

Comparative Evaluation of Vermicompost and Vermicompost Leachate on the Growth Performance, Soil Properties and Nutrient Content of Field-Cultivated *Clinacanthus nutans*

Japri, Norsyuhada^{1,2}; Yusuf, Nor Azma^{1*};
Abdullah, Rosazlin^{2,3} & Mahmood, Noor Zalina⁴

¹Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Cawangan Melaka, Kampus Jasin, 77300 Merlimau, Melaka, Malaysia

²Institute of Biological Sciences, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

³Centre for Research in Biotechnology for Agriculture (CEBAR), Institute of Biological Sciences, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

⁴Centre of Excellence – Environmental, Social and Governance (UMCoE-ESG), Universiti Malaya, 50603 Kuala Lumpur, Malaysia

*Correspondence: azma_yusuf@uitm.edu.my

ABSTRACT

The prolonged overuse of inorganic fertilizers has raised environmental concerns, prompting the search for alternative nutrient management strategies that can maintain crop productivity. Vermicompost (VC) and vermicompost leachate (VCL) show promise as organic nutrient sources; however, their effectiveness under field conditions for medicinal crops remains inadequately documented. This study evaluated the effects of VC and VCL on the growth performance, soil properties, and nutrient assimilation of *Clinacanthus nutans*, a high-value medicinal herb with antiviral, wound-healing and anticancer agent. A field experiment was conducted using a randomized complete block design (RCBD) with four treatments: (T1) control; (T2) recommended rate of chemical fertilizer; (T3) vermicompost (15 t ha⁻¹); and (T4) an integrated treatment combining 50% chemical fertilizer with foliar VCL (2 mL L⁻¹). Results indicated that the full CF treatment produced the highest biomass, whereas T4 achieved statistically comparable yields despite a 50% reduction in synthetic fertilizer input. Vermicompost significantly enhanced soil organic carbon and potassium availability but exhibited a slower nitrogen release. The T4 treatment also reduced soil alkalinity while promoting greater nutrient accumulation. Correlation analysis showed that plant nutrient concentrations, particularly nitrogen and potassium, were more closely associated with growth and biomass accumulation than total soil nutrient levels. These findings demonstrate that combining reduced chemical fertilization with vermicompost leachate represents a viable and bio-economic strategy for sustainable cultivation of *C. nutans* without compromising yield.

Keywords: *Clinacanthus nutans*; vermicompost leachate; integrated nutrient management; nutrient use efficiency; sustainable agriculture

INTRODUCTION

Global agriculture continues to depend strongly on fertilizers to sustain the increasing of food production demands, with chemical fertilizers prevailing due to their rapid nutrient release and demonstrated effectiveness in boosting crop yields (Al-Joboory, 2025). However, the prolonged and excessive use of chemical fertilizers has generated serious concerns regarding soil degradation, environmental contamination, and contributions to climate change where such practices have been reported to cause plant toxicity, nutrient imbalances, and health risks arising from nitrate accumulation in edible crops, while also lowering soil pH, suppressing beneficial microbial communities, and inhibiting vital enzymatic processes (Liu *et al.*, 2024; Tayoh, 2020). In response to these challenges, organic fertilizers have

emerged as a more sustainable alternative that not only improves soil fertility but also enhances plant resilience and promotes long-term environmental sustainability (Liu *et al.*, 2024; Li *et al.*, 2025; Palazzolo *et al.*, 2019).

Among organic fertilization approaches, vermicomposting has gained increasing attention as an efficient and environmentally sustainable production of high-quality organic fertilizers (Mohite *et al.*, 2024). The process employs earthworms to biologically decompose organic materials, converting them into nutrient-rich compost through synergistic interactions between earthworms and associated microorganisms (Rehman *et al.*, 2023; Mor *et al.*, 2024; Singh *et al.*, 2023). The synergistic relationship between earthworms and decomposer microbes promotes rapid organic matter mineralization while alleviating the environmental burden of organic waste (Kumari & Kumari, 2025). Numerous studies have demonstrated that vermicompost application enhances root proliferation, nutrient uptake efficiency, and overall crop productivity, with notable improvements observed in crops such as pineapple (Mahmud *et al.*, 2018) cowpea, banana, cassava (Ambasta & Kumari, 2013) and pepper (Sivasakhthivelan *et al.*, 2021).

During the vermicomposting process, a liquid byproduct known as vermicompost leachate (VCL) is also generated. This leachate contains a high concentration of soluble nutrients, organic acids, and plant growth-promoting compounds that contribute to enhanced plant development (Kandari *et al.*, 2011). Empirical evidence indicates that the application of VCL significantly improves key growth parameters, including plant height, leaf expansion, and root system development (Kandari *et al.*, 2011; Tilth *et al.*, 2021). For instance, cucumber plants treated with VCL exhibited superior vegetative growth and yield performance compared to those cultivated in nutrient-deficient soils (Tilth *et al.*, 2021).

In this study, *Clinacanthus nutans* (Burm. f.) Lindau (*C. nutans*), commonly known as Sabah snake grass or “Belalai Gajah,” was selected as the experimental species to evaluate the effects of vermicompost and vermicompost leachate applications. *C. nutans* is indigenous to Malaysia and has been identified as a priority medicinal species in the NKEA Malaysian Herbal Monograph 2012 due to its therapeutic potential (Kong *et al.*, 2016). Scientifically, these plants have potential in medicine such as anticancer, antidiabetic, antibacterial, anti-inflammatory, anti-neurological inflammation, antimicrobial, antitumor and antioxidant, antibiotic, and antiapoptotic (Panjaitan *et al.*, 2023). Previous studies have demonstrated that organic fertilizer application can enhance the quality of medicinal plants by improving their nutritional composition and stimulating the accumulation of bioactive secondary metabolites, which an important consideration in the cultivation of medicinal and aromatic species, where phytochemical quality determines therapeutic efficacy (Hendawy *et al.*, 2020). Furthermore, Ismail-Embong (2021) highlighted that an optimal root-zone environment is critical for achieving maximum growth performance in *C. nutans*. However, the effects of organic fertilization, particularly vermicompost and vermicompost leachate, on the growth and physiological performance of *C. nutans* remain insufficiently explored. Therefore, this study aims to evaluate the effects of vermicompost and vermicompost leachate applications on growth performance and physiological attributes of *C. nutans* under field conditions.

