International Journal of Multidisciplinary Research and Growth Evaluation.

Growth Response of Java Long Pepper Seedlings to the Application of Jakaba, Ferinsa, and Rice Washing Water

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Article Info

ISSN (online): 2582-7138
Volume: 06
Issue: 04
July - August 2025
Received: 09-05-2025
Accepted: 10-06-2025
Published: 08-07-2025
Page No: 677-685

Abstract
Java long pepper (*Piper retrofractum* Vahl) is classified as a medicinal and spice plant. It can be propagated through seeds, climbing branch cuttings, ground branch cuttings, and fruit branch cuttings. The success rate of propagation using climbing branch cuttings is around 75%, ground branch cuttings 66%, and fruit branch cuttings 38%. The use of growth stimulants and fertilizers also plays an important role in the propagation process. This study aims to analyze the growth response of Java long pepper seedlings using biological resources such as rice washing water, Jakaba, and fermented cow urine. The study employed a Completely Randomized Design with DMRT for data analysis. The results showed that the application of these biological resources had a significant effect on the growth response of Java long pepper seedlings in all treatments, influencing parameters such as plant height, number of leaves, number of nodes, internode length, and root length, with the use of auxin hormone, Jakaba, fermented cow urine, and rice washing water.

Keywords: Java Long Pepper, Jakaba, Fermented Cow Urine, Rice Washing Water

Introduction

Java long pepper (*Piper retrofractum* Vahl) is classified as a medicinal and spice plant. The main production areas for Java long pepper in Indonesia are East Java and Lampung provinces. In 2010, the cultivation area for Java long pepper was recorded at 4,211 hectares, distributed across several regions, including Sumenep Regency on Madura Island with 1,709 ha, Sampang Regency with 1,017 ha, Pamekasan Regency with 715 ha, Bangkalan Regency with 356 ha, Lamongan Regency with 276 ha, and Lampung Province with 630 ha (Evizal, 2013) [12].

In Sumenep Regency, the largest cultivation area for Java long pepper is found in Bluto District, with a total area of 687.83 hectares, accounting for approximately 26.48% of the total Java long pepper cultivation area in Sumenep Regency, which spans 2,617.95 hectares (BPS Kabupaten Sumenep, 2023) [9]. Java long pepper is commonly cultivated as an intercrop in community forests using various climbing trees such as coconut, fruit trees, coffee, and sengon. It is also cultivated intensively in open monoculture systems using Gliricidia stakes or Erythrina trees as supports, which also serve as protective shade for the Java long pepper plants (Djauhariya & Rosman, 2009) [11].

In Bluto District, the majority of Java long pepper growers use moringa trees as climbing supports, which are propagated vegetatively through cuttings (Hasanah & Setiawan, 2016) [13]. Java long pepper can be propagated using seeds, climbing branch cuttings, ground branch cuttings, and fruit branch cuttings (Evizal, 2013) [12]. To achieve high production levels, planting materials are selected from mother plants that are considered superior, healthy, and highly productive. The success of Java long pepper cuttings is supported by key growth and development factors, including water availability and sunlight (Nurkhasanah *et al*., 2013) [19]. The success rate of Java long pepper propagation through climbing branch cuttings is approximately 75%, ground branch cuttings 66%, and fruit branch cuttings 38% (Djauhariya *et al*., 1992) [10]. The use of growth stimulants and fertilizers also plays an important role in the propagation process. According to Febriyani (2012) in (Rahmawati *et al*., 2023) [25], one way to increase crop production is by improving soil fertility through fertilization, aiming to maintain, improve, and sustain soil fertility by providing nutrients that ensure the availability of essential elements in the soil.

One potential source of nutrients that can be utilized is rice washing water, which is often considered household waste (Azhari *et al*., 2021) [8]. Rice washing water contains 0.015% nitrogen, 16.306% phosphorus, 0.02% potassium, 2.944% calcium, 14.252% magnesium, 0.027% sulfur, 0.0427% iron, and 0.043% vitamin B1. According to Risman (2022) [26] in (Apriyanto *et al*., 2023) [6], rice washing water contains bacteria such as Pseudomonas fluorescens, pectolytic pectin, and Xanthomonas maltophilia, which play roles in synthesizing carbohydrates and amino acids to produce growth hormones and synthesize metabolites that inhibit pathogen development.

