

Evaluation of indigenous fungal entomopathogens and aqueous leaf extract of *Annona muricata* against *Polyphagotarsonemus latus* infesting *Jatropha curcas* in Indonesia

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Abstract. Puspitarini RD, Afandhi A, Fernando I. 2021. Evaluation of indigenous fungal entomopathogens and aqueous leaf extract of *Annona muricata* against *Polyphagotarsonemus latus* infesting *Jatropha curcas* in Indonesia. *Biodiversitas* 22: 2648-2655. The broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) has been known to cause a great deal of injury on *Jatropha curcas* (L.) plantation in Indonesia. Isolation of indigenous fungal entomopathogens from rhizosphere soils, followed by pathogenicity assay was conducted to find an effective isolate for controlling *P. latus*. Additionally, the potential combination of the selected isolate and soursop (*Annona muricata* L.) aqueous leaf extract (SLE) was investigated. A total of 24 isolates were obtained by using the insect bait method. Four fungal isolates, namely *Paecilomyces* sp. 1., *Lecanicillium* sp., *Beauveria* sp., and *Fusarium* sp. 1, showed high conidial viability and were chosen as representatives to assess their pathogenicity against *P. latus*. Among the tested isolates, *Beauveria* sp. which had the highest conidial viability among the tested isolates, needed a shorter period to completely kill the tested mites. However, the compatibility test revealed the deleterious effect of SLE on *Beauveria* sp. SLE at all concentrations showed a very toxic effect on the fungus, therefore the two must be applied separately. Our results provide useful information on the effectiveness of indigenous entomopathogenic fungi *Beauveria* sp. and aqueous leaf extract of *A. muricata* as an alternative tool to control the broad mite *P. latus* on the *J. curcas* plantation.

Keywords: *Beauveria*, insect bait method; *Jatropha curcas*, *Polyphagotarsonemus latus*, rhizosphere soils

INTRODUCTION

Renewable biofuels derived from agricultural biomass are much needed as the global supply of fossil fuels is in crisis (Baral et al. 2020). Among numerous biomass crops, *Jatropha curcas* (L.) represents a promising candidate, and therefore a lot of researches has been conducted to maximize its exploitation (Sidibe et al. 2020). Due to its hardiness and adaptability to wide agroclimatic conditions, *J. curcas* is cultivated throughout the world (Ntaribi and Paul 2018). In Indonesia, *J. curcas* oil is one of the leading non-petroleum sources of biodiesel, and its plantation is growing progressively (Silitonga et al. 2011). However, during field observation at *J. curcas* plantations in Central Java, it was observed that phytophagous mites severely attacked several plants. Nearly 4,000 mites per leaf were found that consisting mainly of three species, namely *Tetranychus* sp. (Tetranychidae), *Brevipalpus* sp. (Tenuipalpidae), and *Polyphagotarsonemus latus* (Banks) (Tarsonemidae). Amongst, *P. latus* was found to be ubiquitous and responsible for most of the damage. Thus, this problem must be addressed, and a proper control strategy should be established quickly.

The mite *P. latus*, also known as the broad mite, is an important pest with global distribution in tropical and subtropical regions (Weintraub et al. 2003). More than 60 different plant families are registered as its host (Gerson 1992). A recent study indicated that *P. latus* was the most

abundant mite species inhabiting *J. curcas* (Lofego et al. 2013), and become a potential pest of the plant (Rosado et al. 2014, 2015). In *J. curcas*, the mite infestation usually begins in the shoots and the mite population resides in the lower leaf surfaces. The feeding of *P. latus* causes the leaves to become smaller with a leathery texture, twisted edges, and glassy appearance on the abaxial surface. Additionally, injured new leaves and buds become distorted, eventually, flowers are also malformed or fail to develop (Sarmiento et al. 2011).

Due to the heavy infestation of mites, synthetic acaricides have become the conventional practice and perceived to be the cornerstones of an effective control method (van Leeuwen et al. 2015). The repeated use of acaricides is dangerous as they possess indirect hazardous effects on non-target organisms, among other issues (Mills et al. 2016; Meftaul et al. 2020). Consequently, eco-friendly natural acaricides are increasingly needed. Since a decade ago, the ability of fungal entomopathogens in pest control has been explored, and they have become one of the prime biological agents to manage phytophagous mite populations (Mascarin et al. 2019). It has been reported that entomopathogenic fungi can also be used synergistically with botanical acaricides, and their bio-efficacy is bolstered when compatibility is achieved (Hernández et al. 2012). In perspective, the combined application of fungal entomopathogens with botanical pesticides may provide a more powerful tool for pest management strategy (Ribeiro et al.

