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Population Test and Chemical Characteristics of The Rhizosphere Microbial Consortium Enriched with Palm Oil Olein Biosurfactant

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ABSTRACT

The optimization of soil microbes as biological agents in biofertilizers plays a crucial role in enhancing plant production. One of the primary functions of biofertilizers is biocontrol, which can be optimized by adding natural antibiotics, such as azadirachtin compounds from neem plants. Additionally, biofertilizers' effectiveness can be enhanced by improving their spreadability on plants and growing media. Palm olein-based diethanolamide (DEA) biosurfactant, a derivative of palm oil, has the potential to serve as a biofertilizer carrier while also enhancing its spreadability. This study aims to evaluate the viability of a bacterial consortium isolated from the rhizosphere at various propagation stages and after adding neem extract and DEA biosurfactant. It began with the rejuvenation of isolates from a previous study, followed by bacterial population testing at each propagation stage—200 mL, 1000 mL, and 5000 mL—to ensure optimal microbial populations before applying additional treatments. Additionally, chemical characterization tests were conducted to assess the quality of the resulting biofertilizer. The results showed that the total microbial population during isolation and propagation met the standard microbial population threshold, with an average colony count above 10^7 CFU. The combination of biofertilizer with neem extract resulted in a bacterial population of 7.495×10^9 CFU. However, chemical characterization tests indicated that the tested parameters did not meet the biofertilizer standards set by Indonesia's Ministry of Agriculture Regulation No. 261/KPTS/SR.310/M/4/2019.

Keywords: Biofertilizer, total bacterial population, rhizosphere, biosurfactant, neem

INTRODUCTION

The optimization of soil microbes as biological agents in biofertilizers plays a crucial role in enhancing plant production. Biofertilizers are biologically active products containing live microbes that improve fertilization efficiency, soil fertility, and health. According to Raimi *et al*. (2021), biofertilizers are inoculants with living organisms that fix and supply nutrients for plants. They promote plant growth and development by increasing nutrient availability and producing growth-promoting hormones. To achieve these benefits, applying biofertilizers to growing media enhances soil's physical, chemical, and biological properties. This creates a balanced ecosystem with an optimal bacterial population, supporting effective plant growth. Achieving this optimization requires understanding the conditions that foster the development of beneficial soil microorganisms, such as nitrogen-fixing and phosphate-solubilizing bacteria (Antralina, 2015).

Biofertilizers also help enhance nutrient absorption, suppress soil-borne diseases, accelerate composting, improve soil structure, and produce biologically active substances that support plant growth. Their application in growing media is expected to improve fertilization efficiency and reduce dependence on inorganic fertilizers. These benefits stem from the presence of functional microbial groups, which are involved in various biological processes, such as nitrogen fixation and phytohormone production (*Azotobacter* sp., *Azospirillum* sp.), phosphate solubilization (*Bacillus* sp., *Aspergillus* sp.), and biocontrol (*Trichoderma* sp.,

Streptomyces sp.). These microbes fix atmospheric nitrogen, produce growth hormones, solubilize unavailable phosphate in the soil, and generate metabolites that suppress pathogenic microorganisms. With these functional microbes, biofertilizer application is expected to optimize and reduce inorganic fertilizer use while promoting more sustainable crop production (Setiawati *et al*., 2016).

In addition to improving fertilization efficiency and nutrient availability, biofertilizers also have the potential to act as biocontrol agents by suppressing pathogenic microorganisms harmful to plants. This function can be optimized by adding natural antibiotic substances, one of which is azadirachtin, a compound derived from the neem plant. Fernandes *et al*. (2019) stated that among the various compounds isolated and characterized from the neem plant, azadirachtin (Aza) is identified as the main bioactive compound. The neem plant has long been recognized for its unique properties, both in controlling pathogenic insects and promoting human health (Nisbet, 2000). Therefore, in this study, neem leaf extract will be added to the composition of biofertilizers based on a soil microbial consortium to test its effectiveness as a biocontrol agent.

