Optimum Concentration of Using Combination of *Laurentia longiflora*, *Carica papaya*, and *Cymbopogon citratus* as a Biocide on *Bactrocera carambolae*

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Abstract. *Bactrocera carambolae* is a pest in agriculture, citrus farming. The purpose of this true- experimental research is as a preliminary test to determine the optimal concentration of using a combination of *Laurentia longiflora* leaves, *Carica papaya* leaves, and *Cymbopogon citratus* as a biocide against Bactrocera carambolae pests. The technique used in the manufacture of this biocide extract is maceration, while testing for pests uses an olfactometer. The concentration of each type of biocide using a ratio (solvent: extract) there are (2:1, 3:1, 4:1, and 5:1) were positioned as independent variables, while the avoidance of Bactrocera carambolae was positioned as the dependent variable. The results of the two-way ANOVA test showed that the types of biocides and differences in concentrations of biocides had a significant effect. The best concentration of biocides that can work optimally for use is a concentration of 2:1 to 3:1 with a repulsion percentage of 90%.

1 Introduction

Bactrocera is a popular pest in fruit farming, especially citrus farming. This type of fruit fly attack can cause damage to the fruit, namely premature rot, so that the infected fruit cannot be consumed [1] . Damage to fruit is caused by penetration and investment of eggs, this triggers physiological disorders in the fruit, for example color changes and other fruit development disorders [2]. The fruit produced is smaller than normal, and on the other hand, the amount of production has dropped drastically. The impact caused by the presence of *Bactrocera* sp. causes fruit around the plantation to rot more quickly due to the behavior of fruit flies the female lays her eggs in the fruit [3].

The entry of the ovipositor (egg laying device) into the fruit causes puncture symptoms on the citrus fruit, resulting in a blackish brown spot appearing. 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 [4]. *Bactrocera* sp. have a mutual symbiotic relationship with bacteria, so that during the process of laying eggs, the bacteria will be carried away and

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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 [5] . Based on data obtained from the Research and Development Agency of the Ministry of Agriculture in 2018, the symptoms of *CVPD disease* that can be seen externally are part or all of the leaf crown turning yellow; the texture of the leaves becomes stiffer and thicker, and the veins of the leaves stand out green; irregular yellowish spots (*blotching mottle) or chlorosis appear on the leaves;* as well as many other non-specific symptoms that point to rotting of fruit and other citrus plant parts [6–7]. Efforts to control pests need to be carried out so that orange farmers and business actors do not suffer further losses due to the behavior of pests (*Bactrocera* sp.) which like to move around orange plantations. The last few years have shown public interest in natural phenolic compounds produced from plants [8–9]. The use of plant-based biocides is recommended because the production costs are relatively affordable and can be used sustainably to manage pests that are starting to become resistant to synthetic insecticides [10][11],[12]. Biocides themselves are chemical substances that are toxic to the target living organisms.

Based on the results of phytochemical tests carried out, it shows that all organs in the kitolod plant contain secondary metabolite compounds such as alkaloids, saponins, polyphenols, flavonoids, tannins and steroids which can inhibit cell growth [13]. These compounds are believed to have the potential to repel the presence of pests, so they can be used as candidates for vegetable biocides. Based on research conducted, it is also known that papaya plants also contain active compounds, namely phenolics, alkaloids, flavonoids and benzyl glucosinolate [13–14]. The leaves of the papaya plant also contain the same content as the kitolod plant, namely alkaloids, flavonoids, and phenols, but with a higher percentage than the kitolod plant.

Meanwhile, the lemongrass plant (*Cymbopogon citratus)* contains essential oils which are usually used as a vegetable biocide for mosquitoes or other insects [15]. The use of lemongrass as a vegetable biocide is based on the nature of the active ingredient contained in it, which is not toxic to plants, is systemic, and is compatible with other types of compounds, is easily decomposed (leaving no residue), and is more environmentally friendly. Several previous studies used kitolod (*Laurentia longiflora)*, papaya (*Carica papaya)*, and lemongrass (*Cymbopogon citratus)* plants without combining them, and the results were still very effective for use [16][17][18]. However, in this research it is important to carry out a combination because of the behavior of the target insect and other organisms which work in symbiosis to damage the fruit.

