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Composted Rabbit Manure as Organic Matrix for Manufacturing Horticultural Growing Media: Composting Process and Seedling Effects

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Abstract: This study investigated composted rabbit manure as an organic matrix to replace peat for manufacturing horticultural growing media, where three kinds of rabbit manures were composted with temperatures > 50 °C lasting > 7 days, with a germination index > 70%. The heavy metal contents in rabbit manure were far lower than the thresholds in Chinese standards for safe use as horticultural growing media. Then, different ratios of compost and peat were mixed with perlite and vermiculite to manufacture growing media, which were evaluated in a 28-day seedling experiment with cabbage. The manufactured growing media characteristics could satisfy the seedlings' requirements; air spaces in particular were improved from 15.7% to up to 28.7% by mixing composted manure. Seedling emergence percentages > 90% and good seedling qualities (including enhanced root length, seedling height, and chlorophyll content) indicated that composted rabbit manure could effectively replace peat to manufacture horticultural growing media. The best seedling performance was obtained with the following ratio of composted manure, peat, perlite, and vermiculite: 3:3:2:2. However, the mixing ratio could be further optimized, and other methods to reduce the salinity may be required for the different requirements of specific crops in further research.

Keywords: rabbit manure; compost; growing media; seedling quality; heavy metal

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1. Introduction

For future vertical farming systems, rabbits are considered the promising animal compared with poultry and fish. Plant food wastes can be fed to rabbits directly and rabbit manure can be used for plant production, thus realizing the cycle of planting and raising [1]. At present, rabbits have become the second largest farmed species in Europe from the perspective of the breeding quantity. Similarly, China also has a large rabbit breeding volume of 313 million, corresponding to significant rabbit manure production of approximately 8 million tons [2]. However, unfortunately, the treatment of rabbit manure lagged behind in the past compared to other animal manures. Currently, not only in China but also in Spain [3], most rabbit manure is still treated through primitive and rough methods, that is, through natural air-drying. This method has many disadvantages, such as increasingly expensive land costs, serious pollution to the surrounding environment, and excessive dependence on the weather. In facing current problems and potential future applications, it is necessary to explore the value and application scenarios of rabbit manure.

Composting technology has been advocated by the government in the treatment of rabbit manure. Some existing studies have primarily verified the feasibility of composting rabbit manure (e.g., [4]). Composting technology uses aerobic microorganisms to decompose the organic matter in the manure, followed by self-raising the temperature and killing harmful materials (e.g., roundworm eggs, pathogenic bacteria, and weed seeds) to achieve harmless products. At the same time, due to the formation of beneficial substances such as humus and plant hormones during the composting process, the agronomic effects could also be improved [5]. Hence, composted manure has the potential to be used to manufacture organic fertilizer [6].

Compost has also been explored to replace peat for manufacturing growing media to meet the great and potential needs of protected agriculture [7] and internal green wall systems [8]. Peat is an indispensable material for the manufacture of growing media, but the extraction of natural peat has been restricted in many countries due to its non-renewable characteristics [9]. Therefore, using composted manure to replace peat would have good business and environmental prospects. Islas-Valdez et al. [3] reported that the liquid biofertilizer obtained from the anaerobic digestion of rabbit manure could increase the grain yield of barley because of the essential nutrients and biostimulants. Similarly, good agronomic performance of rabbit manure compost was observed when it was used as organic fertilizer for okra [10] and organic tomato [11] or used as substrate for lettuce [12]. In addition, the results from Cabanillas et al. [13] indicate that vermicompost from rabbit manure was an effective alternative to urea in basil cultivation. Nevertheless, it is still unclear whether rabbit manure compost can be used to replace peat for growing media. This is because the requirements for use as fertilizer or growth media are different: the former is more concerned about nutrient effects (e.g., N, P, K-element contents), while the latter has special requirements for some physical and chemical properties (e.g., pH value, salinity, density, permeability, and water-holding capacity) [14]. This has not been comprehensively reported in previous studies, and there are still knowledge gaps concerning the feasibility and agronomic effects of composted rabbit manures to replace peat as an organic matrix for manufacturing growing media.

In this content, the dynamic composting performance of three kinds of rabbit manure was monitored and the safety of the products (including heavy metal, germination index, and hygienic characteristics) was analyzed. Furthermore, the composts with the best performance were manufactured as growing media, and their physicochemical properties were evaluated, including pH value, electrical conductivity (EC), bulk density, and porosity. Then, the growing media were used for a seedling experiment in which the effects on the seedling emergence percentages and the growth status of cabbage seeds were evaluated. Finally, we provide some suggestions for further study and commercial applications of rabbit manure treatments.

2. Materials and Methods

2.1. Rabbit Manure

The rabbit manure was collected in a large-scale rabbit breeding herd in Jiyuan, Henan Province, China (35°08′ N, 112°57′ E). Three kinds of rabbit manure were compared and analyzed in this study to comprehensively evaluate their composting process. They were collected from a pregnant doe room (labeled as "R1"), an early-fattening rabbit (after weaning) room (labeled as "R2"), and a late-fattening rabbit room (labeled as "R3"), respectively. Urine was not included because the breeding herd used manure–urine separation technology.