In addition to enhancing biocontrol functions, another approach to improve the quality and function of biofertilizers is by improving their spreading ability on the application medium, both on plant organs and directly on the growing medium. Therefore, additives used to improve the spreading ability of biofertilizers need to be carefully selected to avoid compromising the viability of bacteria in the biofertilizer suspension. One natural substance that can be used as a carrier for biofertilizers while enhancing their spreading ability is the biosurfactant Dietanolamida (DEA) from palm oil olein. Yusriah *et al*. (2017) explained that surfactants can function as additives to disperse and spread active ingredients and increase their adhesion to target plant organs. Research by Nurmayulis *et al*. (2023) showed that DEA biosurfactant from palm oil olein can improve the effectiveness of biofertilizers. Additionally, Nurmayulis *et al*. (2023) reported that treatment with biofertilizer supplemented with 20 ml/bibit of DEA biosurfactant from palm oil olein affected leaf count and total chlorophyll parameters, with an interaction between fertilization intervals that influenced plant height, leaf count, stem diameter, and dry weight of cacao seedlings.

In this study, rejuvenation of isolates from previous research was conducted, followed by bacterial population testing at each propagation stage of the soil microbial consortium with volumes of 200, 1000, and 5000 mL. This step is crucial to ensure that the population of the soil microbial consortium is at an optimal level before the addition of neem extract and DEA biosurfactant. Additionally, chemical characteristic testing was carried out to assess the quality of the biofertilizer from the rhizosphere-origin microbial consortium, which was then adjusted to meet the Minimum Technical Requirements (MTR) in accordance with Indonesian Ministry of Agriculture Regulation No. 261/KPTS/SR.310/M/4/2019.

MATERIALS AND METHODS

Materials

The equipment and materials used in this study were carefully selected to ensure accurate experimentation and analysis. The equipment included test tubes, Erlenmeyer flasks, Petri dishes, measuring cylinders, a stopwatch, test tube racks, incubation racks, micropipettes, graduated pipettes, an autoclave, a laminar airflow (LAF) cabinet, a magnetic stirrer, spatulas, an incubator, stirring spoons, a digital scale, a hand sprayer, markers, glass slides, cover slips, a Bunsen burner, an inoculation loop, a microscope, documentation tools, and stationery. Meanwhile, the materials used were 70% alcohol, sterile distilled water, Nutrient Agar (NA), Nutrient Broth (NB), plastic wrap, rose bengal, glycerol, $MgSO_4$, KH_2PO_4, a pH meter, crystal violet, Lugol's iodine, safranin, immersion oil, cotton, and matches.

A descriptive study was employed, where data was obtained through direct measurements. The collected data were analyzed and compared with the national biofertilizer standards outlined in Indonesia's Ministry of Agriculture Regulation No. 261/KPTS/SR.310/M/4/2019.

Rejuvenation of rhizosphere bacteria

The rejuvenation of bacterial isolates from the rhizosphere of plantation crops began with retrieving stock isolates from the Agroecology Laboratory, Faculty of Agriculture, Universitas Sultan Ageng Tirtayasa. The isolates were then inoculated onto petri dishes containing Nutrient Agar (NA) medium using the pour plate method. All procedures were conducted in a Laminar Air Flow (LAF) chamber to prevent contamination. After inoculation, the petri dishes were stored in sterile plastic to minimize contamination and placed upside down to prevent condensation. Bacterial growth was observed over 1 to 3 days at room temperature. The fastest-growing isolates were selected for further propagation in a liquid Nutrient Broth (NB) medium.

Bacterial isolate propagation

Bacterial isolates were propagated in Nutrient Broth (NB) medium with volumes of 200 mL, 1000 mL, and 5000 mL. The process lasted for 2 to 3 days for each volume, with bacterial suspensions incubated in a shaker incubator at room temperature (21–24°C). The initial propagation stage began with preparing the NB medium in Erlenmeyer flasks according to the required volume. For the 200 mL volume, two 100 mL Erlenmeyer flasks were used; for the 1000 mL volume, two 500 mL Erlenmeyer flasks were used; and for the 5000 mL volume, five 1000 mL Erlenmeyer flasks were used. The propagation was carried out by transferring bacterial colonies from the rejuvenation medium using an inoculation needle and placing them into the Erlenmeyer flasks containing the NB medium. The bacterial suspensions were then incubated in the shaker incubator for 48 hours at each propagation stage to ensure homogeneity and bacterial growth. The success of bacterial propagation was indicated by the appearance of turbidity and a fermentation odor in the NB medium. To confirm the viability of the bacteria at each propagation stage, bacterial population tests were conducted regularly.

Chemical property testing of biofertilizer suspension

The chemical characteristics of the biofertilizer were analyzed at the Indonesian Center for Biodiversity and Biotechnology (ICBB) Laboratory in Bogor, Indonesia. The analyzed chemical characteristics included Organic Carbon (C), Total Nitrogen (N), Total P_2O_5, Total K_2O, and pH. These chemical parameters serve as the primary indicators of the quality of liquid biofertilizers, determining their suitability for widespread use in agriculture. Additionally, supplementary testing was conducted to assess the role of bacteria in the biofertilizer, ensuring the presence of nitrogen-fixing and phosphate-solubilizing bacteria in the liquid biofertilizer suspension that had been propagated.

