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Application of *Bacillus* sp Liquid Formula Using Root Infusion Technique To Control VSD (*Vascular Streak Dieback*) Disease In Cocoa

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Abstract: This study aims to explore the potential of liquid organic waste formula made from *Bacillus* sp to suppress the severity of vascular streak dieback (VSD) disease in cocoa plants. The research was conducted on smallholder cocoa plantations in Patimbe Village, Palolo Subdistrict, Sigi Regency, Central Sulawesi. The research was arranged in a non-factorial Randomised Group Design (RAK) with 5 treatments and 5 replications. The treatments consisted of control (P0=no formula); coconut water + *Bacillus* sp (P1); rice washing water + *Bacillus* sp (P2); tofu water + *Bacillus* sp (P3); and a combination of coconut water, rice washing water, and tofu water + *Bacillus* sp (P4). Each experimental unit consisted of 10 cocoa plants infected with VSD disease. The liquid organic waste formula was applied using the root infusion technique in months 1 and 3 at a dose of 250 ml/root. Observations of disease symptoms, disease development, disease severity, and disease suppression efficacy were conducted monthly. The results showed that the combination of organic waste of coconut water, tofu water, and rice washing water with *Bacillus* sp active ingredient was more effective in suppressing VSD disease than liquid organic waste alone with an effectiveness rate of 81.7%.

Keywords: *Bacillus* sp.; Formula; Organic waste; Root infusion; VSD disease

Introduction

Vascular streak dieback (VSD), caused by the pathogenic fungus *Ceratobasidium theobromae* (syn. *Oncobasidium theobromae*) and cocoa pod rot (BBK), are important diseases of the cocoa crop (Ali et al., 2019). These diseases pose a serious threat to cocoa production. However, VSD disease is considered more dangerous than BBK disease because infected plants will become weak, resulting in reduced productivity, branch death or even death of productive cocoa plants. While BBK disease only causes damage to cocoa pods. Yield loss due to VSD disease can reach 40-50% (Assad et al, 2017). Besides cacao, avocado and orchids are possible hosts of this pathogenic fungus (Bryceson et al., 2023).

So far, farmers have controlled the fungus *C. theobromae* through pruning infected branches and using resistant planting materials. The control technique of frequent and regular pruning has proven effective in cocoa plantations but is very labour intensive. While the use of resistant cocoa is also effective, the availability of resistant clones is very limited. Some triazolic systemic fungicides have been shown to be effective in controlling VSD disease, but their high cost makes them uneconomical (McMahon et al, 2018) and not necessarily successful when applied in the field. This is because the pathogenic fungi are located in the xylem network (Bryceson et al., 2023) so that pesticide applications are unable to reach the location of the pathogen. The *C. theobromae* fungus colonises and survives in the xylem tissue (Rosmana et al., 2019) so that it easily spreads to

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all parts of the plant and is difficult to reach by fungicides. Pathogenic fungi that live in xylem tissue will disrupt and reduce the transport of water and nutrients to the leaves. This disruption causes leaf fall and twig death (Parawansa et al, 2022). In advanced attacks, tissue death can spread to branches or even to the main stem. This will affect cocoa crop production. In addition, the continuous use of synthetic fungicides causes environmental pollution that can damage the ecosystem in the long run. The use of fungicides can also leave harmful chemical residues in cocoa beans that will be processed for consumption by consumers, and cause pathogens to become more resistant. Therefore, environmentally friendly control methods such as the use of antagonistic microbes as biological agents are needed.

The use of antagonistic microbes as biological agents to control VSD disease has been widely reported such as the use of *Pseudomonas* spp., *Bacillus* spp., *Enterobacter* spp.; *Trichoderma asperellum* (Vanhove et al., 2015), *Trichoderma* spp (Harni et al., 2017), *T. harzianum* (Susiyanto et al., 2017), *Curvularia* sp., *Gliocladium* sp., and *Aspergillus* sp. (Asman et al., 2018). The application of biological agents must be able to reach the cocoa plant tissue either through stem injection or root infusion in the field because the VSD pathogenic fungus is in the xylem network of the plant. This is to make it easier for biological agents to enter the xylem through the stem or roots and spread to all parts of the cocoa plant. One of the biological agents that can enter the plant network is Bacillus sp. Eid et al. (2021) stated that Bacillus sp is an endophytic diazotropic bacterium that can enter plant tissues in the intercellular space and xylem. Bacteria live colonised in plant tissue without having a pathogenic effect on the host plant.

