Effectiveness test of Eucalyptus leaf extract (*Eucalyptus* sp.) and Lemongrass extract (*Cymbopogon citratus* (DC) Stapf.) on mortality of leaf roller pests (*Strepsicrates sp*) in vitro

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Abstract. *Strepsicrates sp* pests in eucalyptus plants are a major constraint that can reduce plant productivity. This study aimed to test the effectiveness of a combination of eucalyptus leaf extract and lemongrass extract on leaf roller pest mortality in vitro and to determine its effective concentration. The study used an experimental method with a completely randomized design consisting of seven treatments with three replications. The application of eucalyptus and lemongrass leaf extracts by wetting the test larvae's food leaves was carried out. To determine their effectiveness, observations were made on larval mortality, the percentage of pupae and imago formation, and other symptoms found in *Strepsicrates sp*. The results showed that the combination of eucalyptus leaf extract and lemongrass as a botanical pesticide was effective against mortality, pupae and imago formation of *Strepsicrates sp*. The best treatment was at a concentration of 300 mL L^{-1}, but it was not significant with a concentration of 250 mLL^{-1} so that the recommended concentration is 250 mL L^{-1} because it caused 83.33% larval mortality, 16.67% pupae formation and no imago formation. Sublethal effects were in the form of developmental disorders, namely, morphological malformations in the pupae and imago stages.

1 Introduction

Eucalyptus is a fast-growing plant species that is crucial for the pulp and paper industry. Eucalyptus's advantages as a fast-growing seedling include its short rotation and high economic value [1,2]. This plant is susceptible to pest attacks, including leaf rollers. Eucalyptus leaf rollers are a major pest of eucalyptus plants, attacking them by feeding on shoot tips and flowers. Their attack can reduce photosynthetic capacity, inhibit plant growth, and even lead to eucalyptus death. At PT ITCI Hutani Manunggal, leaf roller caterpillar attacks are worrying for young plants, particularly those under six months old. There is no definitive data on the attack level at this time; attacks typically depend on

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conditions. The company has established an economic threshold based on the intensity of the attack. If the attack exceeds the economic threshold, control measures using synthetic pesticides are required. The determination of the attack intensity level varies depending on the age of the plant. For plants under three months old, the economic threshold is at an attack level of 4.8%, for plants over three to less than five months old, the attack level is 6.4%, for plants over five to six months old, the attack level is 8%, and for plants over six months old, the attack level is 12.8%. The Open Growing Area (OGA) report states that leaf roller infestations reach 0.04% [1].

Control of eucalyptus leaf rollers still relies on chemical pesticides. Excessive use of chemical pesticides will pollute the environment, leave residues on plants, increase pest resistance and harm humans, and continuous use of chemical pesticides can be a threat to plants and non-target organisms [3].

The use of botanical pesticides is an environmentally friendly alternative control method. Eucalyptus plants can be used as botanical insecticides because they contain phytochemical compounds such as eugenol, alakaloids, polyphenols, tannins, and saponins, which can function as botanical insecticides. Lemongrass is one type of plant that can be used as a botanical insecticide. Lemongrass is a light green grass with a rough texture and a distinctive aroma [4,5]. Lemongrass contains effective compounds such as citronella, geraniol, citronellol, granyl acetate, citronellyl acetate, citral cavicol, vanillin, eugenol, cadinol, elemol, cadinene, camphene, and limonene. One of the compounds in lemongrass, citronella, is disliked by mosquitoes and various types of insects, making it potentially useful as a botanical insecticide. Lemongrass also contains high levels of essential oils, making it an alternative inorganic pesticide that functions as a bactericide, insecticide, and nematicide [6-9].

Previous research has shown that a concentration of 30 gL-1 of eucalyptus leaf extract used as a botanical pesticide can cause 90% mortality of mealybugs (*Paracoccus marginatus*) in acacia (*Acacia mangium*) nurseries [10]. A concentration of 30 gL-1 of lemongrass stem extract, used as a botanical pesticide, caused 68% mortality against *Plutella xylostella* pests on mustard greens (*Brassica juncea* L.) [11]. Based on this description, it is necessary to test the effectiveness of the combination of eucalyptus leaf extract and lemongrass extract against eucalyptus leaf roller pests in vitro.

