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Drought stress and selective manure on the growth and yield of Chaya (*Cnidoscolus aconitifolius*) in a tropical climate

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Abstract. Chaya (*Cnidoscolus aconitifolius*) is a nutrient-dense, perennial leafy vegetable with great potential to support food security, especially in tropical regions. As a drought-tolerant plant, chaya is suitable for cultivation under changing climate conditions, including prolonged dry seasons. However, limited research has examined its specific response to varying levels of drought stress and organic fertilizer application. This study aimed to assess the growth and yield response of chaya under different irrigation volumes and manure types. The experiment was conducted from June to September 2024 in the greenhouse of the Faculty of Agriculture, Sriwijaya University, using a factorial randomized block design with two factors: irrigation volume (250 mL, 500 mL, 750 mL per two days) and manure type (no manure, chicken manure, and goat manure). Each treatment combination was replicated three times. Results showed that severe drought stress (250 mL) significantly inhibited plant growth, particularly in terms of shoot length and leaf area. Chicken manure improved soil moisture retention, maintaining levels up to 25% under drought conditions. In contrast, goat manure was more effective in supporting plant growth and yield under limited water availability. These findings suggest that combining appropriate organic amendments with optimized irrigation can enhance the resilience of chaya cultivation in tropical dryland environments.

Key words: climate change, dry season, manure, perennial vegetables.

INTRODUCTION

Chaya (*Cnidoscolus aconitifolius*) is a type of indigenous perennial vegetable or a perennial vegetable that is not yet widely known. Chaya is native to the Yucatan Peninsula in Mexico and Central America, and it grows well in tropical climates. Chaya is a vegetable with slightly woody stems and palmate leaves resembling papaya leaves. (Gustiar et al., 2023b). According to Totakul et al. (2021), chaya is a leafy vegetable rich

in nutrients and offers numerous benefits. The beneficial compounds found in chaya leaves can be utilized for medicinal purposes, as they contain fats, carbohydrates, proteins, calcium, phosphorus, iron, thiamine, riboflavin, niacin, and ascorbic acid, along with high levels of several flavonoids (Schwarcz et al., 2022; Gustiar et al., 2024). Other active compounds, including tannins, saponins, and alkaloids, further support these benefits. Chaya is easy and inexpensive to cultivate, with a semi-woody tree form that makes it more drought-resistant (Gobena et al., 2023; Gustiar et al., 2023a).

Climate change has made the distribution between the rainy and dry seasons increasingly difficult to predict (Mendez et al., 2020; Esariti et al., 2022). According to Padrón et al. (2020), drier dry seasons have been occurring in several regions of the world, especially in extratropical latitudes, due to increased evapotranspiration compared to precipitation. Prolonged dry seasons cause plants to experience drought stress, which subsequently leads to a decline in vegetable production, including perennial vegetables. Drought stress is a condition in which plants suffer from water deficiency, characterized by a total water capacity of less than 30% (Ria et al., 2020; Bogati & Walczak, 2022). The direct impact of drought stress is a reduction in plant turgor. Indirectly, it affects physiological processes such as photosynthesis, nitrogen metabolism, nutrient absorption, and the translocation of photosynthates. According to Bogati & Walczak (2022), drought-stressed plants undergo morphological changes, including reduced size, leaf area, and leaf number, as well as alterations in root growth and shoot length. If prolonged, it can accelerate plant death (Xiao et al., 2020; Yang et al., 2021).

The application of manure to the soil is one method to reduce the impact of drought. In addition to serving as a nutrient source for plants, manure also enhances the physical and biological properties of the soil, mitigating water deficiency by increasing the soil's water retention capacity (Hadi et al., 2021). Several types of manure can be used. Chicken manure is relatively easy to obtain and contains higher nutrient levels compared to other kinds of manure. Additionally, goat manure has a high pore volume, which helps improve soil porosity (Okorogbona & Adebisi, 2012; Situmeang et al., 2019). The addition of chicken manure and goat manure to the soil can help alleviate drought stress in plants, as higher organic matter content increases the soil's water-holding capacity (Turfan, 2021).

According to Gobena et al. (2023), Chaya is a drought-tolerant plant. Conversely, research by Lakitan et al. (2024) found that chaya does not grow well under water-saturated conditions. Although Chaya is known to be drought-tolerant, it is essential to test how this plant responds to different levels of water availability and manure application under real conditions that reflect the environmental challenges in Indonesia. The results of this study will provide valuable information to optimize Chaya cultivation in tropical areas with variable water conditions. Its growth and yield perform better when cultivated under optimal conditions that are neither waterlogged nor excessively dry. The specific tolerance limits of chaya to drought stress and the role of manure application in its growth and yield have not been thoroughly studied. This research aims to evaluate Chaya's response to drought stress and manure application, with the findings expected to provide recommendations for Chaya cultivation techniques during the dry season.

MATERIALS AND METHODS

This research was conducted from June to September 2024 in the Greenhouse of the Faculty of Agriculture, Sriwijaya University, Indonesia. Under the climatic conditions of the study area, the average temperature was 29.3 °C, and the relative humidity was 76.2%. Data during the study period are presented in Fig. 1. Chaya was propagated using stem cuttings obtained from plants of the same age and size. The variety used is the picuda variety. The cuttings used were taken from the middle part of the stem, measuring 25 cm in length. The growing media was prepared using a 1:4 ratio of manure to topsoil. The planting was carried out using pots with a top diameter of 30 cm and a height of 30 cm, with an approximate growing media volume of 20 liters.

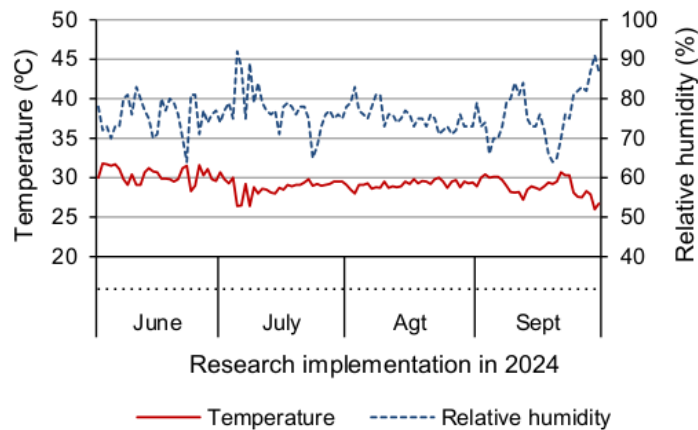


Figure 1. Air temperature and relative humidity at the nearest climatological station to the research location.