Bacillus sp is known to have an advantage in competing for nutrients and space to prevent the growth and spread of pathogens. These bacteria are able to produce antimicrobials such as surfactin, iturin, and fengisin which are antifungal and antibacterial compounds (Karacic et al., 2024). The bacteria also produce various enzymes such as chitinase, β-1,3-glucanase, protease, lipase, amylase, and cellulase that play a role in degrading the cell walls of plant pathogens (Ajuna et al., 2023). In addition, *B. subtilis* can stimulate the plant immune system by activating plant-induced systemic resistance (Bahramisharif & Rose, 2019). *Bacillus* sp also colonises the inner tissues of plants, making it more effective in protecting plants (Lubyanova et al., 2023) from attack by xylem tissue-dwelling pathogenic fungi such as *C. theobromae*.

The application of biological agents in cocoa plants so far is still in the form of live cell suspensions without a formula by spraying antagonists to the entire surface of young cocoa leaves (Vanhove et al., 2015; Susiyanto et al., 2017; Harni et al., 2017), watered into the soil (Rosmana et al., 2018), through stem injection (Christiadi, 2021), or in the form of secondary metabolite suspensions through root infusion (Harni et al., 2019b). Single application of *Bacillus* sp. bacteria without a formula can cause the biological agent to not survive longer and its ability as a biological agent or as a bioactivator is not optimal (Oktrisna et al., 2017). To support the growth and development of these antagonistic bacteria, it is necessary to make a formula as a provider of nutrients and energy sources for *Bacillus* sp.

The successful application of biological agents in the field is strongly influenced by the availability of nutrients in the formula materials used. So far, the application of *Bacillus* sp. singly in the form of a formula (a combination of coconut water waste, tofu water, and rice washing water) through root infusion to control VSD disease in cocoa has never been done. Therefore, *Bacillus* sp as a biocontrol needs to be packaged in the form of a formula so that it can survive longer and is stable when stored. This will increase the effectiveness of the active ingredient in controlling pathogens. Oktrisna et al. (2017) stated that if *Bacillus* sp. bacteria are formulated from materials such as liquid organic waste, the waste will provide nutrients for *Bacillus* sp. to optimise its working power. Nutrients contained in organic waste become an additional food source for bacteria. Liquid organic waste as a carrier material for the formula can be in the form of tofu water waste (Sugiharti et al., 2022), coconut water waste (Abna et al., 2018), and rice washing water waste (Abba et al., 2021).

This study aims to determine the potential of liquid organic waste formulas made from *Bacillus* sp as a single and combination in suppressing the severity of VSD disease applied using the root infusion technique on cocoa plants.

Method

The research was conducted from May to December 2023. The research was conducted at the People's Cocoa Farm in Patimbe Village, Palolo District, Sigi Regency. Antagonistic bacteria rejuvenation and formula making were carried out at the Plant Disease Laboratory, Faculty of Agriculture, Tadulako University.

Tools and Materials

Materials used included plastic sugar bags, plastic ropes, litre measures, labels, *Bacillus* sp isolates, NA medium, brown sugar, shrimp paste, tofu water waste, coconut water, and rice washing water. Equipment consisted of measuring cups, erlenmeyers, test tubes, tube racks, beakers, petri dishes, bunsen, micropipettes, Laminar Air Flow (LAF), autoclaves, tweezers, scissors, hot plates, spatulas, hole-making shovels, hoes, knives, scissors, jerry cans, stoves, buckets, pots, and stationery.

Research Methods

The field test was arranged in a non-factorial Randomised Group Design (RAK) with 5 treatments and 5 replications. Treatments consisted of control (P0=no formula); coconut water + *Bacillus* sp (P1); rice washing water + *Bacillus* sp (P2); tofu water + *Bacillus* sp (P3); and a combination of coconut water, rice washing water, and tofu water + *Bacillus* sp (P4). Each experimental unit consisted of 10 VSD-infected cocoa plants.

Research Implementation

Rejuvenation of Bacillus sp *isolates*

The stored pure culture of *Bacillus* sp was transferred to a new nutrient agar (NA) medium. A bacterial culture of 1 ose was inoculated into the NA medium using the streak plate method. This process was done aseptically in LAF (Laminar Air flow), then incubated at 37^{O}C for 1 x 24 hours in an incubator. Single colonies of bacteria that grew were inoculated on solid NA medium and used as test bacteria.