MATERIALS AND METHODS

Experimental site and land preparation

The study was conducted at the Glami Lemi Biotechnology Research Centre (PPBGL), Jelebu, Negeri Sembilan (3°3'13.76"N, 102°3'50.72"E). This site is characterized by a warm

tropical climate, with mean monthly precipitation ranging from 70 to 215 mm and average temperatures between 23°C and 33°C. Prior to planting, the site underwent thorough ploughing to ensure optimal soil aeration and tilth. Sixteen (16) experimental plots, each measuring 1m×5m, were delineated and covered with silver-shine mulching film to suppress weed growth and conserve soil moisture. The entire experimental area was fenced to prevent intrusion by vertebrate pests.

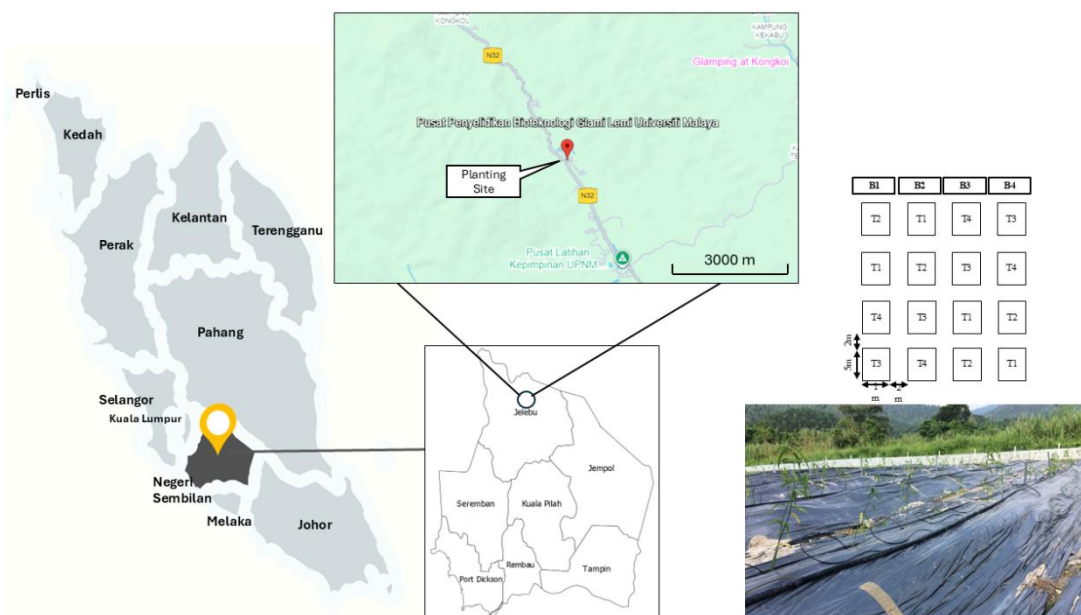


Figure 1. Location and experimental layout at Glami Lemi Biotechnology Research Centre (PPBGL), Jelevu, Negeri Sembilan, Malaysia.

Plant Materials

Clinacanthus nutans (Burm.f.) Lindau plants were obtained from TKC Herbal Nursery Sdn. Bhd., Seremban. Uniform stem cuttings (20 cm length, <1 week old) were transplanted into the prepared plots with a planting distance of 30 cm between plants. The crop was maintained for a duration of 56 days (8 weeks).

Experimental Design and Treatments

The experiment employed a randomized complete block design (RCBD) with four replications. The four treatments were: (T1) unfertilized control; (T2) chemical fertilizer at the recommended rate; (T3) vermicompost at 15 t ha⁻¹; and (T4) reduced chemical fertilizer at 250 kg ha⁻¹ supplemented with foliar vermicompost leachate at 2 mL L⁻¹. The chemical fertilizer utilized was NPK (15:15:15), with dosage rates adapted from the Department of Agriculture Malaysia (2013). Soil amendments were applied basally and incorporated two weeks prior to planting to ensure nutrient stabilization. For T4, the foliar VCL concentration was based on effective rates reported by Singh et al. (2010) with minor modifications, and foliar spray applications were delivered via a hand pressure sprayer (1000 L ha⁻¹) at weekly intervals (days 14, 21, 28, 35, 42, and 49 after planting). To prevent treatment cross-contamination, plots were spaced for 2 m apart. Field management was uniform across all blocks, consisting of manual weeding every two weeks and drip irrigation twice daily.

Measurement of growth and yield parameters

Non-destructive growth parameters (plant height, stem diameter, leaf number, and SPAD chlorophyll content) were assessed at 7-day intervals on five randomly tagged plants per plot. Plant height was measured in centimetres (cm) using a meter ruler, stem diameter in millimetres (mm) using a vernier calliper, and leaf number was counted manually. Additionally, leaf chlorophyll content was recorded using a portable chlorophyll meter (SPAD-502, Minolta Co. Ltd., Osaka, Japan). Destructive sampling was conducted at 56 days after planting (DAP) for harvesting and yield quantification. Prior to harvest, the plants were watered to maintain turgidity, harvested in the morning and cleaned of soil debris. Fresh yield parameters (total fresh weight, leaf weight, and stem weight) were recorded, and the leaf samples were subsequently oven-dried for 48 h at 60°C.

Plant nutrient content analysis

Leaf samples of *C. nutans* were collected for chemical analysis to assess the impact of fertilizer treatments on nutritional composition. To ensure sample integrity, leaves were cleaned using a damp lint less cloth. Heavily soiled leaves were treated with 0.2% phosphate-free detergent followed by a thorough rinse, taking care to avoid soaking to prevent nutrient leaching. The samples were cut and dried in an air-draught oven at 70 ± 10 °C until a constant crisp texture was achieved, then finely ground and stored in airtight containers following the guidelines of the Department of Standards Malaysia (1980). Total nitrogen (TN) content was determined via the dry combustion method (Dumas Method, 2003) using a LECO Nitrogen Analyzer FP-528 (United Kingdom). Ground samples were combusted at 950 °C in an oxygen-rich atmosphere, and the resulting nitrogen gas was quantified via thermal conductivity. For the determination of total phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), a dry ashing and acid digestion method was employed. Briefly, 0.5 g of sample was oven-dried at 105 °C, ignited at 200 °C for two hours, and subsequently ashed at 550 - 600 °C for five hours. The ash was dissolved in HCl and re-ignited before undergoing digestion with nitric acid. The final filtrate was analyzed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Varian, Australia). Instrument calibration was performed using multi-element standards (Merck, Darmstadt, Germany) for K, Ca, and Mg (0 - 100 ppm) and P (0 - 50 ppm).

