Synergism of Lecanicillium lecanii (Zimm) and Chromolaena odorata L. Leaf Extract to Control Aphis gossypii (Glover) in Chili Plants

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Abstract
The purpose of the study was to determine the effect of the appropriate and effective application of the L. lecanii fungus and C. odorata L. leaf extract in increasing the mortality of A. gossypii. The study was divided into two stages. The first stage was in vitro test of L. lecanii synergism plus C. odorata L. leaf extract in PDA media with four treatments, namely LK₁₀ = L. lecanii 10⁻⁷ conidia.mL⁻¹ plus 0% C. odorata L. leaf extract, LK₁₀ = L. lecanii 10⁻⁷ conidia.mL⁻¹ plus 10% C. odorata L. leaf extract, LK₁₀ = L. lecanii 10⁻⁷ conidia.mL⁻¹ plus 25% C. odorata L. leaf extract; LK₁₀ = L. lecanii 10⁻⁷ conidia.mL⁻¹ plus 40% C. odorata L. leaf extract. The second stage was the toxicity test of the application of L. lecanii suspension and C. odorata L. leaf extract on mortality of A. gossypii. The toxicity test was based on the results of the synergism test, where the addition of C. odorata L. leaf extract to L. lecanii growing media showed incompatible results. Therefore, the toxicity test was carried out separately with five treatments, namely H₀ = Control (aqua dest); H₁ = Conidia suspension L. lecanii 10⁻⁷ conidia.mL⁻¹; H₂ = 10% C. odorata L. leaf extract; H₃ = 25% leaf extract of C. odorata; H₄ = 40% C. odorata L. leaf extract. The results showed that the compatibility test of L. lecanii with leaf extract of C. odorata L. was incompatible and classified as toxic. The addition of C. odorata L. leaf extract in concentrations of 10%, 25%, and 40% could significantly inhibit colony growth, sporulation, and conidia viability of L. lecanii, with a higher level of inhibition as the concentration of C. odorata L. leaf extract, was added. The toxicity test of a separate application of C. odorata L. leaf extract and L. lecanii suspension had a significant effect on mortality of 3rd instar nymph A. gossypii, with the highest mean mortality found in a single application of 40% C. odorata L. leaf extract with an average mortality of 100% at 96 HAA(Hours After Application) observations.

Keywords: A. gossypii, C. odorata L. leaf extract, L. lecanii, synergism.

INTRODUCTION
Chili is one of the important horticultural commodities in Indonesia. Data on the projected level of chili consumption in Indonesia is expected to continue to increase, seen from 2016 at 2.90 kg.capita⁻¹, an increase of 3.05 kg.capita⁻¹ in 2019 [1]. The increasing chili consumption has not been matched by a stable production level and low productivity. One of the factors causing the low productivity of chili is the presence of pests and diseases [2]. One of the pests that often cause damages to chili plantations is aphid from the Aphis gossypii species.

Aphis gossypii is a commonly found pest on chili plants because this pest has a wide host range, such as the Fabaceae, Solanaceae, Cucurbitaceae, and Asteraceae families. This pest has a wide distribution area from tropical to temperate climates, so A. gossypii can survive on almost all cultivation plants and populations of aphids that always exist in every season [3]. Aphids can cause damage to plants because they attack by sucking plant fluids, causing loss of plant nutrients and damaged plant cells and tissues [4]. Aphids also produce honeydew in the form of a sweet liquid that can cover the surface of plant leaves, resulting in inhibition of the photosynthesis process. Aphis gossypii also acts as a vector for disease-causing viruses, such as CMV and ChiVMV viruses in chili plants, with symptoms of stunted plants and fall before fertilization [5].

The losses caused by A. gossypii attacks are in line with the level of population on the host plant. The higher the A. gossypii population, the higher the damage to plants. A high and uncontrolled aphid population can cause damage to chili plants up to 65% [6]. Control of aphids currently generally still uses synthetic insecticides, which have the potential to cause the death of natural enemies, pest resistance, and environmental pollution [7]. In addition, the use of chemical pesticides also has affected human health because of the chemical content of pesticides left on agricultural products [8]. There is a need for alternative pest control that is more environmentally friendly and safe for consumer health, one of which is by using biological agents or botanical pesticides.