2012). Plant-derived acaricides themselves are good candidates for mite control in integrated pest management (IPM) (Venzon et al. 2008; de Assis et al. 2011). Therefore, the discovery of new effective entomopathogenic fungi and botanical acaricide is necessary to suppress the phytophagous mite populations. Given the potential economic damage caused by *P. latus* on *J. curcas*, the present study attempts to find an effective indigenous entomopathogenic fungus, elucidate its efficacy against the mite, and evaluate its acaricidal activity plant extract derived from soursop leaves (*Annona muricata* L.). Subsequently, the effects of co-formulation between the plant extract with entomopathogenic fungus were determined. Entomopathogenic fungi were isolated from the rhizosphere soils of *P. latus*-infested crops.

MATERIALS AND METHODS

Mite rearing

The mite *P. latus* was obtained from infested *J. curcas* during field investigations. Infested leaves were harvested and put in a cooler box. The mites were then brought to the Plant Pests Laboratory, Department of Plant Pests and Diseases, University of Brawijaya, Indonesia. In the laboratory, mites were reared on leaves of *Citrus* sp. and placed in an 'arena'. To make an arena, a water-soaked sponge (5 cm x 5 cm x 1.5 cm) was placed inside a Petri dish (d = 9 cm). The sponge was covered with moistened cotton, and then, a citrus leaf was placed over the cotton with the abaxial surface facing up (Martins et al. 2016). Regular watering was done to maintain the humidity in Petri dishes, keep the leaf in turgid condition, and prevent the desiccation of the mites (Puspitarini et al. 2021). The rearing was performed under laboratory conditions at 25 ± 1°C, RH 68 ± 2%, and photoperiod of L12:D12 h. Mites were also maintained in a greenhouse on citrus plants aged 1-2 years.

Collection of rhizosphere soil samples

Soil samples were collected from two different host plants, namely *Citrus* sp. and *J. curcas*. For each host plant, four different fields, located in Malang and Batu (East Java, Indonesia), were selected as sampling sites (Table 1). Each field comprised of 10 sampling units, located using a diagonal pattern. Rhizosphere soil was taken at 10-15 cm depth with a garden spade. The soil sample was then placed into a plastic bag and kept in an airtight cooler box. The garden spade was cleaned with 70% ethanol after being used to minimize the cross-contamination of fungal entomopathogens among the sampling sites. A total of 80 soil samples were collected as the source of fungal entomopathogens.

Isolation of fungal entomopathogens from rhizosphere soils

Isolation was based on the insect bait method by using the fourth instar larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (Sharma et al. 2018). Briefly, 500 g of sieved soil was put in a plastic tray and remoistened. After

that, 25 *T. molitor* larvae were placed in the tray. Trays were kept under laboratory conditions and covered with a black cloth to achieve a complete darkness condition. During two weeks of the incubation period, insect cadavers were observed daily. The obtained cadavers were then washed with 1% sodium hypochlorite (NaOCl) for three minutes and rinsed three times with distilled water. The clean insect cadavers were cultured on Sabouraud dextrose agar with yeast (SDAY) and incubated under the same aforementioned laboratory conditions for 7 days. Subculturing was done to obtain a pure fungal culture. In addition, all the isolated fungi were subjected to Koch's postulates to ascertain their pathogenicity.

Fungal identification

The identification of the obtained entomopathogenic fungi was based on their morphological characteristics using a taxonomic key as described by Domsch et al. (2007).

Fungal conidia viability assay

Conidia viability was tested as the primary criteria determining the selection of entomopathogenic fungi used for the further assay. Initially, each isolate was grown on SDAY in Petri dishes for 7 days until conidia were produced (Shi and Feng 2004). Conidia were further inoculated into potato dextrose broth (Oliveira et al. 2015). Chloramphenicol (1%) was added to the media to hinder contamination from other microorganisms. Before being used, conidial concentration was adjusted to 10⁷ conidia/ml using a hemocytometer. The viability assay was conducted as per Herlinda (2010) using potato dextrose agar (PDA) as the medium.