Fermenting rice washing water over a certain period can produce a jelly-like brown fungus resembling coral reefs, known as the "eternal lucky mushroom" (Jakaba). The nutrient content found in rice washing water can also be found in the Jakaba soaking water (Ani *et al*., 2023). Considering the nutrient content and benefits of rice washing water and Jakaba derived from fermented rice washing water, as well as the potential of Java long pepper cultivation in Sumenep Regency, it is necessary to conduct research to evaluate the growth performance of Java long pepper seedlings using biological resources such as rice washing water and Jakaba in Bluto District, Sumenep Regency.

Methods

Location and Time of Research

This research will be conducted in Bluto District, Sumenep Regency. The location was selected purposively because Bluto District has the largest area of Java long pepper cultivation in Sumenep Regency. According to data from the (BPS Kabupaten Sumenep, 2023) [9], the Java long pepper cultivation area in Bluto District is 687.83 hectares, accounting for approximately 26% of the total Java long pepper cultivation area in Sumenep Regency.

The research will be conducted from January to April 2025, with the detailed planned schedule of research activities as follows:

Table 1: Time for Conducting Research

No	Activity	Month			
		January	February	March	April
1	**Preparation**				
	a. Seedling nursery preparation	x			
	b. Installation of shading structures	x			
	c. Preparation of planting media	x			
	d. Preparation of materials	x			
2	Planting of cuttings	x			
3	Maintenance, observation, and data collection	x	x	x	
4	Data processing and analysis				**x**

Materials and Equipments

1. Materials

The materials used in this research include Java long pepper cuttings from the lower part of the vine for propagation, a seedling medium consisting of a mixture of soil and bokashi (manure) in a 3:1 ratio, Jakaba rice washing water, plant growth regulators (PGR), fermented cow urine, water, fungicides, and insecticides.

2. Equipments

The equipemts used in this research include 20 x 25 cm polybags, knives, pruning shears, wool thread, bamboo stakes, shading nets (paranet), hoes, trowels, and watering cans.

Research Method

This research was conducted using a Randomized Block Design (RBD). The experimental design consisted of six treatments as follows:

P0: No application (control)
P1: Application using rice washing water stored for 24 hours
P2: Application using rice washing water stored for 7 days
P3: Application using Jakaba
P4: Application using plant growth regulator (PGR)
P5: Application using fermented cow urine

The application volumes for treatments P1, P2, and P3 were 500 ml each, as (Sinaga *et al*., 2024) [27] reported that the application of 500 ml of Jakaba provides the best dosage for plant growth. For treatment P4, auxin hormone was applied at a concentration of 1 ml/l, based on (Apriyatna *et al*., 2024) [7], who stated that the application of auxin hormone at 1 ml/l is the optimal dosage for chili cutting propagation. For treatment P5, fermented cow urine was applied at a dosage of 20 ml/l, following (Manurung *et al*., 2022) [17], who found that the application of 20 ml of fermented cow urine effectively influences plant height, stem circumference, and the number of leaves in oil palm seedlings. These treatments were applied once every seven days in the morning. Each treatment was replicated five times, resulting in 30 experimental units. Each unit contained five Java long pepper seedlings, resulting in a total of 150 Java long pepper cuttings used for propagation in this study.

The schematic layout of the experimental design used in this study is presented in Figure 1 as follows.

