

*Original Research*

# Enhanced Seedling Growth Effect of Copeat by Supplementation with Earthworm Manure as Nutrient Amendment

Yiqi Wu<sup>1,2,3</sup>, Runze Chen<sup>1,2,3</sup>, Fenglu Wang<sup>1,2</sup>, Xinyu Liu<sup>1,3\*</sup>, Huaju Chi<sup>4,\*\*</sup>

<sup>1</sup>Institute of Organic Recycling (Suzhou), China Agricultural University, Suzhou 215100, China

<sup>2</sup>College of Resources and Environment, China Agricultural University, Beijing 100091, China

<sup>3</sup>Jiangsu Kunshan Fruit and Vegetable Science and Technology Centre, Suzhou 215300, China

<sup>4</sup>Xinjiang Institute of Technology Two Mountains Theory and Research Center for High Quality Green Development in Southern Xinjiang, Akesu 843100, China

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## Abstract

To investigate the impact of earthworm manure supplementation on cucumber seedling growth in copeat, various concentrations of earthworm manure were prepared in 20% volume increments. Six treatment groups were established: 100% copeat (CK), T1 (20% earthworm manure + 80% copeat), T2 (40% earthworm manure + 60% copeat), T3 (60% earthworm manure + 40% copeat), T4 (80% earthworm manure + 20% copeat), and T5 (100% earthworm manure). Seed germination and seedling growth were evaluated for each treatment, along with an assessment of the physicochemical characteristics of the seedling substrate, to ascertain the optimal earthworm manure dosage. Findings revealed that the optimal range for earthworm manure supplementation was between 40% and 80%, with the total porosity of the earthworm manure emerging as the primary constraint on seedling growth. Based on the study findings, additional enhancements to the physicochemical properties of the seedling substrate composed of “earthworm manure-copeat” are advised to optimize cucumber.

**Keywords:** copeat, earthworm manure, seedling substrate, cucumber

## Introduction

In the realm of agricultural production, the role of seedling substrates is paramount [1]. A well-crafted seedling substrate can significantly enhance seedling quality, thereby positively impacting crop yield and quality [2]. Moreover, as agricultural mechanization advances, the utilization of seedling substrates aids in improving

sowing and transplanting efficiency, consequently reducing overall agricultural production costs [3]. Hence, the development and application of seedling substrates have garnered increasing attention from agricultural practitioners. Despite the myriad formulations available, peat has historically served as a primary raw material for seedling substrate preparation owing to its exceptional physicochemical properties [4]. However, being a non-renewable resource, extensive peat extraction can lead to severe ecological damage [5]. Recognizing this issue, numerous scholars have devoted significant efforts to

\*e-mail: e-mail: liuxinyu@slaas.cn

\*\*e-mail: 2022126@xjit.edu.cn

identifying alternative materials for seedling substrates [6]. Research on peat substitutes, such as straw compost [7], livestock manure compost [8, 9], and municipal solid waste compost [1], is abundant, with cocopeat being particularly favored by researchers [10-12]. Indeed, our previous studies have highlighted cocopeat's prominence as a primary raw material in Chinese vegetable seedling substrate patents. Nevertheless, cocopeat is not devoid of shortcomings when used in seedling substrates. Its high salt content necessitates extensive rinsing with water before incorporation into substrate formulations, resulting in the inevitable loss of essential nutrients during the rinsing process [13]. Consequently, the substantial substitution of cocopeat for peat without adequate nutrient supplementation could significantly hinder seed germination and seedling growth. Therefore, ensuring appropriate nutrient supplementation for cocopeat is imperative in seedling substrate preparation.

In the realm of nutrient supplementation for cocopeat, organic fertilizers hold an advantage over their chemical counterparts owing to their enriched organic matter content, gradual nutrient release, and eco-friendly attributes [14, 15]. Among these organic options, earthworm manure stands out for its significant specific surface area and porous structure, which facilitate nutrient absorption and utilization by microorganisms such as bacteria, actinomycetes, and fungi. Consequently, it enhances crop growth-promoting effects [16]. Hence, employing earthworm manure for cocopeat nutrient supplementation appears promising. While previous studies have explored earthworm manure as a substrate material, the focus has predominantly been on its direct substitution for peat [17], with limited attention to its role in nutrient supplementation within substrates. Thus, our study seeks to investigate the nutrient supplementation of cocopeat with earthworm manure and its effects on seed germination, seedling growth, and the physicochemical properties of the substrate. The objective is to furnish robust theoretical and practical insights for the effective substitution of peat with cocopeat in seedling substrate preparation.

## Experimental

### Materials

The experimental materials were obtained from Taiyuan Earthworm Farm in Shanxi for the earthworm manure and from Xiamen Xian Tu Horticultural Technology Co., Ltd. for the cocopeat. The cucumber cultivar employed

was 'Shenqing No.1' with a germination rate of 100.00%. The physicochemical properties of the experimental materials are comprehensively outlined in Table 1.

### Methods

The experiment took place between March 23 and April 10, 2024. Six treatments were formulated, varying in volume fractions by increments of 20%. These treatments comprised: pure cocopeat (CK), 80% cocopeat with 20% earthworm manure (T1), 60% cocopeat with 40% earthworm manure (T2), 40% cocopeat with 60% earthworm manure (T3), 20% cocopeat with 80% earthworm manure (T4), and pure earthworm manure (T5). Cocopeat and earthworm manure were meticulously blended according to the designated treatments to yield 10 L of seedling substrate for each treatment. Samples of the seedling substrate for each treatment were procured using a quadrant sampling method and subsequently stored at 4 °C for future use.

