



Comparison of the Effectiveness of Biobactericides from Beef Bones (Limousin, Simmental, and PFH) as Anti Bacterial (*Ralstonia Solanacearum*) in Chili Plants

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Abstract. This research aims to evaluate the effectiveness of biobactericides from beef bones, especially from the Limousin, Simmental, and PFH cattle types, in increasing growth and reducing symptoms of wilt disease in chili plants caused by *Ralstonia solanacearum*. Biobactericide is made through the process of deproteinizing beef bones, then applied to infected chili plants. Parameters measured included plant height, number of leaves, and observation of disease symptoms for 28 days. The results showed that biobactericide from Limousin cattle bones gave the best results, with an increase in plant height of up to 26 cm and the number of leaves reaching 15, as well as a complete suppression of disease symptoms at the end of the observation. The Simmental beef bone treatment also showed noticeable improvement, but not as good as Limousin, while the PFH beef bone treatment gave the lowest results. It is important to note that these observations are based on visual comparisons and not on statistical significance. The higher effectiveness of Limousin beef bone is likely due to its higher mineral content, particularly calcium and phosphorus, helping to strengthen plant cell walls and increase resistance to pathogens. These findings suggest that cattle bone waste, especially from Limousin beef bone, has great potential to be developed as a low-cost, sustainable biobactericide for eco-friendly agricultural disease management.

Keywords: Biobactericide, beef bone, *Ralstonia solanacearum*, chili, wilt disease, plant growth.

1 Introduction

Bacterial wilt disease caused by *Ralstonia solanacearum* is one of the serious problems in chili plants (*Potatoes*), especially in tropical and subtropical areas. These pathogens can spread quickly through the soil and attack the plant's vascular system, causing the plant to wilt and eventually die [1]. The conventional use of chemical pesticides to control this disease has raised concerns regarding its impact on the environment and human health, such as persistent chemical residues and pathogen resistance to the active

pesticide ingredients. Therefore, there is an urgent need to develop alternative, more environmentally friendly and sustainable methods for managing plant diseases.

One potential solution is the use of biobactericidal agents derived from animal bone waste, particularly beef bones. Beef bones contain high levels of inorganic minerals, especially calcium and phosphorus, which contribute to their antibacterial properties [2]. These minerals can disrupt bacterial cell integrity and inhibit pathogen growth [3]. Cell membranes in microbes have a negative charge, so the interaction between positive and negative charges can cause pressure on the membrane, disrupting osmotic balance and hindering microbial growth.

Beef bones, in particular, are rich in minerals such as calcium, phosphorus, and magnesium, which are known to have antimicrobial properties after undergoing demineralization and deproteination processes [4]. Several studies have shown that beef bone derivative products can inhibit the growth of plant pathogenic bacteria. However, the mineral composition of beef bones can vary depending on the breed of cattle, which may affect the effectiveness of the resulting biobactericide.

This study aims to compare the effectiveness of biobactericides derived from the bones of three different cattle breeds: Limousin, Simmental, and PFH (Holstein Friesian), against bacterial wilt disease in chili plants. Limousin and Simmental cattle are known for their strong bone structure and high mineral content, while PFH cattle, primarily dairy, have lower bone mineral content. Although male PFH cattle do not lactate, genetic factors, body structure, and feeding patterns still contribute to their relatively lower bone mineral density compared to beef cattle.

This difference in bone composition may affect the effectiveness of the biobactericidal agent [5]. The study aims to explore the potential of cow bone waste as an environmentally friendly alternative for plant disease control. Additionally, it seeks to contribute to sustainable agricultural practices by utilizing local, underused natural resources for antimicrobial products in plant protection.

2 Materials and Methods

This research was carried out from June 2023 to August 2023, located in researcher one's chili garden. The beef bones used were taken from the Malang Slaughterhouse, then carried out a series of deproteination and drying processes at researcher one's house. The process of grinding the bones into powder is carried out at the Big Market in Malang City. Biobactericide spraying from bone powder is carried out 2-3 times per week for each chili plant, with the aim of controlling the symptoms of wilt disease experienced by the plants.

2.1 Research Materials

In this research, the materials used were beef bones from three different types, namely Limousin, Simmental and PFH cattle, each 1 kg. Cow bone was chosen as a source of biobactericidal material because its mineral content has the potential to have antibacterial properties. The bone deproteinization process is carried out using a 35% sodium

hydroxide (NaOH) solution which aims to remove the protein content in the bone so as to produce a powder that is purer and ready to be used in biobactericide formulations. In addition, the chili plants used in this research were plants that showed symptoms of wilt disease, which is caused by bacterial infection *Ralstonia solanacearum*.

2.2 Research Equipment

The materials used in this research include various tools for making biobactericides from cow bones. The beef bones were ground into a fine powder using a grinding machine, then sieved using a 40 mesh sieve. The deproteination process uses a measuring cup with a capacity of 100 mL, a beaker with a size of 1000 mL and 500 mL, a hot plate for the deproteination process, a digital scale to ensure the accuracy of the bone powder, while a manual stirrer is used to mix the ingredients evenly. Filter paper to filter before drying. An oven is used to dry bone powder. The sprayer is used to spray biobactericides onto plants. A ruler is used to measure the height of chili plants. All of these tools are important to ensure the process of making and applying biobactericides runs effectively and efficiently.

