86 punctures. Meanwhile, the highest puncture was found on 10 am application (148 punctures). Further, the application at 4 pm and 1 pm have no significantly different results and carrying impacts on the soybean dried weight, as shown in Table 3. Additionally, surfactant addition did not affect the number of punctures and soybean dried weight. However, of the two-surfactant used in this study, it was observed that using biopesticide with active alkylaryl polyglycol ether resulted in the lowest puncture than the alkyl glycerol phthalate and control. The obtained data on puncture and soybean dried weight affected by the application time and surfactant addition are shown in Table 3.

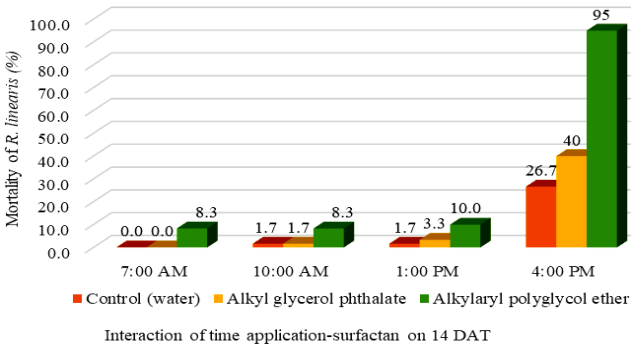

Figure 2. Comparison of the efficacy of *Lecanicillium lecanii* in the treatment of interaction time of application-surfactant at 14 DAT observations

Table 1. Mortality of *Riptortus linearis* imago in different application times

Application times	Average mortality of *R. linearis* imago (%) on n DAT[1)]*						
	2	4	6	8	10	12	14
7 am	1a	2a	3a	3a	3a	3a	3a
10 am	1a	1a	1a	1a	1a	1a	2a
1 pm	0,5a	1a	2a	2a	3a	3a	5a
4 pm	10b	11b	13b	13b	13b	16b	54b

Note: 1) DAT (day after treatment). * The data in the arc sin $\sqrt{x}$ before the analysis of variance. The average data followed by the same annotation presents no significant difference (LSD test, p = 0.05)

Table 2. Mortality of *Riptortus linearis* imago after the *Lecanicillium lecanii* + surfactant biopesticide application

Surfactants	Average mortality of *R. linearis* imago (%) on n DAT[1)] *						
	2	4	6	8	10	12	14
B0	2a	3a	4a	4a	4a	4a	8a
B1	1a	1a	1a	1a	1a	1a	11a
B2	7b	8b	9b	10b	10b	12b	29b

Note: [1)] DAT (Day after treatment); * Data transformed into arc sin $\sqrt{x}$ before analysis of variance, the average data followed by the same annotation has no significant difference (LSD test, α = 0.05). Description: B_0 = control or water; B_1 = alkyl glycerol phthalate; B_2 = alkylaryl polyglycol ether

Table 3. Effects of time application and surfactant on the number of punctures and dried soybean weight

Application times	Parameters		Addition of surfactants	Parameters	
	Punctures	Soybean dried weight (g)		Punctures	Soybean dried weight (g)
7 am	101	6.38	B0	122	64.6
10 am	148	6.57	B1	107	86.6
1 pm	94	8.81	B2	94	97.4
4 pm	86	11.38			

Discussion

Application time

The fungal infection on the targeted insect occurs when the conidia successfully stick into the insect's cuticle, then grow, germinate, and penetrate (Dinkwar and Ashwini 2020; Ferreira dan Soares 2023). This process can only appear if the fungi have the appropriate environment (Al-ani 2019; Puza and Tarasco 2023). Xavier-Santos et al. (2011), Dinkwar and Ashwani (2020), and Subramaniam et al. (2021) revealed that best environmental setting for *L. lecanii* development is temperature between 25-30°C and relative humidity between 90-95%. For a fungal infection to be successful, such circumstances must exist for at least 10 to 12 hours each day (Dinkwar and Ashwani 2020).