Soil chemical analysis

Soil samples were collected at 56 days after planting (DAP) from the 0-15 cm soil depth. The samples were composited, air-dried, and sieved to pass through a 2.0 mm mesh. Soil pH and electrical conductivity (EC) were measured using soil-water suspension at ratios of 1:2.5 and 1:5 (w/v) respectively. Cation Exchange Capacity (CEC) was determined using the ammonium acetate leaching method (Chapman, 1965), followed by distillation and titration with 0.01 N HCl. Total organic carbon (TOC) was determined using the Walkley and Black wet oxidation method (Walkley & Black, 1934; Mahmud *et al.*, 2016). Briefly, 0.5 g soil sample was mixed with 10 mL of 1 N $K_2Cr_2O_7$, 20 mL of concentrated H_2SO_4 , and 3 mL of H_3PO_4 , and allowed to react for one hour. The mixture was then diluted with 120 mL distilled water, and diphenylamine indicator was added before titrating the excess dichromate with ferrous sulphate until the blue colour disappeared. TOC was calculated as:

$$\% C = \frac{\text{Vol. of } K_2Cr_2O_7 - \text{Vol. of FAS (f)}}{\text{Weight of soil}} \times \frac{100}{1000}$$

Total nitrogen (TN) was analyzed using the Kjeldahl method (Bremner & Mulvaney, 1982; Mahmud *et al.*, 2016). Samples (0.5 g) were digested with sulfuric acid and a catalyst at 350°C, distilled with 30% NaOH, and titrated with 0.01 N HCl. Nitrogen content was calculated using:

$$\% \text{ N} = \frac{[(\text{Amount of HCL in mL} - \text{Blank}) \times 0.00014 \times 100 \times 10]}{\text{Weight of soil sample (g)}}$$

Total P, K, Ca, and Mg contents were determined using the aqua regia digestion method (Chen & Ma, 2001; Nieuwenhuize *et al.*, 1991). A 0.5 g soil sample (<0.25 mm) was digested with 4 mL of a 3:1 (v/v) HCl-HNO₃ mixture at 110 °C until near dryness. The residue was dissolved in 10 mL of 1.2% HNO₃, reheated, filtered through Whatman No. 42 paper, and diluted up to 50 mL. The suspension was analyzed using an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES; Varian, Australia) for elemental concentrations.

Statistical analysis

Data were analysed using IBM SPSS Statistics version 24 (IBM Inc., Armonk, NY, USA). Normality was tested at the 95% confidence level ($p > 0.05$). Results are presented as mean $\pm$ standard error of the mean (S.E.M). Plant growth parameters were analysed using a general linear model (multivariate and repeated measures). Mean differences were determined using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) at the 5% significance level. Pearson's correlation analysis was conducted to assess relationships between soil properties, plant nutrient concentrations, and growth parameters. Partial Least Squares Regression (PLSR) was performed using XLSTAT version 2021.1 (Addinsoft Inc., Paris, France) to evaluate relationships among plant growth performance, soil properties, and plant nutrient contents. Variable Importance in Projection (VIP) scores were calculated, with values >0.8 considered important (90% confidence level) and >1.0 considered most important (95% confidence level).

RESULTS AND DISCUSSION

Effects of nutrient management on plant growth

The growth performance of *C. nutans* was evaluated under four treatments: (T1) Control; (T2) CF at 500 kg ha⁻¹; (T3) VC at 15 t ha⁻¹; and (T4) Reduced CF at 250 kg ha⁻¹ + VCL at rate 2 mL L⁻¹. The effect of different fertilizer treatment on the vegetative growth of *C. nutans* is illustrated in Figure 2.

As shown in **Figure 2(a)**, plant height increased steadily across all treatments over the 49-day cultivation period. Vermicompost application (T3) resulted showed the highest reading, reaching approximately 55.0 cm at 49 DAP, which was significantly higher ($p < 0.05$) than the unfertilized control (T1). The chemical fertilizer alone (T2) and the integrated treatment (T4) exhibited intermediate growth with no significant difference between the two treatments at the final measurement. These findings align with Wang *et al.* (2017), who reported that organic matter-rich amendments improve soil structure and water-holding

capacity, thereby supporting better root development and more sustained shoot growth compared with inorganic fertilizer inputs alone.