Suspension Preparation

One ose of *Bacillus* sp pure culture that has been rejuvenated on NA media is taken and suspended into a test tube containing 10 ml of sterile 0.9% NaCl solution media. NaCl solution is used because it is isotonic with bacterial cell fluid so that it can maintain bacteria to stay alive before being transferred to a new growth medium. The turbidity of the test bacteria that have been diluted using 0.9% NaCl, is equalised with the comparative turbidity of 0.5 Mc Farland I standard solution. Turbidity equivalent to 0.5 Mc. Farland I has a population of 10^7 - 10^8 CFU/ml (Sari et al., 2022).

Preparation of Bacillus sp.

The liquid formula made refers to the liquid formula according to Asrul et al (2023), The composition of the liquid organic waste formula consists of active ingredients (*Bacillus* sp), carrier materials (organic waste of tofu water, coconut water, and rice washing water), and additives (brown sugar and shrimp paste). The carrier material was obtained from domestic industrial factory waste in Palu City. Each single liquid organic waste was prepared as much as 980 ml, put into jerry cans separately and stirred until evenly distributed. For the combination of liquid organic waste the ratio is 1:1:1. Next, the solution was filtered with gauze to obtain clean organic waste, then boiled in a pot until boiling. After cooling, 20 ml of *Bacillus* sp suspension (10^8 cfu/ml) was added to each solution. Next, 2% sugar and 1% shrimp paste were added, then stirred until homogeneous. The formula was put into jerry cans of 20 litres each and the hole was covered with a jerry can lid. After that, the formula was fermented for 2 weeks. During the fermentation process, the jerry can lid was opened every day for 1 minute so that the gases formed could escape and microbes could grow. The fermentation process is declared successful when the jerry can lid smells like tape and is ready to be applied to plants.

Application of Biological Agents with Root Infusion

The application of *Bacillus* sp biological agents was carried out using the root infusion technique twice, namely month 1 and 3 with the application time in the afternoon at 15.00-18.00 WIB. Root infusion is done by digging the soil to form a hole at a radius of one metre from the base of the stem. Four root infusion holes were made for each cocoa plant, with the holes located in the west, east, north, and south. Active and healthy roots were selected from each hole, then the tip of the root was cut using a sharp knife. Cocoa roots that look young, light brown in colour, but green at the cut marks indicate healthy and active roots. The roots were cut obliquely to expand the wound surface so that they could absorb the liquid faster. Next, the root tip is inserted into a plastic bag, which has contained the *Bacillus* sp biological agent formula (10^8 cfu/ml) with a 45^{O} tilt position so as not to spill. The application dose of the formula was 250 ml/root and the control treatment was without *Bacillus* sp. The end of the plastic bag was tied with a rope carefully and the root tip was placed at the bottom of the plastic bag. The soil hole containing the infused roots was covered again to avoid direct sunlight and to avoid livestock or human disturbance.

Observation Variables

The observed variables include disease symptoms, disease progression, and VSD disease severity, which were conducted directly and regularly on diseased cocoa farms. Observations of disease symptoms and severity were made before and after formula application for 6 months. Disease severity was calculated once a month by observing the number of infected twigs per tree based on typical symptoms of infected twigs/branches. Infected twigs, when cut longitudinally, will show brownish stripes on the xylem tissue up to the petiole.

Disease Severity

To calculate the severity of VSD disease using the following formula (Khaerati et al., 2016):

$$KP = \sum_{i=0}^{n} \frac{n_i \, x \, v_i}{N \, x \, Z} \, x \, 100\% \tag{1}$$

KP = Disease severity
n_i = Number of plants with symptoms of the i-th scale
v_i = Scale value of each attack category from i=0,1,2,3,4
Z = Highest scale value
N = Number of plants observed

The scoring values obtained were used to calculate disease severity on all cocoa trees as follows (McMahon et al., 2018): 1 (nil), no infection detected; 2 (low), <10% of branches on the tree are infected, infection is generally mild, no significant leaf drop; 3 (medium), 10-25% of branches are infected, moderate infection with some leaf drop; 4 (high), >25% of branches are infected, some severe infection or immediate death is seen with most leaves on some branches infected or dropped.