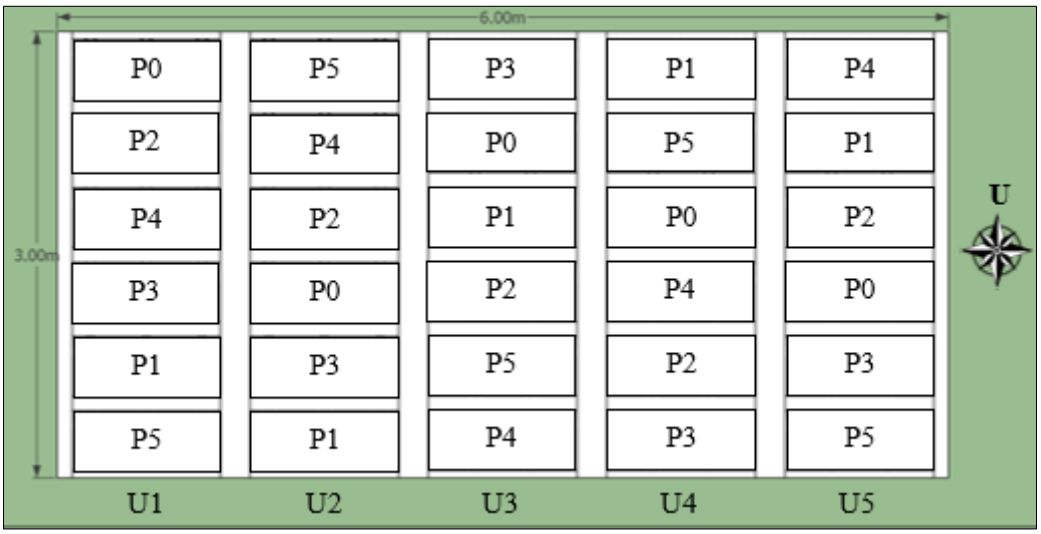

Fig 1: Experimental Plot Layout

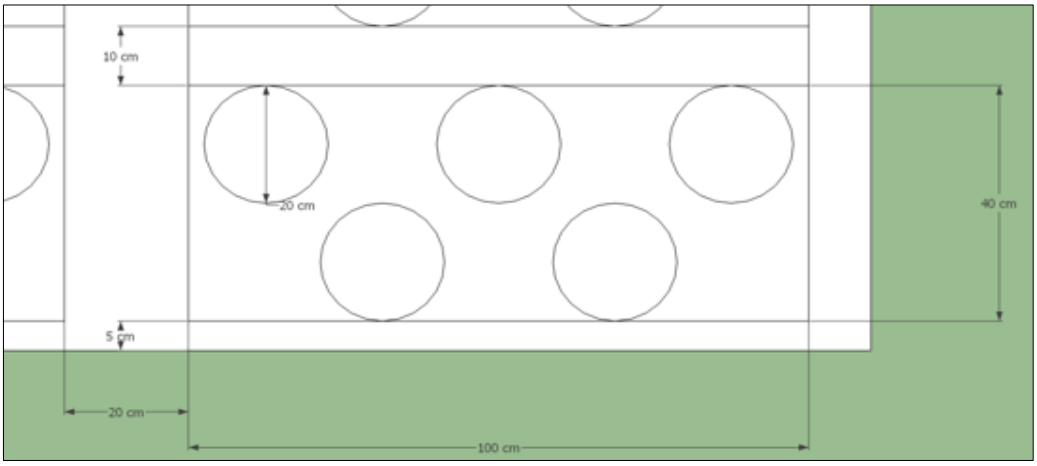

Fig 2: Plant Placement Plan

Research Implementation Stages

The stages in the implementation of this research aim to present the processes and steps carried out during the study. The stages are as follows:

1. Preparation of the Seedling Nursery

The seedling nursery in this study was established at the Greenhouse of the Agricultural Extension Center (BPP) in Bluto District, with a size of 4 meters x 3 meters.

2. Preparation of Planting Media

The planting medium used for the propagation of Java long pepper seedlings was a mixture of soil and bokashi (manure) at a ratio of 3:1. The soil and bokashi were cleaned from debris such as gravel and other impurities before use. They were then thoroughly mixed, and 25 x 25 cm polybags were prepared as containers for the seedling media. Each polybag was filled with the prepared medium up to two-thirds of its volume.

3. Preparation of Propagation Materials

The propagation material used was cuttings from Java long pepper plants. The cuttings were taken from healthy mother plants approximately two years old or with a stem diameter of 0.5–1 cm and containing five nodes. The cuttings used in this study were sourced from ground runners (lower vines) of Java long pepper plants. The use of ground runners is due to their generally higher growth rates and better survival percentages compared to upper vines. (Nurhuda *et al*., 2017) reported that cuttings from ground runners exhibited better growth than those from climbing vines. Additionally, the use of ground runners in propagation resulted in superior shoot diameter, number of nodes, number of shoots, and root length compared to climbing vines and was productive (Prameswari *et al*., 2021).

4. Planting of Cuttings

Stakes were prepared and inserted into the polybags containing the planting media, ensuring the stakes stood firmly and did not wobble during the research period. Water was applied to the planting media before planting to maintain moisture at the time of planting the cuttings. The cuttings were then planted in the moistened media, ensuring that the nodes on the cuttings were properly embedded in the planting medium. The cuttings were tied to the stakes using raffia string to prevent them from falling over.