2.2. Rabbit Manure Composting

2.2.1. Composting Preparation

The composting experiment was conducted in rabbit farms in November 2020. About 400 kg of fresh rabbit manure for each set of manure was piled into a conical stack with a triangular cross-sectional area. The initial moisture contents of the raw rabbit manures were ~60% (Table 1) and thus suitable for composting, so that the moisture was not adjusted. Commercial composting additives (Organic fertilizer fermentation microbial agent, Shandong Lvlong Biological Technology Co., Weifang, China) were used to ensure successful composting, and 0.1 kg/ton fresh weight was added according to the product manual. The piles were manually turned over every two days, and the composting temperatures and ambient temperatures were measured every day. The composting test was completed until the composting temperatures were close to the ambient temperature, that is, 23 days into this study. In addition, some manures were air-dried and served as controls (without manually turning over), which were deposited on a 4–5 cm layer, and the drying test lasted one week and was completed after 8 days according to practice experience. The composting piles and air-dried layers were placed under a ventilated shelter, and the samples were collected after 1, 3, 6, 10, 14, 18, and 23 days, in turn, and were used for determination.

Table 1. Characteristics of the three kinds of raw rabbit manures (R1, R2, and R3).

	R1 [2]	R2	R3	Significance [3]
Rabbit feed label [1]	Jinyu 573	Jinyu 518	Jinyu 572	/
pH Value [4]	8.8 ± 0.0 c [5]	8.9 ± 0.0 b	9.0 ± 0.0 a	***
Dry matter (DM, %)	41.0 ± 2.6 a	38.7 ± 3.0 a	39.7 ± 1.7 a	n.s.
Organic matter (% DM)	84.9 ± 0.3 a	84.0 ± 0.1 c	84.4 ± 0.1 b	**
Carbon/nitrogen ratio	23.2 ± 0.1 c	30.3 ± 0.0 a	25.4 ± 0.3 b	***
N (% DM)	2.0 ± 0.0 a	1.6 ± 0.0 c	1.9 ± 0.0 b	***
P (% DM)	6.8 ± 0.3 b	6.5 ± 0.1 b	8.2 ± 0.3 a	***
K (% DM)	1.2 ± 0.0 b	1.4 ± 1.0 a	1.2 ± 0.1 b	**
Hemicellulose (% DM)	22.3 ± 0.3 b	23.9 ± 0.2 a	23.7 ± 0.2 a	**
Cellulose (% DM)	27.3 ± 0.2 a	28.3 ± 0.5 a	25.7 ± 0.5 b	**
Lignin (% DM)	16.8 ± 0.5 a	15.7 ± 0.4 a	16.9 ± 0.8 a	n.s.

[1] Numbers 573, 518, and 572 (from Jiyuan Jinyu Feed Co., Ltd., Jiyuan, China) are the feed labels for pregnant does, early-fattening rabbits (after weaning), and late-fattening rabbits, respectively. [2] Means with different letters in a row differ significantly ($p < 0.05$). [3] n.s. means no significant difference at the 0.05 probability level; ** and *** signify significance at the 0.01 and 0.001 probability levels, respectively. [4] The pH values were tested at a ratio of manure to distilled water of 1:10. [5] ±means standard deviation.

2.2.2. Analytical Methods

The fresh sample and deionized water were mixed at 1:10 (v/v) to obtain the sample extract, and its pH value was then measured using a pH meter (Sartorius PB-10, Sartorius AG, Göttingen, Germany); its EC was measured using a portable conductivity meter (Leici DDB-303A, Shanghai Oustor Industrial Corp., Shanghai, China). Moreover, the E4/E6 values and germination index (GI) were measured with reference to the reports of [15] and [16], respectively. Briefly, the E4/E6 values were obtained by calculating the ratios of the absorbance at 465 nm and 665 nm in the sample extract using an ultraviolet spectrometer (TU-1901 Ultraviolet-visible Spectrophotometer, Beijing Puxi General Instrument Co., Ltd., Beijing, China). GI were obtained by evaluating the germination of radish seeds (the number of germinated seeds and the length of the roots). The radish seeds were incubated using the sample extract at 25 °C in an incubator (CTHI -150B, STIK Instrument Equipment (Shanghai) Co., Ltd., Shanghai, China) in the dark for 48 h.