Efficacy Level

The efficacy level of the *Bacillus* sp. organic waste formula was calculated by comparing the severity of VSD disease in the control (without treatment) with the formula treatment, following the formula below (Harni & Baharuddin, 2014):

$$TE = \frac{(KP_k - KP_p)}{KP_k} x\ 100\% \quad (2)$$

TE = Efficacy level
KP_k = percentage of disease severity in the control (without formula)
KP_p = percentage of disease severity in formula treatment

The tested formula is said to be effective if the efficacy level value is at least (1/2 n + 1) where n = the total number of observations after application. In this study, observations were made 6 times so that the efficacy rate (EI) ≥ 40% (Abidin et al., 2015).

Primary Data Collection

Primary data collection was carried out by directly measuring air temperature and humidity at the research site using a thermohygometer tool hung on a cocoa tree. Measurements of air temperature and humidity were made at three repetitions, namely morning (07:00 - 08:00), afternoon (12:00 - 14:00) and evening (16:00 - 17:00) every day. Furthermore, the measurement results were averaged, so that the average monthly air temperature and humidity data were obtained.

Data Analysis

The results of the study were analysed with variance analysis (ANOVA), if the analysis showed significantly different results then continued with the BNT test at the 5% level.

Result and Discussion

Description of VSD Disease Symptoms in the Field

Observations of VSD disease symptoms in the field showed necrotic leaves at the leaf tip and chlorotic (yellowing) surrounded by small green spots with clear boundaries, especially on the second or third leaf from the top of the cocoa plant (Figure 1A). The spots appear diffuse against a background of yellowing leaves. Diseased leaves miscarry or detach from the twig a few days after yellowing, leaving the twig without leaves. Twigs often look toothless because the tip and base of the twig still have leaves, while the centre has fallen off. When the twig where the diseased petiole used to stand is cut/folded, three brownish spots can be seen. These spots are wood vessels that have been damaged by VSD disease (Figure 1B). Similarly, when the diseased branches or twigs were cut longitudinally, several brown lines on the vascular streak were seen on the dead branches or twigs from the ends (die back) (Figure 1C). The results of the study were analysed with variance analysis (ANOVA), if the analysis showed significantly different results then continued with the BNT test at the 5% level. The brown line is damage to the xylem tissue due to the attack of the pathogenic fungus *C. theobromae*. If the attack continues, it will cause tissue death to the branches and main stem. The symptoms of VSD are similar to the symptoms reported by McMahon et al (2018), namely pale green-yellow leaves followed by advanced chlorosis with the appearance of blotches, or the development of small necrotic spots usually at the tip or edge of the leaf then expanding, followed by rapid leaf fall.

Figure 1. Symptoms of VSD disease on cocoa plants

The *C. theobromae* fungus enters plant tissues through basidiospores that are carried by the wind and fall on young leaves of cocoa plants. The basidiospores then germinate and penetrate through the epidermis, mesophyll, and into the leaf bone. In infected leaf bones, white fungal colonies (mycelium) will develop and grow outward until they enter the xylem tissue, especially in humid environmental conditions (Harni et al., 2019a). In the xylem, fungi colonise due to the growth of fungal mycelium, thus inhibiting the transport system in plant tissues that carry photosynthetic needs such as water and nutrients from roots to leaves (Hendra et al., 2019). Blockage of woody vessels (xylem) in the stem causes brownish discolouration (necrosis) and yellow colour with green patches (chlorosis) in leaves near the branch tips (McMahon et al., 2018). Sometimes there is also damage to leaf tissue with necrosis between the leaf bones, or the leaf dries from the tip. The fungus, which continues to grow in the xylem, will then make its way to the main branch, killing the branches above it (Harni et al., 2019a).

Development of VSD Disease Severity

The observation results of the development of VSD disease severity can be seen in Figure 1. In the 1st month observation, the development of VSD disease after the application of organic waste formula treatment made from active *Bacillus* sp (P1-P4) and control (P0) showed high disease severity. However, in month 2-6 observations, all formula treatments experienced a decrease in disease severity except the control (no formula). In the control, the development of disease severity increased significantly in months 5 and 6, while in the formula treatments there was a varying percentage decrease. The high increase in disease severity in the control was in line with the increase in observation time. This was due to the fact that the climate during the observation period was very favourable for the development of disease severity because the average temperature and humidity at the research site were in the range of 24.3 - 26.7ºC and 88.9 - 94.6%. The results of research by Harni et al (2017) showed that temperatures ranging from 22 - 27ºC and humidity of 93 - 97% are very supportive of *C. theobromae* infection. At night, *C. theobromae* will circulate by spawning hyphae or mycelium on former leaf mounts that have fallen off, especially in humid conditions (Khaerati et al., 2016; Bryceson et al., 2023). McMahon et al (2018) added that basidiospores of *C. theobromae* will be produced at night under humid conditions. Fungal basidiospores are confined to xylem tissue within leaf and stem bones. If dispersed on young leaves, basidiospores can cause infection and fall off, and shoots or branch tips eventually die, causing symptoms of death. The treatment of combined organic waste formula (P4) showed the highest reduction in disease severity compared to the single formula of coconut water waste (P1), rice washing water (P2), or tofu water (P3). The treatment of coconut water waste (P1) and rice washing water waste (P2) in suppressing the development of VSD disease severity is almost the same but coconut water waste (P1) is better than rice washing water waste (P2).