### Sowing and Management

Seedlings were cultivated in a 50-cell seedling tray, with one seed allocated per cell, and sown at a depth of 0.5 cm beneath the substrate surface. Each treatment received uniform irrigation totaling 1.6 L per tray and was placed in a growth chamber set to controlled conditions of 25°C temperature and 80% humidity for a duration of 10 days. Throughout the experimental phase, equal volumes of water were administered to each treatment according to the growth stage of the seedlings. Irrigation ceased one day prior to sampling to facilitate substrate desiccation for sampling purposes.

### Determination of the Seed Germination Effect

In accordance with the guidelines outlined in Chinese agricultural industry standard NY/T 525-2021 [18] and considering the research insights provided by Kong *et al.* [19], radish seeds were utilized to assess the germination index (GI) of the seedling substrates in each treatment. Ten milliliters of the extraction solution from each treatment's seedling substrate were applied onto Petri dishes lined with 9 cm filter paper. Distilled water was utilized as the control (CKw). Each Petri dish contained ten seeds and was placed in a constant temperature chamber set at 25°C, where they were kept in darkness for 48 hours. Assessment criteria post-incubation comprised the germination rate, total root

Table 1. Physical and chemical properties of the test material.

Test material	Bulk density (g·cm <sup>-3</sup> )	pH	EC (μS·cm <sup>-1</sup> )	Alk. N/ (mg·kg <sup>-1</sup> )	Ava. P/ (mg·kg <sup>-1</sup> )	Ava. K/ (mg·kg <sup>-1</sup> )	Organic matter/%
Earthworm ma- nure	0.60	7.95	421.33	99.16	627.30	2291.66	10.76
Cocopeat	0.11	6.90	652.00	90.12	50.34	21.25	64.37

length, and fresh weight of seedling roots. The germination index was determined by the germination rate and total root length, as per the following formula:

$$GI = \frac{\text{germination rate of treatment group}}{\text{germination rate of CK}_w} \times \frac{\text{Total root length of treatment group}}{\text{Total root length of CK}_w} \times 100$$

#### *Physicochemical Properties of Seedling Substrates*

In accordance with the agricultural industry standard NY/T 2118–2012 [7], the bulk density, water holding capacity, air-filled porosity, and total porosity of the seedling substrate were assessed employing the ring knife method. Furthermore, the air-water ratio was computed as the ratio of air-filled porosity to water holding capacity. The hydrolyzed nitrogen content was determined via the alkali diffusion method, the available phosphorus content through the molybdenum antimony anti-spectrophotometric method, the readily available potassium content via flame photometry, and the organic matter content via the potassium dichromate volumetric method. pH and EC values were measured using electrode methods.

#### *Growth Indicators for Young Cucumber Seedlings*

Fifteen cucumber seedlings were selected from each tray 15 days post-seeding. Their height was measured using a straightedge, while stem thickness was assessed using a vernier caliper. The leaf area of the cucumber seedlings was determined using the length-width coefficient method [20]. Fully expanded leaves were assessed using a chlorophyll content meter, with the chlorophyll content expressed as SPAD values. The substrate adhering to the fifteen cucumber seedlings was removed using a brush and stored in a sealed container at 4°C for subsequent analysis. The root length and surface area of cucumber seedlings were measured using a root scanner (WinRHIZO) [21]. Both above-ground

and below-ground weights of cucumber seedlings were measured employing an electronic balance. Subsequently, the seedlings were euthanized at 90°C and dried at 60°C until reaching a constant weight. The strong seedling index was computed based on plant height, stem thickness, and plant weight, offering a comprehensive assessment of cucumber seedling quality [22]. The formula is presented as follows:

$$\text{Strong seedling index} = \left( \frac{\text{Stem thickness}}{\text{height}} + \frac{\text{belowground dry weight}}{\text{underground dry weight}} \right) \times (\text{belowground dry weight} + \text{underground dry weight})$$

#### *Seedling Nutrient Uptake*

Following drying, the plant seedlings from each treatment were individually ground using a ball mill and subsequently stored at 4°C in a refrigerator for further analysis. Total nitrogen, phosphorus, and potassium contents in the plant seedlings were analyzed using the Kjeldahl method [23], the molybdenum antimony colorimetric method [24], and flame photometry [25], respectively.

#### *Data Statistics and Analysis*

The raw data from the trial were processed with DPS software [26] and subsequently analyzed through one-way ANOVA, following initial compilation in Excel 2019.

## **Results**

### **Seed Germination**

The findings in Table 2 reveal a notable decrease in both seedling root length and germination index across treatments T1-T5, with increasing proportions

Table 2. Effect of substrate infusions of earthworm manure and cocopeat at different levels of rationing on radish seed germination.

Treatments	Germination rate/%	Root length/cm	GI
CK <sub>w</sub>	100.00±0.00a	36.98±0.21a	-
CK	100.00±0.00a	39.31±1.52a	1.07±0.05a
T1	100.00±0.00a	26.37±0.38c	0.71±0.01c
T2	100.00±0.00a	27.39±0.40c	0.74±0.01c
T3	100.00±0.00a	31.12±0.47b	0.84±0.01b
T4	100.00±0.00a	28.42±0.99c	0.77±0.03bc
T5	100.00±0.00a	27.75±0.39c	0.75±0.01bc

Note: CK stands for 100% cocopeat, T1 stands for 80% cocopeat + 20% earthworm manure, T2 stands for 60% cocopeat + 40% earthworm manure, T3 stands for 40% cocopeat + 60% earthworm manure, T4 stands for 20% cocopeat + 80% earthworm manure, and T5 stands for 100% earthworm manure. Different lowercase letters in the same column represent significant differences ( $P < 0.05$ ).