2 MATERIALS AND METHODS

2.1 Materials and Experimental Design

This research was conducted from March to September 2024. Larvae were collected from a plantation at PT. ITCI Hutani Manunggal, Sepaku District, Penajam Paser Utara Regency, East Kalimantan. Extraction of eucalyptus leaves and lemongrass, as well as treatment of leafroller caterpillars, were carried out at the Integrated Laboratory, Mulawarman University, Samarinda, East Kalimantan, Indonesia.

The materials used in this study were fresh eucalyptus leaves, lemongrass plants, eucalyptus leafroller larvae (*Strepsicrates sp*), 5% honey, 70% alcohol, a 140x75x90 cm rearing box, a 300 mL test box, and distilled water. The equipment used included an

evaporator, scales, a blender, a stopwatch, a sprayer, tweezers, stationery, and documentation tools.

The experimental design was a completely randomized design (CRD) with 7 treatments and 3 replications. The treatment was a combination of eucalyptus leaf and lemongrass extract with a ratio of 2:3, namely: 1). Control (P_0), 2). 50 mL L^{-1} (P_1), 3). 100 mL L^{-1} (P_2), 3). 150 mL L^{-1} (P_3), 4). 200 mL L^{-1} (P_4), 5). 250 mL L^{-1} (P_5), 6). 300 mL L^{-1} (P_6).

2.2 Research Procedures

2.2.1 Sampling and Propagation of Leaf Roller Pests.

A land survey was conducted to document land conditions and collect samples of eucalyptus leaf roller pests (*Strepsicrates sp*) on infested eucalyptus trees from PT. ITCI Hutani Manunggal. *Strepsicrates sp* larvae were reared at the Integrated Laboratory, Mulawarman University. The rearing was carried out for 3 months to obtain the number of individuals required for the research. Rearing was carried out in a 1000 mL rearing box for larvae, covered with tile cloth. From the pupal stage to the imago stage and egg-laying, the larvae were fed natural food in the form of guava leaves and 5% honey diluted with distilled water.

2.2.2. Preparation of a Combination of Eucalyptus Leaf Extract and Lemongrass Extract

The preparation of eucalyptus and lemongrass leaf extract solution begins by weighing 1 kg of eucalyptus leaves. Next, they are ground using a blender and 3 L of 70% alcohol is added. The preparation of lemongrass extract is carried out in the same way as the preparation of eucalyptus leaf extract. The blender results are filtered to obtain eucalyptus and lemongrass leaf extract in different containers. After filtering, the solution is put into a bottle and left for 48 hours. Then, it is evaporated at room temperature, as long as there is no further reduction in the volume of the extract and then it is ready to be applied. When it is to be applied, the eucalyptus leaf extract is mixed with lemongrass extract in a ratio of 2:3. The difference in the amount of comparison is based on the results of previous studies, which show that lower concentrations of eucalyptus leaves are effective compared to lemongrass plants.

2.3 Application of Botanical Pesticides

The test larvae were fasted for 6 hours, so that when tested, the larvae were hungry and ate the treated feed leaves. The larvae were placed in a 300 mL test box, each test box containing 20 third-instar larvae, so that 420 test larvae were prepared for the entire treatment. The test larvae feed was eucalyptus leaves that were not too young. The application of a combination of eucalyptus and lemongrass leaf extract was carried out by

soaking the larval feed for 10 minutes as much as 10 mL with a concentration according to the treatment, followed by air-drying the leaves for 5 minutes and the feed was ready to be given to the test larvae.

2.4 Observation Variables

The effectiveness of eucalyptus leaf extract and lemongrass plants against *Strepsicrates sp* larvae was observed by observing the symptoms of death and mortality of larvae, the formation of pupae and imago.