The moisture and organic matter content were determined by drying the sample at 105 °C for 24 h and calcining at 600 °C for 4 h, using an electric heating constant temperature blast drying oven (AL204, Mettler Toledo Instruments Ltd., Zurich, Switzerland) and a smart-box-type resistance furnace (SX2-8-10A, Shanghai Huyueming Scientific Instrument Co., Ltd., Shanghai, China), respectively; the total carbon content (TOC) was calculated according to the methods described in [17]. In addition, the samples were dried and ground to pass through a 1 mm sieve to prepare the solid sample. Then, the solid sample was used to measure the total Kjeldahl nitrogen (TKN) via a modified semi-micro Kjeldahl procedure (KDY-9830, Beijing Tongrunyuan Electromechanical Technology Co., Ltd., Beijing, China) [18]. The elements (including P, K, Cr, As, Cd, Hg, and Pb) were determined using inductively coupled plasma mass spectrometry (Agilent ICPMS7800, Agilent Technologies Co., Ltd., Palo Alto, CA, USA), and lignocellulose content (including cellulose, hemicellulose, and lignin) was determined using an automatic fiber analyzer (ANKOM A2000i, American ANKOM Co., Ltd., Macedon, NY, USA) according to the measures of [19]. As regards hygiene, the mortality of roundworm eggs and the number of fecal coliforms were determined using the Chinese standard issues [20,21].

2.3. Growing Media Preparation

Only the composted R2 was used for the subsequent seedling experiment, due to its good nutritional value, hygiene, and GI compared to R1 and R3. The composted R2 was air-dried at room temperature for 7 days, and was then manually ground and sieved (5 mm). Then, the prepared compost was mixed with perlite (3–5 mm), vermiculite (2–4 mm), and peat in five different ratios to manufacture the growing media. For the five ratios, the composted manure was mixed at ratios of 0% (labeled as T0 treatment), 15% (labeled as T15 treatment), 30% (labeled as T30 treatment), 45% (labeled as T45 treatment), and 60% (labeled as T60 treatment), respectively. For each treatment, the ratios of both perlite and vermiculite were same, at 20%. The rest was mixed with peat. In other words, composted rabbit manure was used in different ratios (15–60%) to replace peat, which is the traditional material for growing media.

The pH and EC values were determined following the description provided in Section 2.2.2. The bulk density, total porosity, and air space of the growing media were measured as described by [22]. Briefly, a ring knife with a volume V was dried at 105 °C to a constant weight (recorded as M_1). Furthermore, the ring knife was filled with the sample and immersed in deionized water for 24 h and weighed (recorded as M_2). After the water was naturally removed by turning the sample upside down with sealing by gauze until no water dripped out, it was weighed (recorded as M_3). Finally, the ring knife was placed in an oven at 105 °C and weighed (recorded as M_4). The bulk density, total porosity, and air space were calculated using Equations (1)–(3), as follows:

$$\text{Bulk density (g/cm}^3\text{)} = (M_4 - M_1)/V \tag{1}$$

$$\text{Total porosity (\%)} = 100\% \times (M_2 - M_4)/V \tag{2}$$

$$\text{Air space (\%)} = 100\% \times (M_2 - M_3)/V \tag{3}$$

2.4. Seedling Assay

The prepared growing media were used in the seedling experiment, which was conducted in a greenhouse at the China Agricultural University. Cabbage seeds (BEIJING XINSANHAO) were used in order to evaluate their agronomic effects; they were purchased from Jingyan Yinong Seed Sci-tech Co. (Beijing, China). For each treatment, seeding assays were repeated in triplicate at a polystyrene tray (72 cells), which means a total of 15 trays (5 treatment × 3 repetition) were conducted. These trays were randomly arranged in the greenhouse, where the temperature was controlled at 20 °C ± 5 °C and the humidity was about 60%. No additional fertilization was added.

At days 10, 15, and 20, the germination counts were performed and used for calculating the emergence rates in each growing medium. The experiment was completed on day 28. The seedling parameters (including the stem thickness, stem length, seedling height, fresh weight, and dry weight of the seedlings) were then determined. Briefly, five seedlings were randomly selected for each growing medium, and the stem diameter, seedling height (the distance from the growing medium surface to the top of the seedling), stem length, and root length were measured using a vernier caliper. The dry weight was determined by drying the samples at 105 °C for 15 min and at 80 °C after reaching constant mass. Chlorophyll (sum of chlorophyll a and chlorophyll b) was extracted and then measured following the method of [23]. Briefly, 1 cm^2 of leaves was soaked in 5 mL of 80% acetone for 24 h; the optical density at 663 and 645 nm were then tested with a spectrophotometer (TU-1901 Ultraviolet-visible Spectrophotometer, Beijing Puxi General Instrument Co., Ltd., Beijing, China), and the total chlorophyll content was calculated using Equations (4)–(6), as follows:

$$\text{Chlorophyll a } (\mu\text{g/mL}) = 12.7 \times \text{the optical density at 663 nm} - 2.69 \times \text{the optical density at 645 nm} \tag{4}$$

$$\text{Chlorophyll b } (\mu\text{g/mL}) = 22.9 \times \text{the optical density at 645 nm} - 4.86 \times \text{the optical density at 663 nm} \tag{5}$$

$$\text{Chlorophyll (mg/dm}^2\text{)} = 5 \text{ (mL)} \times (\text{Chlorophyll a} + \text{Chlorophyll b})/1 \text{ cm}^2 \times 100/1000 \tag{6}$$