The formulation of several plants was carried out to change the habits of fruit farmers who previously used synthetic pesticides to control pests, to switching to vegetable biocides which are expected to have the same effectiveness as synthetic pesticides, but do not leave residue on cultivated plants [19]. The content of alkaloids, flavonoids and tannins are essential compounds that biocide products must have, because they have effects that can attack pests systemically, however, they need to be combined with other compounds that are able to work compatibly to control them. certain pests [20]. Flavonoids work by inhibiting the work of mitochondria in cells, namely where mitochondria are where respiration occurs in the form of the Krebs cycle and electron transport which produces energy in the form of ATP. If the work of mitochondria is hampered, ATP production will be disrupted and result in low oxygen binding and respiratory problems [21]. Alkaloids are one of the insecticidal substances possessed by plants to protect themselves from insect attacks [22], while tannin compounds have a bitter taste that insects do not like. When tannin compounds interact with protein, they will give rise to toxic properties because they can inhibit the work of digestive enzymes, resulting in reduced appetite [23].

There is a lot of research related to the three plants that will be used as candidates for plant biocides. The aim of using a combination of these plants is to maximize the function of the chemical compounds contained in these plants as plant biocides to replace synthetic pesticides. Vegetable biocides are used as transitional formulations for novice farmers who want to switch from using synthetic insecticides to insecticides made from natural ingredients. It is hoped that the combination of compounds contained in these plants can effectively combat pests.

2 Method

2.1 Experimental Design

This experimental study used *Bactrocera carambolae* as the research subject. The independent variables in this study were biocide that we used (*Laurentia longiflora, Carica papaya,* and *Cymbopogon citratus)* and the concentration of biocide that we used (10%, 20%, 30%, and 40%). The dependent variable in this study was the number of fruit flies perched. Each treatment was repeated third.

2.2 Repellent Making

The manufacture of biocides extract was carried out using a conventional extraction method, namely the maceration method. The solvent used in this study was distilled water. The biocide solution is then allowed to stand for 1 x 24 hours so that the active substances contained in the plant candidate are dissolved in the solvent. After the soaking process for 1 x 24 hours, the plant candidate solution was then filtered to separate the pulp and extract. The extract from plant candidate was then dissolved with distilled water, for a concentration of 10% obtained from 10 ml of extract and 90 ml of distilled water, for a concentration of 20% obtained from 20 ml of extract and 80 ml of distilled water, for a concentration of 30% obtained from 30 ml of extract and 70 ml of distilled water, and for a concentration of 40% obtained from 40 ml of extract and 60 ml of distilled water.

2.3 Repellent Tests

This test was carried out with the help of a four-arm Olfactometer used in insect preference tests (Figure 1). The four-armed olfactometer was then modified by adding one branch so that it was called the five-armed olfactometer. Then the olfactometer was connected to the Y tube with the help of a clear hose. This test adapts 2 test mechanisms, namely the Four-arm olfactometer and the Y-tube olfactometer (Figure 2) in the hope of getting more valid research results. A small fan is placed at each end of the Y tube arm, which functions to circulate air flow to each tube arm.

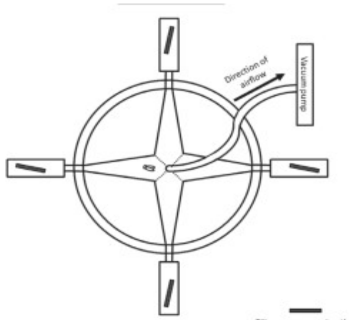

Fig. 1. Four-arm olfactometer by [24]

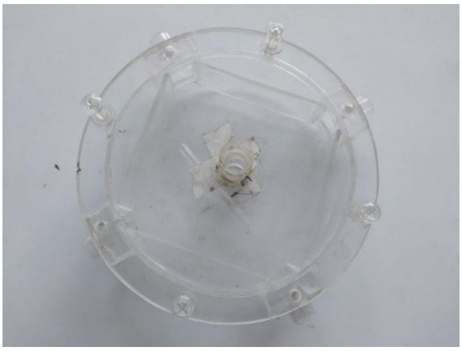

Fig. 2. Five-arm olfactometer that have modify to be used in collecting data in this research.