2.4.1 Larval Mortality Symptoms

Observation of death symptoms was carried out visually by observing behavior after being given treatment for 24 hours from the larval phase to adult insects.

2.4.2 Larval Mortality of Strepsicrates sp.

Observations were conducted daily, continuing until the larvae died completely, according to the concentration used. Larval mortality of *Strepsicrates* sp. was calculated using the formula:

$$P = \frac{a}{b} \; x\; 100\% \qquad (1)$$

Where: P = Percentage of test larvae mortality, a = Number of test larvae that died, b = Total number of test larvae.

2.4.3. Lethal Concentration 50 (LC_{50}).

The LC_{50} calculation was based on observations of the mortality percentage of *Strepsicrates* sp. larvae on the seventh day of mortality observation.

2.4.4 Percentage of Pupae Formation

Observations were conducted on test larvae that had transformed into pupae. The percentage of pupae formation was calculated using the formula:

$$P = \frac{v}{p}\; x\; 100\% \qquad (2)$$

Where, P = Percentage of pupae formation, v = Number of pupae formed, p = Number of larvae tested.

2.4.5 Percentage of Imago Formation

Observations were made on test pupae, continuously observed until they transformed into adults. The percentage of adult development was calculated using the formula:

$$P = \frac{A}{B}\; 100\% \qquad (3)$$

Where: P = Percentage of imagoes formed, A = Number of imagoes formed, B = Number of pupae tested.

2.5 Data Analysis

The data obtained were analyzed using analysis of variance. If there were significant differences, the Least Significant Difference (LSD) test was performed at the 5% level.

3 RESULTS AND DISCUSSION

3.1 Larval Mortality Symptoms

Symptoms of *Strepsicrates sp* larvae sprayed with eucalyptus leaf and lemongrass extracts indicate that when touched, the larvae become immobile, weaken, and shrink/shrivel, with color changes. The larvae change color to a pale cream, have black spots on the underside of their abdomen, and excrete a greenish-yellow fluid. Some larvae shrivel and stiffen, and the bodies of dead larvae dry out, starting from the abdomen, and their bodies turn black.

The combination of eucalyptus leaf and lemongrass extracts contains various bioactive phytochemical compounds such as eugenol, polyphenols, tannins, saponins, citronella, and geraniol. Eucalyptus extract contains eugenol and phenolic compounds that can damage the insect's nervous system, causing metabolic disorders and death [9]. Lemongrass extract contains citronella and geraniol compounds, which disrupt the respiratory and nervous systems of larvae and act as a [6,11,12]. The combination of these compounds produces a stronger synergistic effect than using either extract alone, resulting in a lower effective dose, resulting in significant mortality and developmental inhibition.

3.2 Percentage of Larval Mortality of *Strepsicrates* sp.

The data analyzed is the original data that has been transformed to Arcsin √x. The results of the analysis of variance of *Strepsicrates* sp. larval mortality data from the application test of eucalyptus leaf extract and lemongrass extract, showed a very significant difference. The higher the concentration of the extract applied, the higher its effectiveness on *Strepsicrates* sp. larval mortality. This indicates a higher content of metabolic compounds that can cause larval death. The development of the average percentage of *Strepsicrates* sp. larval mortality after the application of eucalyptus extract and lemongrass extract can be seen in the Table 1.

Research on the use of eucalyptus leaf extract and lemongrass plants to control *Strepsicrates* sp has not been widely conducted, but several studies to control other pests have been reported. Moniharapon et al. [11] in their study on the effectiveness of lemongrass stem extract against *Plutella xylostella* pests in mustard greens (*Brassica juncea* L.) stated that a concentration of 30 gL^{-1} of the extract used as a botanical pesticide can cause mortality of up to 68% in larvae within 24–48 hours. The main active compounds such as citronellal and geraniol show toxic effects that disrupt the nervous system and metabolism of insects. This indicates that lemongrass extract alone has sufficient potential as a pest larval mortality agent. Putra and Zein [8] stated that a concentration of 30 gL^{-1} of eucalyptus leaf extract can cause mortality of up to 90% in

mealybug pests (*Paracoccus marginatus*) in acacia nurseries. Phytochemical compounds such as eugenol and tannin are believed to be the main components that cause damage to the physiological system of target insects, both through contact and stomach poison. The performance of eucalyptus extract as a botanical insecticide is not only lethal but also affects the feeding process and development of pests. According to Younoussa et al [13], who studied the combination of Eucalyptus camaldulensis extracts against *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes, the combination of extracts was more effective in causing high mortality in larvae compared to single applications, with a combined working mechanism in the form of stomach poison, contact poison, and growth disorders.