RESULTS AND DISCUSSION

The results of bacterial isolate rejuvenation and colony count calculations at each propagation stage are crucial in ensuring the success of increasing the microbial population used in biofertilizer. The isolation process in this study began with the rejuvenation of isolates on a Nutrient Agar (NA) medium to ensure the bacteria were in optimal condition before propagation. Colony counting is one method to determine the number of bacterial colonies present in a medium (Rosmania and Yanti, 2020). Total Plate Count (TPC) is a commonly used method for analyzing microbial population numbers. The number of microbes can be directly counted after they grow and proliferate, forming colonies. The isolates used in this study were the result of isolation and characterization conducted by Nurmayulis *et al*. (2023), which

identified three bacteria from the same genus and two different species, namely *Bacillus amyloliquefaciens* and *Bacillus subtilis*. Subsequently, total colony counting was performed at each propagation stage. The results of bacterial isolate rejuvenation are presented in **Table 1**.

Table 1. Total colony count of rejuvenated bacterial isolates

Bacterial isolate	Dilution					CFU
	-2	-4	-5	-6	-7	
Bacillus amyloliquefaclens	1	TNTC	TNTC	148	18	1.66×10^9
Bacillus subtilis 1	TNTC	TNTC	110	27	7	2.25×10^8
Bacillus subtilis 2	0	149	TNTC	TNTC	0	1.49×10^7

Calculation of Total Colony of Rejuvenated Bacterial Isolates

Bacillus amyloliquefaclens

$$cfu = \frac{colony\ count}{inoculant} = \frac{166}{0.1(10^{-6})} = 1.66 \times 10^9$$

Bacillus subtilis 1

$$cfu = \frac{colony\ count}{inoculant} = \frac{110}{0.1(10^{-5})} = 1.1 \times 10^8$$

Bacillus subtilis 2

$$cfu = \frac{colony\ count}{inoculant} = \frac{149}{0.1(10^{-4})} = 1.49 \times 10^7$$

$$cfu = \frac{34}{0.1(10^{-6})} = 3.4 \times 10^9$$

*TNTC = exceeds the colony count limit (30-300 colonies per petri dish medium)

The results of the bacterial population count showed that the highest colony population was found in *Bacillus subtilis* 2 at a 10^{-4} dilution, with a total of 149 colonies, while the lowest colony count was found in *Bacillus subtilis* 1 at a 10^{-7} dilution, yielding only 7 colonies. These results indicate that dilutions of 10^{-4}, 10^{-5}, and 10^{-6} produce colony counts within the ideal range for bacterial population calculation. According to Suharman *et al*. (2023), colony counts greater than 300 (>300 colonies) on agar media are not considered valid for counting, as this increases the possibility of counting errors. Conversely, fewer colonies (<30 colonies) are also considered invalid for statistical counting due to the lack of precision in the count. Too few colonies may lead to inaccurate results, while too many colonies may result in competition that disrupts colony development. Postgate (1969) stated that an organism can proliferate effectively when in optimal conditions specific to the species and bacterial strain. Based on the average CFU from the rejuvenation phase, the highest colony count was found in *Bacillus amyloliquefaciens*, with a total of 1.66 x 10^9, while the lowest colony count was found in *Bacillus subtilis* 2.

The next step involved propagation with a volume of 200 mL, starting from a dilution of 10^{-2} to 10^{-7} to ensure the counting results met the standards. This propagation process aimed to ensure that the bacterial isolates could grow well to reach a large population and that the consistency of bacterial growth was maintained at each propagation stage. In general, dilutions are performed between 10^{-1} to 10^{-5} or more (Hastuti, 2018). At a 10^{-4} dilution, the *Bacillus amyloliquefaciens* and *Bacillus subtilis* isolates showed results with too many colonies to be counted (TNTC). The complete results regarding the bacterial population in the 200 mL propagation can be seen in **Table 2**.