The fungus Lecanicillium lecanii is an entomopathogenic fungus that has the potential to control insects. The fungus L. lecanii can infect several types of host insects from the orders
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Orthoptera, Hemiptera, Lepidoptera, Thysanoptera, and Coleoptera [9]. The use of the fungus L. lecanii with a conidia density of 5 x 10^7 ml^-1 is pathogenic and causes A. gossypii mortality with an average mortality of 59.00±3.83% [10].

Chromolaena odorata L. plant has potential as a botanical pesticide, whereas C. odorata L. contains active sesquiterpene compounds effective against termite mortality [11]. Weed of C. odorata L. is also reported to have pyrrolizidine alkaloids as active compounds which are toxic [12]. The application of C. odorata L. leaf extract with a concentration of 40% was effective in killing A. gossypii with 100% mortality, and the application of botanical pesticides of C. odorata L. leaf extract could cause A. gossypii to move slowly and eventually die [13]. Chromolaena odorata L. leaf extract with a concentration of 80 g.L^-1 of water was effective in controlling Aphis gossypii (Glover) with total mortality of 95% at an LC50 of 7.7% [14].

The application of pest control combination using biological agents and botanical pesticides has been done before. The combination of vegetable insecticides such as Aglaia odorata, Annona squamosa, and Jatropha curcas is synergistic because it can increase the growth of L. lecanii colonies. It also increased the control efficacy of brown ladybug eggs up to 77% compared to the single application [15]. The combination of the fungus M. anisopliae with 2 mL and 3 mL of babadotan leaf extract can increase the mortality of N. viridula compared to a single application [16]. The combination of entomopathogenic fungi and botanical pesticides was able to increase pest mortality. Therefore, the purpose of this study was to determine the effect of the most effective application of the entomopathogenic fungus L. lecanii and C. odorata L. leaf extract in increasing the mortality of aphids (A. gossypii) on chili plants.

MATERIAL AND METHOD

The research was carried out at the HPT Laboratory, Faculty of Agriculture, University of Jember, and in the Patrang greenhouse, Jember Regency. The study started from February to September 2021.

The study consisted of two stages arranged in a completely randomized design. The first stage was the compatibility test of the fungus L. lecanii and C. odorata L. leaf extract. It was carried out in vitro with the technique of mixing C. odorata L. leaf extract concentrations of 10%, 25%, and 40% on PDA media through colony growth calculations, sporulation, and viability with four treatments and five replications. The second stage was the toxicity test on the application of the fungus L. lecanii and C. odorata L. leaf extract to the mortality of A. gossypii with five treatments and four replications. The data obtained were analyzed by ANOVA and continued with the DMRT test with a 95% confidence level.

Data Collection
Compatibility Test

Colony Diameter Growth L. lecanii

Measuring the diameter of the fungus colonies on the 7th day after application (DAA) with a ruler. The calculation was done by making vertical and horizontal lines on the petri dish then the measurement results were entered into the following Equation 1 [17]:

\[
D = \frac{d_1 + d_2}{2}
\]

Description:
D = diameter of L. lecanii colony (cm)
\(d_1\) = vertical diameter of L. lecanii
\(d_2\) = horizontal diameter of L. lecanii

The percentage decrease in colony growth was calculated by the Equation 2 [18]:

\[
Nr = \frac{N_1 - N_2}{N_1} \times 100\% 
\]

Description:
Nr = percentage decrease in colony growth
\(N_1\) = growth of fungal colonies on media that was not given C. odorata L. leaf extract
\(N_2\) = growth of fungal colonies on media that was given C. odorata L. leaf extract

