Utilization of Liquid Organic Waste as a Formulation of Biopesticide with *Bacillus* sp. DB12 for Controlling *Fusarium oxysporum* f.sp. *cepae*

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Abstract - Synthetic (inorganic) carrier materials are expensive and their use is very limited, so they become an obstacle for the development of biopesticide formulas made from active biocontrol agents. Therefore, formula innovation technology is needed that can reduce research costs through the use of alternative carriers of liquid organic matter that are cheap and easy to obtain but still effective in controlling plant pathogens. The purpose of this study was to determine the right combination of liquid carrier formulas in maintaining population density and inhibition of *Bacillus* sp. DB12 against the pathogenic fungus *Fusarium oxysporum* f.sp. *cepae* in-vitro as a basis for the manufacture of biological biopesticides. This study was designed in a completely randomized design (CRD) using 9 treatments of carrier composition with the addition of the biological agent *Bacillus* sp DB12. The waste used is tofu liquid waste (LCT), coconut liquid waste (LCK), and rice washing liquid waste (LCB). The treatment is as follows LCT 100% (L1), LCT 75% + LCK 25% (L2), LCT 50% + LCK 50% (L3), LCT 25% + LCK 75% (L4), LCK 100% (L5) , 75% LCT + 25% LCB (L6), 50% LCT + 50% LCB (L7), 25% LCT + 75% LCB (L8), and 100% LCB (L9). The results showed that the combination of 75% LCT + 25% LCK carrier was able to provide the highest population density and inhibition after being stored for 12 weeks, and was significantly different from all carriers except 50% LCT + 50% LCK carrier. The antifungal activity ability of *Bacillus* sp DB12 from 75% LCT + 25% LCK carrier was categorized as strong based on the inhibition area..

Keywords - *formulas, carrier material, Bacillus sp DB12, organic wastewater*

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I.INTRODUCTION

One of the biological agents used to control the fungus *Fusarium oxysporum* f.sp. *cepae* (Smith) that causes root rot disease in shallot plants is *Bacillus* sp. [1]. These bacteria are the most widely used biocontrol agents because they can suppress the development of various plant pathogens through competition, antibiosis and induction of plant resistance. In addition, bacteria can act as plant growth promotions by increasing N uptake, phosphate solubilization, and production of siderophores and phytohormones [2], [3].

Bacillus sp isolate DB12 is an antagonistic bacterium isolated from the rhizosphere of wakegi shallots. This bacterium has been tested to inhibit the growth of colonies of *F. oxysporum* f.sp. *cepae* in-vitro and succeeded in suppressing the incidence of shallot base rot disease in-planta (screening house). *Bacillus* sp. DB12 applied to plants shows high effectiveness in suppressing the disease up to 78.04% [4]. The ability of DB12 isolate as a biological agent can be exploited and developed as a biopesticide formula so that it can last longer, is easy to apply and remains effective in controlling pathogens.

Bacillus sp can form biopesticides with active ingredients in solid or liquid form using carriers and additional ingredients [3]. Carriers play a role in increasing the stability of the population of biological agents during production, transportation and storage, so they effectively control plant diseases [5]. Most formulas of biological agents still use synthetic carriers, or semi-synthetics, as ingredients for the mixture of biopesticide formulas. The instant medium made by the manufacturer is in a ready-to-use dosage form. However, it is expensive and can only be obtained in certain places. The limited availability of carrier material technology packages that are cheap, easy to obtain, and highly nutritious is the main obstacle to the use of Bacillus sp DB12 in the field.

Bacillus sp is widely known as a saprophytic bacterium that can survive and reproduce on the remains of organic matter [6]. The availability of liquid organic waste, which is relatively abundant in Palu City, has encouraged researchers to find alternative carrier materials that are cheap, easy to obtain, and environmentally friendly for the manufacture of biopesticide formulas. This research focused on developing a formula based on organic waste from tofu water, coconut water, and rice washing water with various combinations as carriers for *Bacillus* sp DB12 bacteria. This study aims to determine the appropriate combination of liquid carrier formulas in maintaining population density and inhibition of *Bacillus* sp. DB12 against the pathogenic fungus *F. oxysporum* f.sp. *cepae* in vitro.