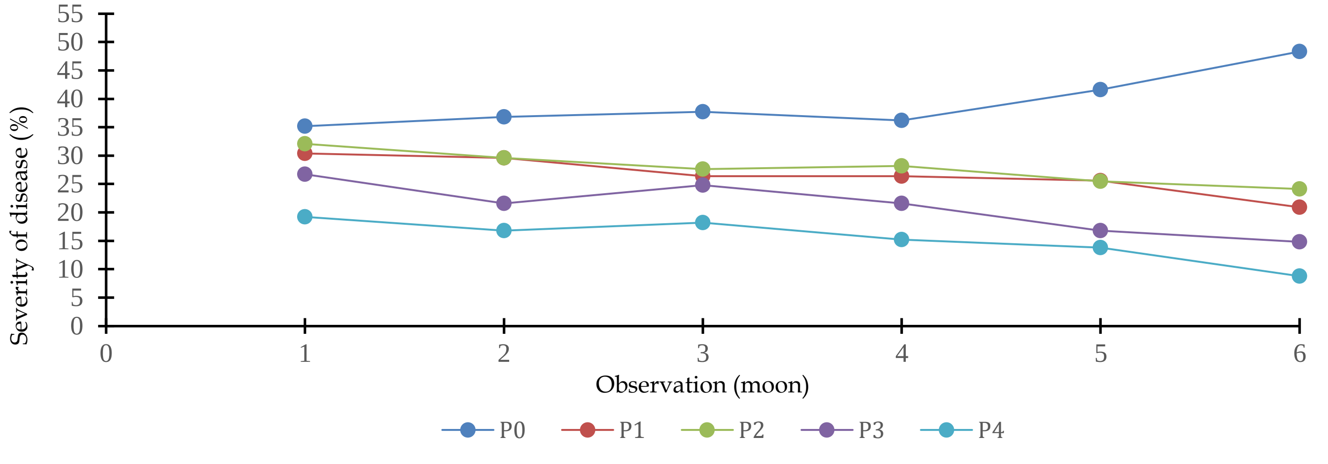

Figure 2. The development of VSD disease on cocoa plants after being treated with a formula of organic waste formula of coconut water (P1), rice washing water (P2), tofu water (P3), a combination of coconut water, rice washing water, and tofu water (P4), and control (P0, no formula).
(P4), and control (P0, no formula).

The development of VSD disease is not only influenced by climatic conditions around cocoa farms, but also by physical plant factors, such as the use of susceptible cocoa clones. The development of the disease that continues to increase in cocoa plants without formula (control) is thought to be because farmers still use susceptible cocoa clones from previous cocoa cultivation. During disease observation in the field, many infected leaves were found falling around the cocoa trees. Parawansa et al (2022) stated that the severity of the disease is determined by the number of infected or fallen leaves. In susceptible cocoa clones, the proportion of infected or fallen leaves is higher than in resistant cocoa clones. This indicates that the study site has more susceptible cocoa clones than resistant ones. In susceptible cocoa clones, the *C. theobromae* pathogen easily clogs the xylem network or spreads within the branch to other leaves, resulting in many leaves being shed.

Disease severity

Observations of disease severity after 6 months of formula application ranged from 8.8 - 48.3% (Table 1). The results of statistical analysis showed that the treatment of liquid organic waste made from *Bacillus* sp gave a significant effect on the average percentage of VSD disease severity. The lowest disease severity was found in the combination of organic waste of coconut water, rice washing water, and tofu water (P4) at 8.83%, while the highest was found in the control /P0 (48.37%). The high disease severity in the single treatment of rice washing water, coconut water, and tofu water is likely due to the lack of nutritional elements that can support each other to meet the needs of bacterial life. This has an impact on the low activity of antagonistic bacteria in producing various secondary metabolite compounds to suppress pathogen growth. In Table 1, there are differences in the percentage of VSD disease severity in each liquid organic waste treatment.