Table 1: Percentage mortality of eucalyptus leafroller caterpillar larvae.

Treatm.	Days after application (%)													
	1		2		3		4		5		6		7	
	Trans.	Orig.	Trans.	Orig.	Trans.	Orig.	Trans.	Orig.	Trans.	Orig.	Trans.	Orig.	Trans.	Orig.
P_0	0.40^{c}	0.0	0.40^{c}	0.0	0.40^{c}	0.0	0.40^{c}	0.0	0.40^{d}	0.0	0.40^{e}	0.0	0.40^{d}	0.0
P_1	1.37^{b}	6.7	2.28^{b}	16.7	3.31^{b}	33.3	3.78^{b}	43.3	3.85^{c}	45.0	4.13^{d}	51.6	4.33^{c}	56.7
P_2	1.20^{b}	5.0	2.33^{b}	16.0	3.48^{ab}	36.7	3.86^{b}	45.0	4.13^{bc}	51.7	4.33^{cd}	56.7	4.70^{bc}	66.7
P_3	1.69^{ab}	8.3	2.58^{ab}	20.0	3.24^{b}	31.7	3.57^{b}	38.3	3.78^{c}	43.3	3.90^{d}	46.7	4.36^{c}	58.3
P_4	1.69^{ab}	8.3	2.64^{ab}	21.7	3.54^{ab}	38.3	4.05^{ab}	50.0	4.38^{bc}	58.3	4.73^{bc}	68.3	4.79^{bc}	70.0
P_5	1.69^{ab}	8.3	2.77^{ab}	23.3	3.44^{ab}	36.7	4.12^{ab}	51.7	4.57^{ab}	63.3	4.97^{ab}	75.0	5.24^{ab}	83.0
P_6	2.33^{a}	11.8	3.40^{a}	35.0	4.25^{a}	55.0	4.62^{a}	65.0	5.13^{a}	80.0	5.55^{a}	93.3	5.55^{a}	93.3
BNT 5%	0.88		0.91		0.85		0.71		0.67		0.73		0.73	

Notes: Trans.=transformed data, Orig.=original data. Numbers followed by the same letter in the same column indicate no significant difference in the 5% BNT test.

3.3 Lethal Concentration 50 (LT_{50})

The LC_{50} calculation uses data on the percentage of *Strepsicrates* sp. larvae mortality on the seventh day of observation after application. The LC_{50} of the combination of eucalyptus leaf extract and lemongrass plants was obtained by calculating the regression of the treatment, the result was the equation y = -0.044 + 3.063x. In the Probit Table, 5 = 50%, so the calculation result of the equation is x = 1.616 then the antilog is 41.33. So, the LC_{50} of the combination of eucalyptus leaf extract and lemongrass plants is 41.33 mL L^{-1} (LC_{50} = 41.33 mL L^{-1}). This indicates that the treatment with a concentration of 50 mL L^{-1} of the combination of eucalyptus leaf extract and lemongrass plants with the extraction method using 70% alcohol solvent was effective in controlling *Strepsicrates* sp. larvae in vitro on the seventh day after application.

3.4 Pupa Formation

The results of the analysis of variance (ANOVA) on the percentage of *Strepsicrates sp* pupa formation following the application of the combination of eucalyptus and lemongrass extracts showed a highly significant difference. The percentage of pupa formation is shown in the table below.

Table 2. Results of the effectiveness test of eucalyptus leaf extract and lemongrass extract on the average percentage of pupa formation.