Source: <https://www.bmkg.go.id>

The study was conducted using Factorial Randomized Block Design (RBD-F) with two factors. The first factor was drought stress, applied using an irrigation volume approach every two days, i.e., 250 mL (C1), 500 mL (C2), and 750 mL (C3). The second factor was the addition of manure, i.e., control (without manure) (M1), chicken manure (M2), and goat manure (M3). Each treatment combination was replicated three times, and each replicate contained three plant units. Thus, the total number of plant sample units was 81 (eighty-one).

The irrigation volume treatments were applied two weeks after planting (WAP). Watering was carried out every 2 days in the afternoon. Before watering, soil moisture was measured first to determine the field capacity of the growing media. The treatment with the lowest irrigation volume (250 mL, C1) was considered as the drought stress condition, as it provides a limited water supply to simulate water deficit. The intermediate volume (500 mL, C2) represents moderate moisture availability, while the highest volume (750 mL, C3) was treated as an optimum moisture condition, ensuring no water limitation for the plants.

Data collection

Data collection was conducted when the plants were 4 weeks old. Soil moisture was monitored daily after irrigation as an indicator of treatment effects using a Lutron PMS 714 soil moisture meter. The observed plant growth variables included shoot length (cm), shoot diameter (mm), number of leaves, and leaf area expansion (cm²), which was measured using a linear regression method (Lakitan et al., 2022). Yield parameters were observed when the plants were 65 days old. The observed yield parameters included canopy area (cm²) and leaf area (cm²) using the Easy Leaf Area method (Easlon & Bloom, 2014), root length (cm), leaf weight (g), stem weight (g), and root weight (g). At harvest, edible and non-edible parts were separated, with edible leaves defined as the plant's shoot section consisting of the stem, lamina, and petiole, including the top five leaves (Gustiar et al., 2023b).

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Data analysis

All collected data were analyzed using two-way Analysis of Variance (ANOVA). For data showing significant effects, multiple comparison tests were conducted using the Least Significant Difference (LSD) test at a significance level of $P < 0.05$. The entire data analysis was carried out using RStudio software

RESULTS AND DISCUSSION

Soil Moisture

Soil moisture monitoring revealed that both irrigation volume and manure application had a significant impact on soil moisture levels. Irrigation with a volume of 750 mL consistently resulted in higher soil moisture compared to lower irrigation volumes. Soil moisture measurements were used as key indicators to assess the degree of soil drought stress. Among the manure treatments, chicken manure proved more effective in retaining soil moisture than other types of manure. Notably, the combination of chicken manure application and an irrigation volume of 750 mL was most successful in maintaining optimal soil moisture levels (Fig. 2).

Both internal and external factors influence the growth of the Chaya plant. Internal factors originate from within the plant itself, while external factors include environmental conditions surrounding the plant (Sugai et al., 2023). One critical external factor is water availability, particularly in the context of drought conditions. Soil moisture can be used as an indicator to measure soil dryness. Irrigation with higher water volumes increases soil moisture, whereas irrigation with lower volumes results in drier soil. Besides irrigation volume, the soil's organic matter content also plays a significant role in determining soil moisture levels. The application of manure helps maintain soil moisture because it contains organic matter that improves soil structure, making the soil more porous and able to retain water effectively (Okorogbona & Adebisi, 2012). Soil moisture levels under chicken manure application tend to be higher than those under goat manure due to the finer texture of chicken manure. This finer texture allows chicken manure to mix more thoroughly with soil and form more stable soil aggregates, thereby increasing the soil's water-holding capacity (Sudita et al., 2021). However, in general, organic matter provides limited benefits in mitigating severe drought stress due to its slow decomposition rate and limited long-term moisture retention capacity (Ria et al., 2025).

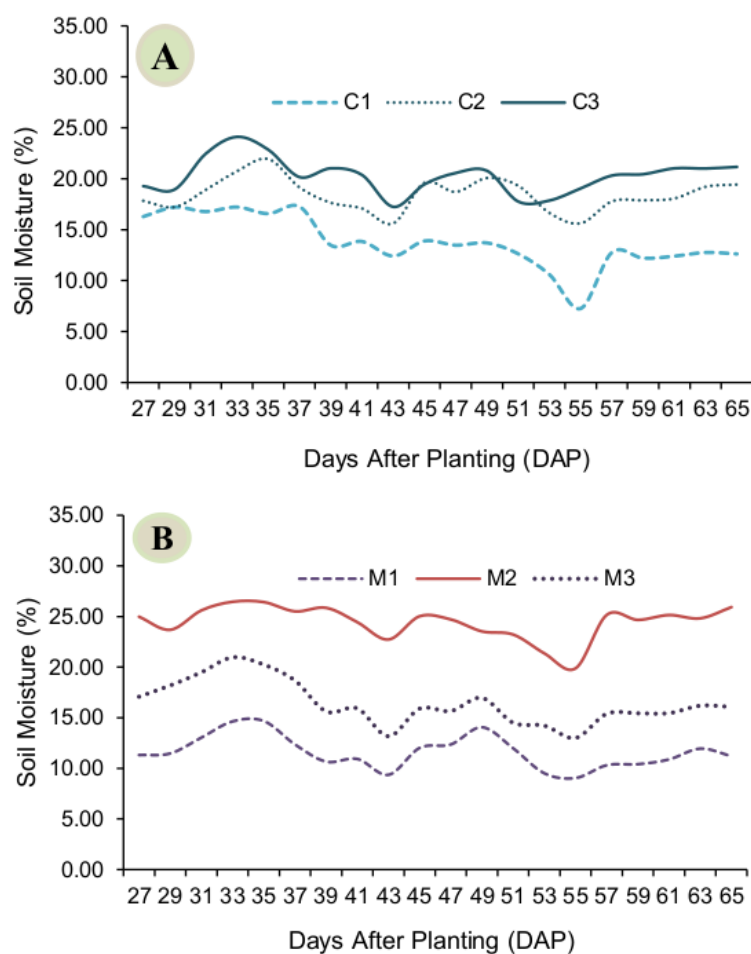


Figure 2. Soil moisture conditions at different drought levels (A) and manure application (B).