In addition, the root/shoot ratio and the seedling vigor index were calculated using Equations (7) and (8), as follows:

$$\text{Root/shoot ratio} = \text{belowground dry weight/aboveground dry weight} \quad (7)$$

$$\text{Seedling vigor index} = (\text{stem diameter/plant height} + \text{belowground dry weight/aboveground dry weight}) \times (\text{aboveground dry weight} + \text{belowground dry weight}) \quad (8)$$

Finally, the seedling quality of the growing media in each of treatments was comprehensively evaluated using the membership function [24]:

$$X\,(f) = (X - X_{min})/(X_{max} - X_{min}) \quad (9)$$

where f is the specific parameter in the growing media, including the stem diameter, stem length, root length, seedling height, aboveground fresh weight, belowground fresh weight, aboveground dry weight, belowground dry weight, and chlorophyll. X is the measure value of the specific parameter; X_{max} and X_{min} are the maximum and minimum values of the parameter in all treatments.

All dates were the mean of three replicates, and the significant differences were analyzed through the Duncan test using SPSS 17.0. All figures were drawn using Origin 2021.

3. Results and Discussion

3.1. Properties of Raw Rabbit Manure

The properties of different types of raw rabbit manure are shown in Table 1. The dry matter (DM) content in the three kinds of rabbit manures were 41.0%, 38.7%, and 39.7%, respectively. High organic matter contents were observed in the raw manures with contents of ~84% DM. Among them, lignocellulose (sum of hemicellulose, cellulose, and lignin) was dominant with the contents of >66% DM. Moreover, the contents of N, P, and K were in the range of 1.6–2.0% DM, 6.5–8.2% DM, and 1.2–1.4% DM, respectively. Most of these contents were comparable with the range reported in existing studies (e.g., [10,25]). Furthermore, some differences between the three types of manure were observed; for example, the N content of R1 was significantly ($p < 0.05$) higher than that of R2 and R3, which should be attributed to the difference in their feeds (Table A1) and to different animal age [25].

3.2. Rabbit Manure Composting and Air-Drying

3.2.1. Temperature and Moisture Content

Temperature and moisture content variations during the composting process are shown in Figure 1a,b. The temperatures of the three types of manure rapidly increased within the first day and then remained at >50 °C for 7, 9, and 10 days in R1, R2, and R3, respectively; finally, all the temperatures slowly decreased to ambient temperature on day 23 (Figure 1a). In addition, their moistures were constantly decreasing and were approximately 25% on day 23 (Figure 1b). The composting temperature decreased between day 10 and day 12 (Figure 1a), and this may have been due to the sharp decrease in moisture content between day 6 and day 10 (Figure 1b) [26]. However, the results of the composting temperature and final moisture indicate that the composting process could be terminated referring to the quality requirement (temperature $\geq$ 50 °C lasting $\geq$ 7 days, moisture content $\leq$ 30%) in the Chinese standard document [27]. Relatively lower composting temperatures were observed in R1 compared to R2 and R3, which may have been because its lower C/N ratio (Table 1) resulted in weaker microbial activity during composting, as in the result of [28,29]. Compared to the composted manure, the final moistures of 9.1–21% in air-dried manure were lower (Figure 1c), which means that more water was lost in the air-drying process.

