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Effectiveness of A Combination of Kitolod Leaves (*Laurentia longiflora*), Papaya Leaves (*Carica papaya*), and Lemongrass (*Cymbopogon citratus*) as Candidates for Biocides on *Bactrocera* Pests in *Citrus sinensis* Plants

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ABSTRACT

Bactrocera is a pest that is detrimental to citrus farming. *Bactrocera* attacks cause developmental disorders in citrus fruit, including changes in fruit color (black spots appear from *Bactrocera* infection) and premature rot. *Bactrocera* also has a symbiotic relationship with *Liberibacter asiaticus* which causes *Citrus Vein Phloem Degeneration* (*CVPD*) disease which attacks phloem tissue in citrus plants. *Bactrocera* pest control was carried out by combining plants that have the potential to be used as biocides, namely kitolod, papaya, and lemongrass plants. This combination was used to switch the habits of fruit farmers who previously used synthetic pesticides to control pests. Natural biocides are expected to have the same effectiveness as synthetic pesticides, but leave no residue on cultivated plants. A combination biocide from the three plants was made using the maceration method to obtain an infusion from each plant. Before being tested on Citrus plantations, an assay was carried out using an olfactometer to determine the effectiveness of the biocides. The research revealed that the combination of these three plants has the potential to be used as a natural biocide without leaving residueand causing damage or other developmental disorders to citrus plants.

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INTRODUCTION

Batu is one of the cities that produces the most *Citrus* fruit on the island of Java. The harvested *Citrus* are exported to several large cities in Java, especially in East Java. However, based on information obtained from the Agricultural Research and Development Agency in 2023, *Citrus* farming in several regions of Indonesia experienced a decline in production from 2016-2022 due to increasing attacks by the Bactrocera pest on Citrus plantations. The impact of *Bactrocera* attacks is that *Citrus* fruit rots more quickly due to infection of the female *Bactrocera* which pierces its ovipositor under the surface of the *Citrus* peel (Anggraini et al., 2019). *Bactrocera* females tend to penetrate and invest their eggs in citrus fruit, which ultimately trigger physiological disorders in the fruit, such as color changes and disruption of fruit development (Alzanado et al., 2022; Amoabeng et al., 2020). The penetration of the ovipositor (egg-laying device) into the fruit causes puncture symptoms on the citrus fruit, resulting in the appearance of a blackish brown spot. The eggs in the fruit then hatch into larvae, which then eat the flesh of the fruit and develop inside it so that the fruit becomes rotten. After the eggs hatch, the larvae make holes in the fruit, making it easier for bacteria and fungi to enter (Wijaya & Adiartayasa, 2018).

Bactrocera sp. has a mutual symbiotic relationship with bacteria, so that during the process of laying eggs, the bacteria will be carried away and followed by fungi which ultimately causes rot. One of the bacteria that has symbiosis with *Bactrocera* sp. is *Liberibacter asiaticus* which causes *Citrus Vein Phloem Degenation* (*CVPD*) disease (Wijaya et al., 2017). Based on data obtained from the Research and Development Agency of (Ministry of agriculture Indonesia 2018), the symptoms of

CVPD disease that can be seen externally include part or all of the leaf crown turning yellow; the texture of the leaves becoming stiffer and thicker, and the veins of the leaves sanding out green; irregular yellowish spots (blotching mottle) or chlorosis appearing on the leaves; and many other non-specific symptoms indicating rot in fruit and other parts of citrus plant. Symptoms of chlorosis caused by *Liberibacter asiaticus* infection indicate a physiological disorder in the plant (Rustiani et al., 2016). This physiological disturbance occurs because the mass of bacteria inhibits nutrient transport in the phloem tissue, resulting in cell abnormalities (Tuwo et al., 2024).