Table 3. Physical properties of seedling substrates with different levels of earthworm manure and cocopeat rationing.

Treatments	Bulk density/ (g·cm <sup>-3</sup> )	Water-holding porosity/%	Aeration porosity/%	Total porosity/%	Air-water ratio/%
CK	0.11±0.00f	54.29±0.91c	26.97±0.22a	81.26±0.71a	0.50±0.01a
T1	0.27±0.0e	56.44±0.28bc	18.34±0.90b	74.79±1.05b	0.33±0.02b
T2	0.32±0.00d	59.73±0.91ab	13.08±1.59c	72.81±1.82bc	0.22±0.03c
T3	0.41±0.00c	59.26±0.45ab	10.49±0.94cd	69.75±0.60c	0.18±0.02cd
T4	0.55±0.00b	57.48±1.78abc	7.64±1.37de	65.13±0.93d	0.14±0.03de
T5	0.60±0.01a	60.66±1.11a	5.21±1.07e	65.87±0.56d	0.09±0.02e

Note: CK stands for 100% cocopeat, T1 stands for 80% cocopeat + 20% earthworm manure, T2 stands for 60% cocopeat + 40% earthworm manure, T3 stands for 40% cocopeat + 60% earthworm manure, T4 stands for 20% cocopeat + 80% earthworm manure, and T5 stands for 100% earthworm manure. Different lowercase letters in the same column represent significant differences ( $P < 0.05$ ).

Table 4. Chemical properties of seedling substrates with different levels of earthworm manure and cocopeat rationing.

Treatments	pH	EC ( $\mu\text{S}\cdot\text{cm}^{-1}$ )	Alk. N/(mg·kg <sup>-1</sup> )	Ava. P (mg·kg <sup>-1</sup> )	Ava. K (mg·kg <sup>-1</sup> )	Organic mat- ter/%
CK	6.90±0.02d	652.00±9.28a	91.98±0.29d	54.74±3.75f	22.42±39.39e	62.21±1.45a
T1	7.49±0.01c	635.00±2.17b	97.57±0.06c	384.11±0.76e	1318.08±7.95d	33.17±0.29b
T2	7.75±0.04b	578.67±1.09c	100.79±0.07b	573.56±0.97d	2063.35±10.13c	16.47±0.37c
T3	7.77±0.09ab	532.00±1.97d	100.62±0.07b	563.49±0.87c	2023.74±9.17c	17.36±0.34c
T4	7.84±0.06ab	515.00±2.74e	101.29±0.09a	602.83±1.23b	2178.48±12.93b	13.89±0.48d
T5	7.95±0.05a	421.33±2.02f	101.68±0.11a	625.66±1.44a	2268.32±15.11a	11.88±0.56d

Note: CK stands for 100% cocopeat, T1 stands for 80% cocopeat + 20% earthworm manure, T2 stands for 60% cocopeat + 40% earthworm manure, T3 stands for 40% cocopeat + 60% earthworm manure, T4 stands for 20% cocopeat + 80% earthworm manure, and T5 stands for 100% earthworm manure. Different lowercase letters in the same column represent significant differences ( $P < 0.05$ ).

of earthworm manure in the substrate, as compared to the control treatment (CK) ( $P < 0.05$ ). Within the treatment groups, seeds subjected to treatment T3 displayed the greatest root length and germination index, whereas those in treatment T1 exhibited the lowest metrics. More precisely, the root length in treatment T3 decreased by 20.83% compared to CK and rose by 18.01% compared to T1. Likewise, the germination index in treatment T3 decreased by 21.50% compared to CK and increased by 18.31% compared to T1. No significant differences in germination rates were observed among the treatments, as each exhibited a rate of 100.00%.

#### Physical Properties of Seedling Substrates

Table 3 presents the physical properties of seedling substrates subjected to various different treatments. It is evident that as the proportion of earthworm manure increases continuously, there is a significant trend of increasing bulk density (BD) across treatments T1 to T5. In comparison to the control treatment (CK), the bulk density in T5 increased by 445.45%. Concerning porosity, the total porosity (TP)

and air-filled porosity (AFP) of the seedling substrate exhibited a significant decreasing trend with the addition of earthworm manure. Nevertheless, no significant differences were observed in AFP between treatments T2 and T3, or between treatments T3 and T4. Similarly, no significant differences were found in TP between treatments T1 and T2, or between treatments T2 and T3. Additionally, treatments T4 and T5 exhibited no significant differences in AFP and TP. Regarding water-holding porosity (WHP), there was a modest increase in the WHP of the seedling substrate with the addition of earthworm manure, with T5 showing a 6.37% increase compared to CK. Nonetheless, no significant differences were observed in WHP among treatments T2 to T5, nor between CK and T1, or between treatments T1 and T2.

#### Chemical Properties of Seedling Substrates

Table 4 illustrates the substantial influence of earthworm manure addition on the chemical composition of seedling substrates. Notably, pH and levels of available nutrients demonstrate a considerable rise with the incorporation