Sporulation or Number of Conidia

Count number of spores in each treatment after the fungus was conducted at the 7th DAA. Calculation of the conidia number was carried out by harvesting conidia in a petri dish first, harvesting conidia by adding 5 mL of aqua dest then leveling and placing it in a test tube containing 5 mL of aqua dest, then vortexing the suspension and taking 1 mL of L. lecanii suspension and placing it on a hemocytometer.

\[
J = \frac{t \times d}{0.25 \times n} \times 10^6
\]

Description:
J = calculated conidia density (conidia ml^-1)
t = the number of conidia in the calculated sample box
d = dilution rate
n = number of observed sample boxes (80)
0.25 = correction factor
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The number of conidia was counted using a compound microscope with a magnification of 400 times. The conidia were counted in five sample boxes. The results of observations of the number of conidia were added up and calculated by the Equation 3 [19]. Meanwhile, the percentage decrease in sporulation was calculated by the Equation 4 [18]:

$$ S_f = \frac{S_1 - S_2}{S_1} \times 100\% $$. \hspace{1cm} 4

**Description:**
- \( S_f \) = percentage decrease in sporulation
- \( S_1 \) = the number of spores produced by the fungus on media not given \textit{C.odorata} L. leaf extract (control)
- \( S_2 \) = the number of spores produced by the fungus in the media given the \textit{C.odorata} L. leaf extract

**Viability or Number of Germinating Conidia**
Harvesting conidia on PDA media according to the treatment, then taking a suspension of \textit{L.lecanii} conidia and incubating on glass slides and placing them on a damp tissue placed on a tray and covered with plastic wrap for 24 hours. Conidia germination was observed using a microscope with a magnification of 400 times. Germination percentage was calculated from 100 conidia, where conidia were said to germinate if the length of the germination tube had exceeded the diameter of the conidia. Calculation of germination (viability) of spores is calculated using the Equation 5 [20]:

$$ V = \frac{g}{g+u} \times 100\% $$. \hspace{1cm} 5

**Description:**
- \( V \) = conidia germination
- \( g \) = number of germinated conidia
- \( u \) = number of conidia that did not germinate

The percentage decrease in sporulation was calculated by the Equation 6 [18]:

$$ M_f = \frac{M_1 - M_2}{M_1} \times 100\% $$. \hspace{1cm} 6

**Description:**
- \( M_f \) = percentage decrease in germination
- \( M_1 \) = germination of conidia in media that was not given \textit{C.odorata} L. leaf extract (control)
- \( M_2 \) = germination of conidia in media that was given \textit{C.odorata} L. leaf extract

**Compatibility Value Calculation**
The results of the compatibility observations are entered into the \( T \) formula as following Equation 7 [18]. The \( T \) value is divided into categories, namely 0-30 very toxic, 31-45 toxic, 46-60 less toxic, and >60 non-toxic (compatible).

$$ T = \left( \frac{20(PK)+80(SP)}{100} \right) $$ \hspace{1cm} 7

**Description:**
- \( T \) = compatibility value (%) C. odorata L. leaf extract addition had the highest average diameter of 5.27 cm, compared to the diameter of \textit{L.lecanii} colonies with \textit{C.odorata} L. leaf extract. The addition of \textit{C.odorata} L. leaf extract at a concentration of 10% showed an average
Synergism of L. lecanii and C. odorata L. Extract for Controlling A. gossypii (Glover) (Nurhayati & Haryadi)

diameter of L. lecanii of 2.48 cm, and the percentage decrease in diameter was 52.94%. Then the addition of C. odorata L. leaf extract with a concentration of 25% showed an average value of L. lecanii diameter of 2.34 cm with a percentage a decrease in the diameter of 55.60%, while the addition of C. odorata L. leaf extract with the highest concentration was 40% showed that the inhibition of the growth of L. lecanii colonies was much greater, for 2.23 cm, which means that the growth of L. lecanii colonies decreased by 57.69% (Fig.1).