II. MATERIALS AND METHODS

This research was carried out at the Plant Disease Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Tadulako University, from March to October 2022. This research was carried out in an experimental laboratory using a Completely Randomized Design (CRD) with a single factor: a combination of liquid waste carriers containing 9 treatments. The liquid waste used is liquid tofu waste (LCT), coconut liquid waste (LCK), and rice washing liquid waste (LCB). Each treatment was repeated 10 times so that the total number of experiments was 90 experimental units. The treatment is as follows LCT 100% (L1), LCT 75% + LCK 25% (L2), LCT 50% + LCK 50% (L3), LCT 25% + LCK 75% (L4), LCK 100% (L5), 75% LCT + 25% LCB (L6), 50% LCT + 50% LCB (L7), 25% LCT + 75% LCB (L8), and 100% LCB (L9).

Liquid organic waste is obtained from the tempe/tofu manufacturing industry, the coconut milling industry, and household waste around the Palu City area. Collected organic waste as a carrier material using jerry can containers, as much as 5 litres each, then taken to the Plant Disease Laboratory.

The carrier material that has been shaken and filtered is put into a 250 ml Erlenmeyer as much as 99 ml (mixed or single according to treatment) separately. The carrier material is then sterilized using an autoclave at 121°C with a pressure of 1 atm for 15 minutes [7], [8]. After cooling, acetic acid is added so that the degree of acidity of the carrier is at pH 7 (neutral) [9] to achieve optimum bacterial growth. Additives are 2% granulated sugar and 1% shrimp paste. All tested formula compositions were mixed homogeneously. Next, 1 ml of pure isolate culture suspension of Bacillus DB12 (10^8 cfu/ml) was transferred to the carrier in an Erlenmeyer and incubated in a shaker. After 1 x 24 hours, the formula in Erlenmeyer is packaged and labelled according to the treatment, then stored at room temperature for 12 weeks to test the viability of the bacteria.

Population density is calculated using the formula population density (cfu⁄ml)= /(p+v),

where x is the average number of colonies growing in the dish; p, dilution factor; υ, the volume of suspension (ml) that is spread on the cup. The acidity level of the carrier material was measured using an ATC digital pH meter tester for each storage period.

The inhibition test was carried out using a modified dual culture method. The inhibition power (DB) of the growth of pathogenic fungi was calculated using the formula: DB=(C-T)/C x 100%, where C = the diameter of the fungal colony in control (cm), T = the diameter of the fungal colony in the treatment (along with the antagonist) (Asthana *et al*., 2016). Data were analyzed statistically using ANOVA (Analysis of Variance). If there is a significant difference, proceed with the BNJ (Honest Significant Difference) test at the 5% level.

III. RESULTS AND DISCUSSIONS

A. Population Density

The results of statistical analysis showed that the liquid organic carrier formula had a significant effect on the average population density of *Bacillus* sp. DB12. The highest population density (8.93 x 10^9 cfu/ml) was found in the L2 carrier combination and was significantly different from the L1, L3, L4, L5, L6, L7, L8, and L9 carriers. The lowest population density was found in single carrier L9 (6.09 x 109 cfu/ml) but not significantly different from carriers L1, L4, L5, L7, and L8 (Table 1).

TABLE 1. AVERAGE POPULATION DENSITY (CFU ML^{-1}) AND INHIBITION (%) OF *BACILLUS* SP. DB12 ON VARIOUS CARRIERS AFTER 12 WEEKS OF STORAGE

Treatments	**Population Density (10^9 cfu/ml)**	**Inhibition (%)**
L1 (100% tofu water)	7,24ab	72,59a
L2 (75% tofu water + 25% coconut water)	8,93c	81,41c
L3 (50% tofu water + 50% coconut water)	7,47b	75,31b
L4 (25% tofu water + 75% coconut water)	7,21ab	78,71bc
L5 (100% coconut water)	7,22ab	72,08a
L6 (75% tofu water + 25% rice washing water)	7,39b	72,10a
L7 (50% tofu water + 50% rice washing water)	6,26a	69,02a
L8 (25% tofu water + 75% rice washing water)	6,17a	67,931a
L9 (100% rice washing water)	6,09a	66,614a

Note: the numbers in the same column followed by the same letter show results that are not significantly different according to the BNT test at the 0.05 level of confidence.

B. Inhibition

The results of statistical analysis showed that the formula of the carrier material had a significant effect on the percentage of inhibition of Bacillus sp DB12 bacteria. The highest average inhibition was found in the combination of L2 carriers (81.41%) but not significantly different from L4 carriers. In contrast, the lowest average inhibition was found in single carrier L9 (66.61%) but not significantly different from L1, L5, L6, L7, and L8 (Table 1).

Carriers L1, L2, L3, L4, L5, and L6 used to prepare the formula showed good inhibition because they could reach more than 70%. According to Wibisono *et al*. (2014), the quality standard for good inhibition tests is when a biological agent has >70% inhibitory ability against the growth of pathogens in vitro. However, if the percentage of inhibition only reaches 30%, the microbial antagonist can be categorized as having a minimal inhibitory effect (Hartanto and Heni, 2016).