Fungal pathogenicity assay against *P. latus*

Treatments of this assay consisted of the four selected fungal isolates obtained from the previous assay, and control containing distilled water alone. Each treatment was replicated seven times. Fungal conidia concentration was adjusted to 10⁷ conidia/ml, after that a leaf disc of citrus (approximately 3 cm x 3 cm) was sprayed with each treatment at a 1 ml rate per leaf. The application was done manually using a calibrated hand sprayer. Ten adults of *P. latus* aged 0-24 h, that were initially placed into the arena, were allowed to feed on the treated-leaf disc. To obtain mites of the same age, individuals that enter the last quiescent stage were arbitrarily taken from the stock cultures and transferred into a given arena.

Table 1. Location of the sampling sites

| Host plant | Site | Location |
|------------------------|------|------------------------------------|
| <i>Citrus</i> sp. | 1 | 7° 49' 55.86" S, 112° 31' 39.46" E |
| | 2 | 7° 50' 7.81" S, 112° 31' 31.79" E |
| | 3 | 7° 54' 42.88" S, 112° 32' 25.25" E |
| | 4 | 7° 55' 2.99" S, 112° 34' 8.42" E |
| <i>Jatropha curcas</i> | 5 | 8° 0' 21.22" S, 112° 46' 13.31" E |
| | 6 | 8° 4' 0.95" S, 112° 48' 51.51" E |
| | 7 | 8° 7' 44.63" S, 112° 45' 4.37" E |
| | 8 | 8° 3' 22.12" S, 112° 44' 5.7" E |

Thereafter, mites that emerged on the same day were taken for the experiment. Following the treatments, mortality was checked daily during 6 days of the observation period (Martins et al. 2016). Test mites were assumed dead if there was no movement of the appendages when being touched with a brush. Mortality was then calculated and expressed in percentage.

Collection and extraction of soursop leaves

The soursop (*A. muricata*) leaves were collected from the vicinity of the University of Brawijaya, Indonesia. Collected leaves were cleaned under running tap water and were drained thoroughly. For extraction, 100 g of the leaf along with 100 ml of sterilized distilled water were mixed using an electric blender and subsequently were shaken overnight at 220 rpm. The suspension was filtered with Whatman filter paper, and the clean filtrate was kept in a sterilized glass bottle. Soursop aqueous leaf extract (SLE) was then stored at a 4°C refrigerator for further experiments. Three concentrations that were 0.5, 1, and 1.5% were prepared from the stock solution using the addition of an appropriate amount of distilled water.

Compatibility assessment between *Beauveria* sp. and SLE

Entomopathogenic fungus *Beauveria* sp. was the most virulence isolate, hence it was used as the sole fungal isolate for this assay. To evaluate the compatibility between botanical acaricide with the fungus, SLE at various concentrations was incorporated into the PDA following the procedure described by Depieri et al. (2005). *Beauveria* sp. inoculum was prepared by suspending fungal spores in a falcon tube containing 10 ml of distilled water with 0.02% of Tween 20. The tube was centrifuged at 3,000 rpm for 5 min to separate the conidia. The conidia pellet was mixed with distilled water and the conidia concentration was standardized into three levels (10^4 , 10^6 , and 10^8 conidia/ml). An aliquot of each solution was then added into a petri dish with a PDA medium incorporating a given concentration of SLE. A PDA medium without SLE was considered as the control. In total, 12 treatments and 4 replicates were included. During 6 days of the incubation period, the fungal vegetative growth, conidial production, and conidial viability were observed and calculated (Depieri et al. 2015). Compatibility was then determined by calculation of T factor, $T = [20 (VG) + 80 (SP)]/100$, where VG is relative vegetative growth and SP is sporulation values compared to the control. The T values from 0-30 indicate that the botanical acaricide is considered as very toxic; from 31-45 as toxic; from 46-60 as moderately toxic; and >60 represent that botanical acaricide is considered compatible with the fungal entomopathogen.

Toxicity bioassay of *Beauveria* sp. and SLE against *P. latus*

The bioefficacy of *Beauveria* sp. and SLE against *P. latus* was assessed separately. The toxicity assay consisted of five replicates per treatment. Totally seven treatments consist of three different levels of SLE concentration (0, 1, and 1.5%), three different levels of *Beauveria* sp. conidia density (10^4 , 10^6 , and 10^8 conidia/ml), and control containing distilled water alone. The application was

conducted similarly to the previous method described earlier in fungal pathogenicity assay.