2.4 DATA ANALYSIS

Data analysis begins with the Kolmogorov-Smirnov test and is followed by Levene's 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 performed. The type of plant as biocide positioned as factor 1 while the dilution level is positioned as factor 2. If the analysis results indicate that there is a significant effect, the Least Significance Different (LSD) test with a significance level of 5% is carried out.

3 Results and Discussion

The results of testing plant biocide candidates as repellents were calculated using the Repellent Index (*IR*) formula based on Pascual-villalobos and Robledo (1998), with the Equation 1.

$$IR = \frac{(C-T)}{C+T} \times 100\% \quad (1)$$

where *IR* is repellent index, *C* is control, and *T* is treatment.

The results of the Kolmogorov-Smirnov test and Levene.s test inform that the data meets the assumptions of normality and homogeneity. Based on the results of the assumption test, data analysis 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 fruit flies landing ($F = 9.314$, $p = 2.798$). The 40% concentration had a significantly lower average number of perches than the 10 and 20% concentrations. The variable solvent concentration had a significant effect on the number of fruit flies perching ($F = 4.305$, $p = 2.565$). Likewise, the interaction between the type of biocide candidate and solvent concentration was also reported to be significant ($F = 13.949$, $p = 1.960$).

Based on the results of research that has been carried out, the number of *Bactrocera carambolae* that landed on controls had the highest average. This is because Bactrocera carambolae felt that the odor in the control treatment room was neutral, whereas in the treatment of various plant biocides with various candidate plants *L. longiflora, C. papaya, C. citratus*, and a combination of the three, and a concentration of 10%, 20%, 30%, and 40% the number of Bactrocera carambolae that landed began to decrease. The higher the concentration used, the fewer Bactrocera will land. This is due to the presence of volatile compounds originating from plants that are candidate biocides that fruit flies do not like.

TABLE 1. Efectiveness plant biocides with Repellent Index Formula

Plant	Concentration			
	40%	**30%**	**20%**	**10%**
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

The results of the research that has been carried out show hat the combined extract from the three plants *(L. longiflora, C. papaya, and C. citratus*) is effective as a fruit fly repellent. The most effective concentration is a concentration of 40% with a repulsive power of 100%. The potential of several plant 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, such as in the research tested the cytotoxic activity of *Laurentia longiflora leaves* against MCF-7 cells (anticancer drug) [25]. The results of this research show that *Laurentia longiflora* has a cytotoxic effect on MCF-7 cells, which means that *Laurentia longiflora* has the potential to be used as a vegetable biocide. Another research was used essential oil from *Cymbopogon citratus* as a biopesticide to control *Phutella xylostella* [26]. Furthermore, in other research try to tested an environmentally friendly insecticide from *Cymbopogon citratus* against the earthworm pest *Agrotis ipsilon* [27]. Several studies have been carried out using the *Carica papaya plant* as a botanical insecticide, who tested the larvicide of *Carica papaya leaves* as *an antifeedant on Spodoptera litura* larvae [13]. The other research carried out control of the *Argulus ectoparasite* on goldfish (*Cyprinus carpio)* [28], while the other research carried out a larvicide test of *Carica papaya* leaf extract on the mortality of *Aedes aegypti larvae* [29]. These three plant biocide candidates contain volatile compounds, most of which are non-polar compounds. The bioactivity or repulsion caused by this compound can be attributed to its volatility and the presence of functional groups capable of reacting with the pest's sensory receptors.

4 Summary

Based on the research that has been carried out, it can be concluded that plant biocidal candidate plants have the effect of being a repellent with varying percentages of effectiveness. The highest effectiveness was treated with a combination of *Laurentia longiflora*, *Carica papaya* , and *Cymbopogon citratus* with the percentages of effectiveness 100%, next is biocide from *Carica papaya* extract with a percentage of 99% and L. longiflora extract with a percentage of 98%. The lowest effectiveness was treated with *Cymbopogon citratus*. The optimum solution concentration to use is 40% - 20% because it has an effective percentage of 80% - 100%. The higher concentration of extract used, the better the results will be.

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