5. Maintenance

Plant maintenance is a form of care to ensure the cultivated plants can grow properly. The maintenance activities for Java long pepper seedlings in this study included the following:

a. Watering
Watering was conducted once a day in the morning at 06:30 AM using well water, applying water evenly until the surface was saturated. Watering that included the specific treatments was carried out once every seven days throughout the research period.

b. Weeding
Weeding was conducted twice a week or whenever weeds were observed growing around the plants. Weeding was performed manually (mechanically) by hand. Care was taken during the weeding process to avoid damaging the plant root systems.

c. Pest and Disease Control
Pest and disease control was conducted mechanically by removing parts of the plants showing signs of pest or disease attack. If severe infestations occurred, control was carried out using insecticides and fungicides.

Research Parameters
The parameters observed in this study included non-destructive observations, destructive observations, the observation of cutting success rates, and plant growth analysis.

1. Non-Destructive Observations
Non-destructive observations were conducted when the seedlings were 7 days after planting (DAP) at 7-day intervals, with three plant samples observed for each treatment. The variables measured in these non-destructive observations were as follows:

a. Plant Height (cm) per plant, measured from the base of the plant at the soil surface to the highest point of each plant sample.
b. Number of Leaves (leaves) per plant, determined by counting the fully opened leaves on each plant sample.
c. Number of Nodes (units) per plant, counted from the base of the plant at the soil surface to the top of the plant.

2. Destructive Observations
Destructive observations were conducted at 30, 50, 70, and 90 days after planting (DAP). Three plant samples per treatment were observed. The variables measured in these destructive observations were as follows:

a. Internode Length (cm), measured by recording the length of each internode on each plant sample and then calculating the average.
b. Primary Root Length (units), determined by counting the number of main roots directly connected to the stem cuttings planted in the soil.

Data Analysis
The analysis design in this study used the Analysis of Variance (ANOVA) method, with data processing performed using SPSS version 16 software. The results of this analysis were then subjected to the Duncan's Multiple Range Test (DMRT).

The linear model in the randomized block design used in this study is as follows:

$$Yij = \alpha ij + \beta j + Pi + eij$$

Where:
Yij = Observation on the i^{th} treatment in the j^{th} replication
Aij = Population mean
Bj = Effect of the j^{th} replication/block
Pi = Effect of the i^{th} treatment
Eij = Error effect of the i^{th} treatment and the j^{th} replication/block

Result and Discussion
Growth Response of Java Long Pepper Seedlings
The growth response of a plant is influenced by several factors, such as the application of fertilizers and the availability of nutrients in the soil. In addition, seedling growth is not only affected by nutrient availability but also by environmental factors such as CO_2 and H_2O concentrations, which are essential components in the photosynthesis process (Pramuji & Fathurrahman, 2023) [23]. In this study, several treatments were applied to optimize the growth of Java long pepper seedlings, including the use of rice washing water and its derivatives, root growth hormones, and fermented cow urine. The following are the analysis results of the growth response of Java long pepper seedlings under different treatments.

1. Plant Height
Plant height is one of the important indicators used to observe the plant's response to the treatments applied. The measurement of plant height was conducted from the soil surface to the tip of the plant. Measurements were taken at 7, 21, 35, 49, 63, 77, and 91 days after planting (DAP). The following data are the results of the DMRT post-hoc test on the plant height of seedlings under the treatments:
P0: No Application (Control)
P1: Rice Washing Water (24 hours)
P2: Rice Washing Water (7 days)
P3: Jakaba Application
P4: Plant Growth Regulator
P5: Fermented Cow Urine

Table 2. Results of DMRT Analysis on Plant Height of Java Long Pepper Seedlings

Treatment	Plant Height (cm) at Observation Time (DAP)							
	7	21	35	49	63	77	91	Average
P0	24,0	29,3 a	31,5 a	48,4 a	55,4 a	81,8 a	88,8 a	51,3 a
P1	24,0	29,3 a	33,5 ab	50,2 a	57,2 a	89,9 ab	96,9 ab	54,5 a
P2	25,5	29,3 a	34,5 b	52,9 ab	60,0 ab	90,8 ab	97,8 ab	55,8 a
P3	26,1	30,7 b	42,2 c	62,4 cd	69,4 cd	106,3 cd	113,3 cd	64,4 bc
P4	26,2	32,0 c	45,2 d	67,7 d	74,7 d	114,4 d	121,4 d	68,8 c
P5	26,2	30,4 b	41,2 c	58,2 bc	65,2 bc	99,3 bc	106,3 bc	60,9 b
DMRT 5%	ns	*	*	*	*	*	*	*
Note:	Numbers followed by different letters in the same column indicate significant differences between treatments based on the DMRT at the 5% significance level, where * = significantly different, and ns = not significantly							