In the present study, the required temperature and relative humidity for the fungal infection were observed in 4 am application, with 21-29ºC temperature and 90-98% relative humidity. The attainment of these optimum conditions maintains the fungi metabolism to germinate and produce toxic compounds, which play a role in degrading insect cuticles, the central barrier for fungi infection. Maintaining this ideal temperature and relative humidity (4 pm to the next day or 15 hours) was essential for the *L. lecanii* efficacy in controlling the *R. linearis*, compared to the other time application. In the 7 am treatment, biopesticide at the beginning of spraying obtained the temperature and humidity suitable for its metabolism. However, this condition did not last long (no more than 6 hours) because the temperature increased in intensity, and the humidity was below 90% (average 80.14%) during the day until late afternoon. The remarkable efficacy of fungi was confirmed by the higher mortality of *R. linearis* in 4 pm treatment than the other time applications, as shown in Table 1.

Our results are also linear with the findings reported in previous studies. Acheampong (2020) discovered that temperature and humidity significantly affect the efficacy of *B. Bassiana* and *M. anisopliae* entomopathogen fungi in controlling the *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) in the field application. The effect of temperature on the decreased efficacy of fungi has also been reported by Borisade and Magan (2015) and Tumuhaise et al. (2018). Besides, the lower fungi efficacy has also been reported to be affected by the humidity (Fargues and Luz 2000; Xavier-Santos et al. 2011).

Aside from temperature and humidity, the immense mortality of *R. linearis* at 4 pm compared to the other application period is also influenced by the different sunlight duration and intensity exposure received by the fungi. In this study, it was observed that the beginning of treatment becomes the key to successful fungi performance in the field. Applying biopesticide at 4 pm did not cause the fungus to be exposed to sunlight continuously. Additionally, after treatment (first spray), the fungi did not

receive no direct exposure to sunlight, which favors their growth and germination on the pests. The data obtained from the 4 pm treatment differ from those from other treatments with a long period of sunlight exposure. Therefore, the obtained data suggest that (i) a more extended period of sunlight exposure lowers the fungi efficacy toward *R. linearis* and (ii) the period of UV exposure also postpones the fungi-killing ability toward the targeted pest.

The biopesticide applied at 7 am received the most extended sunlight exposure and scored the lowest killing power than those used at 10 am and 1 pm. Until 14 DAT, the biopesticide efficacy toward the *R. linearis* was below 5% on all three treatments. Meanwhile, they had 93% lower results than the biopesticide applied at 4 pm. This different obtained average is possibly induced by the damages in the conidia (the main component during the infection process) due to the temperature and sunlight exposure. Thus, it affects the germination of the pest's body. This finding is linear with the research by Rodrigues et al. (2016), who reported that 5 minutes of exposure to UV rays caused 45 and 44% of germination in the *B. bassiana* and *M. anisopleae*, respectively, in laboratory.

Aside from decreasing germination, UV rays' exposure also triggers a mutation of the gene that regulates chitinase production, *Chit*1 gene (Mulyati et al. 2015). Further, the nucleotide sequence changes within the *Chit*1 gene influence the production of insect cuticle degrading enzymes, chitinase. Mulyati et al. (2015) also reported that exposure to UV rays for one to four hours induces mutation in *Chit*1 gene in laboratory conditions. Unfortunately, no published data have been found regarding the effect of UV light on the genes encoding cuticle-degrading enzymes, both in *L. lecanii* and other entomopathogenic fungi, under field conditions. In the 7 am application, fungi were received the optimum temperature and humidity for infection. However, following the treatment at 7 pm, fungus was exposed to sunlight and high temperature for longer than application in the afternoon at 4 pm. The application of *L. lecanii* at 7 pm allows the fungus to experience UV stress, which intensity increases and is stronger during the day (Couceiro et al. 2021; Khan et al. 2021). This condition lowers the *L. lecanii* efficacy in the morning or noon.