Treatment	Average	
	Percentage of pupae formed (%)	Transformation result
P_0	100.00	5.75[a]
P_1	43.33	3.78[b]
P_2	30.00	3.14[bc]
P_3	38.33	3.40[bc]
P_4	31.67	3.16[bc]
P_5	16.67	2.25[cd]
P_6	6.67	1.33[d]

Notes: Numbers followed by the same letter in the same column show no significant difference in the 5% BNT test (1.44).

Based on the table above, there is a difference in the average percentage of pupa formation in each treatment. The control treatment (P_0) showed the highest average pupa formation, namely 100%. This shows that without the treatment of eucalyptus leaf extract and lemongrass leaf extract, all larvae were able to develop into pupae normally, while in treatments P_1 to P_6, which were given eucalyptus leaf extract and lemongrass extract, there was a decrease in the percentage of pupa formation. The highest decrease in pupa formation was in treatment P_6 with an average of only 6.67%, which was not significantly different from treatment P_5, which was 16.67%. The pupae formed after application of the plant pesticide extract were faster than the control (P_0), which

normally takes 7-8 days in nature. In the P_0 treatment, pupae took 5 days to form, while P_1-P_6 varied between 2-3 days. This indicates that the application of the plant pesticide extract caused the leaf roller caterpillars to accelerate their metamorphosis. Naturally, every living creature strives to maintain the continuity of its life cycle. In this study, the condition of the larvae in the container illustrates the presence of pressure so that in the P_0 treatment, metamorphosis took place 2 days faster than naturally. Larvae given P_1-P_6 treatments metamorphosis into pupae took place 5 days faster than naturally. This illustrates that the content of eucalyptus leaf extract and lemongrass plants causes pressure on the metabolism of the larvae, resulting in accelerated metamorphosis into pupae. The formed pupae also experienced several changes in shape and condition, such as the death of the pupae or failure to form a pupa. The image below shows several pupae forms observed, namely pre-pupae, normal pupae, dead pupae, and failed pupae formation.

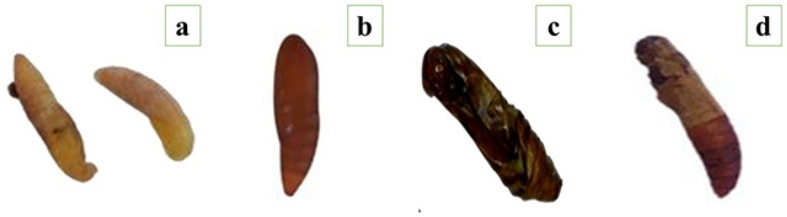

Figure 1. Symptoms of pupa death; a) Pre Pupa, b) Normal Pupa, c) Dead Pupa, d) Failed Pupa.

The results of observations of the pupae shape as shown in the image above are in accordance with Younoussa et al. [3] who stated that the phytotoxic effects of botanical pesticides often require time to have a significant impact on larvae, especially since early-stage larvae still have quite strong physiology. A very clear increase in mortality was seen in treatments P5 and P6 on the second day. This indicates that high extract doses cause faster physiological damage so that many larvae cannot survive for more than two days. In treatments P2 and P3, mortality began to increase significantly on the third day. This is thought to be due to the process of toxin accumulation in the larvae's bodies which begins to reach the physiological tolerance threshold. Residual and accumulative effects often occur with botanical insecticides because larvae are repeatedly exposed either through contact or consumption of leaf tissue that has been coated with the extract [14]. Increasing the extract concentration caused a significant increase in larval mortality, indicating that the phytochemical content in both extracts is effective as a botanical insecticide [15]. The toxic effects of this extract combination are thought to originate from active compounds such as eugenol, alkaloids, polyphenols, tannins, and saponins in eucalyptus, as well as citronella, geraniol, and flavonoids in lemongrass. These compounds act as contact and stomach poisons, disrupting the nervous system and metabolism of larvae, causing death. Increasing mortality with increasing concentration also indicates a strong dose-response relationship, strengthening the effectiveness of the extract combination as a bioinsecticide [14].