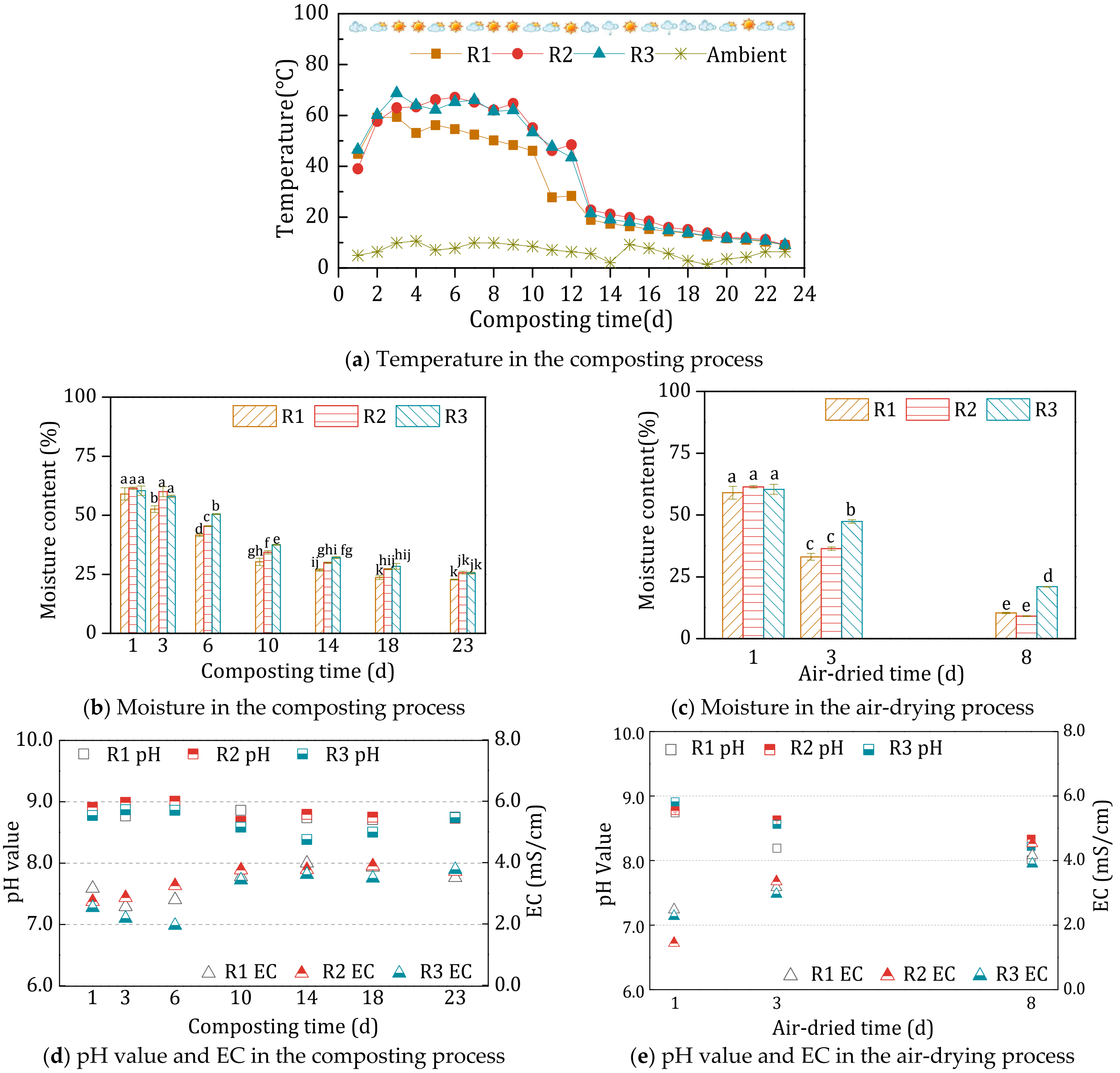


Figure 1. Temperature (**a**), moisture content (**b**,**c**), pH value, and EC (**d**,**e**) variations during composting and air-drying processes of three kinds of rabbit manures (R1, R2, and R3). Note: The manures from R1, R2, and R3 were collected from a pregnant doe room, an early-fattening rabbit (after weaning) room, and a late-fattening rabbit room, respectively. The means with different superscript letters for a bar differ significantly ($p < 0.05$). The pH and EC values were tested at a ratio of manure to distilled water of 1:10.

3.2.2. pH Value and EC

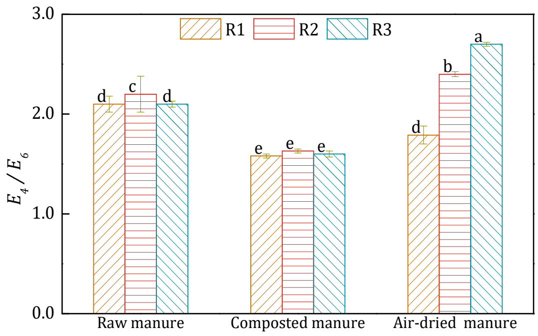
The changes in the pH value and electric conductivity (EC) of R1, R2, and R3 during the composting and air-drying processes are shown in Figure 1d,e. During the composting process, the pH values initially increased slightly from 8.8–8.9 on day 1 to 8.9–9.0 on day 6, then decreased to 8.4–8.7 on day 14, and finally increased to 8.7–8.8 on the final day, day 23. The EC values of 2.5–3.2 mS/cm on day 1 slightly decreased within 6 days and then increased to 3.5–3.8 mS/cm on day 23 (Figure 1d). During the composting process, slight fluctuations in pH values were observed, which were attributed to the combined actions of the mineralization of nitrogen-containing organic matter, the volatilization of ammonia, the generation and dissolution of organic acids, and the nitrification effect [30]. Increases in EC were always attributed to the release of phosphate, ammonium, and potassium ions [31].

Similarly, in the air-drying process, the pH values also decreased, with final values of 8.0–8.3, and EC values increased to about 4.2 mS/cm on the final day, day 8 (Figure 1e). Notably, the EC values of air-dried manure were higher than 4 mS/cm, which means a higher concentration of soluble minerals, but they may sometimes have negative effects on plant growth when they are returned to the farmland [32,33].