Efforts to control *Bactrocera* pests continue among *Citrus* farmers and business actors to avoid further losses, one of which involves using natural plant-based biocides (Rohman & Putra, 2021). The use of natural biocides is recommended because the manufacturing costs are relatively affordable and they can be used sustainably to manage pests that are starting to become resistant to synthetic insecticides (Rioba & Stevenson, 2017). The use of plant extracts as biocides was chosen because they contain plant defense mechanisms due to interactions with plant-eating insects, namely the production of secondary metabolic compounds that act as repellents (Schlaeger et al., 2018). The advantage of using plant extracts as natural biocides is that they do not leave residue, because the compounds in plant extracts can decompose and degrade quickly with the help of sunlight (Kobenan et al., 2021). All organs in the kitolod plant contain secondary metabolite compounds, such as alkaloids, saponins, polyphenols, flavonoids, tannins, and steroids which can inhibit bacterial growth (Haryoto & Hapsari, 2019). These compounds have the potential to repel the presence of pests, so they can be used as candidates for natural biocides. Papaya also contains active compounds, namely phenolics, alkaloids, flavonoids and benzyl glucosinolate (Rahayu et al., 2020).

The leaves of papaya also contain the same content as the kitolod plant, namely alkaloids, flavonoids, and phenols, but with a higher percentage of alkaloids and flavonoids than the kitolod plant, namely 26,11% for alkaloids, 6,83% for flavonoids (Alzanado et al., 2022). Meanwhile, the lemongrass plant (*Cymbopogon citratus*) contains essential oils which are usually used as a natural biocide for mosquitoes or other insects (de Souza et al., 2021). The use of lemongrass as a natural biocide is based on the nature of the active ingredient contained in it, which is non- toxic to plants, systemic, and compatible with other types of compounds, easily decomposed (leaving no residue), and more environmentally friendly. The formulation of several plants was used to change the habits of fruit farmers who previously used synthetic pesticides to control pests, encouraging them to use natural biocides which are expected to have the same effectiveness as synthetic pesticides, but do not leave residue on cultivated plants. Alkaloids, flavonoids, and tannins are essential compounds that biocide products must have, because they can attack pests systemically. However, they need to be combined with other compounds that work compatibly to control certain pests (Suarny, 2017). In this research, we tried to optimize the function of compounds contained in old papaya leaves (which are usually ignored and have low economic value), kitolod leaves (which usually grow wild on riverbanks), and lemongrass (which is believed to have aromatic compounds). These three plants are expected to synergize in pest control efforts with natural products and increase the economic value of these three ingredients.

Alkaloids are one of the insecticidal substances possessed by plants to protect themselves from insect attacks, while tannin compounds in plants have a bitter taste that insects do not like. When tannin compounds interact with protein, they will produce toxic properties because they can inhibit the activity of digestive enzymes, resulting in reduced appetite if the biocide is ingested by insect pests (Yogantara et al., 2017). Several studies have been carried out using the *Carica papaya* plant as a botanical insecticide, including its use as a larvicide which has antifeedant properties for *Spodoptera litura* larvae; control of the *Argulus ectoparasite* in goldfish (*Cyprinus carpio*), and as a larvicide that triggers mortality in *Aedes aegypti* larvae (Azizah et al., 2019; Ilham et al., 2019; Rahayu et al., 2020). Other research used *Laurentia longiflora* because of its alkaloid content such as lobelin and lobelanin, which are toxic (Haryoto & Hapsari, 2019). Meanwhile, the essential oil contained in lemongrass has been widely used because it has the potential to repel the presence of *Aedes aegypti* mosquitoes; *Phutella xylostella*; and the earthworm pest *Agrotis ipsilon* (Li et al., 2021; Moustafa et al., 2021; Najib et al., 2017;).

In this study, the focus was on the combination of three plants as a repellent for insects without causing death to them. The parameters used in this research were different from previous studies. While previous studies typically measured insect mortality, this study assessed the behavior of *Bactrocera* and the impact of biocides on Citrus plant The behavior measured in this research was avoidance and attraction to the repellent.