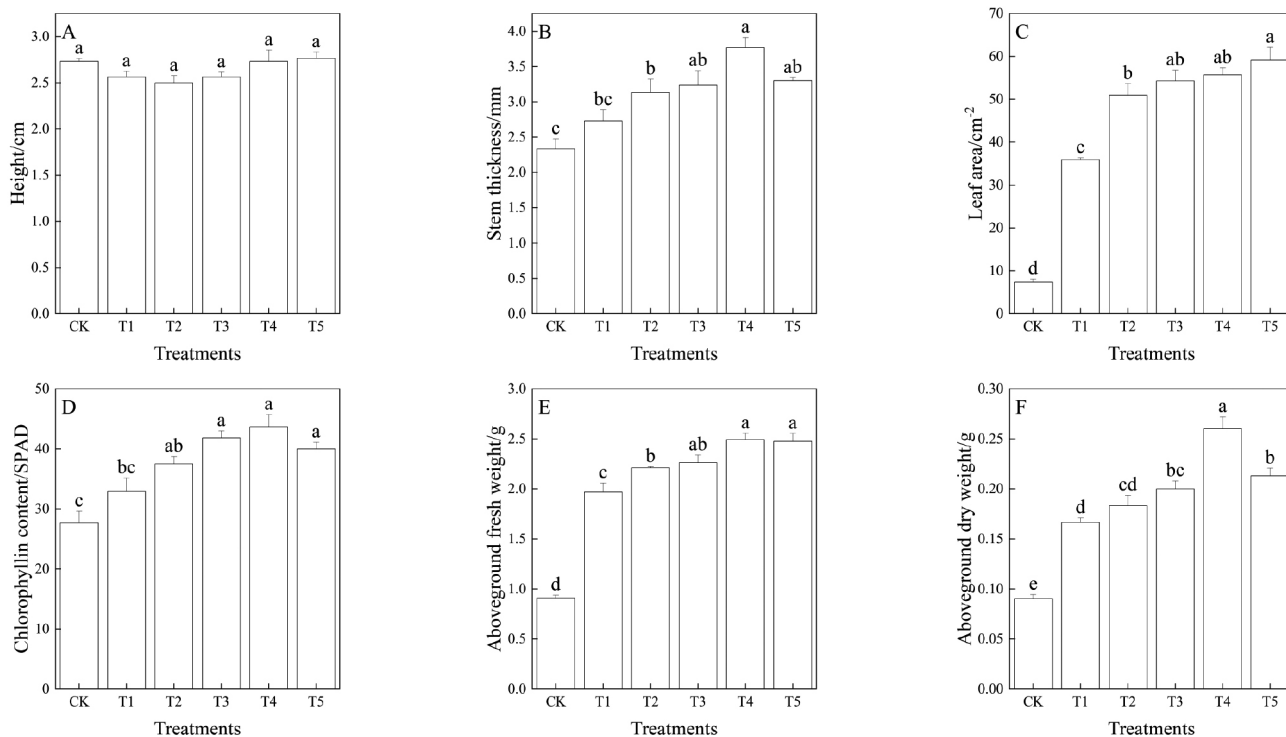


Fig. 1. Effect of the addition of earthworm manure to cocopeat on the aboveground growth of cucumber seedlings.

Note: Different lowercase letters in the same column represent significant differences ( $P < 0.05$ ). CK-T5 represents 100% cocopeat, 80% cocopeat + 20% earthworm manure, 60% cocopeat + 40% earthworm manure, 40% cocopeat + 60% earthworm manure, 20% cocopeat + 80% earthworm manure, and 100% earthworm manure, respectively.

of earthworm manure, whereas electrical conductivity (EC) and organic matter content exhibit a notable decrease. Specifically, in the T5 treatment, pH increased by 15.22% in comparison to the control (CK), while alkaline nitrogen, available phosphorus, and available potassium levels escalated by 10.55%, 1042.97%, and 10018.89%, respectively, relative to CK. Conversely, the EC value decreased by 35.38% and organic matter content dropped by 80.90% in the T5 treatment compared to CK.

### Cucumber Seedling Growth

Based on the findings presented in Fig. 1, it is evident that the introduction of earthworm manure did not exert a notable influence on the height of cucumber seedlings (Fig. 1A). Nevertheless, considerable enhancements were observed in various aboveground parameters due to the application of earthworm manure. Specifically, when the earthworm manure comprised 60% or more of the volume ratio, cucumber seedlings exhibited the most robust levels of stem diameter, leaf area, chlorophyll content, and aboveground fresh weight. Notably, treatments T3-T5 showed no significant deviations from one another, with respective increases of 61.43%, 706.55%, 57.86%, and 173.63% compared to the control (CK) treatment. Additionally, the T4 treatment demonstrated the highest

increment in aboveground dry weight, recording a surge of 188.89% compared to the CK treatment and a 21.88% rise compared to the T5 treatment.

Regarding the subterranean growth of cucumber seedlings, insights from Table 5 are instructive. Relative to the control group, the incorporation of earthworm manure markedly augmented various root growth parameters of cucumber seedlings. Notably, the T4 treatment exhibited the maximal values across all root indicators, showcasing increases of 79.21%, 106.04%, 96.91%, and 117.16% in total root length, total root surface area, total root tip number, and root dry weight, respectively, compared to the CK treatment. However, no significant disparities were detected between the T4 and T5 treatments in terms of total root length. Similarly, there were no statistically significant variances in total root surface area between the T2-T4 treatments or in total root tip number between the T4 and T3 treatments. Likewise, no significant distinctions were observed in root dry weight among the T2-T4 treatments. The seedling vigor index provides a comprehensive assessment of seedling growth status, with the vigor index of the T4 treatment notably surpassing that of all other treatments by 63.64% compared to the CK treatment, albeit without significant differences discerned among the T2-T4 treatments.

Table 5. Effect of cocopeat supplemented with earthworm manure on root growth and strong seedling index of cucumber seedlings.