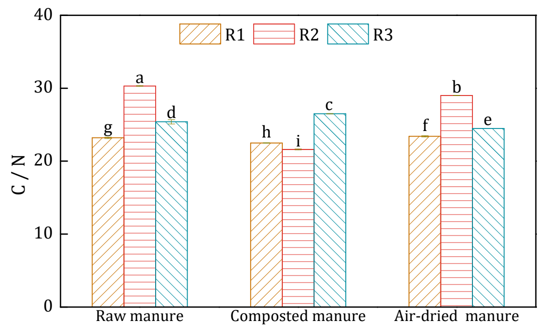
3.2.3. The E_4/E_6 and C/N Ratios

The E_4/E_6 and C/N ratios are indictors for evaluating the maturity of composted manure; they are shown in Figure 2a,b. The E_4/E_6 ratio was reduced to 1.6 from 2.1–2.2 in the three types of manure via 23-day composting, but it increased to 2.4–2.7 (except R1) after 8-day air-drying (Figure 2a). Similarly, the C/N ratios were also reduced, and the values of 23.2–30.3 in raw manure were reduced to 21.6–26.5 after 23-day composting, but they were only slightly reduced to 23.4–29 via 8-day air-drying (Figure 2b).

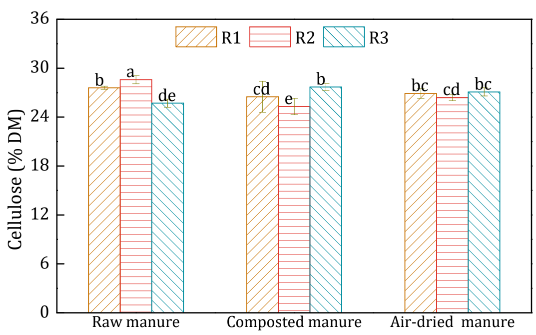
E_4/E_6 is the ratio of the optical densities of humic acids and fulvic acids at 460 and 660 nm, which is always used to reflect the degree of condensation of the aromatic nucleus of humus, indicating the maturity of composted manure [34]. The reduction in the E_4/E_6 ratio in the composted manure indicates that much more stable organic matter such as humus were produced [35], and the reduction in the C/N ratios was attributed to the degradation of organic matter (including organic acids, phenols, cellulose, hemicellulose, etc.) through humification [32]. In particular, the greatest reductions of 0.57 units in E_4/E_6 and 8.7 units in the C/N ratio were simultaneously observed in the R2 treatment, which means that it has the highest composting maturity. The slight reductions in the E_4/E_6 and C/N ratios in the air-dried manure indicates that there was almost no humification in the air-drying process.