Table 1 shows the plant height (in cm) at various observation periods (7 to 91 DAP) for each treatment (P0 to P5) and the mean plant height during the seedling stage of Javanese long pepper. Different letters indicate significant differences according to the DMRT at the 5% significance level. At 7 DAP, no significant differences were observed among treatments, indicating that each treatment did not significantly affect plant height at this early stage. However, from 21 DAP to 91 DAP, significant differences were found among the treatments applied. The best treatment was the application of Plant Growth Regulator (P4) using auxin hormone, which resulted in the highest mean plant height of 68.8 cm. This was followed by the Jakaba treatment (P3) with a mean plant height of 64.4 cm, and the fermented cow urine treatment (P5) ranked third with a mean plant height of 60.9 cm. In contrast, the control treatment without any application (P0) resulted in the lowest mean plant height of 51.3 cm during the seedling period of Javanese long pepper.2**.**

Number of Leaves

The number of leaves was determined by counting the total number of leaves on each Java long pepper seedling. Leaf counting was conducted at 21, 35, 49, 63, 77, and 91 days after planting (DAP). The following data present the results of the DMRT post-hoc test on the number of leaves in seedlings under the following treatments:

P0: No Application (Control)
P1: Rice Washing Water (24 hours)
P2: Rice Washing Water (7 days)
P3: Jakaba Application
P4: Plant Growth Regulator
P5: Fermented Cow Urine

Table 2: Results of DMRT Analysis on the Number of Leaves in Java Long Pepper Seedlings

Treatment	Number of Leaves at Observation (DAP)							
	7	**21**	**35**	**49**	**63**	**77**	**91**	**Average**
P0	7,0	10,0 a	13,0 a	19,0 a	21,8 a	28,0 a	31,0 a	18,5 a
P1	7,0	10,0 a	14,0 b	19,2 ab	22,6 b	32,8 b	35,8 b	20,2 b
P2	7,2	10,4 ab	14,2 b	19,4 ab	22,8 b	33,0 b	36,0 b	20,4 b
P3	7,4	10,4 ab	16,8 c	19,8 bc	24,8 c	35,0 c	44,0 c	22,6 c
P4	7,2	11,0 c	17,0 c	20,4 c	25,8 d	35,8 c	44,8 c	23,1 d
P5	7,4	10,6 bc	16,6 c	19,6 ab	24,6 c	35,0 c	44,0 c	22,5 c
DMRT 5%	ns	*	*	*	*	*	*	*
Note:	Numbers followed by different letters in the same column indicate significant differences between treatments based on the DMRT at the 5% significance level, where * = significantly different, and ns = not significantly							

The data of Table 2 Table 5.2 presents the number of leaves (in units) at various observation periods (7 to 91 DAP) for each treatment (P0 to P5) and the mean number of leaves during the seedling stage of Javanese long pepper. Different letters indicate significant differences according to the DMRT at the 5% significance level. At 7 DAP, no significant differences were observed among treatments, indicating that each treatment did not significantly affect the increase in the number of leaves of Javanese long pepper seedlings at this early stage. However, from 21 DAP to 91 DAP, significant differences were observed among the treatments applied. The best treatment was the application of Plant Growth Regulator (P4) using auxin hormone, which resulted in the highest mean number of leaves at 23.1 leaves. This was followed by the Jakaba treatment (P3) and the fermented cow urine treatment (P5), with nearly similar mean numbers of leaves at 22.6 and 22.5 leaves, respectively. In contrast, the control treatment without any application (P0) resulted in the lowest mean number of leaves, with 18.5 leaves recorded during the seedling stage of Javanese long pepper.