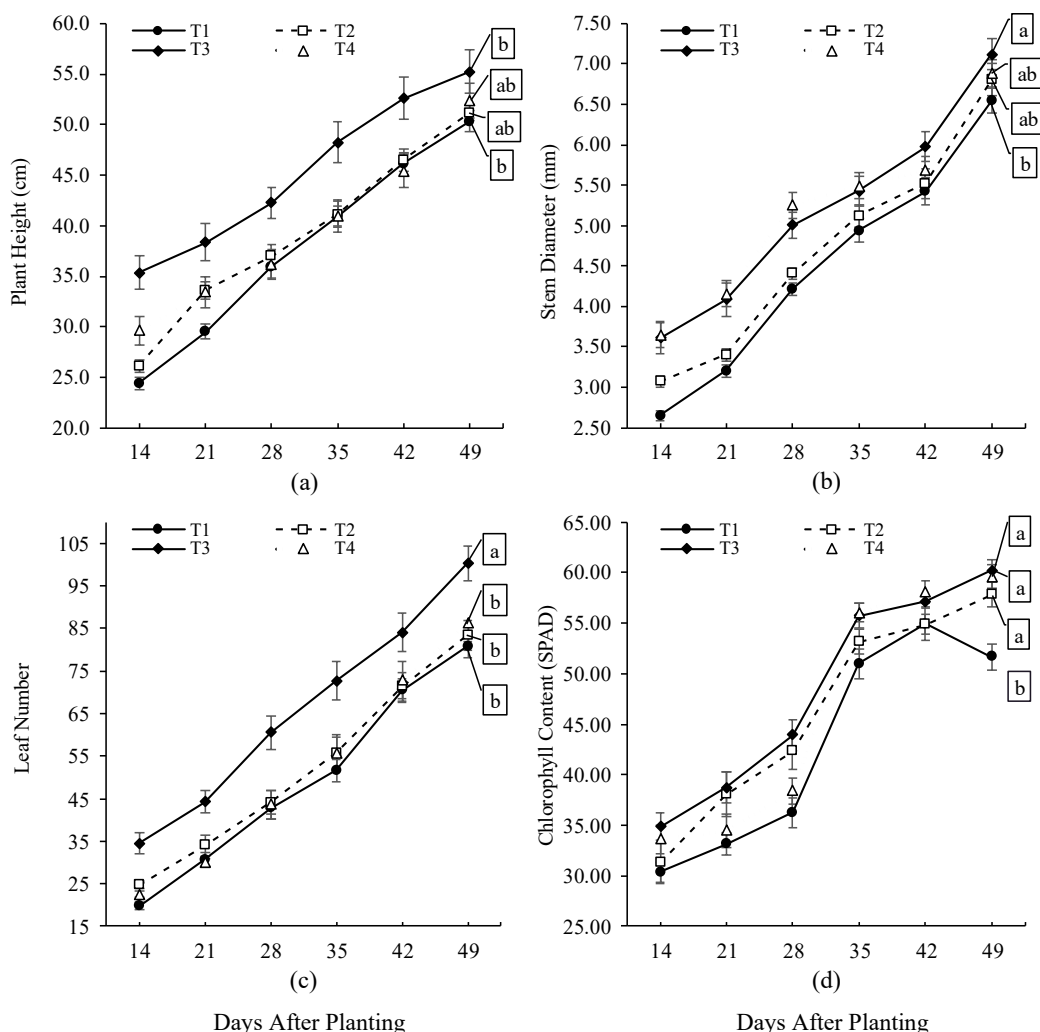


Figure 2. Effects of different treatments on (a) plant height, (b) stem diameter, (c) leaf number, and (d) chlorophyll content (SPAD) of *C. nutans* from 14 to 49 days after planting (DAP).

Note: Error bars represent the standard error of the mean (n=4). Means with different letters at the final sampling date indicate significant differences at *p* < 0.05.

T1: Control; T2: CF at 500 kg ha⁻¹; T3: VC at 15 t ha⁻¹; T4: CF at 250 kg ha⁻¹ + VCL at 2 mL L⁻¹.

A similar trend was observed for stem growth in **Figure 2(b)**. Vermicompost-treated plant (T3) developed significantly thicker stems (7.1 mm) compared with those in the control (6.5 mm). The integrated treatment (T4) showed stem diameters comparable to T3 during the early growth stage (14 - 28 DAP) but exhibited a slight plateau toward 49 DAP. The greater stem thickening under T3 is likely linked to bioactive compounds in vermicompost, particularly humic substances and plant growth-regulating hormones such as auxins and cytokinins, which are abundant in earthworm casts and known to promote cell division and stem tissue development. Increased stem diameter also enhances structural stability, supporting canopy development and facilitating efficient transport of water and photo-assimilates required for sustained plant growth (Pu *et al.*, 2016; Picazo *et al.*, 2025).

Leaf development showed a more pronounced response to nutrient management in **Figure 2(c)**. Vermicompost treatment (T3) consistently produced the highest leaf numbers, exceeding 100 leaves per plant at 49 DAP, which was significantly higher ($p < 0.05$) by approximately 25% compared with T2 and T4. No significant differences were observed among T1, T2, and T4, all of which stabilised at around 80–85 leaves per plant. The limited response under T2 suggests that inorganic nitrogen alone supports vertical growth but may be insufficient to promote lateral meristem activation. Similar findings were reported by Suthar (2009), who attributed increased leaf production under vermicompost application to growth-promoting substances and micronutrients (Cu, Fe, Mn, and Zn), which likely contributed to enhanced leaf initiation in T3.

Chlorophyll content also shows a significantly different among treatments **Figure 2(d)**. SPAD values for T3 and T4 were comparable to those of the full chemical fertilizer treatment (T2), reaching approximately 60.0, and were significantly higher than the control value of 52.0 ($p < 0.05$). This indicates that foliar application of vermicompost leachate in T4 effectively compensated for the reduced synthetic nitrogen input, maintaining chlorophyll levels similar to those achieved with full chemical fertilization. The sustained photosynthetic capacity under T4 is likely supported by essential micronutrients supplied through the vermicompost leachate, particularly magnesium and iron, which play key roles in chlorophyll synthesis and stability (Mahmud *et al.*, 2019).

Effects of vermicompost and integrated fertilization on plant yield and biomass

Harvestable yield and biomass distribution of *C. nutans* were assessed at 56 days after planting (DAP). The effects of the different nutrient management strategies on total leaf number, fresh leaf weight, fresh stem weight, and total shoot biomass are presented in **Figure 3**.

Leaf production, which represents the primary component of *C. nutans*, was significantly influenced by fertilization treatment ($p < 0.05$) (**Figure 3a**). Plants receiving vermicompost (T3) produced the highest number of leaves per plant (118), which was significantly greater than in the control (T1) with 98 leaves, and the chemical fertilizer treatment (T2) with 104 leaves. The integrated treatment (T4) also performed well, producing 110 leaves per plant, and did not differ significantly from T3. These results indicate that organic nutrient inputs, applied alone or in combination with reduced chemical fertilizer, effectively enhanced leaf development. This response is likely associated with the rich microbial communities present in vermicompost, which improve nutrient availability and uptake, supports healthier, more resilient agroecosystems (Prisa & Jamal, 2025; Papathanasiou *et al.*, 2012; Arancon *et al.*, 2006; Ferreras *et al.*, 2006; Arancon *et al.*, 2004). In addition, foliar application of vermicompost tea or leachate supplies readily available nutrients, particularly nitrogen, phosphorus, and potassium, which are essential for leaf initiation and expansion, thereby supporting improved leaf growth and protection against pathogen (Gutierrez-Miceli *et al.*, 2008; Yassen *et al.*, 2020; Prisa & Jamal, 2025).