3.5 Adult Formation

The results of the analysis of variance (ANOVA) showed a highly significant difference in the percentage of adult formation of *Strepsicrates* sp. following the application of eucalyptus leaf extract and lemongrass. Further testing using a 5% LSD showed that the treatment at P_0 was significantly different from all other treatments, as can be seen in the table below.

Table 3. Results of the effectiveness test of eucalyptus leaf extract and lemongrass on the average percentage of adult formation

Treatment	Average	
	Average pupa formation (%)	Transformation result data
P_0	100.00	5.75^{a}
P_1	10.00	1.82^{bc}
P_2	10.00	1.82^{bc}
P_3	18.33	2.14^{b}
P_4	15.00	1.80^{bc}
P_5	0.00	0.40^{c}
P_6	1.67	0.72^{bc}

Notes: Numbers followed by the same letter in the same column show no significant difference in the 5% BNT test (1.65).

The image below shows various forms of imago resulting from the application of eucalyptus and lemongrass leaf extract, such as normal imago, damaged imago, imago that failed to emerge from the pupa and dead imago.

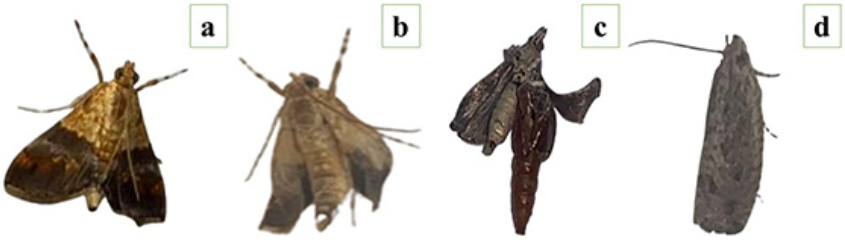

Figure 2. Symptoms of imago death: a) Imago does not show any symptoms of damage (Normal), b) Imago's wings are damaged, c) Imago fails to emerge from the pupa, and d) Imago dies.

Some pupae that were able to develop into adults exhibited malformations, such as smaller body size, small wings, and abnormal wing shapes, resulting in a shorter lifespan. This is suspected to be due to impaired feeding activity during the larval stage due to compounds found in lemongrass extract. This lack of nutrition during the larval stage results in insufficient food reserves during pupal development, resulting in incomplete pupae. Incomplete pupal development also impacts imago development, as energy reserves for the imago stage are reduced due to metabolic disturbances in the insect's body.

The low percentage of imago development from pupae is due to the low number of larvae that develop into pupae. Not all pupae develop into adults; some pupae are normal in shape but do not develop into adults. Those that do not develop into adults show no signs of life and are therefore considered failures to develop into adults. Mortality rates for larvae that develop into pupae and pupae that develop into adults are relatively low, due to the already high mortality rates of larvae. This shows that all effective treatments are able to control eucalyptus leaf roller caterpillar pests (*Strepsicrates* sp.), namely in the imago phase.

4 CONCLUSION

Based on the results of the study testing the effectiveness of eucalyptus leaf extract (*Eucalyptus* sp.) and lemongrass (*Cymbopogon citratus* (DC.) Stapf) on the mortality of eucalyptus leaf rollers (*Strepsicrates* sp.) in vitro, the following conclusions can be drawn:

1. The combination of eucalyptus leaf extract and lemongrass effectively caused larval mortality, failure of pupae and imago formation in Strepsicrates sp.
2. The best concentration was 300 mL L-1, but was not significantly different from the concentration at 250 mL L-1, where larval mortality was 83.33%, pupae formation was only 16.67%, and no imago formation was observed. Therefore, the 250 mL L-1 concentration is recommended for further research.
3. Symptoms of larval mortality included shriveling, shortening of the body, a change in color from pale cream to black, and the discharge of fluid from the larvae. Sublethal effects occurred in the form of disruption of the pest's life cycle, resulting in minimal population regeneration.

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