Statistical analysis

All assays were designed in a completely randomized design. Obtained data were initially subjected to the Shapiro-Wilk test to validate that the normality assumption was met. The data were then subjected to analysis of variance (ANOVA) and the mean values were compared by using Duncan's test at $P < 0.05$. R statistics was employed as the software.

RESULTS AND DISCUSSION

Fungal entomopathogens isolated from *Jatropha curcas* and *Citrus* sp. rhizosphere soils and their conidial viability

In this study, rhizosphere soil was chosen as the source of fungal entomopathogens. It is known that soil is a natural reservoir of entomopathogenic fungi. Results showed that a total of 24 different fungal isolates were isolated from the collected rhizosphere soils. These fungal isolates belong to ten genera, namely *Metarhizium* sp., *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Cladosporium* sp., *Paecilomyces* sp., *Lecanicillium* sp., *Acremonium* sp., and *Beauveria* sp. (Table 2). The entomopathogenic fungus *Metarhizium* was the most common in the sampled areas, both in *J. curcas* or *Citrus* sp. plantations. Several studies indicate that *Metarhizium* is the most commonly found fungus in agricultural fields (Quesada-Moraga et al. 2007; Sánchez-Peña et al. 2011). *Metarhizium* compared with the other fungal entomopathogens, is more tolerant to pesticides therefore it persisted in cultivated habitats (Vänninen et al. 2000; Bruck 2004). It was also observed that pesticides regularly applied the selected fields during the growing season.

The sampled areas were selected because of the prevalence of *P. latus* infestation in those locations, therefore our first hypothesis was that the indigenous fungal entomopathogens act as the natural enemies of *P. latus* in the field. Moreover, their presence might be considered as an environmental service and potentially suppress the *P. latus* population. However, the efficacy of each isolate is certainly varied depending on individual innate virulence and genetic variability (Xiao et al. 2012). One of the main parameters used to determine the virulence of entomopathogenic fungi is conidial viability (Faria et al. 2015). Out of 24 isolates, *Paecilomyces* sp. 1., *Lecanicillium* sp., *Beauveria* sp., and *Fusarium* sp. 1, were the isolates with the relatively higher conidial viability ranging from 39.04 to 55.3 % (Table 1). This result is in agreement with previous works reporting that conidial viability is strongly species- and strain-specific (Herlinda 2010; Xavier-Santos et al. 2011). Moreover, Jin et al. (1992) suggested that propagules with a slow-germinating capability are not efficiently being utilized as biocontrol agents. Therefore, only the four isolates were tested for their pathogenicity against *P. latus*. Although *Metarhizium* sp. was the most prevalent species, it had a low conidial viability and therefore not used in the pathogenicity assay.

***Beauveria* sp. has higher pathogenicity to *P. latus* compared with the other fungal entomopathogens**

All entomopathogenic fungi caused mortality of *P. latus* since the first day of observation, and the lethal effects were more profound afterward (Table 3). *Beauveria* sp. exhibited the highest control efficacy causing complete mortality of adults *P. latus* on the fourth day of observation. This result was consistent with the results of the conidial viability assay which showed that *Beauveria* sp. had greater conidial viability. The ability of *Beauveria* sp. to kill phytophagous mites has been addressed by a myriad of researches, such as against the two-spotted spider mite *Tetranychus urticae* Koch (Wu et al. 2016), citrus rust mite *Phyllocoptruta oleivora* (Ashmead) (Alves et al. 2005), and false spider mite *Brevipalpus phoenicis* (Geijskes) (Rossi-Zalaf and Alves 2006). Similarly, Nugroho and Ibrahim (2004) found that *Beauveria bassiana* significantly caused the highest mortality rate on *P. latus*. In addition, it was also observed that there was a large variation in the mite mortality among the tested entomopathogens (*B. bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea*) which was in accordance with our results.

Compatibility of SLE with *Beauveria* sp.

