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The Effect of Botanical Nutrients Enriched with *Trichoderma* sp. on the Growth of Cocoa Seed Radicles (*Theobroma cacao* L.)

Mahyatul Qomariyah[1)], Imam Mudakir[2)], and Niken Puspita Sari[*3)]

[1)]Student of the Department of Biology Education, Faculty of Teacher Training and Education, University of Jember, Jl. Kalimantan, Jember, Indonesia
[2)]Lecturer, Department of Biology Education, Faculty of Teacher Training and Education, University of Jember, Jl. Kalimantan, Jember, Indonesia
[3)]Soil Researcher, Plant Production Science Group, Indonesian Coffee and Cocoa Research Institute, Jl. PB. Sudirman 90, Jember, Indonesia
[*)]Corresponding author: niken.iccri@gmail.com
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Abstract

Cocoa (*Theobroma cacao* L) is a plant that is widely cultivated by the Indonesian people. Many cocoa farmers experience crop failure due to pests and diseases. One effort that can be made to reduce this problem is to create high-quality cocoa seedlings that are able to survive when attacked by pests and diseases. This study aims to investigate the effect of adding nutrients in the form of herbal nutrient and *Trichoderma* sp. to cocoa seed soaking water on radicle length. The treatment used three different formulas: A: control, B: formula 1 (a mixture of weeds and water shoots in a 1:1 ratio), B: formula 2 (a mixture of weeds and water shoots in a 2:1 ratio), and C: formula 3 (a mixture of weeds and water shoots in a 1:2 ratio). Each nutrient solution was mixed with *Trichoderma* sp. at a dilution of 10-1, and the treatment was repeated three times, with three cocoa seeds in each replicate. The results obtained indicate that the best formula for cocoa seed radicle length, spore count, and spore density of *Trichoderma* sp. is the nutrients with a weed and water sprout ratio of 2:1 mixed with *Trichoderma* sp. at a dilution of 10 L.

Keyword: Botanical nutrients, cocoa, *Trichoderma* sp.

INTRODUCTION

Cocoa (*Theobroma cacao* L) is a widely cultivated crop in Indonesia. This is because cocoa has high economic value, thereby boosting the economy of both the Indonesian people and government. Cocoa beans have high market demand, both domestically and internationally. Its relatively stable selling price and export market potential make cocoa an important source of income for farmers, cooperatives, and processing industries. Indonesia itself is one of the world's largest cocoa producers. The world's main cocoa producing countries based on ICCO data in 2022 are Ivory Coast, Ghana, Ecuador, Cameroon, Nigeria, Brazil, and Indonesia (Rohmadona *et al*., 2023).

Cocoa cultivation in Indonesia faces several challenges. The main issues during cocoa cultivation are pests and diseases, as well as inconsistent seed quality. This can reduce cocoa productivity in various regions (Bertha., 2023). To address these challenges, one of the key steps is to produce high-quality cocoa beans that can withstand pest and disease attacks. The efforts undertaken are expected to help maintain the soil ecosystem. Therefore, it is advisable to use biological agents in these efforts.

Cocoa seedlings are obtained through the germination process of cocoa seeds. These cocoa seeds are the starting point for the growth and development of cocoa plants. If the seeds planted are of good quality, they will positively influence subsequent growth. Cocoa seeds come from the fruit on the main trunk, branches, and twigs. Seeds used as seeds should not be too small. The quality of cocoa seeds affects the growth of cocoa seedlings. Good cocoa plants that are resistant to pests and diseases can produce high-quality cocoa fruits and seeds (Hotimah, *et al* 2024).

Farmers generally still use local (random) seed sources that are susceptible to disease and have low productivity (Ariningsih *et al.*, 2021). This process is done because it is considered easier, faster, and does not require special tools or skills. Seeds that are too small or not fully mature are often used in the sowing process. However, small seeds tend to have lower nutrient reserves such as carbohydrates, proteins, and fats, which are crucial for supporting germination and early growth, particularly root or radicle formation. This affects the quality of cocoa seedlings during growth, leading to a decrease in cocoa productivity (Siahaan *et al.*, 2024).