MATERIALS AND METHODS

This research was carried out at the Greenhouse of the State University of Malang for rearing *Bactrocera* to olfactometer tests and at the Center for Standardization of Citrus and Sweetener Crops Agricultural Instruments in Batu City to test the effects of biocides on citrus plants. The research was carried out from February 2023 to July 2023. Phytochemical tests were carried out to ensure the content of active compounds in plants, confirming their suitability as biocides. The maceration method with water was used to obtain infusions from each plant. There was no method for solidifying the extract from the maceration results in this research, so that the biocide solution can be used by anyone with a simple and practical manufacturing method.

The biocide ratios used in this study were 2:1; 3:1; 4:1; and 5:1. these comparison treatments were carried out to find out whether the concentration of the biocide solution affected its effectiveness. A repellency test using an olfactometer was carried out to determine the effectiveness of the biocide of each treatment. There were 5 treatments in the repellency test, namely control; kitolod biocide; papaya leaf biocide; lemongrass biocide; and a combined biocide of the three plants (Figure1). We adapted the working principle of the 4-arm olfactometer and modified it into a 5-arm olfactometer with additional modifications (Figure 2). The olfactometer was connected to a flow meter to control the biocide discharge entering the inner filter. This test optimized the inlet and outlet air filtration system to obtain more valid research results.

A small fan was placed at the bottom of the olfactometer to circulate air flow to each tube arm. The olfactometer used was made of acrylic and had 5 branches which indicated the presence of 5 treatments. The olfactometer circuit consisted of an adapter; air pump; filtration 1; flow meters; filtration 2; input; and air output. Each treatment required 30 *Bactrocera* specimens with 3 repetitions. Field tests on citrus plants were carried out on *two-year-old Citrus trees* that were already producing fruit ith 80 ml of biocide sprayed on each tree. The treated *Citrus* trees were observed for 10 days to find out whether any changes had occurred

Data analysis began with the *Kolmogorov-Smirnov* test and continued with the *Levene test* to analyze the normality and homogeneity of the data. After the data was declared to be normally distributed and the variance between groups was homogeneous, a *two-way ANOVA* test was carried out. The type of plant as a biocide was positioned as factor 1, while the level of dilution was positioned as factor 2. If the analysis results showed a significant effect, a Least Significant Difference (LSD) test was carried out at a 5% significance level.

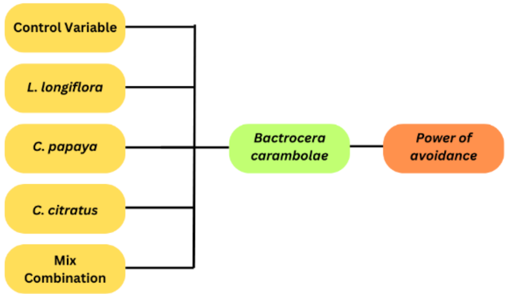

Figure 1. Treatment diagram

The results of testing plant biocide candidates as repellents were calculated using the Repellent Index (IR) formula as described by Pascual-villalobos & Robledo (1998), with the following formula:

$$IR = 100\ \%\ x\ \frac{(C - T)}{(C + T)}$$

Information:

IR : *Repellent Index* ***T*** : *Treatment*

C : *Control*

RESULTS AND DISCUSSION

Based on the results of phytochemical tests carried out on kitolod plants (*Laurentia longiflora*), some results were obtained. In the alkaloid compound test using water as the solvent (H_2O), positive results were obtained using the Mayer reagent and Dragendorf reagent methods, indicated by the presence of a brownish-red precipitate. In the flavonoid compound test, positive results were indicated by the formation of a *Citrus* color in the extract from the kitolod plant. In the phenol test, positive results were obtained from the water solvent treatment, indicated by a change in the color of the extract to blue, green, red, purple, or blackish.

The saponin test showed positive results, indicated by the presence of foam in the kitolod plant extract. The terpenoid test also showed positive results, indicated by a brownish color. The steroid test showed positive results, marked by a color change in the extract to bluish-green. The final phytochemical test, namely the tannin compound test, also obtained positive results, marked by a color change in the extract to dark blue. The results of the phytochemical test can be seen in Table 1.