2.3 Research Method

The procedure for making biobactericides consisted of several stages. First, beef bones from Limousin, Simmental, and PFH cattle were washed, sun-dried, ground into powder, and sieved using a 40-mesh sieve. Second, the sifted powder was deproteinized by reacting it with 35% NaOH (1:10 ratio) in a beaker, heated for 1 hour using a hot plate, then cooled, filtered, and neutralized with distilled water. The resulting powder was oven-dried for 4 hours. For application, 50 grams of bone powder were mixed with 300 mL of sterile water and sprayed onto chili plants infected with *Ralstonia solanacearum*. The study used 20 chili plants per treatment group, including a control group, with three replications for each treatment. The plants were arranged without randomization or blocking, as the experimental setup was simple and exploratory in nature. The aim was to observe trends between treatments through descriptive observation. Control plants did not receive any biobactericide; they were only watered regularly with sterile water and were used to monitor the natural progression of the disease without any treatment intervention. Data were analyzed based on direct visual observations of plant growth and disease symptoms.

2.4 Data Analysis

Analysis of data on the use of beef bone powder as a biobactericide on chili plants affected by wilt disease was carried out by observing three main parameters: plant height, number of leaves, and symptoms of wilt disease [6]. Measurements are taken every week to observe noticeable changes over time. Data obtained from groups without treatment and three treatments (Limousin, Simmental, and PFH beef bone powder) were analyzed descriptively to compare the effectiveness of each treatment in increas-

ing plant growth and suppressing disease symptoms. Each change is recorded and compared to see growth trends and decrease in disease symptoms, with a focus on visual observation results, such as increases in height, number of leaves, and reduction in wilting symptoms. The data from the analysis can then be presented in the form of tables or simple graphs to clarify comparisons between treatments.

3 Results and Discussion

3.1 Plant Height

The results showed that biobactericide from beef bones had a noticeable effect on the growth of chili plants affected by wilt disease. As shown in Table 1, at the start of the study, all plants had the same initial height, namely 10 cm. However, after treatment, plants treated with biobactericide from Limousin cow bones grew faster, reaching 15 cm on the 7th day, while plants without treatment only grew up to 12 cm. In the Simmental and PFH cattle bone groups, the plants grew to 14 cm and 13 cm, respectively. On the 28th day, plants treated with Limousin bone biobactericide reached a height of 26 cm, higher than Simmental bone (23 cm) and PFH (21 cm), while plants without treatment only reached 15 cm. Biobactericide from Limousin beef bone showed higher effectiveness than Simmental beef bone and PFH in supporting growth and increasing the resistance of chili plants infected with pathogens.

Table 1. Weekly observations of chili plant height (cm) under biobactericide treatments from a control group (no treatment), Limousin, Simmental, and PFH beef bone.

Day	No Treatment	Treatment A (Biobactericide from Limousin Beef Bone) (cm)	Treatment B (Biobactericide from Simmental Beef Bone) (cm)	Treatment C (Biobactericide from PFH Beef Bone) (cm)
0	10	10	10	10
7	12	15	14	13
14	13	18	17	15
21	14	22	20	18
28	15	26	23	21

One of the main reasons is the high content of calcium and phosphorus minerals in beef bones such as Limousin, which are important in maintaining the strength and health of plant tissue. Calcium, which is richly present in bones, helps strengthen plant cell walls and increases their resistance to infection, while phosphorus supports important metabolic processes in plant growth, including photosynthesis and cell division [7]. PFH cattle, especially those bred for milk production, tend to have lower mineral content in their bones than beef cattle. This is due to the metabolic priorities of dairy cows, which allocate more minerals to milk production than to forming a dense and strong bone structure. Bones consist of elements such as calcium and phosphorus,

which are important for the strength of bone structures and the tissues that depend on them, including in agricultural applications [8].

3.2 Number of Leaves

The results showed that biobactericide from beef bones had a noticeable effect on increasing the number of leaves on chili plants infected with wilt disease. At the start of the study, all plants had the same number of leaves, namely 4 leaves. However, after being treated with biobactericide, especially from Limousin beef bones, there was a clearly observable increase. On the 28th day, plants treated with biobactericide from Limousin beef bones had 15 leaves, compared to plants without treatment which only had 8 leaves. An increase in the number of leaves was also seen in the treatments with Simmental beef bone and PFH, which produced 12 and 10 leaves respectively. As shown in Table 2, this increase in leaf number indicates that the biobactericide contributes directly to plant health and growth. The mineral content in biobactericides, such as calcium and phosphorus, provides the nutrients plants need to develop properly. Calcium plays an important role in strengthening plant cell walls, which helps increase plant resistance to stress and disease [9]. Meanwhile, phosphorus supports the photosynthesis process and helps in the formation of stronger roots, so that plants can absorb more water and nutrients from the soil [10].

Table 2. Weekly observations of chili plant leaf number under different biobactericide treatments from a group control (no treatment), Limousin, Simmental, PFH beef bone.