Table 2. Total colony count of bacterial isolates from 200 mL propagation

Bacterial isolate	Dilution					CFU
	-2	-4	-5	-6	-7	
Bacillus amyloliquefaclens	0	TNTC	18	10	68	9.6×10^7
Bacillus subtilis 1	0	TNTC	284	136	14	8.92×10^8
Bacillus subtilis 2	0	24	90	30	160	5.44×10^9

Calculation of Total Colony of Bacterial Isolates from the 200 mL Propagation

Bacillus amyloliquefaclens

$$CFU = \frac{colony\ count}{inoculant} = \frac{96}{0.1(10^{-5})} = 9.6 \times 10^7$$

Bacillus subtilis

$$CFU = \frac{colony\ count}{inoculant} = \frac{284}{0.1(10^{-5})} = 2.84 \times 10^8$$

$$CFU = \frac{150}{0.1(10^{-6})} = 1.5 \times 10^8$$

$$CFU = \frac{(2.84\times10^8)+(1.5\times10^8)}{2} = 8.92 \times 10^8$$

Bacillus subtilis

$$CFU = \frac{colony\ count}{inoculant} = \frac{144}{0.1(10^{-4})} = 1.44 \times 10^7$$

$$CFU = \frac{30}{0.1(10^{-6})} = 30 \times 10^7$$

$$CFU = \frac{160}{0.1(10^{-7})} = 1600 \times 10^7$$

$$CFU = \frac{(1.44\times10^7)+(30\times10^7)+(1600\times10^7)}{2} = 5.44 \times 10^9$$

*TNTC = exceeds the colony count limit (30-300 colonies)

Based on **Table 2**, after calculating the CFU, the average CFU values for each bacterial isolate were found to be 9.6×10^7 for *Bacillus amyloliquefaciens*, 8.92×10^8 for *Bacillus subtilis* 1, and 5.44×10^9 for *Bacillus subtilis* 2. These three isolates met the standards for the total microbial population. According to the Indonesian Ministry of Agriculture Standard No. 261 of 2019, the population of functional bacteria must not be less than 10^7. Further propagation was carried out with a 1000 mL volume. **Table 3** shows the results of the total colony count of bacterial isolates during the 1000 mL propagation, which indicates that the total colony count exceeded the counting limit or was too numerous to count (TNTC) at dilutions 10^{-2}, 10^{-6}, and 10^{-5}. The detailed results of the bacterial population calculation for the 1000 mL propagation are presented in **Table 3**.

Table 3 shows the CFU calculation results, where the average CFU value for the *Bacillus amyloliquefaciens* isolate is 2.205×10^9, the highest colony count compared to *Bacillus subtilis* 1, which has an average CFU value of 8.5×10^7, and *Bacillus subtilis* 2, with 3.52×10^7. The propagation up to a 1000 mL volume indicates good bacterial viability, with CFU counts exceeding 10^7, in accordance with the established standards. Based on these results, the study proceeded to the next propagation stage.

In the 5000 mL propagation, the total bacterial colony count obtained exceeded the counting limit or TNTC, with a higher number compared to the previous propagations. This could be due to several factors, including the antagonistic properties between species. The more colonies that grow on the surface of the medium, the greater the likelihood of interactions between colonies, either through inhibition or stimulation of neighboring colony growth. Additionally, there is competition for nutrient absorption, and two colonies may merge into one larger colony (Pradhika, 2018).

Table 3. Total colony count of bacterial isolates from 1000 mL propagation

Bacterial isolate	Dilution					CFU
	-2	-4	-5	-6	-7	
***Bacillus amyloliquefaclens*ov**	TNTC	0	31	5	36	2.205 x 10^9
Bacillus subtilis 1	50	TNTC	164	TNTC	6	8.5 x 10^7
Bacillus subtilis 2	TNTC	94	53	5	3	3.52 x 10^7

Calculation of Total Colony Count of Bacterial Isolates from the 1000 mL Propagation

Bacillus amyloliquefaclens

$$CFU = \frac{colony\ count}{inoculant}$$
$$= \frac{31}{0.1(10^{-5})}$$
$$= 3.1 \times 10^7$$
$$CFU = \frac{41}{0.1(10^{-6})}$$
$$= 41 \times 10^7$$
$$CFU = \frac{(3.1\times10^7)+(41\times10^7)}{2}$$
$$= 2.205 \times 10^8$$

Bacillus subtilis 1

$$CFU = \frac{colony\ count}{inoculant}$$
$$= \frac{50}{0.1(10^{-2})}$$
$$= 0.05 \times 10^6$$
$$CFU = \frac{170}{0.1(10^{-5})}$$
$$= 170 \times 10^6$$
$$CFU = \frac{(0.05\times10^6)+(170\times10^6)}{2}$$
$$= 8.5 \times 10^7$$