In general, all carrier formulas could support the growth of *Bacillus* sp DB12 as indicated by their ability to maintain a bacterial population density of 10^9 cfu/ml at 12 weeks of storage. This population density meets the minimum amount of active ingredients present in the carrier, namely 10^7 cfu/ml or cfu/g. This means that all of these carriers, both in combination and individually, have the potential to be used as an alternative medium for mass multiplication and to maintain the life of *Bacillus* sp DB12 bacteria because they contain elements needed for bacterial growth.

The ability of the carrier material to maintain the highest population density is related to the nutrient content. According to Juariah and Sari [8], the most significant nutritional component in tofu wastewater is protein, so it can be used as an alternative medium for the growth of *Bacillus* sp. Meanwhile, the most abundant

nutrient content in coconut liquid waste is carbohydrates in dissolved form so that they are easily absorbed by bacteria [10]. These carbohydrates and proteins are essential media compositions for bacterial growth [11], [12].

The population density of antagonistic bacteria on each carrier differed, although not significantly (Table 1). This difference is thought to be due to the amount of nutrient content in each carrier, both in combination and individually, which is also different. The amount of different nutrient content has an impact on the growth of the bacterial population is also different. This follows the statement of Yelti *et al*. [13] that differences in population density in carrier materials are due to differences in the nutrient content of each carrier material.

LCT is known to contain as much as 25 – 50% carbohydrates, 40 – 60% protein, and fats ranging from 8 – 12% [14]. and the remainder is in the form of nitrogen, phosphorus, C-organic, iron, potassium, calcium, unsaturated fats. Saturation, calories, phosphorus, vitamin E, B-complex vitamins such as thiamin, riboflavin, and vitamin B12 [15]. This nutrient is suitable for use as an alternative medium for the growth of *Bacillus* sp bacteria [8]. The nutritional content of LCT, which has excellent potential as a medium for microbial growth, is protein, fat, vitamins, and minerals (calcium, magnesium, and iron). Meanwhile, LCK has a total carbohydrate content of 1.92%, 0.64% protein, 0.10% fat, and around 96.79% water (Jermwongruttanachai *et al*., 2021). Abna (2018) added LCK also contains sugar, nitrogen, amino acids, minerals, vitamins, and growth regulators, so it can be a suitable medium for bacterial growth.

Bacillus sp DB12 bacteria must be supported with nutrients containing carbon (C) and nitrogen (N) to increase and maintain population density. Wulandari *et al.* (2013) reported that bacterial cultures could live in the presence of C and N sources, where a low C/N ratio would increase the growth rate of bacteria.

All organic carrier ingredients in the formula can influence antagonistic bacteria in inhibiting the development of pathogenic colonies of *F. oxysporum* f.sp *cepae*. However, only 6 out of 9 carriers containing antagonistic bacteria had an inhibition percentage above 70% in the in vitro antagonist test. The magnitude of this inhibition indicated that the six combination formulas had the potential to be used and developed as alternative carriers for *Bacillus* sp DB12 bacteria. The ability to maintain and maintain the percentage of inhibition of antagonistic bacteria over a long period is one of the characteristics that a carrier material must possess.

Therefore, the combination of LCT and LCK carriers has nutritional content that complements each other and synergizes to meet the needs of bacterial life compared to other carriers so that the bacterial metabolic process takes place optimally for growth and development. Djaenuddin *et al*. [16] proved that the population density of *Bacillus cereus* and *Brevundimonas diminuta* bacteria was relatively higher (10^9 cfu/g) in the combination of sago and compost carrier material. The high bacterial population density is thought to have influenced the amount of secondary metabolite secretion, causing high inhibition. Another study showed that the increasing population density of lactic acid bacteria *Lactobacillus plantarum* was in line with the increased production of secondary metabolites by these bacteria. The number of microorganisms (population density) affects the diameter of the pathogen growth inhibition zone. The higher the antagonistic microbial population density, the wider the inhibition zone is formed to inhibit the growth of pathogenic colonies. The level of inhibition was influenced by the concentration of antifungal compounds, population density, temperature, time, type of microbe, pH and dissolved organic substances or materials.

IV. CONCLUSION

The combination of 75% LCT + 25% LCK with the addition of the antagonist *Bacillus* sp DB12 was able to maintain population density (8.93 x 10^9 cfu/ml) and inhibition (81.41%) for 12 weeks in storage.

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