Growth Performance of Chaya Plant

During the early growth stage of chaya, irrigation volume had no significant influence on shoot length. However, after 7 Week after planting (WAP), plants irrigated with the lowest volume (250 mL) exhibited significantly reduced shoot length compared to those receiving higher water volumes (Table 1). This suggests that at later developmental stages, Chaya requires increased water availability to support optimal shoot elongation, and insufficient water supply restricts shoot growth. Similarly, manure application had no significant effect on shoot length during the initial growth phase, but its impact became significant after 6 Week after planting (WAP). These findings indicate that the effects of manure on growth manifest after a specific period, while the type of manure applied did not result in significant differences in shoot length. Other factors, such as drought stress and organic matter content, also contribute to shoot growth, especially influencing shoot diameter (Table 2).

Table 1. The effect of drought stress and manure application on the shoot length (cm) of *Cnidioscolus aconitifolius*

Week after planting (WAP)							
	4	5	6	7	8	9	10
Drought stress							
C1	6.09 ^b	11.32 ^a	18.84 ^a	24.12 ^b	30.18 ^b	36.05 ^b	39.72 ^b
C2	7.56 ^a	12.99 ^a	21.52 ^a	27.77 ^{ab}	34.83 ^{ab}	44.64 ^a	50.64 ^a
C3	8.13 ^a	14.38 ^a	21.05 ^a	28.43 ^a	35.91 ^a	45.22 ^a	52.02 ^a
LSD _{0.05}	1.38	2.69	3.66	4.07	5.27	6.73	7.57
Manure application							
M1	5.97 ^b	14.69 ^b	21.44 ^b	26.75 ^b	32.35 ^b	37.64 ^b	8.01 ^b
M2	8.74 ^a	18.83 ^a	26.52 ^a	33.69 ^a	40.66 ^a	47.53 ^a	10.99 ^a
M3	7.07 ^b	17.15 ^{ab}	25.21 ^{ab}	33.37 ^a	42.48 ^a	50.87 ^a	9.52 ^a
LSD _{0.05}	1.38	2.69	3.66	4.07	5.27	6.73	7.57
Interaction of drought stress and organic matter							
C1M1	5.38 ^d	10.61 ^c	17.03 ^b	21.31 ^c	25.15 ^d	28.71 ^d	30.33 ^d
C1M2	7.26 ^{bcd}	11.70 ^{abc}	20.65 ^{ab}	27.13 ^{abc}	34.08 ^{abcd}	41.28 ^{bc}	47.56 ^{bc}
C1M3	5.63 ^{cd}	11.63 ^{abc}	18.83 ^{ab}	23.91 ^{bc}	31.30 ^{bcd}	38.15 ^{bcd}	41.20 ^{cd}
C2M1	6.30 ^{cd}	11.20 ^{bc}	19.58 ^{ab}	25.01 ^{abc}	31.73 ^{bcd}	39.93 ^{bcd}	44.43 ^{bc}
C2M2	8.78 ^{bcd}	14.85 ^{abc}	23.51 ^a	30.28 ^{ab}	35.61 ^{abc}	46.28 ^{abc}	52.75 ^{abc}
C2M3	7.60 ^{bcd}	12.91 ^{abc}	21.45 ^{ab}	28.00 ^{abc}	37.15 ^{ab}	47.71 ^{ab}	54.75 ^{ab}
C3M1	6.21 ^{cd}	11.61 ^{abc}	17.15 ^b	21.80 ^c	27.75 ^{cd}	35.91 ^{cd}	42.48 ^{bcd}
C3M2	10.18 ^a	16.10 ^a	23.25 ^{ab}	31.80 ^a	38.58 ^{ab}	44.25 ^{abc}	54.75 ^{ab}
C3M3	7.93 ^{abc}	15.43 ^{ab}	22.75 ^{ab}	31.70 ^a	41.38 ^a	55.48 ^a	64.13 ^a
LSD _{0.05}	2.40	4.65	6.33	7.04	9.12	11.65	13.11

Table 2. The effect of drought stress and manure application on the shoot diameter (mm) of *Cnidioscolus aconitifolius*

Week after planting (WAP)							
	4	5	6	7	8	9	10
Drought stress							
C1	5.33 ^a	6.56 ^a	7.57 ^a	8.29 ^a	9.07 ^a	9.59 ^b	10.26 ^b
C2	4.92 ^a	6.73 ^a	7.60 ^a	8.31 ^a	9.16 ^a	9.74 ^{ab}	10.77 ^a
C3	5.75 ^a	6.51 ^a	7.77 ^a	8.74 ^a	9.51 ^a	10.37 ^a	11.07 ^a
LSD _{0.05}	0.86	0.74	0.61	0.73	0.79	0.74	0.57
Manure application							
M1	5.20 ^a	6.35 ^a	7.24 ^b	7.90 ^b	8.70 ^b	9.40 ^b	9.96 ^b
M2	5.88 ^a	6.74 ^a	7.88 ^a	8.62 ^{ab}	9.31 ^{ab}	9.95 ^{ab}	10.83 ^a
M3	4.93 ^a	6.71 ^a	7.83 ^{ab}	8.81 ^a	9.73 ^a	10.35 ^a	11.32 ^a
LSD _{0.05}	0.86	0.74	0.61	0.73	0.79	0.74	0.57
Interaction of drought stress and organic matter							
C1M1	5.78 ^{abc}	6.84 ^{ab}	7.69 ^{ab}	8.14 ^{ab}	8.97 ^{ab}	9.61 ^{abc}	9.89 ^{dc}
C1M2	5.20 ^{abc}	6.10 ^{ab}	7.18 ^{bc}	7.84 ^{ab}	8.81 ^{ab}	9.33 ^{bc}	10.49 ^{bcd}
C1M3	4.99 ^{bcd}	6.74 ^{ab}	7.84 ^{ab}	8.88 ^a	9.41 ^a	9.81 ^{ab}	10.40 ^{cd}
C2M1	4.59 ^{cd}	6.34 ^{ab}	7.62 ^{ab}	8.63 ^a	9.20 ^{ab}	10.12 ^{ab}	10.79 ^{bcd}
C2M2	6.49 ^a	6.99 ^{ab}	8.05 ^{ab}	8.92 ^a	9.56 ^a	10.53 ^{ab}	11.00 ^{bc}
C2M3	3.66 ^d	6.19 ^{ab}	7.63 ^{ab}	8.65 ^a	9.76 ^a	10.46 ^{ab}	11.41 ^{ab}
C3M1	5.21 ^{ab}	5.86 ^b	6.39 ^c	6.92 ^b	7.92 ^b	8.47 ^c	9.18 ^c
C3M2	5.93 ^{abc}	7.12 ^{ab}	8.39 ^a	9.08 ^a	9.54 ^a	9.98 ^{ab}	10.97 ^{bc}
C3M3	6.12 ^{ab}	7.19 ^a	8.00 ^{ab}	8.90 ^a	10.00 ^a	10.78 ^a	12.15 ^a
LSD _{0.05}	1.48	1.27	1.05	1.26	1.36	1.27	0.99