In addition to selecting cocoa seeds, another way to improve cocoa seed quality is by adding nutrients during the seed soaking process. The addition of botanical nutrients can serve as an alternative nutrient supplement during the cocoa seed soaking process. Botanical nutrients is elicitors are chemical compounds made from extraction of some weeds that can trigger physiological responses and improve plant morphology, providing positive signals to root cell membranes, leading to greater productivity (Napitupulu, 2023). Botanical nutrients is a new innovation in agriculture. Its primary function is to enhance plant resistance against biotic factors such as fungi and bacteria, as well as abiotic factors like humidity and air temperature (Reflis *et al.*, 2023).

Botanical nutrients is made from several types of wild plants that are healthy and free from pests and diseases. The plants used are generally weeds that are considered a nuisance in agriculture. The production of botanical nutrients can also be utilized for weed control efforts. In its production process, almost all types of wild grasses and leaves available in the surrounding environment can be used as the main ingredient, with the exception of plants that have thorns or toxic properties that could potentially damage cultivated plants. This diversity of raw materials makes botanical nutrients a practical and economical solution (Azhimah *et al.*, 2023).

Another biological agent that can be used in water mixtures during cocoa seeds soaking is *Trichoderma* sp. *Trichoderma* sp. is a type of fungus that plays an important role in agricultural systems due to its ability to control pests and pathogens biologically without polluting the environment. In addition to functioning as an effective biological control agent in suppressing the growth of various types of plant disease-causing pathogens, *Trichoderma* sp. also plays a significant role in supporting plant vegetative growth, particularly through stimulating the growth of a stronger and healthier root system. With these capabilities, this fungus not only plays a role in protecting plants from pest attacks but also directly contributes to improving nutrient absorption efficiency by plants, ultimately positively impacting overall plant productivity (Krisdayani, *et al.,* 2020).

This study aims to determine the effect of adding botanical nutrients and *Trichoderma* sp. to cocoa seed soaking water on the quality of the seedlings produced. The addition of these two substances is expected to improve seedling quality from the early stages of growth. This is important because high-quality cocoa seedlings have the potential to produce plants that grow optimally and are resistant to environmental stress and pest attacks.

Thus, improving seedling quality will directly impact the productivity of cocoa plants in the future.

MATERIALS AND METHOD

Time and Place

This experiment was conducted at the Indonesian Coffee and Cocoa Research Institute (ICCRI), Nogosari Village, Rambipuji District, Jember Regency, East Java Province, Indonesia. The research was conducted in February 2024.

Materials and Equipment

The materials used in this study included cocoa seeds, water, *Trichoderma* sp. at a dilution of 10-1, several types of weeds, namely *Cleome rutidosperma*, *Rivina humilis* L., *Elephantopus scaber*, *Cyperus rotundus*, *Phyllanthus urinaria*, and cocoa shoots. The tools used in this study include a ruler, containers, sieves, funnels, measuring cups, scales, writing tools, and buckets.

Research Design

This study will test the botanical nutrient formulation of several types of weeds mixed with *Trichoderma* sp. in granular form with a density of 10-8 diluted with water at a dilution of 10-1 on cocoa seeds. This experiment uses a Completely Randomized Design (CRD) consisting of four treatments and three replications, with each replication containing three cocoa seeds. The treatments performed are: Formula A soaking using water. Formula B botanical nutrients with a 1:1 ratio, where the ratio of weed to cocoa shoots is equal at 125 g each weed 50 g. Formula C: botanical nutrients with a 1:2 ratio, where the weed quantity was 167 g each weed 33,4 g, which was more than the cocoa water sprout quantity of 83 g. Formula D: botanical nutrients with a 2:1 ratio, where the weed quantity was 83 g each 16,6 g, while the cocoa water sprout quantity was 167 g.