Proper understanding of the compatibility of fungal entomopathogens with other pest control techniques is necessary to maximize their potential or hinder the unwanted contradictory effects. In all tested concentrations, aqueous extract derived from soursop leaves (SLE) only slightly reduced the vegetative growth (Table 4) and conidiogenesis (Table 5) of *Beauveria* sp. The decreases in the mycelial growth and conidia production of entomopathogenic fungi by botanical pesticides have been demonstrated by several researchers (Mohan et al. 2007; Jamali et al. 2021). Ribeiro et al. (2012) found that bioactive compounds of botanical pesticides may directly affect the growth and development of entomopathogenic fungi, or indirectly influence the pH of the culture medium. However, the effect of botanical pesticides on entomopathogenic fungi is strongly dependent on the concentrations and formulation used and the quantitative and qualitative composition of bioactive compounds contained in the products (Forim et al. 2010; Diniz et al. 2020). Additionally, the genetic profile of each species or strain of entomopathogenic fungus also determines its susceptibility in response to botanical pesticide exposure (Mohan et al. 2007).

Table 2. Fungal entomopathogen isolates found in rhizosphere soil of *Citrus* sp. and *Jatropha curcas* by using the insect bait method, and their conidial viability after incubation for 24 h

| Host plants | Fungal isolate | % Conidial viability |
|----------------------------|---------------------------|--------------------------|
| <i>Citrus</i> sp. | <i>Metarhizium</i> sp. 1 | 27.78 |
| | <i>Metarhizium</i> sp. 5 | 22.83 |
| | <i>Metarhizium</i> sp. 8 | 21.59 |
| | <i>Aspergillus</i> sp. 1 | 24.19 |
| | <i>Aspergillus</i> sp. 2 | 14.29 |
| | <i>Aspergillus</i> sp. 4 | 18.74 |
| | <i>Fusarium</i> sp. 3 | 14.20 |
| | <i>Penicillium</i> sp. 1 | 27.08 |
| | <i>Cladosporium</i> sp. 2 | 19.98 |
| | <i>Paecilomyces</i> sp. | 29.41 |
| | <i>Acremonium</i> sp. | 26.13 |
| | <i>Metarhizium</i> sp. 3 | 14.21 |
| | <i>Metarhizium</i> sp. 4 | 40.20 |
| | <i>Jatropha curcas</i> | <i>Metarhizium</i> sp. 6 |
| <i>Metarhizium</i> sp. 7 | | 39.044 |
| <i>Fusarium</i> sp. 4 | | 35.08 |
| <i>Penicillium</i> sp. 2 | | 15.13 |
| <i>Cladosporium</i> sp. 1 | | 23.53 |
| <i>Lecanicillium</i> sp. 1 | | 41.70 |
| <i>Beauveria</i> sp. | | 55.35 |
| <i>Fusarium</i> sp. 2 | | 23.30 |
| <i>Trichoderma</i> sp. | | 17.37 |
| <i>Aspergillus</i> sp. 3 | | 25.64 |
| <i>Fusarium</i> sp. 1 | | 27.36 |

Table 4. Mean of colony diameter of *Beauveria* sp. on PDA supplemented with different concentration of SLE at 6 days after inoculation

| Treatments | Colony diameter (cm ± SD) | Reduction at 6 DAI (%) |
|--|---------------------------|------------------------|
| <i>Beauveria</i> sp. 10 ⁴ and SLE 0 | 4.73 ± 0.74 | - |
| <i>Beauveria</i> sp. 10 ⁶ and SLE 0 | 4.75 ± 0.13 | - |
| <i>Beauveria</i> sp. 10 ⁸ and SLE 0 | 4.83 ± 0.73 | - |
| <i>Beauveria</i> sp. 10 ⁴ and SLE 0.5 | 3.70 ± 0.30 | 0.22 |
| <i>Beauveria</i> sp. 10 ⁶ and SLE 0.5 | 4.40 ± 0.23 | 0.07 |
| <i>Beauveria</i> sp. 10 ⁸ and SLE 0.5 | 4.73 ± 0.85 | 0.02 |
| <i>Beauveria</i> sp. 10 ⁴ and SLE 1 | 3.60 ± 1.07 | 0.24 |
| <i>Beauveria</i> sp. 10 ⁶ and SLE 1 | 3.83 ± 0.19 | 0.19 |
| <i>Beauveria</i> sp. 10 ⁸ and SLE 1 | 4.50 ± 0.23 | 0.07 |
| <i>Beauveria</i> sp. 10 ⁴ and SLE 1.5 | 3.18 ± 0.24 | 0.33 |
| <i>Beauveria</i> sp. 10 ⁶ and SLE 1.5 | 3.80 ± 0.45 | 0.20 |
| <i>Beauveria</i> sp. 10 ⁸ and SLE 1.5 | 4.43 ± 0.76 | 0.08 |

Note: *Beauveria* sp. concentration was in conidia/ml and SLE concentration was in %. Means were not statistically different among the treatments.