Day	No Treatment	Treatment A (Biobactericide from Limousin Beef Bone)	Treatment B (Biobactericide from Simmental Beef Bone)	Treatment C (Biobactericide from PFH Beef Bone)
0	4	4	4	4
7	5	7	6	5
14	6	9	8	7
21	7	12	10	9
28	8	15	12	10

In this case, a greater number of leaves means the plant has a higher photosynthetic capacity, which contributes to overall growth and potentially better crop yields. The Simmental and PFH beef bone treatments also showed an increase in leaf number, but not as large as that seen in the Limousine treatment [11]. This suggests that there may be differences in the mineral composition or specific properties of each type of bone that influence the effectiveness of these biobactericides [12]. Overall, the results of this study emphasize the importance of biobactericides from beef bones as an effective alternative for increasing the growth of chili plants, especially in situations where the plants are experiencing disease attacks. Apart from cow bones, the growth of the number of leaves is also influenced by other factors, one of which is hormones. Plant growth

hormones were discovered to enhance the growth and physiological activities of plants [13].

3.3 Symptoms of Wilt Disease

The research results relate to observing disease symptoms in infected chili plants *Ralstonia solanacearum* shows that biobactericides from cow bones have an observable effect in suppressing the symptoms of wilt disease. At the beginning of observation (day 0), all treatment and control groups showed early symptoms of disease, with the number of symptoms ranging from 1 to 2. However, after being given biobactericide treatment, especially from Limousin cattle bones, a clear reduction in disease symptoms was observed. On the 7th day, plants treated with Limousin cattle bones showed no symptoms of disease, while control plants still showed 2 symptoms of disease. Plants treated with biobactericide from Simmental cattle bones also experienced decline, with only 1 symptom remaining. In contrast, plants treated with biobactericide from PFH cattle bones still showed 2 persistent symptoms. Further observations on the 14th and 21st days showed that plants treated with Limousin and Simmental cattle bones were completely free of disease symptoms, while plants treated with PFH cattle bones and controls continued to show symptoms. At the end of the observation (day 28), plants treated with PFH beef bones still showed 1 symptom, while plants without treatment experienced an increase in symptoms to 6 symptoms. As shown in Table 3, these results demonstrate the observable impact of biobactericides in reducing disease symptoms in chili plants infected with *Rolstonia solanacearum*.

Table 3. Weekly observations of wilt disease symptoms in chili plants under different biobactericide treatments from a group control (no treatment), Limousin, Simmental, and PFH beef bone.

Day	No Treatment	Treatment A (Biobactericide from Limousin Beef Bone)	Treatment B (Biobactericide from Simmental Beef Bone)	Treatment C (Biobactericide from PFH Beef Bone)
0	2 symptoms	1 symptom	1 symptom	2 symptoms
7	2 symptoms	0 symptoms	1 symptom	2 symptoms
14	4 symptoms	0 symptoms	0 symptoms	2 symptoms
21	5 symptoms	0 symptoms	0 symptoms	1 symptom
28	6 symptoms	0 symptoms	0 symptoms	1 symptom

PFH cattle bones (Holstein Friesian breeds) are considered less effective in treating symptoms of wilt disease in chili plants, especially those caused by infection. *Ralstonia solanacearum*, compared to bones from other cattle breeds such as Limousin and Simmental. This can be explained by the mineral and nutritional composition present in PFH bones. PFH bones have lower calcium and phosphorus content, which are important minerals for the healing process and plant defense against pathogens. These

minerals contribute to bone strength and bioactive properties that can suppress infection. PFH cattle often lack important nutrients because the availability of feed ingredients in Indonesia is still lacking [14]. Therefore, it is possible that bones from PFH cattle have lower effectiveness in preventing wilt plant disease. In this context, calcium has a vital role in increasing the resilience of plant cell walls, while phosphorus functions in the process of photosynthesis and healthier root growth. Limousine beef bones provide better protection against infection due to their higher mineral content and ability to produce antimicrobial compounds [15]. PFH cattle often lack nutrients in the form of minerals.

4 Conclusion

This research shows that biobactericides from cattle bones, especially from Limousin cattle, have a notable effect in increasing growth and reducing symptoms of wilt disease in infected chili plants. *Ralstonia solanacearum*. Plants treated with biobactericide from Limousin cattle bones showed the best results, with an increase in plant height, a greater number of leaves, and a drastic reduction in disease symptoms compared to the control group and other treatments, such as Simmental cattle bones and PFH. This effect can be explained by the higher mineral content, especially calcium and phosphorus, in Limousine beef bones which help strengthen plant cell walls and increase resistance to bacterial infections. Although bones of Simmental cattle also gave good results, bones of PFH cattle showed lower effectiveness, possibly due to the lower mineral content in PFH bones, which are more focused on milk production rather than the formation of a strong bone structure. Thus, the use of cattle bone waste, especially from beef cattle such as Limousin, as a biobactericide has proven to be an environmentally friendly alternative that is effective in controlling plant diseases, while also utilizing resources that were previously underutilized.

Disclosure of Interests. The authors have no competing interests to declare that are relevant to the content of this article.

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