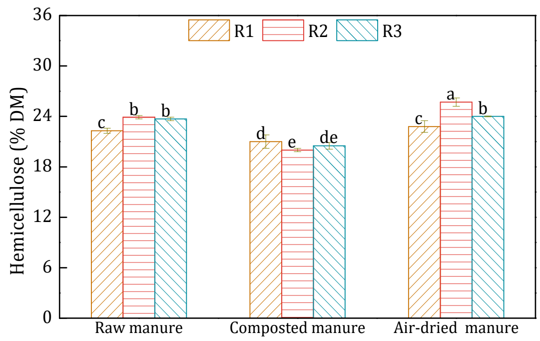
(**a**) *E₄/E₆*



(**b**) C/N ratio

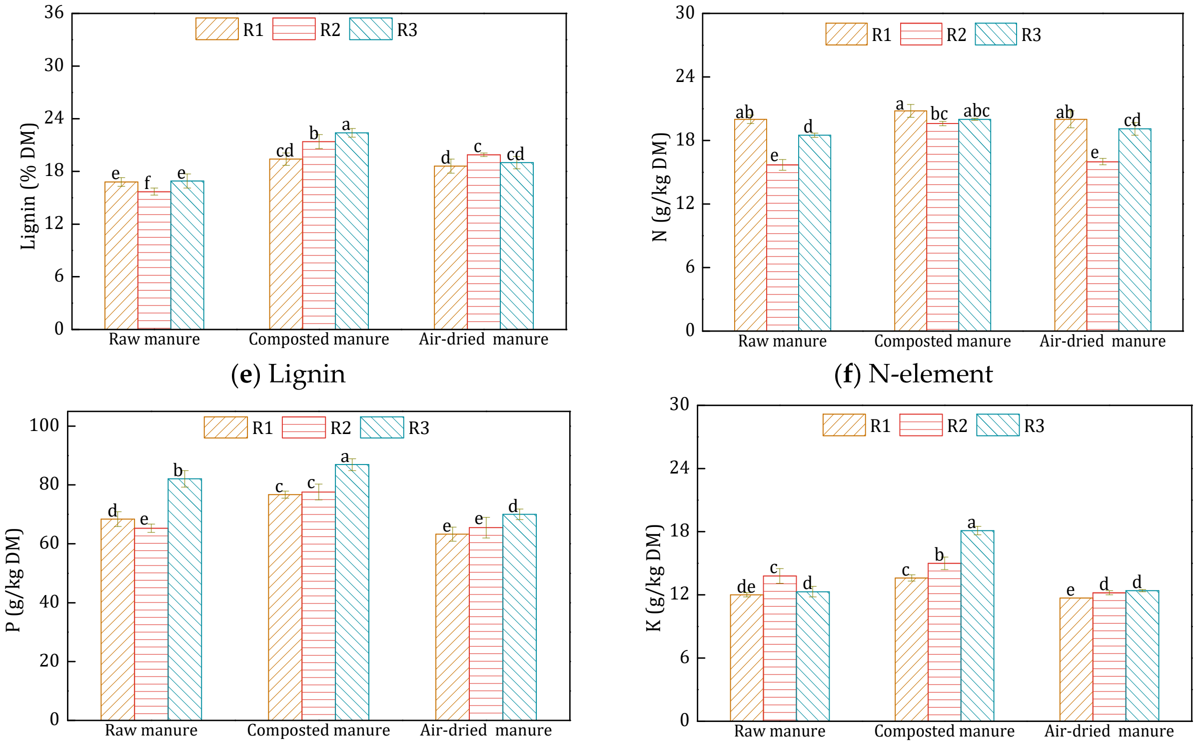


(**c**) Cellulose



(**d**) Hemicellulose

Figure 2. *Cont.*



(**e**) Lignin (**f**) N-element (**g**) P-element (**h**) K-element

Figure 2. Changes in physicochemical parameters during the composting and air-drying processes of three kinds of rabbit manure (R1, R2, and R3). Note: The manures from R1, R2, and R3 were collected from a pregnant doe room, an early-fattening rabbit (after weaning) room, and a late-fattening rabbit room, respectively. The means with different superscript letters for a bar differ significantly ($p < 0.05$).

3.2.4. Lignocellulose

Figure 2c–e show the lignocellulose content (including cellulose, hemicellulose, and lignin contents) before and after 23-day composting and 8-day air-drying. After 23-day composting, the hemicellulose contents were reduced to 20.0–21% from 22.3–23.9% in raw manure (Figure 2d). Moreover, the cellulose contents of 27.6% and 28.6% in R1 and R2 were also reduced to 26.5% and 25.3%, except for a slight increase observed in R3 (Figure 2c). By contrast, the contents of lignin increased from 15.7–16.9% to 19.4–22.4% (Figure 2e). The decrease in lignocellulose content was due to the degradation activity of aerobic microorganisms during the composting process [36]. Furthermore, because of a concentration effect, the relative concentration of lignin significantly increased in this study (Figure 2e) [37]. Nevertheless, the greatest reduction of ~1.5% in lignocellulose content was observed in the R2 treatment, which was consistent with the results regarding the reductions in the E_4/E_6 and C/N ratios.

3.2.5. N-, P-, K-Elements

The element contents of N, P, and K in the three manures before and after 23-day composting and 8-day air-drying are shown in Figure 2f–h. After 23-day composting, the N-element contents increased to 19.6–20.8 g/kg DM from 15.7–20.0 g/kg DM, the P-element contents increased to 76.7–86.9 g/kg DM from 65.3–82.1 g/kg DM, and the K-element contents increased to 13.6–18.1 g/kg DM from 12.0–13.8 g/kg DM. The largest increase in the total contents of N-, P-, and K-elements were observed in R2 (manure of early-fattening rabbits), where the total content increased by 18.4%. However, after 8-day air-drying, the contents of all three elements were reduced or no significant change was observed. N-, P-, and K-elements are important ingredients of organic fertilizer [38]. The increase in N-, P-, and K-element contents was due to a "concentration effect" [37], which means that other matter (e.g., C-element) was degraded in a larger proportion. Compared to the reductions in N-, P-, and K-elements in the air-dried rabbit manure, the increase in composted rabbit manure has outstanding advantages and would be useful for subsequent application in circular agriculture.

3.2.6. Safety Evaluation

Safety evaluation, including of heavy metals, GI, and hygienic indicators (mortality of ascaris egg and fecal coliforms), is a crucial step before manure is applied to farmland [39]. The heavy metal contents of rabbit manure before and after composting and air-drying are shown in Table 2. The heavy metal content of other animals' manure reported in existing reports and in Chinese standard documents involving organic fertilizer, growing medium for flowering trees and shrubs, and organic media for greening were also reviewed and are shown in Table 2. The contents of Cr, As, Cd, Hg, and Pb in raw manure decreased slightly in the composted manure but increased slightly in the air-dried manure (except Pb content). Referring to the Chinese standard documents on growing media for flowering trees and shrubs [40] and organic media for greening [41], these heavy metals in rabbit manure, whether before or after composting or air-drying, all met the threshold requirements, which means that the application of rabbit manure may not cause heavy metal pollution in the soil. In addition, the heavy metal contents in rabbit manure are evidently lower than those in other manures; for example, there are high contents of Cr in chicken manure [42], high contents of Cd in cattle manure [43], and high contents of Cd, Hg, and Cr in broiler manure, swine manure, layer manure, and dairy cattle manure [44].

Table 2. Heavy metal content in different kinds of manure [1,2,3].