Table 1. Mean percentage of disease severity, efficacy, and assessment of the efficacy of liquid organic waste in suppressing VSD disease in cacao

Liquid organic waste	Disease severity (%)	Disease suppression efficacy (%)	Efficacy level assessment
P0 (control)	48.37a	-	
P1 (coconut water)	20.91b	56.30	effective
P2 (rice washing water)	24.16b	50.10	effective
P3 (tofu water)	14.83c	69.40	effective
P4 (coconut water, rice washing water, and tofu water)	8.83d	81.70	effective

Note: numbers followed by the same letter in the same column show no significant difference in the BNT test at the 5% level.

The difference is thought to be influenced by the nutrient content contained in each liquid organic waste as a growth medium that affects the growth and development of antagonistic bacteria. According to Putri et al (2016), differences in nutrients available in growth media affect the formation of microorganism cells. Media containing high nutrients can support bacterial growth for a longer time (Harris, 2016).

In general, formula treatments applied to cocoa roots either singly or in combination, can cause a decrease in the severity of VSD disease. This indicates that the liquid organic waste formula made from *Bacillus* sp has the ability to inhibit the development of VSD disease well. Presumably, the applied liquid organic waste is able to keep the viability (population density) of *Bacillus* sp stable so as to support the antagonistic activity to suppress the population development and activity of pathogenic fungi while in the xylem network. The complete nutrient content in the liquid organic carrier material causes the bacterial metabolic process to take place faster so that the cell division process to form new cells is also easier. This has an impact on the growth of antagonist colonies that are more fertile and the population density of antagonists is more so that the ability to colonise space and nutrients in plant tissues to suppress the growth of pathogenic fungi is better. Kim et al (2021) stated that the antagonistic activity of *Bacillus* sp. bacteria to suppress pathogenic fungi will be greater if the bacterial population density is high enough (108 cfu/ml). In this study, the population density of Bacillus sp applied through root infusion was 108 cfu/ml. This high population density allows the antagonist to aggressively colonise the xylem space, limiting the development and spread of *C. theobromae* fungus in cocoa plant tissues. Antagonists grow faster colonising plant tissues to compete with the pathogen so that pathogen growth is suppressed. In the xylem network, antagonists take all the nutrients derived from plant metabolic products while plants get nutrient derivatives and active compounds needed during their life from antagonistic bacteria (Purwaningsih & Wulandari, 2021). The colonisation of nutrients for growth and antagonist activity in the tissue results in limited nutrient sources available for the life needs of the fungus *C. theobromae*. In the xylem network, antagonists also undergo a self-propagation process so that they fill the space that will inhibit the growth of pathogenic fungi. Saputri et al (2020) reported that *Bacillus* sp generally colonises the intercellular part of the host plant tissue, the woody vascular system (xylem), and can be translocated systemically to all parts of the plant. Bacillus sp colonisation in plant tissues occurs through migration into the intercellular space by secreting cell wall degrading enzymes such as cellulase and pectinase (Vandana et al., 2021). Shafi et al (2017) stated that rapid colonisation of plant tissues by *Bacillus* sp is a key element for successful biological management of plant diseases. Plant organs and tissues that have been colonised by antagonists cannot be entered by the pathogenic fungus *C. theobromae* even though xylem tissue is a colonisation site for the pathogen. This is possible because the antagonistic bacteria *Bacillus* sp are in the same ecological space as the pathogen so that control can be more effective (Giyanto et al., 2020). The support of organic matter from the organic waste formula as a source of nutrients causes antagonistic bacterial cells to live and multiply quickly, and colonise xylem vessels early so that they can win the competition for space and nutrients in the same location. This antagonistic colonisation results in the suppression of destructive pathogens in plant tissues. Therefore, the ability of antagonists to colonise plant tissues is an important factor in suppressing pathogen development. Shafi et al (2017) reported that the rapid colonisation ability of B. subtilis NJ5 at high population densities could significantly reduce *Verticillium* wilt disease of cotton plants.