Treatments	Total root length/cm	Total root area/cm <sup>2</sup>	Total root tips	Root dry weight/mg	Strong seedling index
CK	363.01±26.03c	32.73±1.58c	669.67±63.24c	18.07±3.95c	0.11±0.01d
T1	493.17±45.48b	51.66±4.83b	935.33±16.82b	22.80±0.49c	0.13±0.01cd
T2	497.81±16.53b	57.19±0.47ab	963.33±12.16b	36.50±2.00ab	0.17±0.00ab
T3	522.40±14.37b	58.39±0.90ab	1261.67±81.91a	37.47±5.19a	0.17±0.01ab
T4	650.55±31.97a	67.44±1.56a	1318.67±2.83a	39.23±1.61a	0.18±0.01a
T5	570.44±19.01ab	65.66±4.52a	1040.67±61.66b	25.87±2.75bc	0.15±0.00bc

Note: CK stands for 100% cocopeat, T1 stands for 80% cocopeat + 20% earthworm manure, T2 stands for 60% cocopeat + 40% earthworm manure, T3 stands for 40% cocopeat + 60% earthworm manure, T4 stands for 20% cocopeat + 80% earthworm manure, and T5 stands for 100% earthworm manure. Different lowercase letters in the same column represent significant differences ( $P < 0.05$ ).

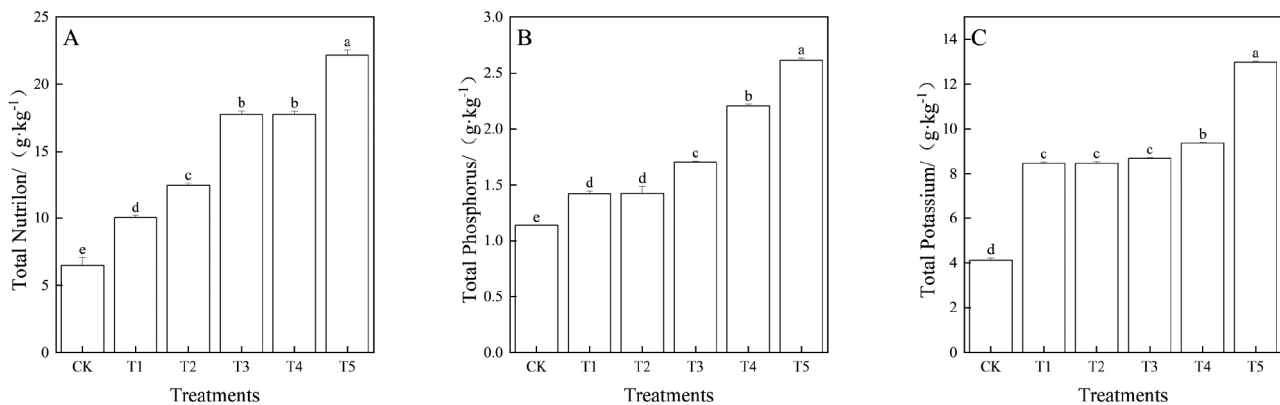


Fig. 2. Effect of the addition of earthworm manure to cocopeat on the aboveground growth of cucumber seedlings

Note: Different lowercase letters in the same column represent significant differences ( $P < 0.05$ ). CK-T5 represents 100% cocopeat, 80% cocopeat + 20% earthworm manure, 60% cocopeat + 40% earthworm manure, 40% cocopeat + 60% earthworm manure, 20% cocopeat + 80% earthworm manure, and 100% earthworm manure, respectively.

### Nutrient Uptake in Cucumber Seedlings

The nutrient absorption capacity of plants serves as an indicator of the growth-enhancing properties of seedling substrates. It is evident that the incorporation of earthworm manure led to a notable augmentation in the uptake of nitrogen (N), phosphorus (P), and potassium (K) by cucumber seedlings, with the highest nutrient accumulation observed in plants subjected to the T5 treatment. Specifically, compared to the control (CK) treatment, the T5 treatment resulted in substantial increases in plant N, P, and K content, registering increments of 241.65%, 130.35%, and 214.36%, respectively (Fig. 2).

### Correlation Analysis

Due to considerable disparities in the growth status of cucumber seedlings across various treatments, a correlation analysis was conducted to examine

the relationship between seedling growth indicators and the physicochemical properties of their substrates, as illustrated in Fig. 3. The analysis revealed that there is no significant correlation between the physicochemical properties of the substrates and the plant height of cucumber seedlings.

Bulk density, air-filled porosity, electrical conductivity (EC), and organic matter content display notable negative correlations with the stem thickness of cucumber seedlings. Conversely, the remaining physicochemical properties, excluding water-holding porosity, exhibit positive correlations with stem thickness. This pattern extends to other growth indicators such as leaf area, root length, root surface area, shoot fresh weight, and shoot dry weight.

The number of root tips shows a predominantly positive correlation with bulk density and pH, while root dry weight and seedling vigor index are primarily influenced by the total porosity of the substrate.