Increased water availability promotes vegetative growth, whereas limited watering induces drought stress, leading to stunted growth. Water deficit during the vegetative phase commonly causes reduced plant height and leaf wilting, symptoms linked to inhibited cell expansion and accelerated leaf senescence. Drought stress restricts cell elongation and disrupts mitotic activity, thereby limiting plant height (Yang et al., 2021). Additionally, plant growth is strongly affected by nutrient availability and soil physical properties; improved nutrient supply and favorable soil conditions enhance shoot development (Widjajanto et al., 2023). Manure application plays a critical role in enhancing soil fertility by supplying essential nutrients and improving soil physical structure (Situmeang et al., 2019). Organic matter from manure enhances soil aggregation, which positively influences water infiltration, retention, aeration, and root penetration - all crucial factors for healthy plant growth (Maryana & Suwardi, 2022).

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Table 3. The effect of drought stress and manure application on the number of leaves of *Cnidioscolus aconitifolius*

Week after planting (WAP)	4	5	6	7	8	9
Drought stress						
C1	12.78 ^a	17.89 ^a	21.17 ^a	25.89 ^a	27.56 ^a	29.17 ^b
C2	16.28 ^a	20.50 ^a	24.56 ^a	31.00 ^a	32.94 ^a	35.83 ^a
C3	12.78 ^a	17.39 ^a	20.39 ^a	26.06 ^a	27.89 ^a	31.11 ^{ab}
LSD 0.05	5.56	5.83	5.84	6.66	6.19	6.55
Manure application						
M1	13.72 ^a	17.17 ^a	20.39 ^a	25.50 ^{ab}	26.61 ^b	28.67 ^b
M2	13.22 ^a	16.89 ^a	19.94 ^a	25.33 ^b	26.56 ^b	28.56 ^b
M3	14.89 ^a	21.72 ^a	25.78 ^a	32.11 ^a	35.22 ^a	38.89 ^a
LSD 0.05	5.56	5.83	5.84	6.66	6.19	6.55
Interaction of drought stress and organic matter						
C1M1	13.33 ^a	16.50 ^a	20.16 ^{ab}	24.33 ^{ab}	25.50 ^{bc}	26.67 ^c
C1M2	11.00 ^a	14.17 ^a	16.83 ^b	21.66 ^b	23.16 ^c	25.00 ^c
C1M3	14.00 ^a	23.00 ^a	26.50 ^{ab}	31.66 ^{ab}	34.00 ^{ab}	35.83 ^{abc}
C2M1	15.00 ^a	18.83 ^a	22.66 ^{ab}	29.16 ^{ab}	30.16 ^{abc}	33.50 ^{abc}
C2M2	15.33 ^a	19.33 ^a	22.33 ^{ab}	29.00 ^{ab}	30.33 ^{abc}	31.83 ^{abc}
C2M3	18.50 ^a	23.33 ^a	28.66 ^a	34.83 ^a	38.33 ^a	42.16 ^a
C3M1	12.83 ^a	16.16 ^a	18.33 ^b	23.00 ^b	24.17 ^{bc}	25.83 ^c
C3M2	13.33 ^a	17.16 ^a	20.66 ^{ab}	25.33 ^{ab}	26.16 ^{bc}	28.83 ^{bc}
C3M3	12.17 ^a	18.83 ^a	22.16 ^{ab}	29.83 ^{ab}	33.33 ^{abc}	38.66 ^{ab}
LSD 0.05	9.64	10.10	10.12	11.53	10.71	11.35

At the early growth stage, drought stress treatments did not significantly affect the number of leaves on chaya plants. However, from 9 Week after planting (WAP) onward, differences between treatments became increasingly apparent. This trend likely corresponds to the plant's increasing water demand as it matures and metabolic activity intensifies. Under severe drought stress (C1), plants exhibited the fewest leaves, whereas the highest leaf number was observed under moderate drought stress (C2) (Table 3). Optimal growth of chaya requires balanced soil moisture conditions - not too dry nor excessively wet. Both water deficit and waterlogging can induce stress that inhibits growth. Drought reduces water availability necessary for physiological processes, while

waterlogged conditions limit oxygen availability to roots, impairing plant function (Kumar, 2020). Application of goat manure has been shown to improve soil physical properties (Odedina et al., 2011), which helps maintain adequate drainage and soil moisture levels suitable for chaya growth. Consequently, treatments maintaining moderate soil moisture supported a higher number of leaves compared to other treatments.

Plants subjected to drought stress exhibited a reduced rate of leaf area expansion compared to those receiving adequate irrigation (Table 4). Consequently, leaves of water-deficient plants were smaller in size than those under non-stress conditions. This reduction is attributed to the inhibitory effect of water deficit on physiological processes essential for leaf cell expansion. Manure application had a positive influence on leaf area growth, indicating that the nutrients supplied by organic fertilizers enhanced the development of chaya leaves. Among the manure types tested, chicken manure produced the most significant increase in leaf area, likely due to its higher nutrient availability compared to goat manure.