Table 3. Mean mortality (%) of adult *P. latus* after the application of fungal entomopathogen isolates

| Fungal entomopathogens | Day(s) after application | | | | | |
|----------------------------|--------------------------|----------|---------|----------|----------|----------|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Control (distilled water) | 0.00 a | 0.00 a | 0.00 a | 0.00 a | 0.00 a | 0.00 a |
| <i>Fusarium</i> sp. 1 | 8.57 b | 31.43 b | 50.00 b | 80.00 b | 97.14 b | 100.00 b |
| <i>Paecilomyces</i> sp. 1 | 14.29 bc | 34.29 bc | 64.29 c | 84.29 b | 98.57 b | 100.00 b |
| <i>Lecanicillium</i> sp. 1 | 17.14 c | 42.86 c | 67.14 c | 88.57 b | 97.14 b | 100.00 b |
| <i>Beauveria</i> sp. | 37.14 d | 80.00 d | 95.71 d | 100.00 b | 100.00 b | 100.00 b |

Note: The concentration of fungal entomopathogens was 10⁷ conidia/ml. Means followed by different letters on the same row indicate significant differences (ANOVA followed by Duncan's test at P < 0.05)

Spore germination has a pivotal role in determining the effectiveness of entomopathogenic fungi under field conditions, and laboratory assessment is primarily undertaken to elucidate the effect of products on conidial viability. In the present study, it was observed that SLE showed a deleterious effect on *Beauveria* sp. germination. The reduction of conidial viability was significant and depended on concentration, with SLE at 1.5% greatly suppressed the conidia germination (Table 6). Soursop extract has been known to contain alkaloids, flavonoids, triterpenoids, and acetogenins (Ravaomanarivo et al. 2014). Those secondary metabolites found in SLE may serve as fungi toxic compounds against *Beauveria* sp. A recent study indicates that some plant derivatives, such as alkaloids, although confer pesticidal activities for pest control, also negatively affect fungal entomopathogens (Ribeiro et al. 2012).

Although the effect of SLE on mycelial growth and conidiogenesis was not statistically different, the T value indicated that SLE at all concentrations was not compatible with *Beauveria* sp., and was very toxic (Table 7). A higher concentration of SLE elicited a lower T value, and it was ranged as low as 1.79 (*Beauveria* sp. 10^4 conidia/ml with SLE at 1.5%) to the highest of only 3.52 (*Beauveria* sp. 10^8 conidia/ml with SLE at 0.5%). Supporting this statement, Saharayaj et al. (2011) reported that among the five tested plant extracts used in their study, only *Annona* extract showed significant inhibition on the tested fungal entomopathogens, especially *B. bassiana*, *I. fumosorosea*, and *Lecanicillium lecanii*. Hence, it clearly indicates that *Annona* extract is incompatible with entomopathogenic fungus. These results are similar to those reported by Hirose et al. (2001), who reported a significant reduction in spore germination of *B. bassiana* when combined with neem oil at 2%. We suggest that the combined application of plant extract and entomopathogenic fungi must be avoided if contradictory interactions were detected.

Acaricidal activity of SLE on *P. latus* is comparable with *Beauveria* sp.

Soursop is traditionally employed as biopesticidal agents and topical insect repellents throughout the world (Moghadamtousi et al. 2015). A plethora of researches prove the anti-pest feature of the aqueous extract of soursop such as to control stored product pests, aphids, thrips, lepidopteran larvae, and mosquito (Leatemia and Isman, 2004; Adeoye and Ewete, 2010; Asmanizar et al. 2012; Ravaomanarivo et al. 2014; Ribeiro et al. 2014). Nonetheless, to date, the application of soursop extract on phytophagous mites is still obscure, especially on *P. latus*. Considering the local abundance of this plant in Indonesia, we attempted to exploit the plant as a promising candidate for botanical acaricide. In this present work, the acaricidal activity of SLE and *Beauveria* sp. against *P. latus* was tested separately at various concentrations (because of their incompatibility). Results of the toxicity assay evidenced the potent acaricidal activity of SLE. On the last day of observation, SLE at all concentrations gave higher mortality on *P. latus* than the *Beauveria* sp. at 10^4 , 10^6 conidia/ml (Table 8).