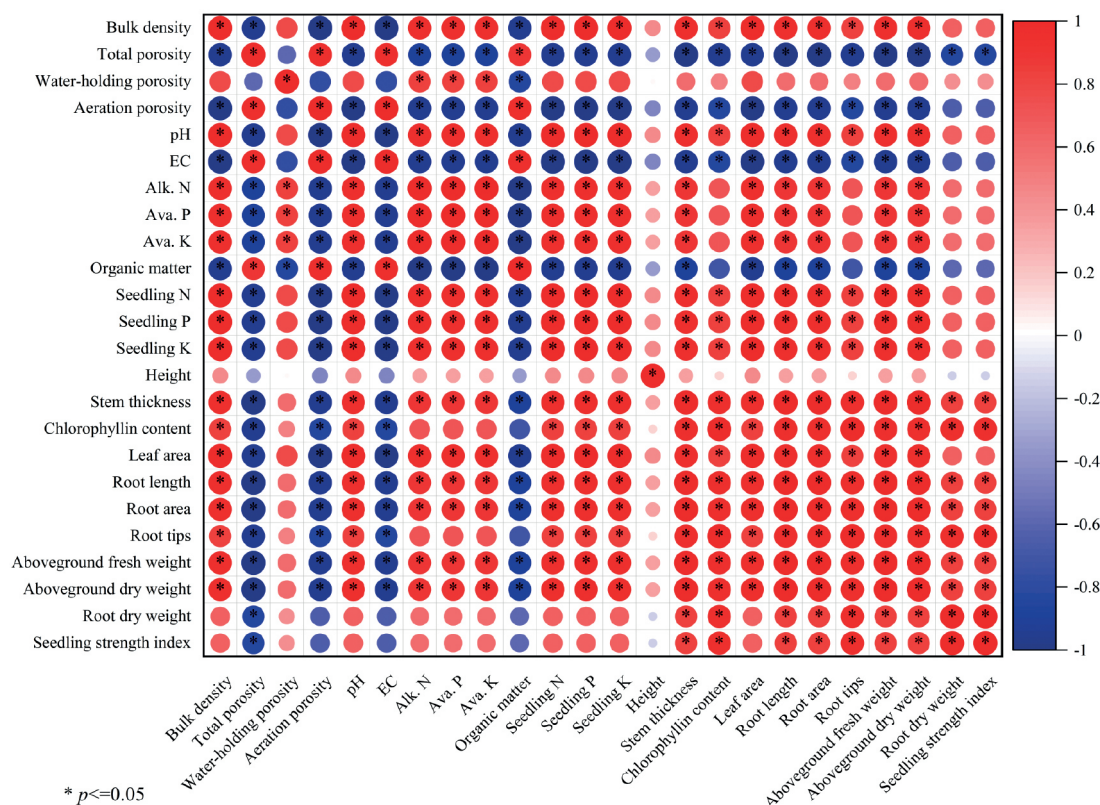


Fig. 3. Analysis of key factors influencing the growth of cucumber seedlings with different levels of earthworm manure and cocopeat rationing.

### Discussion

Cocopeat is widely recognized as one of the most suitable raw materials for seedling substrates due to its exceptional physical properties. Nevertheless, desalinated cocopeat often suffers from nutrient deficiencies, necessitating the supplementation of nutrients, typically in the form of organic fertilizers. In this study, earthworm manure was selected as a nutrient source to supplement cocopeat, and the germination index was initially assessed following the preparation of the seedling substrate. According to the pertinent formula, it can be inferred that a germination index exceeding 100% is indicative of promoting seed germination [27]. However, as depicted in Table 2, except for the treatment utilizing 100% cocopeat, all treatments incorporating earthworm manure exhibited germination indices reaching 100%. In line with the definition of the germination index, this observation suggests an inhibitory effect on seed germination relative to distilled water. Nonetheless, subsequent seedling experiments revealed that even cucumber seedlings cultivated in the 100% earthworm casting treatment exhibited significantly superior growth compared to those in the 100% cocopeat treatment. This disparity between the outcomes of the two experiments warrants analysis. We posit two potential explanations for this incongruity. First, while the seedling substrate

supplemented with earthworm manure may inhibit seed germination, the presence of abundant nutrients within the earthworm manure may afford cucumber seedlings improved nutritional conditions during the seedling growth stage, thereby offsetting the inhibitory effect on seed germination. Second, the prevailing method for determining the germination index of organic fertilizers, commonly employed for assessing seedling substrates due to the absence of a standardized method in China's agricultural industry, may not be wholly suitable for this purpose, potentially resulting in experimental deviation.

Subsequent seedling experiments revealed a significant promotion in the growth of cucumber seedlings with the addition of earthworm manure to the substrates. Following a comprehensive evaluation of the seedling index, we propose that a volume ratio of earthworm manure in cocopeat seedling substrates ranging between 40% and 80% is optimal. Notably, a 100% earthworm manure ratio, while offering the highest nutrient content for seedling growth, resulted in significantly higher pH and bulk density compared to other treatments, accompanied by a notable reduction in total porosity. Correlation analysis indicated that total porosity serves as a significant limiting factor influencing the seedling index. In the studies conducted by other scholars, a proportion of 30% earthworm manure might be considered appropriate [28]. However, it is notable

that other researchers typically utilize a variety of materials alongside vermicompost in preparing seedling substrates, such as biochar, straw, and manure compost. In contrast, our study only employed two types of materials, which could result in different optimal proportions of earthworm manure [29, 30]. Typically, the pH of seedling substrates should fall within the range of 5.5 to 7.5, while the bulk density is preferably situated between  $0.2 \text{ g}\cdot\text{cm}^{-3}$  and  $0.6 \text{ g}\cdot\text{cm}^{-3}$ , with a total porosity exceeding 60% [2]. Following this standard strictly, a seedling substrate with a 20% volume ratio of earthworm manure would be deemed most suitable. However, contrary to expectations, this ratio did not yield optimal results in the seedling experiments, highlighting the importance of evaluating seedling substrate quality not solely based on physicochemical properties but rather on its ability to foster optimal crop seedling growth. Furthermore, as auxiliary indicators, the nutrient content of cucumber seedlings cultivated in each treatment seedling substrate was determined. The results substantiated that the addition of earthworm manure led to increased nutrient absorption by crops, aligning to some extent with the growth indicators of plant seedlings.