In this study, in comparison to chemical insecticide use, the application of *L. lecanii* requires a more extended period to deprive *R. linearis* in the field trial. Fifty percent killed pests were not observed on the twelfth day after the spraying. In the 4 pm application, more than 90-100% of the pests were killed on the 14th day after the spraying. Meanwhile, even on 14th day, pest kills in the other treatment remained below 5%. A similar study also uncovered the success of *L. lecani* in annihilating 54.67% of *Nilaparvata lugens* in 14 days after the spraying (Atta et al. 2019), along with 43.25% suppression of *Tetranychus cinnabarinus* population (Kale et al. 2021). In addition, Sapteshwriya and Barad (2020) also reported that 50% of the mealybug *Ferrisia virgata* population decreased on 7th day after the spraying. This study is also supported by the finding from a previous study by Rumbos and Athanassiou (2017) that reviewed several entomopathogen fungi. The study suggested that the entomopathogenic fungi need 6-26 days to kill the pests, depending on their isolate and host.

Surfactant

During field application, the efficacy of fungus can also be increased by adding surfactants to the fungal suspension. Ramayanti et al. (2022) suggested using synthetic oil surfactants in entomopathogenic fungi applications. No studies have examined the efficacy of *L. lecanii* in regulating the soybean pod-sucking bugs *R. linearis* using the addition of alkylaryl polyglycol ethers and alkyl glycerol phthalates synthetic oil. However, several previous studies have reported adding oil formulation to improve the *L. lecanii* efficacy in laboratory or greenhouse tests. Ritika et al. (2019) examined three strains of *L. lecanii* fungi toward *Lipaphis erysimi* (KALT) nymph. Ten days of observation suggested that three strains of *L. lecanii* (NIPHM, MTCC 956, and MTCC 2056) with gliserol+tween 80 adjuvant showed 73.16, 66.63 and 45.23% efficacy, respectively, higher than the control (water). Nithya and Rani (2019) reported that addition of a surfactant combination (polyethylene glycol, polyoxyethylene, glycerol, and tween-80) on three formulations (I: chitin enriched groundnut oil/CGNO + adjuvant combination/AC 1; II: -CGNO + AC 2; and III: CGNO + AC 3) are capable of improving *L. lecanii* biopesticide efficacy toward *Bemisia tabaci*, *Amrasca biguttula*, and *Tetranychus* sp. Similar studies on the surfactant addition into the oil formulation to enhance the effectiveness of entomopathogenic fungi have also been extensively reported (Oliveira et al. 2018; Arnosti et al. 2019; Renkema et al. 2020).

Surfactants with an oil-in-water emulsion (O/W) system protect the conidia from evaporation, maintaining their humidity. Besides, surfactant addition accelerates the biopesticide adhesion on the targeted object. Saputro et al. (2019) also uncovered that the addition of surfactant increases conidia viability. These conditions enhance the fungi germination on the insect's cuticle (Arnosti et al. 2019). The surfactant's capacity to keep humidity and increasing viability of conidia optimize the fungi's metabolism, influencing the production of insects' cuticle-degrading enzymes (Nithya and Rani 2019, Ritika et al. 2019; Ferreira and Soares 2023). Nguyen et al. (2015) also discovered a 25% increase in chitinase enzymes in *L. lecanii* from adding Tween 20, Tween-80, and Triton X-100. The increase in other cuticle-degrading enzyme production, such as lipase, lignase, and α-amylase due to the surfactant addition has also been reported previously (Woertz and Kinney 2004; Silva et al. 2005). Therefore, conidia in the O/W emulsion increases the fungi efficacy compared to the conidia in water suspension (Oliveira et al. 2018). Additionally, the low efficacy of *L. lecanii* on the control sample (water) is caused by the rapid increase of water level in the conidia on the water suspension, making it more susceptible to temperature stress (Paixão et al. 2017). In contrast, the conidia formulation within the oil formulation also forms a biofilm capable of conserving the conidia from UV radiation (Oliveira et al. 2018; Rodrigues

et al. 2016). Further, Nithya and Rani (2019) suspect that surfactant's role as a UV protector is due to its oil content acting as the lubricating agent protecting the layer of mucilaginous conidia in *L. lecanii*.