Table 4. Increase the leaf area (cm²) of *Cnidoscolus aconitifolius* under drought stress conditions and manure application

Days After Blade Unfolded	1	2	3	4	5	6	7	8	9	10	11	12
Drought stress												
C1	22.77 ^a	32.38 ^a	51.20 ^a	60.72 ^a	74.64 ^a	84.62 ^a	90.94 ^b	101.97 ^a	112.12 ^a	119.22 ^a	124.94 ^b	128.39 ^b
C2	29.89 ^a	39.95 ^a	55.30 ^a	71.66 ^a	89.79 ^a	104.89 ^a	118.40 ^a	130.97 ^a	142.49 ^a	155.48 ^a	162.64 ^a	171.58 ^a
C3	29.04 ^a	41.49 ^a	52.12 ^a	64.73 ^a	76.98 ^a	90.73 ^a	101.40 ^b	118.53 ^a	138.04 ^a	154.80 ^a	165.48 ^a	175.11 ^a
LSD 0.05	9.55	12.28	12.83	15.12	18.02	20.28	21.58	29.78	32.88	35.19	34.81	34.46
Manure application												
M1	25.63 ^a	38.94 ^a	54.90 ^a	64.25 ^a	76.69 ^a	85.94 ^a	91.26 ^b	100.54 ^b	109.76 ^b	120.18 ^b	125.25 ^b	130.40 ^b
M2	31.56 ^a	41.67 ^a	52.41 ^a	65.24 ^a	77.89 ^a	90.34 ^a	100.27 ^{ab}	115.13 ^{ab}	127.21 ^{ab}	135.32 ^b	140.72 ^b	147.59 ^b
M3	24.53 ^a	33.20 ^a	51.29 ^a	67.62 ^a	86.83 ^a	103.97 ^a	119.19 ^a	135.80 ^a	155.66 ^a	173.99 ^a	187.09 ^a	197.08 ^a
LSD 0.05	9.55	12.28	12.83	15.12	18.02	20.28	21.58	29.78	32.88	35.19	34.81	34.46
Interaction of drought stress and organic matter												
C1M1	21.54 ^a	36.41 ^a	61.59 ^a	67.27 ^a	77.19 ^a	84.91 ^{abc}	90.89 ^{abc}	95.83 ^{abc}	101.60 ^b	104.67 ^b	106.55 ^c	108.26 ^c
C1M2	27.34 ^a	35.38 ^a	48.30 ^a	57.24 ^a	64.49 ^a	74.24 ^{bc}	78.15 ^{bc}	94.54 ^{bc}	104.88 ^b	139.71 ^{ab}	117.66 ^c	121.34 ^c
C1M3	19.45 ^a	25.35 ^a	43.71 ^a	57.63 ^a	82.23 ^a	94.71 ^{abc}	103.75 ^{abc}	115.53 ^{abc}	129.87 ^b	141.01 ^{ab}	150.59 ^c	155.56 ^{bc}
C2M1	32.28 ^a	45.06 ^a	58.37 ^a	68.07 ^a	91.49 ^a	107.07 ^{ab}	114.15 ^{ab}	126.85 ^{abc}	133.73 ^{ab}	146.36 ^{ab}	154.36 ^{bc}	156.25 ^{bc}
C2M2	34.45 ^a	44.05 ^a	55.88 ^a	74.11 ^a	87.18 ^a	101.40 ^{ab}	114.36 ^{ab}	120.98 ^{abc}	130.38 ^{ab}	139.71 ^{ab}	146.27 ^{bc}	157.35 ^{bc}
C2M3	22.95 ^a	30.74 ^a	51.64 ^a	72.79 ^a	90.70 ^a	106.21 ^c	126.67 ^a	145.08 ^{ab}	163.34 ^a	180.36 ^a	187.30 ^b	201.14 ^b
C3M1	23.07 ^a	35.34 ^a	44.74 ^a	57.40 ^a	61.39 ^a	65.82 ^c	68.73 ^a	78.93 ^c	93.94 ^b	109.50 ^{ab}	154.35 ^{bc}	126.69 ^c
C3M2	32.85 ^a	45.59 ^a	53.07 ^a	64.34 ^a	82.00 ^a	95.37 ^{abc}	108.29 ^{ab}	129.87 ^{abc}	130.38 ^{ab}	104.67 ^{ab}	158.23 ^{bc}	164.08 ^{bc}
C3M3	31.21 ^a	43.51 ^a	58.54 ^a	72.44 ^a	87.54 ^a	110.99 ^{ab}	127.15 ^a	146.79 ^a	173.78 ^b	106.55 ^{ac}	223.38 ^a	234.54 ^a
LSD 0.05	16.54	21.27	22.22	26.19	31.22	35.13	37.38	51.59	56.95	60.94	106.55	59.70

Drought stress impacts not only leaf number but also leaf size. Previous studies have shown that chaya leaves expand until approximately 10–12 days after unfolding, with drought stress inhibiting growth from the ninth day onward (Gustiar et al., 2023b). The reduction in leaf area is a typical plant response to drought, aimed at minimizing water loss by decreasing transpiration surface area (Toscano et al., 2019; Darmanti et al., 2021). Notably, chaya plants treated with goat manure exhibited larger leaf sizes, which may be associated with improved soil moisture conditions. Organic matter from manure enhances soil physical properties such as structure, water retention capacity, and

infiltration rate (Washaya & Washaya, 2023). These improved soil characteristics are crucial in dry and drought-prone environments, supporting optimal plant growth.

The relative drought levels did not significantly affect the SPAD values, indicating that chlorophyll content in chaya leaves increased steadily with plant age regardless of water stress conditions (Fig. 3, A). The SPAD values rose until approximately 55 days after planting, after which a decline was observed, reflecting the natural senescence process of the leaves. This pattern suggests that chlorophyll concentration reaches its peak at a specific developmental stage before decreasing due to leaf aging. During the early growth stages, plants treated with chicken manure exhibited lower SPAD values compared to other treatments. However, as the plants matured, the SPAD values in the chicken manure treatment increased more markedly than in the different treatments (Fig. 3, B). This trend suggests that chicken manure has a positive influence on chlorophyll accumulation over time, likely due to its higher nitrogen content and faster mineralization rate compared to goat manure, which enhances chlorophyll biosynthesis and overall leaf greenness.