3. Number of Nodes

The number of nodes was determined by counting the total nodes on each Java long pepper seedling. Measurements were taken at 21, 35, 49, 63, 77, and 91 days after planting (DAP). The following data present the results of the DMRT post-hoc test on the number of nodes in seedlings under the following treatments:

P0: No Application (Control)
P1: Rice Washing Water (24 hours)
P2: Rice Washing Water (7 days)
P3: Jakaba Application
P4: Plant Growth Regulator
P5: Fermented Cow Urine

Table 3: Results of DMRT Analysis on the Number of Nodes in Java Long Pepper Seedlings

Treatment	Number of Nodes at Observation Time (DAP)				
	7	**35**	**63**	**91**	**Average**
P0	8,0	14,0 a	22,0 a	31,4 a	22,0 f
P1	8,0	15,0 b	22,6 a	33,8 b	23,7 e
P2	8,2	15,2 b	22,8 a	34,0 b	26,4 d
P3	8,4	17,8 c	24,8 b	36,0 c	30,9 b
P4	8,2	18,0 c	25,8 c	36,8 c	32,8 a
P5	8,4	17,6 c	24,6 b	36,0 c	28,4 c
Uji DMRT 5%	ns	*	*	*	*
Note:	Numbers followed by different letters in the same column indicate significant differences between treatments based on the DMRT at the 5% significance level, where * = significantly different, and ns = not significantly.				

Based on Table 3 presents the number of nodes at various observation periods (7 to 91 DAP) for each treatment (P0 to P5) and the mean number of nodes during the seedling stage of Javanese long pepper. Different letters indicate significant

differences according to the DMRT at the 5% significance level. At 7 DAP, there were no significant differences among the treatments, indicating that each treatment did not significantly affect the increase in the number of nodes on Javanese long pepper seedlings at this early stage. The P4 treatment resulted in the highest mean number of nodes (22.2 nodes), showing a significant difference compared to other treatments. The P3 and P5 treatments also exhibited relatively high mean numbers of nodes (21.7 and 21.6 nodes, respectively), while the control treatment (P0) had the lowest mean number of nodes, with 18.8 nodes recorded during the seedling stage of Javanese long pepper.

4. Internode Length

Internode length was measured by recording the length of each internode on Java long pepper seedlings. Measurements were taken at 7, 35, 63, and 91 days after planting (DAP). The following data present the results of the DMRT post-hoc test on internode length under the following treatments:
P0: No Application (Control)
P1: Rice Washing Water (24 hours)
P2: Rice Washing Water (7 days)
P3: Jakaba Application
P4: Plant Growth Regulator
P5: Fermented Cow Urine

Table 4: Results of DMRT Analysis on Internode Length in Java Long Pepper Seedlings

Treatment	Internode Length (cm) at Observation Time (DAP)				
	7	**35**	**63**	**91**	**Rata-Rata**
P0	2,0	2,2	2,5 a	2,8 a	2,4 a
P1	2,0	2,2	2,5 a	2,9 ab	2,4 a
P2	2,1	2,3	2,6 ab	2,9 ab	2,4 a
P3	2,1	2,4	2,8 ab	3,1 bc	2,6 ab
P4	2,2	2,5	2,9 b	3,3 c	2,7 b
P5	2,1	2,3	2,7 ab	2,9 ab	2,5 ab
Uji DMRT 5%	ns	ns	*	*	*
Note:	Numbers followed by different letters in the same column indicate significant differences between treatments based on the DMRT at the 5% significance level, where * = significantly different, and ns = not significantly.				

Table 4 presents the internode length at various observation periods (7 to 91 DAP) for each treatment (P0 to P5) and the mean internode length during the seedling stage of Javanese long pepper. Different letters indicate significant differences according to the DMRT at the 5% significance level. At 7 DAP and 35 DAP, there were no significant differences among treatments, indicating that each treatment did not significantly affect the increase in internode length of Javanese long pepper seedlings at these stages. The P4 treatment resulted in the highest mean internode length (2.7 cm), showing a significant difference compared to the control (P0 = 2.4 cm). The P3 (2.6 cm) and P5 (2.5 cm) treatments also exhibited higher mean values compared to the control treatment.