Fresh leaf weight shows decreased in the order of T3, T4, T2, and T1 (**Figure 3b**). The highest fresh leaf weight was recorded under T3 (20.5 g per plant), which was significantly greater than that of the unfertilized control (18.2 g per plant). Leaf yields under T4 and T2 were statistically comparable to T3, indicating that a 50% reduction in chemical fertilizer in T4 did not significantly reduce leaf yield when supplemented with vermicompost leachate. This comparable performance suggests that foliar applied vermicompost leachate maintained nutrient availability and photosynthetic capacity, as reflected by similar chlorophyll content among T2, T3, and T4. Agronomically, these results demonstrate the

potential of integrated fertilization strategies to sustain yield while reducing reliance on synthetic fertilizers (Srinivasan *et al.*, 2017).

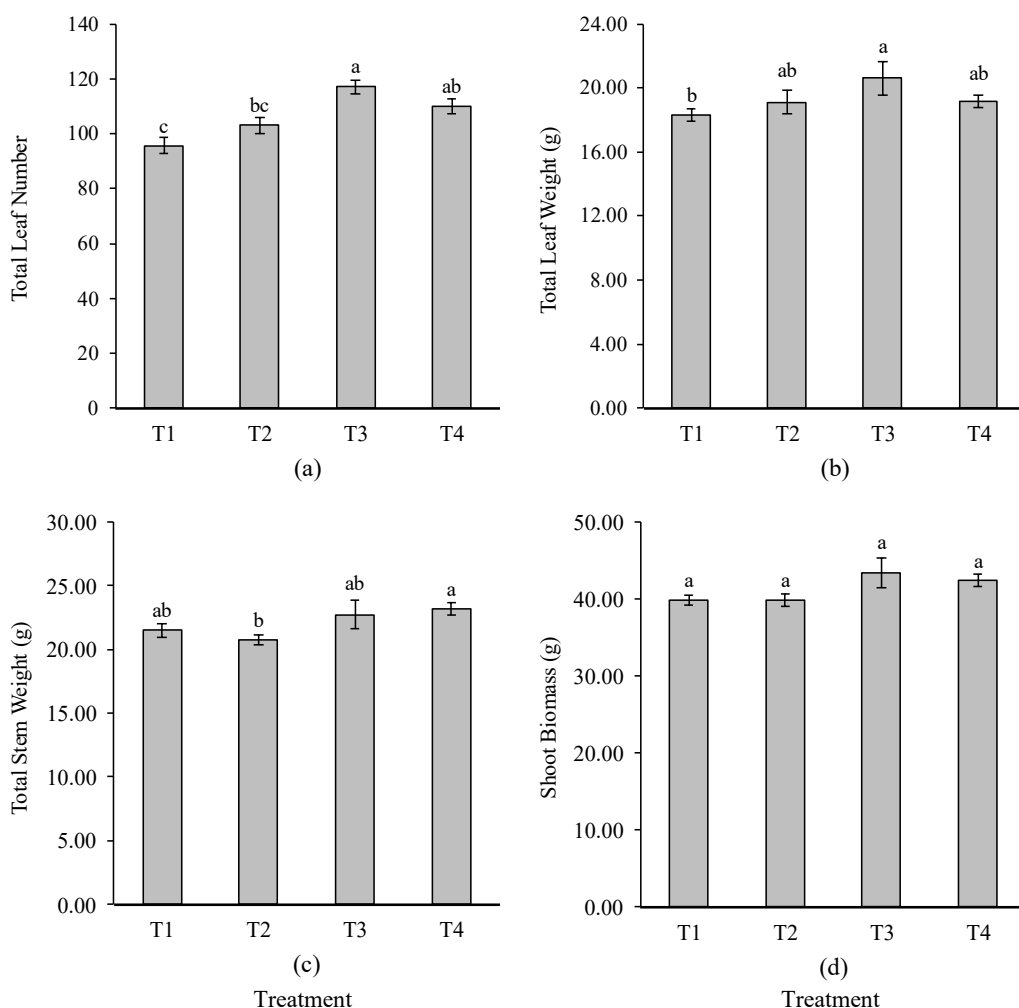


Figure 3. Effects of different nutrient management strategies on (a) total leaf number, (b) total leaf weight, (c) total stem weight, and (d) total shoot biomass of *C. nutans* at harvest (56 DAP).

Note: Vertical bars represent the standard error of the mean (n=4). Means with different letters indicate significant differences at $p < 0.05$.

T1: Control; T2: CF at 500 kg ha⁻¹; T3: VC at 15 t ha⁻¹; T4: CF at 250 kg ha⁻¹ + VCL at 2 mL L⁻¹.

Meanwhile, the stem biomass responded differently to nutrient management strategies (**Figure 3c**). The integrated treatment (T4) produced the highest stem weight (23.0 g), which was significantly greater than that observed in T2 (20.5 g). The comparatively lower stem biomass under T2 suggests that inorganic fertilizer alone may preferentially promote rapid leaf expansion rather than structural growth. In contrast, treatments incorporating organic amendments (T3 and T4) likely provided a more sustained and balanced nutrient supply, promoting coordinated development of both leaves and stems. This balanced growth indicates more efficient nutrient utilization, likely due to the synchronized nutrient release characteristic of organic amendments (Ibrahim & Fatondji, 2020).

Total shoot biomass (**Figure 3d**) which reflects the overall harvestable yield, highlights the practical relevance of the integrated approach. Although no statistically

significant differences were detected among treatments, the biological response is noteworthy. The integrated treatment (T4) produced biomass levels comparable to those achieved with the full chemical fertilizer treatment (T2), even though synthetic fertilizer input was reduced by 50%. This indicates that the yield reduction commonly expected when chemical fertilizer rates are lowered was effectively offset by the application of vermicompost leachate.

Effects of fertilization on soil and plant chemical analysis

Soil physico-chemical properties

Table 2 presents the soil chemical properties at 56 days after planting (DAP). Fertilization treatments significantly influenced soil pH and electrical conductivity (EC), whereas cation exchange capacity (CEC) did not differ significantly among treatments.

Table 2. Soil physico-chemical properties at harvest (56 DAP).

Total Element	T1	T2	T3	T4
pH	8.7 ± 0.0 ^a	8.6 ± 0.1 ^a	8.6 ± 0.0 ^a	5.5 ± 0.2 ^b
CEC (cmol/kg)	3.77 ± 0.31 ^a	3.59 ± 0.20 ^a	4.17 ± 0.27 ^a	3.68 ± 0.06 ^a
EC (µS/cm)	88.68 ± 2.07 ^b	94.13 ± 2.63 ^{ab}	79.08 ± 1.48 ^c	97.03 ± 1.32 ^a

Note: Values represent Mean ± S.E.M (n=4). Means within a row followed by the same letter are not significantly different at $p > 0.05$ according to Duncan's Multiple Range Test (DMRT).