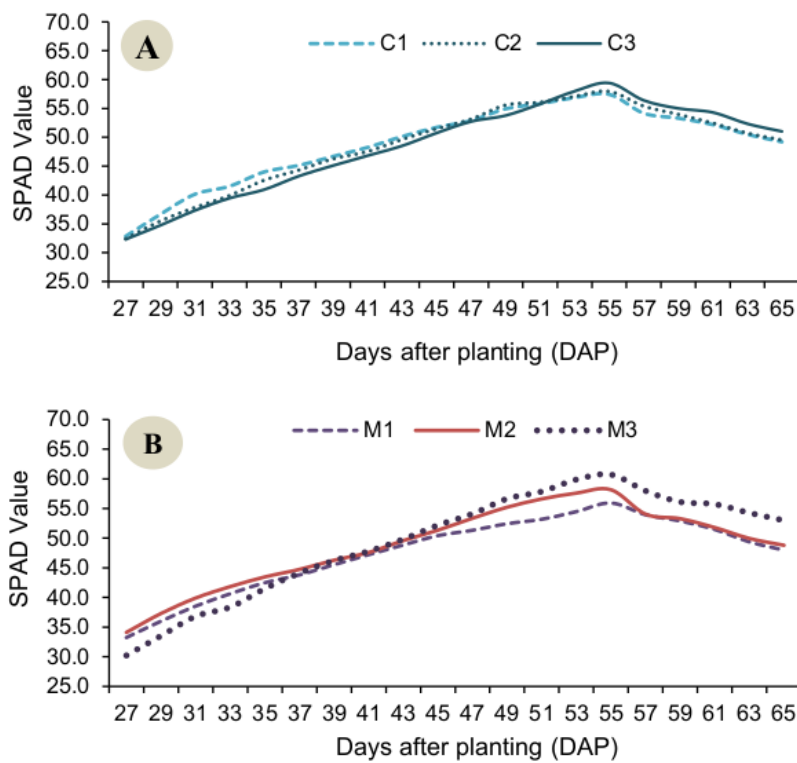


Figure 3. SPAD value of chaya under different drought stress levels (A) and organic material application (B).

When plants experience drought stress, their initial response includes both morphological changes and internal physiological adjustments. One key response to water deficit is the closure of stomata (Zhu et al., 2020). Stomatal closure limits carbon dioxide

uptake, disrupting photosynthesis and consequently reducing chlorophyll synthesis. This physiological mechanism explains why SPAD values, which reflect chlorophyll content, tend to decrease under drought stress (Syamsia et al., 2018).

The application of goat manure was found to influence leaf growth rate and leaf greenness positively. According to Washaya & Washaya (2023), goat manure typically contains higher nitrogen levels compared to other types of manure. Nitrogen is essential for leaf development as it contributes to chlorophyll synthesis and photosynthate accumulation, which supports the initiation of new leaf shoots. This relationship is supported by Li et al. (2019), who reported a positive correlation between leaf nitrogen content and SPAD values.

Leaf Area and Rooting of Chaya Plant

The C1 treatment (lowest irrigation volume) resulted in the smallest canopy area, consumable leaf area, and non-consumable leaf area, indicating that severe drought conditions significantly restrict plant growth. In contrast, treatments C2 and C3 (moderate and higher irrigation) showed no significant differences in canopy and leaf area, suggesting that chaya plants can tolerate mild to moderate drought without significant growth reductions. Notably, the combination of moderate drought (C3) with chicken manure application (M3) resulted in the most extensive canopy and leaf area, demonstrating that organic amendments can optimize plant growth under water-limited conditions (Table 5).

Table 5. Comparison of Destructive Harvest Yields of Chaya Plants Under Drought Stress Treatment with Different Irrigation Volumes and Growing Media

	CA (cm ²)	NELA (cm ²)	ELA (cm ²)	LR (cm)	FWR (g)	DWR (g)
Drought stress						
C1	855.27 ^b	735.27 ^b	204.57 ^b	29.63 ^a	1.73 ^b	0.59 ^b
C2	1,463.87 ^a	1,392.90 ^a	404.44 ^a	27.84 ^a	2.40 ^{ab}	0.96 ^a
C3	1,760.79 ^a	1,358.95 ^a	388.75 ^a	32.76 ^a	3.14 ^a	0.98 ^a
LSD 0.05	454.47	327.37	135.14	6.59	0.88	0.26
Manure application						
M1	1,055.23 ^b	876.54 ^b	301.83 ^{ab}	31.16 ^a	2.13 ^b	0.74 ^b
M2	1,266.90 ^b	1,142.74 ^{ab}	260.31 ^b	22.61 ^b	1.91 ^b	0.66 ^b
M3	1,757.78 ^a	1,467.85 ^a	435.63 ^a	36.47 ^a	3.23 ^a	1.12 ^a
LSD 0.05	454.47	327.37	135.14	6.59	0.88	0.26
Interaction of drought stress and organic matter						
C1M1	650.32 ^d	528.13 ^c	201.32 ^{cd}	27.35 ^{abcd}	1.47 ^c	0.58 ^{cd}
C1M2	805.32 ^{cd}	707.49 ^{de}	117.52 ^d	23.57 ^{cd}	1.38 ^c	0.44 ^d
C1M3	1,110.16 ^{bed}	970.20 ^{cde}	294.86 ^{bed}	37.98 ^a	2.35 ^{bc}	0.75 ^{bed}
C2M1	1,305.94 ^{bed}	1,164.12 ^{bed}	398.09 ^{abc}	35.47 ^{ab}	2.89 ^{abc}	0.90 ^{bc}
C2M2	1,411.38 ^{bed}	1,413.20 ^{abc}	372.81 ^{abc}	25.52 ^{bed}	2.64 ^{abc}	0.84 ^{bed}
C2M3	1,674.29 ^b	1,601.38 ^{ab}	442.43 ^{ab}	37.30 ^a	3.90 ^a	1.13 ^{ab}
C3M1	1,209.45 ^{bed}	937.36 ^{cde}	306.08 ^{bed}	30.65 ^{abc}	2.04 ^{bc}	0.75 ^{bed}
C3M2	1,584.02 ^{bc}	1,307.54 ^{abc}	290.60 ^{bed}	18.75 ^d	1.71 ^c	0.72 ^{bed}
C3M3	2,488.90 ^a	1,831.96 ^a	569.58 ^a	34.13 ^{abc}	3.45 ^{ab}	1.46 ^a
LSD 0.05	787.16	567.02	234.08	11.42	1.52	0.45

Note: CA (Canopy area), NELA (Non-edible leaf area), ELA (edible leaf area), LR (length Root), FWR, and DWR.

While drought stress under C1 stunted chaya shoot growth (Fig. 4), root development was not significantly affected by drought treatments (Fig. 5), suggesting possible root adaptation mechanisms to water deficit.

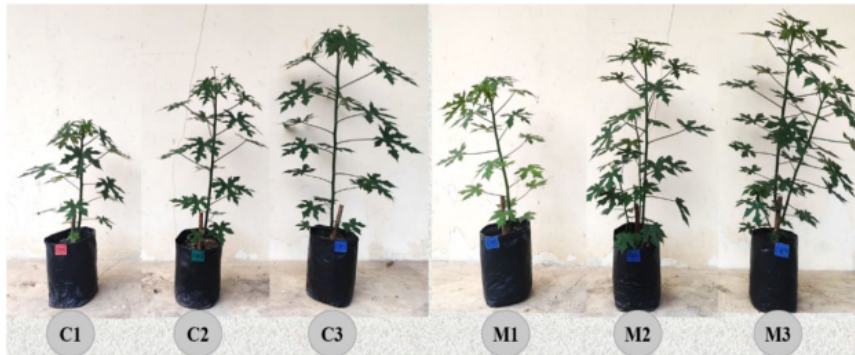


Figure 4. Performance of Chaya with various drought stress (A) and manure application (B).