From the investigated surfactants, the alkylaryl polyglycol ether was the more effective surfactant sheltering and enhancing the efficacy of *L. lecanii* than alkyl glycerol phthalate. The alkyl glycerol phthalate surfactant had no significant different role than the tap water (control). The role of alkylaryl polyglycol ether as the conidia protector of fungi was observed two days after treatment. It remained to appear in the 14 days after treatment, as shown in Figure 2. Both alkylaryl polyglycol ether and alkyl glycerol phthalate surfactants are non-ionic adjuvant that can be added to biopesticide. This non-ionic feature is essential to avoid chemical reactions between the surfactants that may alter the pesticide's chemical structure and the biopesticide efficacy. However, following its properties, alkyl glycerol phthalate is a surfactant with an adhesive feature that facilitates the (bio)pesticide adhesion. Meanwhile, alkylaryl polyglycol ether offers an adhesive nature and a penetrant feature. Thus, it can penetrate chitin in insects. Their different characteristics may induce the distinct efficacy of the biopesticide containing active *L. lecanii* added with alkyl glycerol phthalate and alkylaryl polyglycol ether.

Soybean crop yield

The application period and surfactant addition showed different protection for the fungi during field application. The most accurate application of *L. lecanii* bioinsecticide (at 4 pm) was induced by the optimum environmental condition (temperature and humidity) for its growth and development. Besides, treatment in the evening also obviates the fungi from sunlight exposure, the abiotic factor damaging the conidia. Additionally, the protection from the adhesive agent was in the form of biofilm from oil emulsion. This biofilm formed by the adhesive agent protects the conidia from UV and temperature exposure, similar to the application time treatment. The proper environmental condition supported by the surfactant addition optimized the *L. lecanii* biopesticide in regulating the *R. linearis*, escalating the soybean yield.

In this study, the fungi infection was through the *R. linearis* integument. Conidial adhesion on the pest's cuticle was facilitated by surfactant. In the proper environment, conidia germinate and penetrate the insect's cuticle, as helped by cuticle degrading enzymes. The cuticle penetration occurs up to the hypodermic, constructing the blastospores and disseminating across the insect's body (Altinok et al. 2019; Mantzoukas et al. 2022). Consequently, it induces tissue, organ, and system circulation damage. These obstructions trigger changes in insect behaviors, including reduced eating behavior (Mantzoukas et al. 2022). Herlinda et al. (2020) proved that the application of the entomopathogenic fungus *Metarhizium* spp. able to reduce the appetite of *Spodoptera frugiperda* larvae compared to controls. This condition also possibly decreases the obstruction on the soybean pods, as proven by the significant results in enhancing the fungi efficacy.

In general, finding of present study also suggest that the period of application/spraying time, as well as the usage of adjuvant (surfactant), were the crucial factors during biopesticide usage in the field. Therefore, biopesticide application (the ones with active *L. lecanii*) in the evening (4 pm) by adding an active surfactant using alkylaryl polyglycol ether showed best results to control the pest in the field. As suggested from the research results, the surfactant-added biopesticide effectively improves *L. lecanii* efficacy in open field application in controlling soybean pod-sucking bugs *R. linearis*, strengthening the regulation of pests using an environmentally friendly agent.

However, the high *L. lecanii* strain Probolinggo efficacy in controlling *R. linearis* on the field trial requires further treatment and analysis to increase its killing capacity. In this study, the efficacy of *L. lecanii* was limited to observing the mortality of *R. linearis* in the imago phase only. Further research is needed to examine the efficacy of the fungus in other developmental phases of *R. linearis* (eggs and nymphs). In addition to insect mortality, further research is also recommended to examine the influence of abiotic environmental factors on conidial viability. The insect development phase and conidial viability are two crucial variables that affect the level of pathogenicity of the fungus. Future studies can also use synthetic chemical pesticides to be applied along with *L. lecanii* biopesticide for better efficacy improvement.

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