The inhibition of the growth of L. lecanii colonies was thought to be due to the content of C. odorata L. leaves, which act as fungistatic antimicrobial compounds that can temporarily inhibit the growth of the fungus L. lecanii. Leaf extract of C. odorata L. has potential as an antifungal because it has antifungal activity against the growth of Aspergillus flavus fungus [22]. The addition of C. odorata L. leaf extract with a concentration of 0.5% was effective in suppressing the growth of the Colletotrichum capsici and had a very significant effect on the colony diameter and the percentage of inhibition of C. capsici [23]. Therefore, it is better not to combine it with C. odorata L. leaf extract because the C. odorata L. leaf extract has antifungal compounds that can inhibit the colony growth of the L. lecanii fungus.

The effectiveness of an antifungal substance is influenced by the concentration of the substance given [24]. It is in line with the observations which showed that increasing the concentration of C. odorata L. leaf extract results in a high content of active ingredients. It has function as antifungals so that the ability to inhibit fungal growth will be greater. So, the higher the concentration of C. odorata L. leaf extract given will also increase the anti-fungal properties that can inhibit the growth of the fungus L. lecanii.

Sporulation, and Viability of L. lecanii

Table 1 shows that the addition of C. odorata L. leaf extract in various concentrations also significantly affected the number of spores (sporulation) of L. lecanii conidia. The addition of C. odorata L. leaf extract significantly reduced the ability of the fungus to sporulate compared to the control treatment without the addition of C. odorata L. leaf extract.

**Table 1. Mean Diameter, Sporulation, and Viability of L. lecanii Fungus Colonies with the Addition of C. odorata L. Leaf Extract**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diameter 7th DAA (cm)±SD</th>
<th>Decrease in Diameter (%)</th>
<th>Sporulation of conidia (10⁸)</th>
<th>Decrease in Sporulation (%)</th>
<th>Conidia viability (%)±SD</th>
<th>Decrease in viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LK₁</td>
<td>5.27 ± 0.92ᵇ</td>
<td>0</td>
<td>3.66 ± 1.01ᵇ</td>
<td>0</td>
<td>75.20 ± 2.86ᵃ</td>
<td>0</td>
</tr>
<tr>
<td>LK₂</td>
<td>2.48 ± 0.21ᵇ</td>
<td>52.94</td>
<td>1.52 ± 0.24ᵇ</td>
<td>58.47</td>
<td>69.00 ± 1.58ᵇ</td>
<td>8.24</td>
</tr>
<tr>
<td>LK₃</td>
<td>2.34 ± 0.16ᵇ</td>
<td>55.60</td>
<td>1.32 ± 0.19ᵇ</td>
<td>63.93</td>
<td>67.60 ± 0.89ᵇ</td>
<td>10.11</td>
</tr>
<tr>
<td>LK₄</td>
<td>2.23 ± 0.29ᵇ</td>
<td>57.69</td>
<td>1.27 ± 0.26ᵇ</td>
<td>65.30</td>
<td>67.00 ± 1.87ᵇ</td>
<td>10.90</td>
</tr>
<tr>
<td>sig</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
<td></td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** LK₀ = L. lecanii + C. odorata L. Extract 0%, LK₁ = L. lecanii + C. odorata L. Extract 10%, LK₂ = L. lecanii + C. odorata L. Extract 25%, LK₃ = L. lecanii + C. odorata L. Extract 40%; SD = Standard Deviation.

**Figure 1.** Colony growth of L. lecanii at 7 days after inoculation (DAI) with the addition of C. odorata L. leaf extract (a) at 0% concentration, (b) 10% concentration, (c) 25% concentration, (d) 40% concentration.

In LK₁ treatment, the addition of C. odorata L. leaf extract with a concentration of 10% significantly inhibited L. lecanii from sporulating with a decrease of 58.47%. In LK₂ treatment, the addition of C. odorata L. leaf extract with a concentration of 25% has a sporulation value of 1.52 x 10⁸ conidia.mL⁻¹, with a decreased percentage of sporulation reaching 63.93%. And in LK₃, the addition of C. odorata L. leaf extract with a concentration of 40% gave a much lower effect, with a sporulat of only 1.27 x 10⁸ conidia.mL⁻¹ with a decrease in sporulation...
percentage of 65.30%. The higher the concentration of *C. odorata* L. leaf extracts given to the growth medium, the lower the number of *L. lecanii* conidia produced.