All of the ingredients are then weighed to a total weight of 250 grams, then mixed with five liters of clean water in a container. The extraction process is carried out manually by squeezing the ingredients for approximately 20 minutes until a brownish solution is produced or a homogeneous appearance is achieved, indicating that the active compounds from the plant have dissolved into the water. After that, the solution resulting from the squeezing is filtered to separate the pulp from the liquid. The filtered botanical nutrients solution can be used immediately, while the remainder can be stored in a closed container such as a plastic bottle to maintain the stability of the active compound content. The botanical nutrients solution is then used as a seed soaking medium for cocoa seeds and combined with *Trichoderma* sp. for 6 days.

Data collection was conducted by measuring the length of the radicle that emerged during cocoa seed soaking. Observations were made twice daily for one week. Data were analyzed using an ANOVA test, followed by a Duncan's Multiple Range Test (DMRT) at the 5% level to indicate differences between treatments. Additionally, observations were made on the number and density of *Trichoderma* sp. spores present in the solution at the beginning and end of the observation period. These observations were conducted by taking the solution used to soak the cocoa seeds, examining it under a light microscope, and counting the spores using a haemocytometer. According to Rohmah *et al*., (2021), the density of spores is calculated using the formula:

$$C = \frac{t \times d}{n \times 0.25} 10^6$$

Note:

C = spore density per ml of solution

T = total number of spores in the sample box observed

N = number of sample boxes (5 large boxes × 16 small boxes)

0,25 = correction factor for using small-scale sample boxes on a haemo-cytometer.

d = dilution factor if dilution is required (d = 1 means no dilution; d = 10 means diluted 1:10).

10^6 = standard spore density (Directorate of Plantation Protection, Ministry of Agriculture, in 2014).

Research Implementation

The research began by preparing cocoa seeds of uniform size and in healthy condition. The outer layer of the cocoa seed, known as the testa, was first cleaned. The cocoa seeds variety used in this study was Sulawesi 01. Next, a solution was prepared for soaking the prepared cocoa seeds. The solution consisted of three different formulas, each of which was mixed with *Trichoderma* sp. at a dilution ratio of 10:1. Each formula was labeled, and the final treatment involved soaking the seeds in water (control). The cocoa seeds were soaked in the solution, and observations were made of the length of the cocoa seeds radicle, the number of spores, and the density of *Trichoderma* sp. spores over a period of one week.

RESULTS AND DISCUSSION

Radicle Length

The treatment of seed soaking using botanical nutrients and *Trichoderma* sp. on the average length of seed radicles on days 2, 4, and 6 showed significant differences (Table 1). Treatment C differed significantly from treatments A, B, and D. Treatments A, B, and D showed no significant difference, indicating that the three treatments had relatively similar effects on cocoa seedling root growth. The results of this study indicate that treatment C has higher efficacy in stimulating initial root growth compared to other treatment formulations.

Radicle growth results on days 2, 4, and 6 after soaking using a mixture of botanical nutrients and *Trichoderma* sp. showed better results compared to seeds soaked using *Trichoderma* sp. solution alone. This indicates that the botanical nutrients elicitor can enhance radicle growth response in cocoa seeds. The treatment of the botanical nutrients elicitor formulation on the average radicle length at 2 days after soaking showed that treatment C was significantly different from treatments A, B, and D. At 4 and 6 days after soaking, treatment C was significantly different from treatments A, B, and D. Treatments A, B, and D showed no significant difference. The results above indicate that treatment C, which is botanical nutrients with a weed-to-water sprout ratio of 2:1, is more effective than botanical nutrients with other ratios.