The seedling substrate formed in our study contains only two materials, earthworm manure and cocopeat, which in a sense achieves a complete replacement of peat in the seedling substrate, whereas similar studies have mostly replaced peat by 50–80% [31, 32]. However, there may be corresponding problems, one of which is that the present study was conducted only for cucumber, but that does not mean that the seedling substrate is also good for other crops, especially for crops of different families such as tomato, sunflower, and strawberry. The problem of the non-universality of seedling substrates has arisen in similar studies by other scholars [33]. The second is that the earthworm manure used in this study may not necessarily agree with the materials used by other researchers in terms of physico-chemical properties. For example, the earthworm manure produced by earthworms reared using cow dung, food waste, and waste paper varies greatly in terms of nutrient content, and its application to the substrate can lead to differences in the growth conditions of the crop, and the coir produced in different regions may vary in terms of physico-chemical properties [34, 35]. Therefore, while reproducing the results of the present study, attention should be paid to the differences in the physicochemical properties of the raw materials that may lead to different results.

## Conclusions

The present study utilized earthworm manure to supplement cocopeat substrate and explored the optimal ratio for cucumber seedling growth. Results indicate that cucumber seedlings exhibited relatively better growth when the volume ratio of earthworm manure in the cocopeat substrate ranged from 40% to 80%. Based on these findings, further improvements in the physicochemical properties of the “earthworm manure-cocopeat” seedling substrate are recommended to achieve optimal cucumber seedling growth.

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## Conflict of Interest

The authors declare no conflict of interest.

## References and Notes

- LIU Z., GE L., LI S., PAN R., LIU X. Study on the Effect of Kitchen Waste Compost Substrate on the Cultivation of *Brassica chinensis* L. Polish Journal of Environmental Studies, **32** (5), 4139, **2023**.
- GE L., LIU Z., LIU X., LI S., ZHOU Y., JIA K., YAN L., DONG X., PAN R. Suitability Analysis of Kitchen Waste Compost for Rice Seedling Substrate Preparation. Polish Journal of Environmental Studies, **33** (3), 3115, **2024**.
- MENG X., WANG Q., LV Z., CAI Y., ZHU M., LI J., MA X., CUI Z., REN L. Novel seedling substrate made by different types of biogas residues: Feasibility, carbon emission reduction and economic benefit potential. Industrial Crops and Products, **184**, 115028, **2022**.
- HULTBERG M., OSKARSSON C., BERGSTRAND K.-J., ASP H. Benefits and drawbacks of combined plant and mushroom production in substrate based on biogas digestate and peat. Environmental Technology & Innovation, **28**, 102740, **2022**.
- LEMPINEN H., VAINIO A. Lost in transition: Peat workers' experiences of Finland's low carbon transition policies. The Extractive Industries and Society, **15**, 101312, **2023**.
- PENG S.-Y., YAN J., LI M., YAN Z.-X., WEI H.-Y., XU D.-J., CHENG X. Preparation of polysaccharide-conjugated selenium nanoparticles from spent mushroom substrates and their growth-promoting effect on rice seedlings. International Journal of Biological Macromolecules, **253**, 126789, **2023**.
- LI J., XU C., ZHANG X., GU Z., CAO H., YUAN Q. Effects of different fermentation synergistic chemical treatments on the performance of wheat straw as a nursery substrate. Journal of Environmental Management, **334**, 117486, **2023**.
- SILLERO L., SOLERA R., PEREZ M. Agronomic and phytotoxicity test with biosolids from anaerobic CO-DIGESTION with temperature and micro-organism phase separation, based on sewage sludge, vinasse and poultry manure. Journal of Environmental Management, **354**, 120146, **2024**.
- LI H., YANG Z., ZHANG C., SHANG W., ZHANG T., CHANG X., WU Z., HE Y. Effect of microbial inoculum on composting efficiency in the composting process of spent mushroom substrate and chicken manure. Journal of Environmental Management, **353**, 120145, **2024**.
- ZHANG R.-H., DUAN Z.-Q., LI Z.-G. Use of Spent Mushroom Substrate as Growing Media for Tomato and Cucumber Seedlings. Pedosphere, **22** (3), 333, **2012**.
- DI LONARDO S., CACINI S., BECUCCI L., LENZI A., ORSENIGO S., ZUBANI L., ROSSI G., ZACCHEO P., MASSA D. Testing new peat-free substrate mixtures for