Based on the results of phytochemical tests carried out on the leaves of the papaya leaves, positive results were also obtained in the alkaloid test using both Mayer and Dragendorf reagents. The indicator for the presence of alkaloid compounds in *Carica papaya* leaves was marked by a brownish-red precipitate. Positive is indicated by a color change in the extract to *Citrus*. In the phenolic compound test, positive results were obtained, indicated by a color change in extract to brownish red. Meanwhile, positive results were obtained in the saponin test, which was indicated by the presence of foam on the top of the papaya leaves extract. Positive results were also obtained in the terpenoid compound test, indicated by a color change in the extract to brownish. In the steroid compound test, a positive result was obtained, indicated by a change in the extract to bluish-green.

Table 1. Phytochemical test results from the *Laurentia longiflora* plant using water as a solvent

Metabolite Compounds	Method	Result Water Solvent (H_2O)
Alkaloid	Mayer's reagent	Positive (+)
	Dragendorf reagent	Positive (+)
Flavonoid	Concentrated 2N HCl (10 mL) + Mg	Positive (+)
Fenol	$FeCl_3$ 5% (5 mL)	Positive (+)
Saponin	HCl (5 mL)	Positive (+)
Terpenoid	Concentrated H_2SO_4 + CH_3COOH	Positive (+)
Steroid	Liberman Buchard reagent	Positive (+)
Tanin	2 - 3 drops $FeCl_3$	Positive (+)

Table 2. Phytochemical test results from the *Carica papaya* plant using water as a solvent

Metabolite Compounds	Method	Result Water Solvent (H_2O)
Alkaloid	Mayer's reagent	Positive (+)
	Dragendorf reagent	Positive (+)
Flavonoid	Concentrated 2N HCl (10 mL) + Mg	Positive (+)
Fenol	$FeCl_3$ 5% (5 mL)	Positive (+)
Saponin	HCl (5 mL)	Positive (+)
Terpenoid	Concentrated H_2SO_4 + CH_3COOH	Positive (+)
Steroid	Liberman Buchard reagent	Positive (+)
Tanin	2 - 3 drops $FeCl_3$	Positive (+)

Table 3. Phytochemical test results from the *Cymbopogon citratus* plant using water as a solvent

Metabolite Compounds	Method	Result Water Solvent (H_2O)
Alkaloid	Mayer's reagent	Negative (-)
	Dragendorf reagent	Negative (-)
Flavonoid	Concentrated 2N HCl (10 mL) + Mg	Positive (+)
Fenol	$FeCl_3$ 5% (5 mL)	Positive (+)
Saponin	HCl (5 mL)	Positive (+)
Terpenoid	Concentrated H_2SO_4 + CH_3COOH	Positive (+)
Steroid	Liberman Buchard reagent	Negative (-)
Tanin	2 - 3 drops $FeCl_3$	Positive (+)

In the tannin compound test, a positive result was obtained, indicated by a a color change in the extract to blackish. The results of the phytochemical test can be seen in Table 2.

Based on the results of phytochemical tests carried out on lemongrass plants (Cymbopogon citratus), negative results were obtained in testing alkaloid and steroid compounds, while positive results were obtained in testing flavonoid compounds as indicated by a color change the extract to *Citrus*. Positive results were also obtained from testing phenolic compounds with the color of the extract changing to red. Positive results were also obtained from testing saponin compounds indicated by the formationof thick foam at the top of the extract. In the results of testing for terpenoid compounds, positive results were obtained, indicated by a color change to brownish. For tannin compounds, positive results were obtained, indicated by a color change to dark blue. The results of the phytochemical test can be seen in Table 3.