5. Primary Root Length

The primary root length of Java long pepper seedlings was measured from the base of the plant to the tip of the root. Root length measurements were taken at 91 days after planting (DAP) or after three months to determine differences among treatments. The treatments included:
P0: No Application (Control)
P1: Rice Washing Water (24 hours)
P2: Rice Washing Water (7 days)
P3: Jakaba Application
P4: Plant Growth Regulator
P5: Fermented Cow Urine

Table 5: Results of DMRT Analysis on Primary Root Length in Java Long Pepper Seedlings

Treatment	Primary Root Lenght (cm) at Observation Time (DAP)
	91
P0	18,6 a
P1	19,4 ab
P2	20,2 b
P3	23,4 c
P4	23,8 c
P5	23,2 c
Uji DMRT 5%	*
Note::	Numbers followed by different letters within the same column indicate a significant difference between treatments according to Duncan's Multiple Range Test (DMRT) at the 5% significance level. * = significantly different.

The data show the mean root length (cm) at 91 DAP across six treatments (P0–P5). Different letters indicate significant differences among treatments based on the DMRT at the 5% significance level. The P4 treatment (23.8 cm) exhibited the highest root length, showing a significant difference compared to the control (P0) and treatments P1 and P2. Treatments P3 (23.4 cm) and P5 (23.2 cm) also demonstrated high root lengths, falling within the same statistical group as P4, indicating that the application of bio-resource technology in these treatments had a positive impact on root elongation. Treatment P2 (20.2 cm) was higher than the control (P0 = 18.6 cm) and was in a significantly different group from the control, although it remained lower than P3, P4, and P5. Treatment P1 (19.4 cm) did not differ significantly from the control, indicating a still limited effect on root elongation.

Discussion

Overall, the treatments applied in the seedling phase of Java long pepper showed significant differences based on the Duncan test at the 0.05 level after the seedlings reached 21

DAP. Each observed parameter of the Java long pepper seedlings exhibited significant differences at 21 DAP, which is presumed to be due to the well-developed root system at this age, enabling effective nutrient absorption. As explained by (Amiroh & Rohmad, 2017) [4], vegetative growth in plants is highly determined by the development of a robust root system and sufficient nutrient availability, which enable meristematic cell division. According to (Mahfudz *et al*., 2006) [16], the application of plant growth regulators on shoot cuttings positively influences shoot height, leaf count, fresh weight, and dry weight of cuttings compared to the control.

The application of bio-resources such as rice washing water (air leri), fermented cattle urine (ferinsa), and plant growth regulators had a significant impact on the growth of Java long pepper seedlings compared to the control (no treatment). This is presumed to be due to the nutrient content found in rice washing water and fermented cattle urine, which serve as a source of nutrition for the seedlings. The significant contribution of bio-resources such as fermented rice washing water on seedling growth has also been highlighted. According to (Okalia *et al*., 2021) [20], the application of liquid organic fertilizer from fermented rice washing water positively affects the height, leaf number, and fresh weight of lettuce plants. The best composition for improving growth and yield in lettuce was found to be 25% fermented rice washing water liquid organic fertilizer applied twice a week at a dose of 250 mL per lettuce plant.

Furthermore, as noted by Supriadji (1985) [1] in (Hendriyanto *et al*., 2019) [14], cattle urine contains auxin, which is one of the substances found in undigested forage within the cattle's digestive system and is eventually excreted through urine. The auxin present in cattle urine has shown positive effects on the growth of areca seedlings (Hendriyanto *et al*., 2019) [14].

Results of Soil Laboratiry Analysis

Soil testing in agriculture is essential to determine soil fertility levels. Soil testing technology is useful in determining appropriate fertilization recommendations, making farming more profitable. Balanced fertilization recommendations according to nutrient status and plant requirements can increase fertilizer efficiency without damaging the soil and help prevent environmental pollution (Al-Jabri, 2013) [2].

In this study, soil testing was conducted to determine the remaining nutrient content of N, P, K, and moisture content in the soil after various treatments were applied to Java long pepper seedlings. The soil analysis was conducted at the Sukosari Sugarcane Research Center, Soil Physics and Chemistry Laboratory, under sample registration number 030.

The method used to determine moisture content was the absolute dry weight method. Nitrogen (N) content was determined using the Kjeldahl method. Phosphorus (P) content was analyzed using the Olsen method, and Potassium (K) content was measured using the ammonium acetate extraction method. Organic carbon (C-Organic) was determined using the Walkley & Black method (Soil Physics and Chemistry Laboratory, Sukosari Research Center, 2025). The results of the soil analysis for the Java long pepper seedling soil samples are presented in Table 6 below.