Table 5. Average amount of *Beauveria* sp. conidia on PDA supplemented with different concentration of SLE at 6 days after inoculation

| Treatments | Conidia amount | |
|---|-------------------------|---------------|
| | (x 10^8 /ml \pm SD) | Reduction (%) |
| <i>Beauveria</i> sp. 10^4 and SLE 0 | 2.51 \pm 0.32 | - |
| <i>Beauveria</i> sp. 10^6 and SLE 0 | 2.99 \pm 0.21 | - |
| <i>Beauveria</i> sp. 10^8 and SLE 0 | 5.15 \pm 1.55 | - |
| <i>Beauveria</i> sp. 10^4 and SLE 0.5 | 2.20 \pm 0.76 | 0.12 |
| <i>Beauveria</i> sp. 10^6 and SLE 0.5 | 2.64 \pm 0.57 | 0.12 |
| <i>Beauveria</i> sp. 10^8 and SLE 0.5 | 3.22 \pm 1.04 | 0.37 |
| <i>Beauveria</i> sp. 10^4 and SLE 1 | 2.08 \pm 0.72 | 0.17 |
| <i>Beauveria</i> sp. 10^6 and SLE 1 | 2.54 \pm 1.17 | 0.15 |
| <i>Beauveria</i> sp. 10^8 and SLE 1 | 2.74 \pm 0.71 | 0.47 |
| <i>Beauveria</i> sp. 10^4 and SLE 1.5 | 1.44 \pm 0.45 | 0.43 |
| <i>Beauveria</i> sp. 10^6 and SLE 1.5 | 2.34 \pm 0.42 | 0.22 |
| <i>Beauveria</i> sp. 10^8 and SLE 1.5 | 2.69 \pm 0.14 | 0.48 |

Note: *Beauveria* sp. concentration was in conidia/ml and SLE concentration was in %. Means were not statistically different among the treatments

Table 6. Mean of conidia viability of *Beauveria* sp. on PDA supplemented with different concentration of SLE

| Treatments | Conidia viability | |
|---|----------------------|---------------|
| | (% \pm SD) | Reduction (%) |
| <i>Beauveria</i> sp. 10^4 and SLE 0 | 89.73 \pm 4.86 cd | - |
| <i>Beauveria</i> sp. 10^6 and SLE 0 | 94.88 \pm 0.49 d | - |
| <i>Beauveria</i> sp. 10^8 and SLE 0 | 95.97 \pm 1.64 d | - |
| <i>Beauveria</i> sp. 10^4 and SLE 0.5 | 73.38 \pm 6.04 abc | 0.18 |
| <i>Beauveria</i> sp. 10^6 and SLE 0.5 | 83.47 \pm 5.94 bcd | 0.12 |
| <i>Beauveria</i> sp. 10^8 and SLE 0.5 | 87.57 \pm 1.13 bcd | 0.09 |
| <i>Beauveria</i> sp. 10^4 and SLE 1 | 69.28 \pm 2.63 ab | 0.23 |
| <i>Beauveria</i> sp. 10^6 and SLE 1 | 81.43 \pm 2.63 bcd | 0.14 |
| <i>Beauveria</i> sp. 10^8 and SLE 1 | 86.03 \pm 5.56 bcd | 0.10 |
| <i>Beauveria</i> sp. 10^4 and SLE 1.5 | 58.62 \pm 8.28 a | 0.35 |
| <i>Beauveria</i> sp. 10^6 and SLE 1.5 | 75.67 \pm 3.09 ab | 0.20 |
| <i>Beauveria</i> sp. 10^8 and SLE 1.5 | 83.55 \pm 3.19 ab | 0.13 |

Note: *Beauveria* sp. concentration was in conidia/ml and SLE concentration was in %. Means followed by different letters on the same row indicate significant differences (ANOVA followed by Duncan's test at $P < 0.05$)

Table 7. SLE compatibility classification with the entomopathogenic fungus *Beauveria* sp.

| Treatments | T | Classification |
|---|------|----------------|
| <i>Beauveria</i> sp. 10^4 and SLE 0.5 | 2.50 | Very toxic |
| <i>Beauveria</i> sp. 10^6 and SLE 0.5 | 2.99 | Very toxic |
| <i>Beauveria</i> sp. 10^8 and SLE 0.5 | 3.52 | Very toxic |
| <i>Beauveria</i> sp. 10^4 and SLE 1 | 2.38 | Very toxic |
| <i>Beauveria</i> sp. 10^6 and SLE 1 | 2.80 | Very toxic |
| <i>Beauveria</i> sp. 10^8 and SLE 1 | 3.09 | Very toxic |
| <i>Beauveria</i> sp. 10^4 and SLE 1.5 | 1.79 | Very toxic |
| <i>Beauveria</i> sp. 10^6 and SLE 1.5 | 2.63 | Very toxic |
| <i>Beauveria</i> sp. 10^8 and SLE 1.5 | 3.04 | Very toxic |