- the cultivation of perennial herbaceous species: A case study on *Leucanthemum vulgare* Lam. *Scientia Horticulturae*, **289**, 110472, **2021**.
12. PAPANIMITRIOU D.M., DALIAKOPOULOS I.N., LOULOUDAKIS I., SAVVIDIS T.I., SABATHIANAKIS I., SAVVAS D., MANIOS T. Impact of container geometry and hydraulic properties of coir dust, perlite, and their blends used as growing media, on growth, photosynthesis, and yield of Golden Thistle (*S. hispanicus* L.). *Scientia Horticulturae*, **323**, 112425, **2024**.
  13. IGHALO J.O., CONRADIE J., OHORO C.R., AMAKU J.F., OYEDOTUN K.O., MAXAKATO N.W., AKPOMIE K.G., OKEKE E.S., OLISAH C., MALLOUM A., ADEGOKE K.A. Biochar from coconut residues: An overview of production, properties, and applications. *Industrial Crops and Products*, **204**, 117300, **2023**.
  14. YANG J., MATTOO A.K., LIU Y., ZVOMUYA F., HE H. Trade-offs of organic and organic-inorganic fertilizer combinations in tomato quality and yield: A global meta-analysis (1992–2021). *European Journal of Agronomy*, **151**, 126985, **2023**.
  15. BERGSTRAND K.-J. Organic fertilizers in greenhouse production systems – a review. *Scientia Horticulturae*, **295**, 110855, **2022**.
  16. PELOSI C., TASCHEEN E., REDECKER D., BLOUIN M. Earthworms as conveyors of mycorrhizal fungi in soils. *Soil Biology and Biochemistry*, **189**, 109283, **2024**.
  17. AYALA L.P., BAROIS I. Development of the earthworm *Pontoscolex corethrurus* in soils amended with a peat-based plant growing medium. *Applied Soil Ecology*, **104**, 131, **2016**.
  18. KONG Y., WANG G., CHEN W., YANG Y., MA R., LI D., SHEN Y., LI G., YUAN J. Phytotoxicity of farm livestock manures in facultative heap composting using the seed germination index as indicator. *Ecotoxicology and Environmental Safety*, **247**, 114251, **2022**.
  19. KONG Y., ZHANG J., YANG Y., LIU Y., ZHANG L., WANG G., LIU G., DANG R., LI G., YUAN J. Determining the extraction conditions and phytotoxicity threshold for compost maturity evaluation using the seed germination index method. *Waste Management*, **171**, 502, **2023**.
  20. CHO Y.Y., OH S., OH M.M., SON J.E. Estimation of individual leaf area, fresh weight, and dry weight of hydroponically grown cucumbers (*Cucumis sativus* L.) using leaf length, width, and SPAD value. *Scientia Horticulturae*, **111** (4), 330, **2007**.
  21. WANG M.-B., ZHANG Q. Issues in using the WinRHIZO system to determine physical characteristics of plant fine roots. *Acta Ecologica Sinica*, **29** (2), 136, **2009**.
  22. LIN L., QIN J., ZHANG Y., YIN J., GUO G., KHAN M.A., LIU Y., LIU Q., WANG Q., CHANG K., MAŠEK O., WANG J., HU S., MA W., LI X., GOUDA S.G., HUANG Q. Assessing the suitability of municipal sewage sludge and coconut bran as breeding medium for *Oryza sativa* L. seedlings and developing a standardized substrate. *Journal of Environmental Management*, **344**, 118644, **2023**.
  23. MARCÓ A., RUBIO R., COMPAÑÓ R., CASALS I. Comparison of the Kjeldahl method and a combustion method for total nitrogen determination in animal feed. *Talanta*, **57** (5), 1019, **2002**.
  24. PAL A., KULKARNI M.B., GUPTA H., PONNALAGU R.N., DUBEY S.K., GOEL S. Portable and Autonomous Device for Real-time Colorimetric Detection: Validation for Phosphorous and Nitrite Detection. *Sensors and Actuators A: Physical*, **330**, 112896, **2021**.
  25. ULLAH R., ABBAS Z., BILAL M., HABIB F., IQBAL J., BASHIR F., NOOR S., QAZI M.A., NIAZ A., BAIG K.S., RAUF A., FATIMA L., AKHTAR I., ALI B., ULLAH M.I., AL-HASHIMI A., ELSHIKH M.S., ALI S., SAEED-UR-REHMAN H. Method development and validation for the determination of potassium (K<sub>2</sub>O) in fertilizer samples by flame photometry technique. *Journal of King Saud University - Science*, **34** (5), 102070, **2022**.
  26. TANG Q.-Y., ZHANG C.-X. Data Processing System (DPS) software with experimental design, statistical analysis and data mining developed for use in entomological research. *Insect Science*, **20** (2), 254, **2013**.
  27. CHANG R., GUO Q., PANDEY P., LI Y., CHEN Q., SUN Y. Pretreatment by composting increased the utilization proportion of pig manure biogas digestate and improved the seedling substrate quality. *Waste Management*, **129**, 47, **2021**.
  28. MANH V.H., WANG C.H. Vermicompost as an Important Component in Substrate: Effects on Seedling Quality and Growth of Muskmelon (*Cucumis Melo* L.). *APCBEE Procedia*, **8**, 32, **2014**.
  29. MENDOZA-HERNÁNDEZ D., FORNES F., BELDA R.M. Compost and vermicompost of horticultural waste as substrates for cutting rooting and growth of rosemary. *Scientia Horticulturae*, **178**, 192, **2014**.
  30. BACHMAN G.R., METZGER J.D. Growth of bedding plants in commercial potting substrate amended with vermicompost. *Bioresource Technology*, **99** (8), 3155, **2008**.
  31. BU X., JI H., MA W., MU C., XIAN T., ZHOU Z., WANG F., XUE J. Effects of biochar as a peat-based substrate component on morphological, photosynthetic and biochemical characteristics of *Rhododendron delavayi* Franch. *Scientia Horticulturae*, **302**, 111148, **2022**.
  32. NOCENTINI M., PANETTIERI M., GARCÍA DE CASTRO BARRAGÁN J.M., MASTROLONARDO G., KNICKER H. Recycling pyrolyzed organic waste from plant nurseries, rice production and shrimp industry as peat substitute in potting substrates. *Journal of Environmental Management*, **277**, 111436, **2021**.
  33. CARMONA E., MORENO M.T., AVILÉS M., ORDOVÁS J. Use of grape marc compost as substrate for vegetable seedlings. *Scientia Horticulturae*, **137**, 69, **2012**.
  34. A'SAF T.S., AL-AJLOUNI M.G., AYAD J.Y., OTHMAN Y.A., ST. HILAIRE R. Performance of six different soilless green roof substrates for the Mediterranean region. *Science of The Total Environment*, **730**, 139182, **2020**.
  35. BHAVISHYA, SUBRAMANIAN K.S., GOPAL M., BHAT R., NAIR S.S., RADHAKRISHNAN M., RAJESH M.K. Nano-potassium intercalated composted coir pith: A slow-release fertilizer suitable for laterite soils of humid tropics of India. *Biocatalysis and Agricultural Biotechnology*, **57**, 103054, **2024**.