Leaf extract of *C. odorata* L. has antifungal properties and is effectively used as a fungicide in suppressing the growth and formation of *Colletotrichum musae* spores [25]. The antifungal properties of *C. odorata* L. leaves are thought to be caused by the content of secondary metabolites in *C. odorata* L. leaves. The results of phytochemical analysis of *C. odorata* L. leaves contain secondary metabolites such as flavonoids, saponins, terpenoid, steroids, phenolics, and tannins [26]. Plants containing secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and glycosides have potential as antifungals, with different antifungal activity mechanisms between one compound and another [27].

Saponins are secondary metabolites that act as antifungals and antimicrobials [28]. Saponins can inhibit the growth of the fungus *L. lecanii*, where saponins as antifungals work by disrupting the stability of cell membranes resulting in the lysis of microbial cells. Saponins will reduce the surface tension of the fungus so that it can increase cell permeability which causes cell leakage, and intracellular compounds in cells will come out [29]. Saponin compounds in the Ambon banana stem also have an antifungal activity that inhibits the growth of the fungus *Candida albicans* [30].

The mechanism of action of flavonoids in inhibiting fungal growth is by causing interference with the permeability of fungal cell membranes. The hydroxyl group contained in flavonoid compounds causes changes in organic components and nutrient transport, which will eventually lead to toxic effects on fungi [31].

The content of tannin metabolites also acts as an antifungal. The antifungal mechanism caused by tannin compounds inhibited chitin synthesis, which is functional in the fungal cell walls formation and can damage cell membranes so that fungal growth is inhibited. Tannin compounds are lipophilic, which means they are easily attached to fungal cell walls [32]. Terpenoid compounds can also inhibit the growth of fungi through the mechanism of decreasing the permeability of the cell membrane of microorganisms; where the compounds contained in terpenoids are bound to protein and lipid molecules so that they can affect the physiological function of cell membrane proteins and enzyme proteins [33].

**Viability of *L. lecanii***

The addition of *C. odorata* L. leaf extract with various concentrations was also significantly affected the conidia germination (viability) of the fungus *L. lecanii*. The addition of *C. odorata* L. leaf extract significantly reduced the ability of the fungus to germinate compared to the control treatment without the addition of *C. odorata* L. leaf extract. In LK1 treatment, the addition of *C. odorata* L. leaf extract with a concentration of 10% significantly inhibited *L. lecanii* germination with a decrease of 8.24% with a viability value of 69.00%. It was followed by LK2 treatment with the addition of *C. odorata* L. leaf extract with a concentration of 25%, which has a viability value of 67.60% with a decreasing percentage of germination 10.11%. Last, the LK3 treatment (*C. odorata* L. leaf extract with a concentration of 40%) was showed a viability value of 67.00% and a percentage decrease in the germination of 10.90%. The higher the concentration of *C. odorata* L. leaf extract added, the lower the germination rate of conidia *L. lecanii*.

Conidia germination rate and high sporulation can be seen from good fungal physiology, where high conidia germination rate will also increase the virulence ability of the fungus [34]. Viability is positively correlated with the level of fungal infection, where the higher the viability of the spores, the faster the fungus produces spores, thus causing the process of infection in insects to be faster [35]. The faster the occurrence of insect infection by fungi, the faster the process of insect death will be [36].