In this study, drought stress significantly reduced the canopy and leaf area of chaya plants, demonstrating that leaves are susceptible to water deficit. Leaves play a critical role in assimilation and transpiration. Under drought conditions, they typically exhibit morphological adaptations such as reduced leaf area, increased thickness, and higher tissue density to minimize water loss (Yang et al., 2021). Leaf area is a key indicator of plant productivity because a larger canopy area allows for greater light interception, thereby enhancing photosynthesis and assimilation processes (Jayalath & Marc van Iersel 2021).

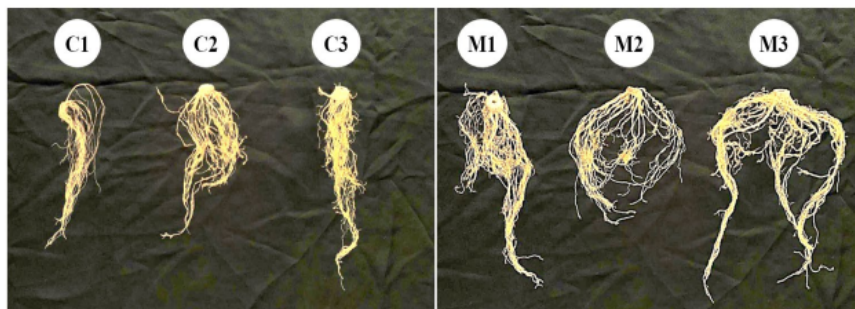


Figure 5. Root conditions of chaya in drought stress conditions (A) and manure application (B).

Morphological changes in the canopy are influenced not only by water availability but also by soil physical properties and nutrient content. Improved soil conditions support greater vegetative growth, resulting in a more extensive canopy. The canopy size depends on both the number and size of leaves. In this research, goat manure application resulted in the highest leaf numbers, leaf lengths, and leaf widths, thereby producing the largest canopy area. While poultry manure generally provides more nutrients, goat manure is particularly effective in improving soil physical properties, which benefits chaya as a perennial species that prefers well-structured soil (Usman, 2015). Drought

stress also influenced root growth, as indicated by reductions in root length and biomass. Roots are vital for water uptake and play a central role in supporting plant metabolism under water-limited conditions (Xiao et al., 2020). Water scarcity typically results in fewer roots, smaller root size, and a more restricted root distribution. Since water is the primary environmental factor affecting root development, modifications in root morphology such as increased root hair density, branching, and overall root density are essential adaptations for optimizing water acquisition in dry environments (Boguszewska-Mańkowska et al., 2020; Yang et al., 2021). Among manure treatments, goat manure produced the most favorable root growth, likely due to its dual role in enhancing soil physical properties and supplying nutrients. Improved soil structure facilitates easier root penetration and development. Furthermore, nutrients from manure stimulate root growth and the formation of a robust root system, which supports faster vegetative growth and ultimately increases crop yield (Homulle et al., 2022).

Biomass of the Chaya Plant

Drought stress significantly influenced both the fresh and dry weights of these edible parts. The highest biomass was observed under treatment C2 (moderate drought), while the lowest biomass occurred in treatment C1 (severe drought).

Table 6. The component fresh of chaya in drought stress treatment and manure applications

Treatment	FWEL (g)	FWNL (g)	FWES (g)	FWNS (%)	TFW (g)
Drought stress					
C1	8.78 ^b	17.78 ^b	8.05 ^b	26.46 ^b	61.07 ^b
C2	16.87 ^a	31.35 ^a	15.12 ^a	44.23 ^a	107.57 ^a
C3	13.94 ^a	32.10 ^a	11.86 ^{ab}	46.25 ^a	104.15 ^a
LSD 0.05	4.58	9.79	5.63	14.78	26.24
Manure application					
M1	12.04 ^{ab}	18.84 ^b	9.80 ^a	26.46 ^b	67.13 ^a
M2	11.05 ^b	28.32 ^{ab}	9.87 ^a	42.95 ^a	92.19 ^{ab}
M3	16.50 ^a	34.08 ^a	15.36 ^a	47.54 ^a	113.47 ^a
LSD 0.05	4.58	9.79	5.63	14.78	26.24
Interaction of drought stress and organic matter					
C1M1	7.99 ^{cd}	13.96 ^c	4.87 ^c	19.29 ^d	46.10 ^d
C1M2	6.25 ^d	17.32 ^{de}	7.25 ^{bc}	29.15 ^{bcd}	59.96 ^d
C1M3	12.12 ^{abcd}	22.07 ^{cde}	12.03 ^{abc}	30.94 ^{bcd}	77.15 ^{cd}
C2M1	14.89 ^{abc}	23.56 ^{bcde}	13.90 ^{abc}	32.89 ^{abcd}	85.24 ^{bcd}
C2M2	16.93 ^{ab}	31.37 ^{abcd}	15.07 ^{ab}	45.96 ^{abc}	109.34 ^{abc}
C2M3	18.79 ^a	39.13 ^{ab}	16.38 ^{ab}	53.84 ^{ab}	128.15 ^{ab}
C3M1	13.25 ^{abcd}	19.00 ^{de}	10.62 ^{abc}	27.20 ^{cd}	70.06 ^{cd}
C3M2	9.97 ^{bcd}	36.28 ^{abc}	7.28 ^{bc}	53.74 ^{ab}	107.27 ^{abc}
C3M3	18.59 ^a	41.02 ^a	17.68 ^a	57.82 ^a	135.11 ^a
LSD 0.05	7.93	16.96	9.76	25.60	45.45

Note: FWEL (Fresh Weight of edible leaves), FWNL (Fresh Weight of non-edible leaves), FWES (Fresh weight of edible stem), FWNS (Fresh weight of non-edible stem), TFW (Total fresh weight).