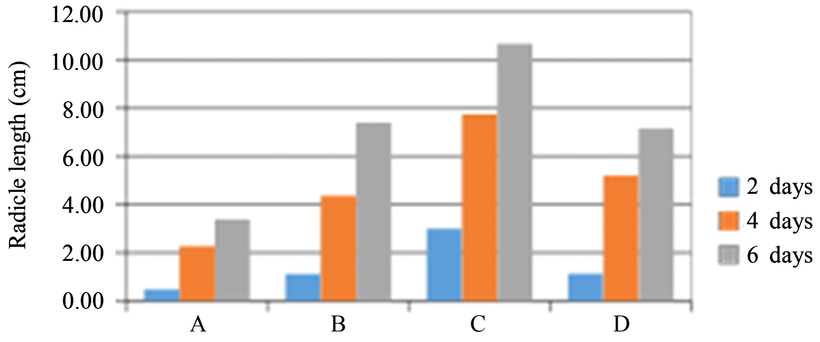
One common example of weeds used is *meniran* (*Phyllanthus urinaria*), which is known to contain active compounds such as flavonoids, tannins, saponins, alkaloids, and lignin. These compounds have important biological activities, particularly in acting as antimicrobial and antifungal agents. Thus, the presence of secondary metabolites from weeds such as meniran significantly contributes to the effectiveness of botanical nutrients in enhancing plant resistance to fungal attacks and supporting more optimal growth (Sophia, 2024).

Flavonoids are useful for protecting plants from pathogen attacks such as fungi and bacteria. Tannins, in addition to protecting against pathogens, also act as biological

Table 1. Average radicle length for several botanical nutrients formulas mixed with *Trichoderma* sp. on cocoa seeds.

Treatment	Average root length (cm)		
	2 days	4 days	6 days
A(control)	0.48 [a] ± 0.11	2.27 [a] ± 0.80	3.69 [a] ± 0.57
B (1:1)	1.12 [a] ± 0.05	4.36 [b] ± 0.32	7.15 [b] ± 0.73
C (1:2)	3.0 [b] ± 0.61	7.74 [c] ± 0.54	10.68 [c] ± 0.07
D (2:1)	1.13 [a] ± 0.30	5.20 [b] ± 0.69	7.38 [b] ± 0.18

Note: Numbers followed by the same lowercase letter in the same column are not significantly different according to the DMRT test at the 5% level.



A = control, B = ratio 1:1, C = ratio 1:2, D = 2:1

Figure 1. Graph showing the average length of radicles for several botanical nutrients formulas mixed with *Trichoderma* sp. on cocoa seeds

antioxidants. These tannins are found in the plant's surface walls, such as in shoots, root tissues, leaves, and stems (Hersila *et al.,* 2023). The compounds present in weeds used for botanical nutrients production help reduce fungal and bacterial growth during the soaking process, thereby optimizing radicle growth.

Cocoa water sprouts used for botanical nutrients production also contain secondary metabolites such as flavonoids, saponins, and tannins, which function as antibacterial agents. These compounds are also effective as antibacterial agents. The presence of these compounds in water sprouts plays a crucial role in suppressing the growth of pathogenic microorganisms that can disrupt plant growth, particularly during the early stages of seedling development (Mandhaki *et al*., 2021).

The weeds used in botanical nutrients production include all parts of the plant, whereas cocoa water sprouts consist only of young leaves and stems. Therefore, the compounds in weeds are more complex than those in cocoa water sprouts. This is why botanical nutrients made with a higher ratio of weeds compared to cocoa water sprouts is more effective for radicle growth during cocoa seed soaking. Additionally, according to Asri (2023), the flavonoid content in sprouts is higher. Therefore, if applied in excessive amounts, it can disrupt plant growth.