Based on the calculation of the T value, the classification of the compatibility of *C. odorata* L. leaf extract with the fungus *L. lecanii* combination treatment showed a classification at a toxic level. It meant that it was not compatible with the fungus *L. lecanii* (Table 2). The results showed that the treatment with the addition of *C. odorata* L. leaf extract in growth media with concentrations of 10%, 25%, and 40% showed compatibility values at the toxic level against the fungus *L. lecanii*. It means that the addition of *C. odorata* L. leaf extract was not compatible with the fungus *L. lecanii*. This compatibility value indicated that the leaf extract of *C. odorata* L. and the fungus *L. lecanii* was not compatible, supported by data on decreased colony growth, sporulation, and significantly decreased germination of conidia of the fungus *L. lecanii*. 

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**Synergism of *L. lecanii* and *C. odorata* L. Extract for Controlling *A. gossypii* (Glover) (Nurhayati & Haryadi)**

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Synergism of L. lecanii and C. odorata L. Extract for Controlling A. gossypii (Glover) (Nurhayati & Haryadi)

Table 2. Leaf Extract of C. odorata L. Compatibility Classification with Entomopathogenic Fungi L. lecanii

<table>
<thead>
<tr>
<th>Treatment of L. lecanii (conidia.ml⁻¹ aqua dest) with EC%</th>
<th>%T (Compatibility Value)</th>
<th>Compatibility Level Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>LK₁ (L. lecanii + C. odorata L. Leaf Extract 10%)</td>
<td>42.64</td>
<td>Toxic (Not Compatible)</td>
</tr>
<tr>
<td>LK₂ (L. lecanii + C. odorata L. Leaf Extract 25%)</td>
<td>37.73</td>
<td>Toxic (Not Compatible)</td>
</tr>
<tr>
<td>LK₃ (L. lecanii + C. odorata L. Leaf Extract 40%)</td>
<td>36.22</td>
<td>Toxic (Not Compatible)</td>
</tr>
</tbody>
</table>

Note: T is the value of the compatibility test results.

Toxicity of Fungus L. lecanii and C. odorata L. Leaf Extract against Mortality of Instar-3 Nymph A. gossypii.

From the compatibility test, it was found that the treatment with the addition of C. odorata L. leaf extract showed incompatible results, so the toxicity test was carried out separately. The results of the toxicity test showed the separate application of the suspension of the L. lecanii fungus with a density of 10⁷ conidia.ml⁻¹ aqua dest and the addition of various concentrations of C. odorata L. leaf extract gave a significantly different effect on the mortality of 3rd instar nymph A. gossypii (Table 3), with a significance value of p<0.05. The mortality percentage of A. gossypii nymphs will increase along with the increasing concentration of C. odorata L. leaf extract given, where the higher the concentration level of C. odorata L. leaf extract, the more effective it is in killing 3rd instar nymphs of A. gossypii, with the best concentration in the H₄ treatment using C. odorata L. leaf extract at a concentration of 40%, with an average mortality of 100% at 96 HAA. In the control treatment that was sprayed with aquadest, none of the aphids nymphs died.

Characteristics of A. gossypii that died due to the application of L. lecanii showed different symptoms from the death due to the application of C. odorata L. leaf extract. It was based on the observation valuation of aphids death symptoms caused by the application of L. lecanii suspension. The valuation was seen from the change in the color of the aphid body, which was black and stiff, the aphid body hardened and hyphae appeared on the body surface, and within a few days, the body surface of the aphids would be covered with mycelium (Fig. 2). Insect death within a few days after application of L. lecanii suspension showed insect’s body becomes hard due to the fungus attack on all tissues and fluids in the insect’s body until it runs out, which makes the insect turn black and stiff. Then the insect’s body will slowly be covered with mycelium [37]. Observation of the symptoms of aphids’ death caused by the fungus L. lecanii was also carried out using the Koch Postulate test to ensure that the pathogen that caused the death of aphids came from the fungus L. lecanii.

Figure 2. Nymphal microscopic observation results of A. gossypii instar-3 infected with the L. lecanii grew hyphae on the body surface of aphids.

The fungus L. lecanii will kill host insects by digesting the host’s body tissues as a source of food or nutrition and producing toxic substances that kill insect pests. The toxic compounds were in the form of dipecolinic acid and cyclosporin which have insecticidal properties so that they have a very toxic effect when applied to the host insect [10].