Table 6: Results of Soil Testing for Java Long Pepper Seedling Nursery

Type	Moisture Content				
	KA (%)	N (%)	P_2O_5 (ppm)	K_2O ($Cmol^+Kg$)	C-Organik (%)
P0	5,25	0,09 (VL)	24,15 (VH)	0,060 (VL)	1,52 (L)
P1	4,47	0,12 (L)	26,74 (VH)	0,054 (VL)	1,40 (L)
P2	4,75	0,11 (L)	19,49 (H)	0,059 (VL)	1,34 (L)
P3	5,71	0,12 (L)	30,26 (VH)	0,060 (VL)	1,18 (L)
P4	4,89	0,11 (L)	30,00 (VH)	0,059 (VL)	1,23 (L)
P5	5,50	0,09 (VL)	30,19 (VH)	0,060 (VL)	1,29 (L)
P	8,02	0,14 (L	26,33 (VH)	0,056 (VL)	1,99 (L)

Note: VL = Very Low, L = Low, M = Moderate, H = High, VH = Very High
(Classification based on the Soil and Fertilizer Instrument Standard Testing Center, 2023)

Nitrogen (N) is a macronutrient that plays a crucial role in plant growth during the vegetative phase. It is involved in shoot formation, leaf development, stem growth, and root development (Sumanto, 2016) [29]. Nitrogen levels in the soil can be well absorbed at around 10 weeks (0.18%) but tend to decrease at 11 and 12 weeks in sugarcane seedling nurseries (Pogon *et al*., 2023) [21]. For Java long pepper seedlings, nitrogen plays an essential role in the development of new roots and shoots. Increased nitrogen uptake by Java long pepper seedlings is indicated by a decrease in nitrogen availability in the soil. Nutrients in new leaf tissues come from the soil or through the reabsorption from aging leaves, both processes requiring energy. Nutrient uptake from the soil tends to decrease as soil nutrient availability increases (Singh *et al*., 2005) [28].

Phosphorus (P) is vital for plant growth, particularly in root formation and stem strengthening in Java long pepper seedlings. It also supports the development of new shoots. Research by (Lukman, 2010) [15] found that phosphorus significantly affects the growth of mangosteen seedlings, especially regarding plant height, branch length, branch number, and leaf count. (Albari *et al*., 2018) [3] also stated that phosphorus increases leaf sheath length, stem circumference, and phosphorus nutrient content in TBM-3 oil palm.

Kalium (K) helps regulate water balance in plants, enhances nutrient absorption, supports photosynthesis, and aids protein formation. In Java long pepper seedlings, potassium helps mitigate excessive nitrogen effects, reducing susceptibility to pests and diseases, and strengthens leaves and stems to prevent easy breakage. (Adji *et al*., 2024) [1] reported that potassium nutrient status significantly affected tomato plant growth, with high potassium levels resulting in the highest total fruit weight. Potassium also positively affects cabbage yield, increasing leaf area, root number, fresh leaf weight, fresh root weight, fresh crop weight, and fresh plot weight (Rahmawan *et al*., 2019) [24].

Organic carbon (C-Organic) is crucial for improving soil fertility and supporting microorganism activity beneficial to

Java long pepper plants. Based on the soil tests conducted, the organic carbon content in the Java long pepper seedling soil was categorized as low. (Supriyadi, 2007) [30] indicated that soil organic matter in Sumenep is generally very low to low due to clay content influencing organic matter levels. Low organic carbon levels are also attributed to the dry climate and limited ground cover vegetation in the sampling locations, leading to higher average temperatures, accelerated organic matter decomposition, and faster loss of soil organic carbon (Risma *et al*., 2023) [26].

Soil organic matter content correlates with soil nitrogen levels (Supriyadi, 2007) [30]. The nitrogen content in the Java long pepper seedling soil was also in the very low to low category, highlighting the importance of organic-based soil management for improving dry soils in Madura, especially in Sumenep.

Conclusion

The use of biological resources in the growth of Java long pepper seedlings, including rice washing water stored for 24 hours and 7 days, Jakaba, auxin hormone, and fermented cow urine, had a significant effect on the growth responses of Java long pepper seedlings. These responses included plant height, number of leaves, number of nodes, internode length, and root length, with the application of auxin hormone consistently resulting in the best performance across all observed parameters.

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