In the treatment without formula (control), there was no decrease in disease severity so that the percentage of disease severity was higher than cocoa plants that received treatment. It is suspected that in cocoa plants without *Bacillus* sp. formula, the pathogenic fungus *C. theobromae* is freely active in growing and developing because there are no obstacles from antagonistic bacteria. Under these conditions, the pathogen colonises and adapts faster in the xylem network to infect plants compared to treated cocoa. Samuels et al (2012) stated that in susceptible cocoa, C. theobromae will grow in the xylem up to the main stem and kill mature cocoa trees. Hyphae of the pathogenic fungus *C. theobromoae* will spread through woody vessels to adjacent leaves, causing leaf drop and eventually killing seedlings or branches, especially susceptible cocoa (McMahon et al., 2018).

The low disease severity in the combined organic waste treatment (P4) compared to the single organic waste indicates the possibility of synergy (compatible) between these organic wastes. The combination of organic waste is complementary and synergistic to support the fulfilment of nutritional needs so as to improve the performance of *Bacillus* sp in controlling pathogens effectively. The results of research by Asrul et al (2023) showed that the combination of nutrients contained in tofu, coconut, and rice washing liquid waste has fulfilled the nutritional needs required by *Bacillus* sp bacteria to grow and develop optimally. In this condition, the organic matter contained in the wastewater will be used as a nutrient in the metabolic process, so that the antagonist works more optimally to form secondary metabolite compounds to suppress the growth of *C. theobromoae* in xylem vessels. Hapsoh et al (2020) stated that liquid organic waste containing organic matter (carbohydrates) and organic nitrogen (proteins and amino acids) will be used as an energy source for bacterial growth. Coconut water waste is known to contain 95.5% water, 4% carbohydrates, 0.1% fat, 0.1% protein, 0.02% calcium, 0.01% phosphorus, 0.5% iron, amino acids (lysine, leucine, and arginine), vitamins C and B complex, enzymes, sugars, cytokinins, auxins and mineral salts (Abna, 2018).

Bacteria require 98.51% water for their metabolic functions and all nutrients are available in solution form so that they are easily absorbed by bacteria. With this very rich content of nutrients, coconut water can be used as an alternative formula material for the growth of these bacteria because it contains the elements needed for their growth (Mayaserli & Renowati, 2015). Meanwhile, tofu water waste has organic content such as protein (40%-50%), carbohydrates (25%-50%), and fat (10%) which are suitable for the growth of *Bacillus* sp bacteria (Juariah & Sari, 2018; Utami & Suprihadi, 2018). Meanwhile, rice washing water waste contains high nutrients and vitamins such as saccharides such as starch as much as 85-90%, glutein, cellulose, vitamin B1 which are dissolved during the rice washing process. The saccharide content contained in rice washing water can be utilised for bacterial growth (Izzati et al., 2019).

The results of previous studies have shown that the use of coconut water waste formula, rice washing water, and tofu water as a medium for bacterial growth is very effective in controlling disease. Yanti et al (2016) reported that *Bacillus thuringiensis* TS2 bacteria grown in the organic waste formula had the ability to suppress the development of bacterial pustule disease in soybean plants. According to Nuraeni & Sebayang (2018), maximum growth can only occur if the nutrients in the formula are in optimal conditions for bacterial life. A complete formula can fulfil nutritional needs and support bacterial survival because bacterial viability is influenced by nutrient content. This liquid organic waste formula contains important components that support the viability and growth of microbes in it. Khaeruni et al (2013) stated that liquid organic waste contains a composition of nutrients that are good for microbial growth such as carbohydrates, proteins, water, amino acids, fats, mineral salts and other nutrients, so it has great potential for making biological agent formulas. The combination of organic waste is in the amount of nutrients that meet the needs of bacterial life so that it has a greater ability to encourage bacterial cell growth. Thus, the application of liquid organic waste formula has the benefit of providing nutrients for Bacillus sp to support bacterial growth in the xylem network. The use of organic waste formula during the application of *Bacillus* sp in root infusion is expected to increase the growth and effectiveness of the biological agent in suppressing pathogenic fungi.

Disease Suppression Efficacy

The efficacy of antagonistic bacteria was inversely proportional to the percentage of disease severity (Table 1). Every increase in the efficacy value of the antagonist, will be followed by a decrease in the percentage value of disease severity. Based on the efficacy level, all organic waste formulas, both single and combined, proved effective in suppressing the development of VSD disease severity in cocoa because the efficacy level was more than 40%. This means that all organic waste formulas made from active *Bacillus* sp applied through root infusion have the same potential as biopesticides to control VSD. The success in suppressing disease severity allows cocoa plants to grow and develop better.