Note: T value was calculated from the formula proposed by Depieri et al. (2005)

Table 8. Toxicity of *Beauveria* sp. and SLE against *P. latus* as represented by the mean of adult mortality at 6 days after application

| Treatments | Mortality (%± SD) |
|---|-------------------|
| Control | 0.00 ± 0.00 a |
| <i>Beauveria</i> sp. 10 ⁴ conidia/ml | 74.00 ± 2.45 b |
| <i>Beauveria</i> sp. 10 ⁶ conidia/ml | 76.00 ± 9.27 b |
| <i>Beauveria</i> sp. 10 ⁸ conidia/ml | 100.00 ± 8.60 c |
| SLE 0.5% | 94.00 ± 13.04 c |
| SLE 1% | 98.00 ± 8.94 c |
| SLE 1.5% | 100.00 ± 4.47 c |

Note: Means followed by different letters on the same rows indicate significant differences (ANOVA followed by Duncan's test at $P < 0.05$)

The high toxicity of SLE reported in this study was in accordance with a previous study (Ravaomanarivo et al. 2014), that under a similar extraction method, the application of an aqueous extract of soursop on two dipteran species showed LC₅₀ values ranged from 1-5% for adults and only 0.5-1% for larvae. Acaricidal activity of soursop was attributed to the various bioactive compound groups found in the plant extract such as flavonoids, fatty acids, alkaloids, and acetogenins (Bermejo et al. 2005; Das et al. 2007).

Alkaloids found in soursop extract have been known to inhibit the active site of acetylcholinesterase, a key enzyme in the nervous system of arthropods (Scott et al. 2008). Plant fatty acids also possess acaricidal activities through a multitude of direct mechanisms or may augment the toxicity of other bioactive compounds (Ravaomanarivo et al. 2014). Acetogenins which are specific to the Annonaceae family, also exhibit diverse biological activities, including act as stomach poisons identical to the rotenone's mode of action (de Cássia Seffrin et al. 2010), or serve as a contact poison and antifeedant (Leatemia and Isman 2004; Maryam et al. 2004; Isman 2006).

Regarding the *Beauveria* sp., one of the determining factors in the infection process of *Beauveria* sp. on its host is the initial number of inoculums. The higher conidia concentration may increase the number of adhered conidia in the host's cuticle. After germinating, the conidia penetrate the host cuticle. The fungus then secretes various enzymes to counter the immune system of its host (Harith-Fadzilah et al. 2021). Additionally, Chandler (2007) observed that the fungus also produces secondary metabolites such as oosporeins, tenellins, isarolides, beauverolides, and beauvericin which are toxic to the arthropod cells.

This study provides useful information on the effectiveness of indigenous entomopathogenic fungi *Beauveria* sp. and aqueous leaf extract of soursop as an alternative tool to control the broad mite *P. latus*. Their application can be implemented in the IPM-based management strategy. Moreover, these control strategies do not require high-tech machinery, and thus are cost-efficient and easily adopted by smallholder farmers, especially in developing countries. Nevertheless, field experiments are necessary to ascertain their efficacy under natural

conditions. In conclusion, 24 fungal entomopathogens belong to ten genera were successfully isolated from rhizosphere soils of *J. curcas* and *Citrus* sp. Based on the conidial viability assessment, four isolates, *Paecilomyces* sp. 1., *Lecanicillium* sp., *Beauveria* sp., and *Fusarium* sp. 1, were selected as potential candidates. There is a positive relationship between fungal conidial viability and pathogenicity as indicated in *Beauveria* sp., which in turn confirms the general theory of which isolate selection could be based solely on the percentage of spore germination. Further experiments revealed that even the lowest concentration of SLE had adverse effects toward *Beauveria* sp., thus SLE was not compatible upon co-application with the fungus. The result of toxicity bioassay indicated promising acaricidal activities of *Beauveria* sp. and SLE for the management of broad mite *P. latus*.

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