Table 3. Average mortality of A. gossypii after application of entomopathogenic fungus L. lecanii and botanical pesticide C. odorata L. leaf extract

<table>
<thead>
<tr>
<th>Treatment</th>
<th>On observation...HAA (%) (X)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td>H₀ = Control (aquadest)</td>
<td>0.00²</td>
</tr>
<tr>
<td>H₁ = L. lecanii suspension 10⁷ conidia.ml⁻¹</td>
<td>3.75²</td>
</tr>
<tr>
<td>H₂ = 38.75¹</td>
<td>53.75²</td>
</tr>
<tr>
<td>H₃ = 60.00²</td>
<td>68.75³</td>
</tr>
<tr>
<td>H₄ = 85.00⁰</td>
<td>95.00⁰</td>
</tr>
<tr>
<td>Sig.</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Description: H₀ = Control (aquadest), H₁ = L. lecanii suspension 10⁷ conidia.ml⁻¹, H₂ = 10% concentration of C. odorata L. Leaf Extract, H₃ = 25% concentration of C. odorata L. Leaf Extract, H₄ = 40% concentration of C. odorata L. Leaf Extract; HAA= Hours After Application.
The fungus *L. lecanii* infects the host through the formation of germ tubes by conidia on the cuticle surface, which will be used to penetrate the insect cuticle, then the fungus will enter through the integument and damage the physiology of the insect host. The surface of the host insect’s body is hydrolyzed more quickly by enzymes produced by fungi, causing insect death [39]. Observations on the characteristics of 3rd instar nymphs of *A. gossypii* that died due to the application of *C. odorata* L. leaf extract showed that at first, the insects experienced changes in behavior and tended to be less active or passive when touched with a brush. The insect’s body also turned dark or blackish brown (Fig.3). Application of botanical pesticides from *C. odorata* L. leaves can cause the movement of *A. gossypii* pests to be slow and the lice will die over time [13].

![Figure 3. Nymph Death Symptoms A. gossypii instar 3 after C. odorata L. Leaf Extract Application; (a) microscopic; (b) macroscopic.](image)

Leaf extract of *C. odorata* L. can cause death in *A. gossypii* because of its active ingredient compounds, *Pyrrolizidine alkaloids* which are toxic [12]. The content of *Pyrrolizidine alkaloids* in *C. odorata* L. leaves can cause the plant to have a pungent smell and taste bitter, so it is insect repellent [26]. In addition, the content of secondary metabolites contained in *C. odorata* L. leaves can also cause death in aphids, with a characteristic the insect’s body turned dark or blackish brown, and activity change that becomes passive. Leaves of *C. odorata* L. contain several secondary metabolites such as saponins, tannins, flavonoids, alkaloids, and phenolics [27]. The content of alkaloid and flavonoid compounds in *C. odorata* L. leaves can be toxic to insects and inhibit appetite or *antifeedant* for pests. The compounds were acted as stomach poisons so that aphids poisoned over time and eventually died.

**CONCLUSION**

The compatibility test of the fungus *L. lecanii* with *C. odorata* L. leaf extract showed incompatibility and was classified as toxic. The addition of *C. odorata* L. leaf extract at concentrations of 10%, 25%, and 40% could significantly inhibit colony growth, sporula, and viability of *L. lecanii* conidia, with a higher level of inhibition as the concentration of *C. odorata* L. leaf extract, was added. Toxicity test of a separate application of *C. odorata* L. leaf extract and *L. lecanii* suspension had a significant effect on mortality of 3rd instar nymph *A. gossypii*, with the highest mean mortality found in a single application of 40% *C. odorata* L. leaf extract with an average mortality of 100% in 96 HAA observations.

**REFERENCES**


Synergism of L.lecanii and C.odorata L. Extract for Controlling A. gossypii (Glover) (Nurhayati & Haryadi)