T1: Control; T2: CF at 500 kg ha⁻¹; T3: VC at 15 t ha⁻¹; T4: CF at 250 kg ha⁻¹ + VCL at 2 mL L⁻¹.

The control (T1), chemical fertilizer alone (T2), and solid vermicompost (T3) treatments maintained strongly alkaline conditions, with pH values ranging from 8.6 to 8.7 and no significant differences among them. In contrast, the integrated treatment (T4) resulted in a substantial decline in soil pH to 5.5 ($p < 0.05$) despite receiving 50% less synthetic fertilizer. This specific pH reduction is attributed to synergistic nitrification, where the microbial-rich vermicompost leachate accelerates the oxidation of co-applied ammonium (Tith *et al.*, 2021). Unlike T2, where high alkalinity likely inhibited native nitrifiers, the introduced microbial activity in T4 promoted rapid ammonium oxidation, releasing protons (Cameron *et al.*, 2013). Furthermore, VCL-enhanced root activity increases ammonium uptake, triggering a compensatory proton efflux, which collectively neutralizes soil alkalinity (Jing *et al.*, 2012).

Despite the notable change in soil pH, CEC remained unchanged across treatments ($p > 0.05$), indicating that the short-term acidification observed under T4 did not alter the soil's underlying exchange capacity or nutrient retention potential. This suggests that pH modification occurred primarily within the rhizosphere rather than through structural changes to soil colloids. Meanwhile, electrical conductivity varied significantly among treatments. Vermicompost alone (T3) resulted in the lowest EC, reflecting its low salt index and gradual nutrient release, which minimizes osmotic stress in the root zone. In contrast, the integrated treatment (T4) recorded the highest EC, likely due to the combined contribution of soluble ions from synthetic fertilizer and vermicompost leachate. Prolonged use of chemical fertilizers may lead to increased soil EC, whereas the addition of organic inputs can mitigate this effect by enhancing nutrient use efficiency and limiting short-term EC buildup (Srivastava *et al.*, 2012; Manivannan *et al.*, 2009).

Soil Nutrients Properties

Soil nutrient contents (N, P, K, Ca, Mg, and TOC) measured at harvest (56 days after planting) are presented in **Table 3**. Soil organic carbon (TOC) showed a strong response to the type of amendment applied. The application of solid vermicompost (T3) significantly increased TOC to 1.26 % compared to control (T1:0.83%), confirming its effectiveness as a soil conditioner that enhances carbon accumulation and improves soil structure (Pan & Huang, 2024; Romaniuk *et al.*, 2011). Consistent with this finding, Azarmi *et al.* (2008) reported that vermicompost application at 15 t ha⁻¹ significantly increased soil total organic carbon in tomato cultivation.

Total nitrogen (N) was highest in the chemical fertilizer (T2) (2.45%) and integrated (T4) (2.17%) treatments, reflecting the direct contribution of mineral nitrogen from inorganic inputs. In contrast, the lower N observed under vermicompost application (T3) (1.68%) is consistent with the slower mineralization and gradual nitrogen release typical of organic amendments (Singh & Wasnik, 2013). Total phosphorus (P) increased to similar levels across all fertilized treatments, indicating that vermicompost was as effective as synthetic fertilizer in maintaining soil phosphorus availability, likely through enhanced solubilization and reduced fixation (Correll, 1998). Soil cation responses varied among treatments. Total potassium (K) concentration was highest under vermicompost application (T3) (0.0366%), supporting evidence that organic amendments are important sources of readily available potassium (Hanč & Pliva, 2013). Conversely, total magnesium (Mg) was greatest under chemical fertilization (T2) (0.0187%), suggesting that inorganic inputs were the primary source of magnesium in this system. Total calcium levels did not differ significantly among treatments, indicating limited short-term sensitivity of soil calcium status to fertilization strategy.

Table 3. Soil nutrients concentration at harvest (56 DAP)

Total Element	T1	T2	T3	T4
TOC (%)	0.83 ± 0.06 ^b	0.72 ± 0.04 ^b	1.26 ± 0.09 ^a	0.91 ± 0.05 ^b
N (%)	1.12 ± 0.20 ^b	2.45 ± 0.14 ^a	1.68 ± 0.43 ^{ab}	2.17 ± 0.09 ^a
P (%)	0.036 ± 0.0013 ^b	0.048 ± 0.0024 ^a	0.048 ± 0.0039 ^a	0.050 ± 0.0026 ^a
K (%)	0.010 ± 0.0002 ^c	0.027 ± 0.0041 ^b	0.037 ± 0.0006 ^a	0.016 ± 0.0019 ^c
Ca (%)	0.098 ± 0.0023 ^a	0.117 ± 0.0023 ^a	0.115 ± 0.0104 ^a	0.113 ± 0.0075 ^a
Mg (%)	0.005 ± 0.0007 ^c	0.019 ± 0.0004 ^a	0.007 ± 0.0008 ^b	0.008 ± 0.0006 ^b

Note: Values represent Mean ± S.E.M (n=4). Means within a row followed by the same letter are not significantly different at $p > 0.05$ according to Duncan's Multiple Range Test (DMRT).

T1: Control; T2: CF at 500 kg ha⁻¹; T3: VC at 15 t ha⁻¹; T4: CF at 250 kg ha⁻¹ + VCL at 2 mL L⁻¹.

Plant nutritional composition

The nutrient composition of *C. nutans* leaves at harvest varied clearly among fertilization treatments (**Table 4**). A clear discrepancy was observed between soil nitrogen availability and nitrogen accumulation in plant tissues. Although the chemical fertilizer treatment (T2) produced the highest residual soil nitrogen levels (**Table 3**), this did not result in enhanced nitrogen uptake by the plants. Leaf nitrogen concentration under T2 (1.68%) was statistically similar with unfertilized control, indicating poor nitrogen use efficiency. In contrast,

treatments receiving organic inputs (T3 and T4) showed significantly higher leaf nitrogen contents, reaching 3.15% and 3.54%, respectively.