The testing process for the target pest (*Bactrocera*) was carried out with using a "five-arm olfactometer". Based on the results of research, the number of *Bactrocera* that land on controls had the highest average, the control treatment had no strong aroma emitted from the biocide vapor, whereas in the *Cymbopogon citratus* biocide treatment, Bactrocera still landed (almost as many as in the control). Meanwhile, biocide treatment that combined *Laurentia longiflora*, *Cymbopogon citratus* and *Carica papaya*, there were no *Bactrocera* landings at all.

The results of the *Kolmogorov-Smirnov* test and *Levene's test* indicated that the data meet the assumptions of normality and homogeneity. Based on the results of the assumption test, data analysis was continued using *two-way ANOVA*. The results of the ANOVA test showed that the types of candidate plants as biocides had a significant effect on the number of *Bactrocera* landings ($F = 9.314$, $p = 2.798$). The 2:1 ratio had a significantly lower average number of perches than the 4:1 and 5:1 ratio. The solvent ratio variable had a significant effect on the number of *Bactrocera* landings ($F = 4.305$, $p = 2.565$). Likewise, the interaction between the type of biocide candidate and solvent ratio was also reported to be significant ($F = 13.949$, $p = 1.960$).

Based on the results of research , the number of *Bactrocera* that landed on the controls had the highest average, because *Bactrocera* perceived that the odor in the control treatment room as neutral, whereas in the treatment of various plant biocides including *L. longiflora*, *C. papaya*, *C. citratus*, and a combination of the three, at the ratios of 2:1; 3:1; 4:1 and 5:1, the number of *Bactrocera* that landed began to decrease. The higher ratio used, the fewer *Bactrocera* landed. This decrease is attributed to the presence of volatile compounds originating from plants biocides that fruit flies do not like. The results of calculating the effectiveness of biocides using the repellent index can be seen in Table 4.

The results of the research showed that the combination of extracts of the three plants (*L. longiflora, C. papaya, and C. citratus*) was effective as a fruit fly repellent. The most effective concentration was a 2:1 ratio with 100% repulsion power. The potential of several biocidal candidate plants as fruit fly repellents reported in this study is in line with several other studies that examined the potential of *Laurentia longiflora* because of its alkaloid content such as lobelin and lobelanin which are toxic. For example, research that tested the cytotoxic activity of *Laurentia longiflora* leaves against MCF-7 cells. [12]. Some studies also used essential oil from *Cymbopogon citratus* as a biopesticide to control *Phutella xylostella* (Li et al., 2021). Furthermore, another study tested the environmentally friendly insecticide from *Cymbopogon citratus* against the earthworm pest *Agrotis ipsilon* (Moustafa et al., 2021). Several studies have been carried out using the *Carica papaya* plant as a natural insecticide, testing papaya leaf larvicide as an antifeedant on *Spodoptera litura* larvae (Rahayu et al., 2020), controlling the *Argulus ectoparasite* on goldfish (*Cyprinus carpio*) (Azizah et al., 2019), and testing *Carica papaya* leaf extract larvicide against the death of *Aedes aegypti* larvae (Ilham et al., 2019). These three plant biocide candidates contain volatile compounds, most of which are non-polar compounds. The bioactivity or repulsion caused by these compounds can be attributed to their volatility and the presence of functional groups capable of reacting with the pest's sensory receptors.

Based on the results of the biocide spraying test on citrus plants, there were no spots or damage observed on the leaves. The leaves also looked healthy and there were no o signs of dryness resulting from the biocide spraying (Figure 2A). Based on the results of these observations, the natural biocides are classified as safe and do not cause damage to plant parts.