Bacillus subtilis 2

$$CFU = \frac{colony\ count}{inoculant}$$
$$= \frac{94}{0.1(10^{-4})}$$
$$= 9.4 \times 10^6$$
$$CFU = \frac{61}{0.1(10^{-5})}$$
$$= 61 \times 10^6$$
$$CFU = \frac{(9.4\times10^6)+(61\times10^6)}{2}$$
$$= 3.52 \times 10^7$$

*TNTC = exceeds the colony count limit (30-300)

Table 4. Total colony count of bacterial isolates from 5000 mL propagation

Bacterial isolate	Dilution					CFU
	-2	**-4**	**-5**	**-6**	**-7**	
Bacillus amyloliquefaclens	TNTC	86	TNTC	183	24	1.04 x 10^9
Bacillus subtilis 1	TNTC	TNTC	40	TNTC	230	1.152 x 10^{10}
Bacillus subtilis 2	TNTC	197	TNTC	1	136	6.95 x 10^8

Calculation of Total Colony Count of Bacterial Isolates from the 5000 mL Propagation

Bacillus amyloliquefaclens

$$CFU = \frac{colony\ count}{inoculant}$$
$$= \frac{86}{0.1(10^{-4})}$$
$$= 0.86 \times 10^7$$
$$CFU = \frac{207}{0.1(10^{-6})}$$
$$= 207 \times 10^7$$
$$CFU = \frac{(0.86\times10^7)+(207\times10^7)}{2}$$
$$= 1.04 \times 10^9$$

Bacillus subtilis 1

$$CFU = \frac{colony\ count}{inoculant}$$
$$= \frac{40}{0.1(10^{-5})}$$
$$= 0.4 \times 10^8$$
$$CFU = \frac{230}{0.1(10^{-7})}$$
$$= 230 \times 10^8$$
$$CFU = \frac{(0.4\times10^8)+(230\times10^8)}{2}$$
$$= 1.152 \times 10^{10}$$

Bacillus subtilis 2

$$CFU = \frac{colony\ count}{inoculant}$$
$$= \frac{197}{0.1(10^{-4})}$$
$$= 1.97 \times 10^7$$
$$CFU = \frac{137}{0.1(10^{-6})}$$
$$= 137 \times 10^7$$
$$CFU = \frac{(1.97\times10^7)+(137\times10^7)}{2}$$
$$= 6.95 \times 10^8$$

*TNTC = exceeds the colony count limit (30-300)

Based on **Table 4**, TNTC values were found in the 10^{-2} and 10^{-5} dilutions for *Bacillus amyloliquefaciens*, the 10^{-2}, 10^{-4}, and 10^{-6} dilutions for *Bacillus subtilis* 1, and the 10^{-2} and 10^{-5} dilutions for *Bacillus subtilis* 2. The highest average CFU value recorded was 1.152 x 10^{10}, while the lowest was 6.95 x 10^8. The results from the 5000 mL propagation indicate that the bacterial consortium's viability is quite good and capable of growing together compatibly. This provides evidence that this biological fertilizer consortium is ready for further enhancement with the addition of DEA biosurfactant and neem extract.

Table 5 provides the calculation of total colonies of bacterial isolates in several combinations of liquid biological fertilizer consortium compositions as follows: First, the biological fertilizer consortium + DEA biosurfactant showed a decrease in total bacteria at the 10^{-5} dilution, but an increase at the 10^{-6} and 10^{-7} dilutions. Second, the biological fertilizer

consortium + neem leaf extract showed total bacteria exceeding the colony counting limit (>300 colonies) at the 10^{-4} and 10^{-5} dilutions, but a decrease in total bacteria at the 10^{-7} dilution. Third, the biological fertilizer consortium + DEA + neem showed that the colony count could be determined starting from the 10^{-5} dilution up to the 10^{-7} dilution. Complete results regarding the microbial population in several compositions of the consortium biological fertilizer can be seen in **Table 5**.

Table 5. Total colony count of bacterial isolates in various biological fertilizer combinations

Isolate with Media	Dilution					*CFU*
	-2	-4	-5	-6	-7	
Biofertilizer + DEA	54	283	75	112	117	2.58×10^9
Biofertilizer + Neem	0	TNTC	TNTC	229	127	7.495×10^9
Biofertilizer + DEA + Neem	TNTC	TNTC	98	110	38	1.66×10^9

*TNTC = exceeds the colony count limit (30-300)

Based on the calculation of the bacterial isolate colony count, the average CFU values for each composition were obtained. The first composition had 2.58×10^9, while the second and the third composition had 7.495×10^9 and 1.66×10^9, respectively. As shown in **Table 5**, the highest average CFU value was found in the second biofertilizer composition, which contains the neem extract. This indicates that the addition of neem extract, which contains Azadirachtin, does not negatively affect the bacterial viability in the biofertilizer consortium. With these results, it is highly likely that the biofertilizer from these three isolates can be enhanced in its potential as a biopesticide.