The low nitrogen uptake under chemical fertilization is likely related to the strongly alkaline soil conditions ($\text{pH} > 8.0$) at the experimental site. Under such conditions, nitrogen from synthetic fertilizers is prone to losses through volatilization, where ammonium is converted to ammonia gas before it can be absorbed by plants (Cameron *et al.*, 2013). In comparison, vermicompost (T3) and the integrated treatment (T4) likely supplied nitrogen more gradually through microbial mineralization, reducing losses and better matching plant demand throughout the growing period.

Total phosphorus (P) levels appeared to be higher under the integrated treatment (T4) (0.59 %) and followed by vermicompost treatment (T3) (0.51%), likely due to the presence of organic acids in the vermicompost leachate that enhance phosphorus solubilization in the soil (Mulatu & Bayata, 2024). However, due to high variability within the samples, there was no statistically significant difference between the highest (T4) (0.59%) and lowest (T1) (0.41%) values.

Leaf potassium (K) concentration differed significantly among treatments, with values decreasing in the order of T4, followed by T3, T2, and T1 ($p < 0.05$). the integrated treatment (T4) produced the highest leaf potassium concentration (4.87%), significantly exceeding all other treatments.

Table 4. Plant nutritional composition at harvest (56 DAP)

Total Element	T1	T2	T3	T4
N (%)	1.773 ± 0.444^b	1.680 ± 0.151^b	3.150 ± 0.131^a	3.540 ± 0.251^a
P (%)	0.413 ± 0.014^a	0.440 ± 0.012^a	0.508 ± 0.023^a	0.590 ± 0.075^a
K (%)	1.490 ± 0.152^d	2.448 ± 0.152^c	3.798 ± 0.286^b	4.868 ± 0.484^a
Ca (%)	0.450 ± 0.136^{bc}	0.273 ± 0.047^c	0.858 ± 0.111^{ab}	0.958 ± 0.225^{ab}
Mg (%)	0.220 ± 0.018^a	0.240 ± 0.053^a	0.800 ± 0.209^a	0.760 ± 0.309^a

Note: Values represent Mean $\pm$ S.E.M (n=4). Means within a row followed by different letters indicate significant differences at $p < 0.05$ according to Duncan's multiple range test (DMRT).

T1: Control; T2: CF at 500 kg ha^{-1} ; T3: VC at 15 t ha^{-1} ; T4: CF at 250 kg ha^{-1} + VCL at 2 mL L^{-1} .

Calcium (Ca) concentration was recoded to have the lowest reading from the chemical fertilizer treatment (T2) (0.27%), compared with the integrated treatment (T4) (0.96%). This reduction is likely attributable to ionic antagonism, where elevated ammonium (NH_4^+) levels from synthetic fertilizers suppress the uptake of divalent cations such as Ca^{2+} at the root surface (Bonomelli *et al.*, 2021; Marschner, 2011). In contrast, the inclusion of organic amendments in T3 and T4 appeared to mitigate this effect. The slower nitrogen release from organic sources likely prevented excessive ammonium accumulation in the rhizosphere, allowing calcium uptake to remain comparable to the control. Magnesium (Mg) followed a similar trend, with vermicompost-based treatments (T3 and T4) exhibiting slightly higher Mg concentrations with reading of 0.80% and 0.76% than the chemical fertilizer treatment; however, these differences were not statistically significant ($p > 0.05$).

Correlations analysis

Soil properties and leaf nutrient relationships

Pearson's correlation analysis was performed to examine the relationships between soil chemical properties and leaf nutrient composition of *C. nutans* (Figure 4). Strong negative relationship was observed between soil pH and leaf nutrient concentrations (**Figure 4**). A strong negative association was observed between soil pH and leaf nitrogen ($r = -0.643, p < 0.01$), phosphorus ($r = -0.589, p < 0.01$), potassium ($r = -0.732, p < 0.01$), and calcium ($r = -0.537, p < 0.01$). These correlations indicate that the strongly alkaline conditions observed in the control and chemical fertilizer treatments (pH 8.7) constrained nutrient availability, likely through phosphorus precipitation and increased nitrogen losses via volatilization, thereby limiting plant uptake (Neina, 2019). In contrast, the reduction in soil pH under the integrated treatment (T4) appears to be a key mechanism enhancing nutrient availability and subsequent accumulation in plant tissues.

Soil organic carbon (TOC) emerged as a significant positive driver of nutrient uptake. TOC was positively correlated with leaf nitrogen ($r = 0.599, p < 0.01$), calcium ($r = 0.552, p < 0.01$), and magnesium ($r = 0.636, p < 0.01$). These findings support the role of organic matter in enhancing nutrient retention and availability through improved cation exchange processes and reducing nutrient losses from the soil system (Oldfield et al., 2019). In contrast, soil total N, P, K, Ca, Mg, EC, and CEC showed weak or non-significant correlations with leaf nutrient concentrations, suggesting that nutrient bioavailability was more strongly regulated by soil chemical conditions and organic matter dynamics than by total nutrient pools alone.

Leaf nutrients and plant growth relationships

Correlations between leaf nutrient concentrations and plant growth parameters are presented in **Figure 5**. Chlorophyll content (SPAD values) exhibited strong positive correlations with leaf potassium ($r = 0.634, p < 0.01$) and magnesium ($r = 0.523, p < 0.05$). These relationships are physiologically consistent, as magnesium is a central component of the chlorophyll molecule, while potassium plays a crucial role in stomatal regulation and the activation of photosynthetic enzymes (Tränkner et al., 2018).

Shoot biomass exhibited significant positive correlations with leaf nitrogen ($r = 0.530, p < 0.05$) and potassium ($r = 0.502, p < 0.05$), indicating that these macronutrients were key drivers of biomass accumulation in *C. nutans*. In contrast, morphological traits such as plant height, stem diameter, and leaf number were not significantly correlated with individual nutrient concentrations ($p > 0.05$). This suggests that vegetative growth responses are governed by the integrated interaction of multiple physiological processes rather than by the availability of a single nutrient alone.

Partial Least Squares Regression (PLSR) analysis

Partial Least Squares Regression (PLSR) was used to examine the relationships between *C. nutans* growth parameters, soil chemical properties, and *C. nutans* leaf nutrients content. From the correlation biplot in **Figure 6a**, most X variables showed positive loadings, including soil total organic carbon, soil potassium, soil electrical conductivity, leaf nitrogen, leaf potassium, and leaf magnesium. In contrast, soil total nitrogen, soil calcium, and soil magnesium displayed weak or negative loadings. Soil pH was positioned toward the negative side of Dimension 1, suggesting that higher pH may limit nutrient uptake and plant growth under the conditions of this study.