The high level of antagonistic efficacy in this study, in addition to being influenced by organic carrier materials contained in wastewater as a growth medium, is also thought to be supported by the presence of *Bacillus* sp bacteria as an active ingredient in the formula to suppress pathogen growth. The suppression mechanism of *Bacillus* sp against the pathogenic fungus *C theobromae* in the xylem network is thought to be through an antibiosis mechanism by producing secondary metabolite compounds in the form of antibiotics and enzymes, as well as inducing plant resistance. The amount of secondary metabolites secreted by bacteria to inhibit pathogen growth is influenced by the high and low population density. This is evidenced by the larger clear zone at high bacterial population density compared to low bacterial population density (Asrul et al., 2023). *Bacillus* sp can produce a variety of antibiotics that exhibit antibacterial and antifungal properties, such as surfactin, iturin, and fengisin (Hashem et al., 2019). Surfactin has antibacterial, antiviral, and antimycoplasma activities, but is not effective against fungi. Iturin is a potent antifungal activity against a wide variety of pathogenic fungi and yeasts. However, the antimicrobial activity of iturin is mainly reserved against fungi. Fengisin exhibits strong antifungal activity and inhibits the growth of various plant pathogens, especially filamentous fungi. When fengisin is combined with surfactin and iturin, it will synergistically increase its antimicrobial effectiveness (Wang et al., 2018). Other types of antibiotics are bacillomycin, mycobacillin, fungistatin, plipastatin, bacillin, biosurfactants, penazine, lipopeptides, hydrogen cyanide (HCN), etc. (Ali et al., 2020), gramicidin, and basitrasin (Chalasani et al., 2015), andrimid, pyoluteorin and amphotericin B (Cesa-Luna et al., 2020). Therefore, *Bacillus* sp can be used as a biopesticide for plant protection.

Bacillus sp can also secrete various enzymes that play a role in controlling plant pathogens. The production of enzymes produced by Bacillus sp, showed strong inhibitory effects against fungi. Hydrolytic enzymes produced by *Bacillus* sp such as chitinase, protease, cellulase, β-1,3-glucanase, endoglucanase, and hemicellulase have the ability to suppress plant pathogens directly by degrading and lysing the fungal cell wall (Shafi et al., 2017; Vandana et al., 2021; Jan et al., 2023). Hydrolytic enzyme activity on fungal cell walls, hyphal tips, and sprout tubes causes hyphae to swell, twist, and rupture resulting in fungal death (Vandana et al., 2021). These enzymes break down specific components of the cell wall of several pathogenic fungi, such as *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Phytophthora* spp, *Pythium ultimum*, and *Botrytis cinerea*. This enzymatic activity can efficiently control the spread of disease by inhibiting the growth of pathogenic fungi (Jan et al., 2023).

In addition to suppressing the activity of plant pathogens through antibiosis mechanisms, Bacillus sp can also induce disease resistance in cocoa plants. Bacillus sp residing in the xylem tissue can cause induced resistance that allows antagonists to inhibit pathogens colonising the plant's xylem tissue (Vandana et al., 2021). The application of antagonistic bacteria will trigger the expression of genes that form certain compounds in plants related to plant defence mechanisms against pathogen attack. According to Mugiastuti et al (2019), *Bacillus* sp can induce resistance by increasing the activity of peroxide enzymes, the production of chitinase and glucanase enzymes, and the accumulation of plant salicylic acid compounds which will affect its ability to suppress disease severity. (Putri et al (2020) reported that *Bacillus* sp. bacteria were able to reduce the severity of fruit rot disease in cocoa through a systemic resistance induction mechanism. In this study, it is likely that the antibiotics and enzymes secreted by *Bacillus* sp., as well as the induction of cocoa resistance by the antagonist, have played an important role in inhibiting the growth of the pathogenic fungus *C theobromae*, resulting in low disease severity and high efficacy values.

Conclusion

Based on the results of this study, it can be concluded that liquid organic waste enriched with *Bacillus* sp active ingredients has the potential to reduce the severity of VSD disease using the root infusion technique of cocoa plants. The combination of organic waste of coconut water, tofu water, and rice washing water made from *Bacillus* sp is more effective in suppressing VSD disease in cocoa plants than liquid organic waste alone with an effectiveness rate of 81.7%.

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review and editing, I.L.; visualization, U.M.; supervision, M.T.; project administration, M.T.; funding acquisition, K.B.

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Conflicts of Interest
The authors declare no conflict of interest.

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