Chemical characteristics of rhizosphere microbial consortium

Biofertilizers are biological products that contain microbes identified at least to the genus level and function to facilitate the supply of nutrients both directly and indirectly. This fertilizer also plays a role in decomposing organic matter, improving fertilization efficiency, and enhancing soil fertility and health. The quality of the fertilizer refers to the legal guarantee of the availability of nutrients, expressed as weight percentage in the fertilizer. The quality standards for biofertilizers in Indonesia are regulated in the Minister of Agriculture Regulation No. 261 of 2019, which includes minimum technical requirements, such as definitions and quality standards (Indonesia's Minister of Agriculture, 2019). The results of the chemical characteristic tests for the biofertilizer are presented in **Table 6**.

Based on the results of the chemical characteristic tests, it was found that all parameters did not meet the standards for liquid organic fertilizer, with organic carbon content of 0.58%, total nitrogen of 0.06%, P_2O_5 of 0.03%, and K_2O of 0.17%. Meanwhile, the pH test results showed a value of 4.2, indicating that the pH was slightly acidic. These chemical characteristics were influenced by the incubation time of the biofertilizer, which was not optimal, affecting the population and growth phase of the microbes. This is because bacteria would continue to multiply if incubation was carried out for a sufficient amount of time. According to Wahyudi (2018), the longer the incubation time, the more microbial cells would increase. The incubation period in a specific substrate allowed microbes to utilize nutrients well until they reached the optimal logarithmic phase, leading to an increase in microbial cell numbers and the chemical quality of the biofertilizer. Ahmad *et al*. (2015) stated that the longer the incubation time, the pH tended to decrease because bacteria released organic acids during their activity. This pH decrease was directly related to the increase in organic acids, while optimal growth of phosphate-solubilizing bacteria occurred at neutral pH and increased as the pH increased.

Biofertilizers, also known as biological fertilizers, essentially do not contain direct nutrients, including N, P, and K. Instead, these fertilizers are rich in various microorganisms that serve important functions, such as nitrogen (N) fixation from the air, solubilizing nutrients (especially P and K), and stimulating plant growth. Some N-fixing microbes form symbiotic relationships with plants, while others are free-living. On the other hand, P-solubilizing microbes (P) play a role in providing phosphorus that is readily absorbed by plants. According to Asrul (2019), biofertilizers utilize the ability of microorganisms, such as bacteria or fungi, to meet the macro-nutrient requirements of plants. Biofertilizers can be in the form of microbial consortia with various capabilities, such as nitrogen-fixing bacteria and P and K solubilizers, or may contain only one type of microbe with a specific ability.

Table 6. Results of the chemical characteristics of biofertilizer

No.	Parameter	Method	Unit	Liquid Organic Fertilizer
1	**C-Organic**	Spectrophotometry	%	0.58
2	**Total N**	Kjeldahl	%	0.06
3	**Total P_2O_5**	$HClO_4$ HNO_3 - Spectrophotometry	%	0.03
4	**Total K_2O**	$HClO_4$ HNO_3 - AAS	%	0.17
5	**pH**	Potentiometry	-	4.2
6	**Functional Testing:**			
	N-Fixing Microbe	Growth on N-free media or the formation of a ring on N-free semi-solid media	-	Positive
	P-Solubilizing Microbe	Clear zone on Pikovskaya media	-	Positive

CONCLUSION

In conclusion, the study found that the total microbial population in both the isolates and serial propagation successfully met the standard for Total Microbial Population, with an average CFU exceeding 10^7. Notably, when neem extract was added to the biofertilizer consortium, the bacterial population reached 7.495 x 10^9, indicating a significant growth boost. However, the results of the chemical characteristic tests on the biofertilizer revealed that none of the parameters met the standards outlined in Indonesia's Ministry of Agriculture Regulation No. 261/KPTS/SR. 310/M/4/2019. This suggests that, while the microbial population met expectations, the chemical composition of the biofertilizer still requires further optimization to align with regulatory standards.

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