No significant differences were found between treatments C2 and C3 (mild drought), suggesting that chaya plants can maintain near-optimal growth and biomass production under mild to moderate water stress conditions. Although manure application did not

result in statistically significant differences in the yield of edible parts, the use of goat manure generally led to higher fresh weight components compared to other treatments (Table 6). Non-edible biomass, which includes older stems and leaves that are not consumed, was found to be greater than the edible biomass (Table 7). On average, the non-edible biomass was approximately twice the amount of the edible biomass. This trend can be attributed to the ability of goat manure to enhance soil physical properties and nutrient availability, thereby supporting improved plant growth and biomass accumulation under drought stress.

Table 7. The component dry weight of chaya in drought stress treatment and manure applications

Treatment	DWEL (g)	DWNL (g)	DWES (g)	DWNS (g)	WCEL (%)	WCNL (%)	WCES (%)	WCNS (%)
Drought stress								
C1	0.79 ^b	3.14 ^b	0.35 ^a	2.98 ^b	88.61 ^a	77.94 ^a	91.33 ^a	71.49 ^a
C2	1.61 ^a	5.37 ^a	0.54 ^a	5.43 ^a	88.03 ^a	71.67 ^a	93.72 ^a	64.33 ^a
C3	1.59 ^a	5.56 ^a	0.39 ^a	5.78 ^a	86.28 ^a	71.79 ^a	94.15 ^a	66.05 ^a
BNT 0.05	0.56	1.47	0.23	1.63	5.04	13.56	2.97	8.41
Manure application								
M1	1.14 ^a	3.43 ^b	0.29 ^b	3.04 ^b	88.02 ^a	72.83 ^a	93.64 ^a	67.63 ^a
M2	1.27 ^a	4.55 ^b	0.46 ^{ab}	5.28 ^a	87.61 ^a	76.81 ^a	91.93 ^a	67.21 ^a
M3	1.59 ^a	6.10 ^a	0.53 ^a	5.87 ^a	87.29 ^a	71.77 ^a	93.63 ^a	67.03 ^a
BNT 0.05	0.56	1.47	0.23	1.63	5.04	13.56	2.97	8.41
Interaction of drought stress and organic matter								
C1M1	0.84 ^{bc}	2.29 ^d	0.24 ^b	2.07 ^d	87.92 ^a	81.87 ^a	92.72 ^{ab}	72.44 ^{ab}
C1M2	0.54 ^c	2.93 ^{cd}	0.35 ^{ab}	3.34 ^{cd}	89.58 ^a	78.32 ^a	88.46 ^b	64.86 ^{ab}
C1M3	1.01 ^{bc}	4.22 ^{bcd}	0.46 ^{ab}	3.53 ^{cd}	88.33 ^a	73.63 ^a	92.80 ^{ab}	77.19 ^a
C2M1	1.20 ^{abc}	3.45 ^{cd}	0.40 ^{ab}	4.03 ^{bcd}	89.65 ^a	65.15 ^a	93.79 ^a	64.28 ^{ab}
C2M2	1.64 ^{ab}	5.31 ^{abc}	0.58 ^{ab}	5.70 ^{abc}	88.93 ^a	83.08 ^a	93.77 ^a	65.99 ^{ab}
C2M3	1.99 ^a	7.35 ^a	0.65 ^a	6.57 ^{ab}	85.51 ^a	66.83 ^a	93.59 ^{ab}	62.72 ^{ab}
C3M1	1.39 ^{abc}	4.54 ^{bcd}	0.23 ^b	3.03 ^{cd}	86.49 ^a	71.47 ^a	94.42 ^a	66.17 ^{ab}
C3M2	1.63 ^{ab}	5.41 ^{abc}	0.45 ^{ab}	6.80 ^{ab}	84.31 ^a	69.08 ^a	93.55 ^{ab}	70.78 ^{ab}
C3M3	1.77 ^{ab}	6.73 ^{ab}	0.50 ^{ab}	7.52 ^a	88.03 ^a	74.83 ^a	94.48 ^a	61.19 ^b
BNT 0.05	0.97	2.55	0.40	2.82	8.74	23.49	5.14	14.57

Note: DWEL (Dry Weight of edible Leaves), DWNL (Dry Weight of non-edible Leaves), DWES (Dry Weight of Edible Stem), WCEL (Water content edible leaves), WCNL (Water content non edible leaves), WCES (Water content edible stem), and WCNS (Water content of non edible stem).

The edible part of the chaya plant consists primarily of young, succulent leaf shoots, which typically contain more than 85% water, highlighting their high moisture content. Additionally, the water content in the non-edible parts was lower than in the edible parts, indicating that these older tissues have a drier and more fibrous structure (Table 7). Treatment C1 (severe drought stress) resulted in the lowest dry weight of biomass production for both leaves and stems compared to other treatments. Meanwhile, treatments C2 and C3 did not show significant differences in biomass, suggesting that chaya plants can maintain biomass production under mild to moderate drought stress conditions. Notably, the combination of moderate drought stress (C3) with goat manure application yielded the highest total biomass among all treatment combinations. This result indicates that under less severe drought stress, the addition of goat manure

effectively supports plant growth, likely through improvements in soil physical properties and nutrient availability.

This study demonstrates that drought stress reduces the fresh weight of both edible and non-edible components, including leaves and stems. Edible components in chaya plants are shoots in the form of stems and young leaves, generally found in the top 5 (five) leaves on each shoot (Gustiar et al., 2023b). Plant weight is influenced by the amount of water absorbed by the plant; the more water absorbed, the higher the plant's fresh weight. Drought stress inhibits dry weight production mainly through ² inhibitory effect (Yang et al., 2021). In the manure application treatment, the highest fresh weight and dry weight were obtained in the goat manure treatment. One of the factors that supports the occurrence of wet weight is the presence of nutrients whose primary function is to improve plant growth, especially in the roots. There is a linear or directly proportional effect between the wet weight of the plant and the dry weight of the plant. In general, the components of the chaya plant that can be eaten as vegetables are lower ¹ than those that cannot be eaten. The biomass produced can be used as animal feed. Chaya leaves are a valuable source of protein and other nutrients for livestock, including both ruminants and poultry (Totakul et al., 2021).

CONCLUSIONS

The results of the study indicate that Chaya is a plant species that can grow well under certain levels of drought stress. When water conditions are too wet, it inhibits several parameters of growth and yield. While the use of fertilizers has a beneficial effect in meeting nutrient needs, it also enhances the physical properties of the soil. The use of goat manure results in greater growth compared to other treatments. This is related to the ability of goat manure to improve the physical properties of the soil, making the soil moisture conditions more suitable for the growth of chaya.

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