Table 4. Effectiveness of plant biocides represented by Repellent Index

Plant	Ratio of Solvent (ml)			
	2:1	3:1	4:1	5:1
Laurentia longiflora	0.98	0.83	0.74	0.53
Cymbopogon citratus	0.89	0.72	0.64	0.42
Carica papaya	0.99	0.91	0.81	0.49
Combination of Biocides	1.00	0.94	0.94	0.41

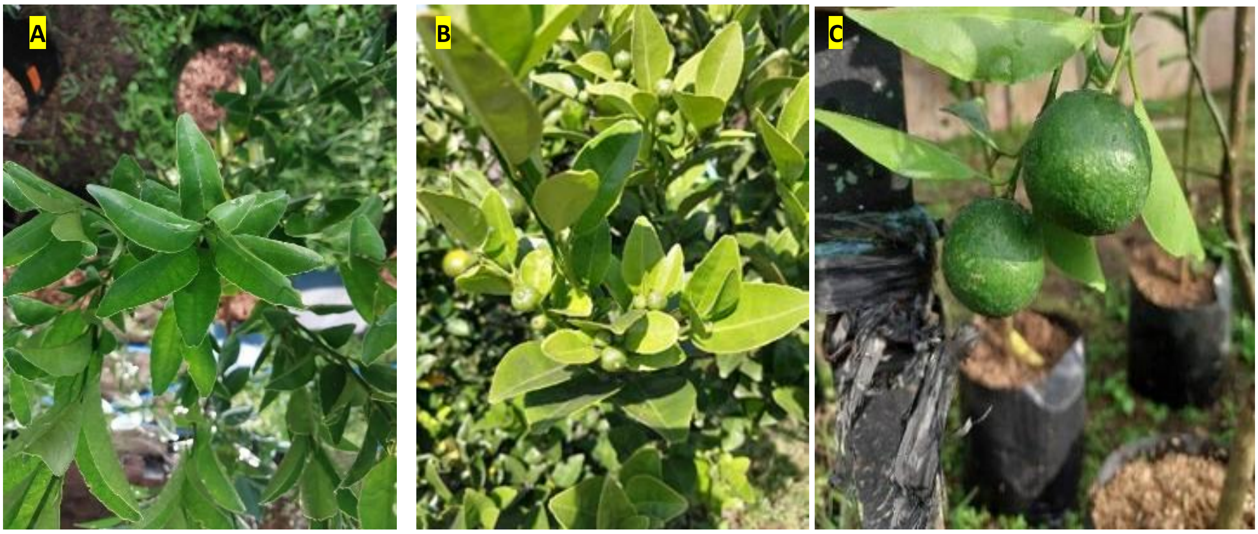

Figure 2. The results of the biocide spraying test on citrus plants. A, B, C healthy *Citrus plan*

This finding is also in accordance with research that successfully tested natural insecticides from *Cymbopogon citratus* on cabbage plantations in Ghana without causing negative effects on cultivated plants and the surrounding environment (Amoabeng et al., 2020). When observing the fruit and ovaries on *Citrus* trees, positive results were obtained. There was no damage to the fruit and ovaries as, indicated by the absence of the spot and color change on the fruit, with the fruit remaining fresh and healthy (Fig 2 B&C).

Based on other research which also succeeded in conducting field tests on the use of natural insecticides from *Carica papaya* on natural farms in Egypt, positive results were achieved without causing resistance effects and damage to agricultural products and the surrounding environment (Mosa et al., 2022). Another study also conducted field tests on the effectiveness of botanical insecticides on banana trees affected by the *Drosophila melanogaster* pest and found that the treatments did not cause damage to the trees or the surrounding environment (Anggraeni et al., 2018). Several studies that have conducted field tests on the effectiveness of biocides made from plants reported very good results, indicating that these biocides are relatively safe for long-term use because they do not leave residue and do not damage the environment and surrounding ecosystem.

CONCLUSION

Biocide candidate plants have an effect as a repellent with varying percentages of effectiveness. The highest effectiveness was achieved with the combination of *Laurentia longiflora*, *Carica papaya*, and *Cymbopogon citratus* with an effectiveness percentage of 100%, followed by biocide from Carica papaya extract with a percentage of 99% and *L. longiflora* extract with a percentage of 98%. The lowest effectiveness was observed in *Cymbopogon citratus*. The optimum solution ratios used were 2:1 and 3:1, because they had effective percentages ranging from 80% - 100%. The higher the infusion concentration used, the better the results.

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