The addition of *Trichoderma* sp. to the botanical nutrients solution has a positive effect on root growth in plants, possibly due to an interaction with the botanical nutrients. This is particularly important during soaking to promote radicle growth in cocoa seeds. Plants with long and strong radicles are expected to develop into plants with good root systems. The better the root system, the wider the root spread. This results in more efficient and optimal uptake of nutrients and water by the plant. If nutrient and water requirements are met, optimal growth will occur, and the resulting cocoa seeds are expected to be of high quality and resistant to pest and disease attacks (Jufri, *et al* 2024). Additionally, *Trichoderma* sp. can stimulate gibberellin hormones, which help increase cell elongation and indirectly promote plant root growth (Nufuza *et al*., 2023).

Number of Spores and Spore Density of *Trichoderma* sp.

The results of this study indicate that the number of *Trichoderma* sp. spores decreased from the beginning to the end of the immersion period. The difference in spore density on the first day was due to the mixture with the botanical nutrients solution. Botanical nutrients with a 2:1 ratio reduced the number of spores more than other ratios. This occurred because water sprouts contain high levels of flavonoids, which are useful as antibacterial agents (Aslamiah *et al*., 2024).

Cocoa water shoots used for botanical nutrients production include the leaves. Cocoa plant leaves (*Theobroma cacao* L.) are known to contain various secondary metabolites with important biological activities, including caffeine, flavonoids, and alkaloids. Caffeine is an alkaloid compound known to inhibit cell wall synthesis in fungal organisms. This mechanism causes the cell wall structure to become imperfect or damaged, making fungal cells fragile, undergo lysis, and eventually die. This process is crucial because the cell wall is an essential component that maintains the stability and shape of fungal cells. Additionally, flavonoids present in cocoa leaves act as antimicrobial compounds by inhibiting the division or proliferation of fungal cells. This means that flavonoids can slow down or halt the fungal cell life cycle, thereby preventing the growth and spread of fungal colonies on a larger scale (Permataningrum *et al*., 2019).

Trichoderma sp. can produce enzymes, antibiotics, and toxic compounds that not only suppress but also eliminate pathogens. This *Trichoderma* produces secondary metabolites with antibacterial and antifungal properties, such as polyketides, pyrroles, and terpenes (Hasanah *et al*., 2023). However, the compounds present in cocoa water sprouts can disrupt or even kill fungi. Although *Trichoderma* sp. is a beneficial fungus for plant roots, flavonoids are non-selective, so both beneficial and harmful fungi can be affected if their concentration is high.

Botanical nutrients with a 2:1 ratio or with less weed material was found to reduce the number and density of *Trichoderma* sp.

Table 2. Number of *Trichoderma* sp. spores in cocoa seed soaking water

Treatment	Number of spores	
	1 days	6 days
A(control)	-	-
B (1:1)	210	108
C (1:2)	207	137
D (2:1)	153	86

Tabel 3. Spore density of *Trichoderma* sp. spores in cocoa seed soaking water

Treatment	Spore density *cels*/ml	
	1 days	6 days
A(control)	-	-
B (1:1)	10.5×10^7	5.4×10^7
C (1:2)	10.35×10^7	6.85×10^7
D (2:1)	7.65×10^7	4.3×10^7

spores for a longer period. This is likely due to the lower flavonoid content in weeds compared to cocoa water sprouts. The chemical composition of weeds is more diverse than that of cocoa water sprouts. Therefore, it is expected that *Trichoderma* sp. in the solution will continue to reproduce and not decrease in spore number and density.

CONCLUSION AND RECOMMENDATIONS

Soaking cocoa seeds using a mixture of *Trichoderma* sp. and botanical nutrients solution from several formulas can stimulate the growth of cocoa seeds radicles. The research results show that the application of a mixture of *Trichoderma* sp. and botanical nutrients with a weed : water sprout ratio of 2:1 produces a significantly different effect compared to the control and other botanical nutrients ratios. Therefore, the selection of botanical nutrients formulations to be used can be chosen from the three formulations that have been studied. If necessary, further research can be conducted to identify the most appropriate botanical nutrients formulation for use in cocoa seeds soaking.

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