The integrated treatment (T4) was closely associated with higher values of soil organic carbon, soil total potassium and EC together with leaf nitrogen, potassium and magnesium, indicating better soil quality and plant nutrient uptake. In comparison, the chemical fertilizer-only treatment (T2) was mainly associated with higher soil nitrogen but showed weak relationships with plant growth variables. These patterns are consistent with the treatment effects observed in the earlier ANOVA results.

	Leaf TN	Leaf TP	Leaf TK	Leaf Ca	Leaf Mg	<i>r</i>
Soil pH	-0.643**	-0.589**	-0.732**	-0.537**	-0.311	1.0
Soil TOC	0.599**	0.376	0.464	0.552**	0.636**	0.8
Soil TN	-0.034	0.180	0.288	-0.175	-0.056	0.5
Soil TP	0.372	0.119	0.462	-0.024	0.076	0.3
Soil TK	0.168	0.174	0.277	0.182	0.295	0.0
Soil Ca	0.221	-0.010	0.245	-0.010	0.304	-0.3
Soil Mg	-0.341	-0.157	-0.104	-0.463	-0.257	-0.5
Soil EC	-0.049	0.183	0.138	-0.098	-0.134	-0.8
Soil CEC	0.313	-0.094	0.023	0.051	-0.041	-1.0

Figure 4. Heatmap of Pearson's correlation coefficient (*r*) between soil chemical properties and *C. nutans* leaf nutrients content.

Note: Red and blue colours indicate positive and negative correlations, respectively. Colour intensity represents the strength of the correlation. Asterisks denote statistical significance (**p* < 0.05, ***p* < 0.01).

	Leaf N	Leaf P	Leaf K	Leaf Ca	Leaf Mg	<i>r</i>
Plant Height	0.216	0.241	0.300	0.256	0.362	1.0
Stem Diameter	0.456	0.224	0.371	0.244	0.264	0.5
Leaf Number	0.431	0.199	0.438	0.274	0.337	0.0
SPAD Chlorophyll	0.389	0.424	0.634**	0.366	0.523*	-0.5
Shoot Biomass	0.530*	0.327	0.502*	0.486	0.428	-1.0

Figure 5. Heatmap of Pearson's correlation coefficient (*r*) between plant growth parameters and *C. nutans* leaf nutrients content.

Note: Red and blue colours indicate positive and negative correlations, respectively. Colour intensity represents the strength of the correlation. Asterisks denote statistical significance (**p* < 0.05, ***p* < 0.01).

The relationships between individual X variables and specific Y variables are further shown in the standardized regression coefficient plots (**Figures 6b - 6f**). Plant height was

positively associated with leaf Ca, Mg, K, N and soil N, P, K, pH, CEC, TOC meanwhile soil leaf P, soil EC and soil Mg showed a negative association on plant height (**Figure 6b**). All parameter shows a positive correlation except for soil EC, calcium and magnesium that shows a negative correlation to stem diameter and leaf number (**Figure 6c** and **Figure 6d**). Soil pH, CEC, EC and magnesium show a negative correlation to plant's relative chlorophyll content (**Figure 6e**). Meanwhile, the same correlation was showed in total biomass except for soil CEC that shows a positive correlation with total biomass (**Figure 6f**).

Based on the variable importance in projection (VIP) values (**Table 5**), only soil organic content, soil potassium, soil electrical conductivity, leaf potassium, nitrogen and magnesium recorded values greater than 1.0, indicating stronger associations with plant growth responses in the PLSR model. In contrast, soil nitrogen, calcium and magnesium showed low importance (VIP=0.505, 0.645 and 0.093), suggesting that total soil nutrient levels alone were weakly related to plant growth. Soil pH had a moderate VIP value (0.754), implying that it may act as a limiting condition rather than a key driver of growth.

The integrated interpretation of the PLSR correlation plot, standardised coefficient profiles, and VIP values demonstrates that plant growth performance of *C. nutans* was primarily governed by internal nutrient accumulation and soil quality related factors rather than by total soil nutrient reserves. The superior performance of vermicompost and integrated fertilization treatments was therefore driven by enhanced nutrient uptake efficiency and availability, while the limited response under chemical fertilizer application alone can be attributed to weak coupling between soil nutrient pools and plant nutrient acquisition.

Table 5. Variable of Importance (VIP) coefficients for the different variables (soil properties and plant nutrients) correspond to the growth performance of *C. nutans*.

Variable	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)
Soil.TOC	1.464	0.422	0.564	2.365
Soil.N	0.505	0.509	-0.581	1.591
Soil.P	0.926	0.508	-0.157	2.009
Soil.K	1.305	0.340	0.580	2.030
Soil.Ca	0.645	0.256	0.099	1.191
Soil.Mg	0.093	0.314	-0.576	0.763
Soil.CEC	0.791	0.472	-0.214	1.796
Soil.pH	0.754	0.277	0.164	1.343
Soil.EC	1.298	0.456	0.327	2.270
Leaf.N	1.124	0.200	0.698	1.551
Leaf.P	0.893	0.154	0.564	1.222
Leaf.K	1.272	0.214	0.817	1.728
Leaf.Ca	0.951	0.209	0.505	1.397
Leaf.Mg	1.073	0.293	0.449	1.697

CONCLUSION

An integrated nutrient management approach using vermicompost and vermicompost leachate proved to be an effective and sustainable alternative to conventional fertilization for *C. nutans*. While the sole application of vermicompost (T3) was most effective in promoting vegetative growth, the integrated treatment (T4) delivered the most balanced agronomic outcome, achieving yields comparable to the standard chemical fertilization treatment (T2) while using only half the amount of synthetic fertilizer. Notably, plants receiving organic-based treatments accumulated higher leaf nitrogen despite lower soil nitrogen levels, suggesting improved nutrient availability and more efficient nutrient utilization within the plant-soil system. Collectively, these findings indicate that combining vermicompost leachate with reduced chemical fertilizer represents a practical, sustainable, and cost-effective fertilization strategy capable of maintaining productivity while enhancing nutrient use efficiency in *C. nutans* cultivation.

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