	Cr	As	Cd	Hg	Pb	Reference
	mg/kg of Dry Matter					
Raw manure	7.1–9.5	4.0–6.0	0.4–0.4	<0.1	12.0–19.6	This study
Composted manure	7.1–8.2	4.0–5.0	0.4–0.4	<0.1	12.6–14.5	
Air-dried manure	5.3–10.2	4.9–8.0	0.3–0.4	<0.1	13.8–18.0	
Chicken manure	153.7 ± 177.0	2.7 ± 2.3	0.3 ± 0.1	/	6.4 ± 4.4	Duan B. and Feng Q., 2021
Broiler manure	1.9–140.2	N–38.1	0.1–14.2	0.1–83.9	0.5–57.2	Wang et al., 2013
Swine manure	1.0–802.4	N–73.9	N–15.3	0.1–505.0	/	
Layer manure	1.1–1601.7	0.0–41.2	0.1–8.9	0.0–81.3	1.2–13.5	
Dairy cattle manure	2.9–678.7	N–8.3	0.1–5.2	0.1–77.3	2.4–14.7	
Cattle manure	N–3.6	0.5–19.4	N–10.5	/	0.5–5.5	Zhang et al., 2012
Growing medium for flowering trees and shrubs	≤400	≤40	≤2	≤2	≤500	National Forestry and Grassland Administration, 2016
Organic media for greening	≤70	≤10	≤1.5	≤1	≤120	General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, 2017

[1] Values are not reported. [2] N means that the content of the sample was below the limit of detection. [3] Different manure means (R1, R2, and R3) were collected from a pregnant doe room, an early-fattening rabbit (after weaning) room, and a late-fattening rabbit room, respectively.

The GI, as another more direct indicator for safety evaluation, is shown in Figure 3. After composting, the GI values of the three kinds of rabbit manure increased significantly to 70.5–78.5% from 41.3–50.5% on day 1 ($p < 0.05$). Moreover, the GI of >70% could be credited as non-toxic to plants [45]. After air-drying, the GI values of R1, R2, and R3 were significantly different and only 46.9%, 63.2%, and 68.5%, which indicates that the effect of air-drying was unstable.

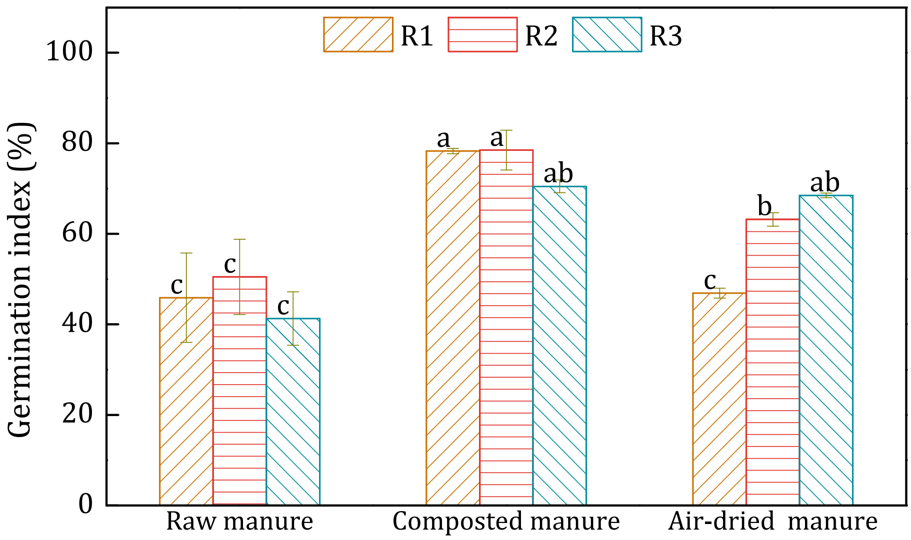


Figure 3. Changes in the germination index during composting and air-drying of three kinds of rabbit manure (R1, R2, and R3). Note: The manure from R1, R2, and R3 was collected from a pregnant doe room, an early-fattening rabbit (after weaning) room, and a late-fattening rabbit room, respectively. The means with different superscript letters for a bar differ significantly ($p < 0.05$).

In addition, the hygienic indicators (shown in Table A2) of the composted manure also meet the relevant requirements (mortality of ascaris egg $\geq$ 95%; fecal coliforms $\leq$ 100 Pcs/g) in Chinese technical specification for animal manure composting [45], except R3, which may have been polluted by the environment.

Based on the above results, it may be concluded that rabbit manure has good compost characteristics. Heavy metal contents and safety indicators in particular showed the superiority of rabbit manure for use in farmland, and provided a basic reference for the farmland utilization of rabbit manure. In this study, composted R2 was selected for the next seedling experiment because of its better performance compared to the others.

3.3. Properties of Growing Media

Good physical and chemical properties in growing media are among the important prerequisites for cultivating high-quality seedlings. The physicochemical properties of the growing media manufactured by composted rabbit manure as an organic matrix to replace peat are shown in Table 3. The bulk density of manufactured growing media with five different ratios of composted rabbit manure had no significant difference and ranged from 0.12 to 0.14 g/cm^3. However, the air space (ranging from 15.7 to 28.7%) of the five growing media was significantly different. Compared to the growing media of T0 without rabbit manure, the air space increased significantly after the mixing of rabbit manure, and the highest value of 28.7% was observed in the growing media of T30, whose ratio of rabbit manure to peat was 1:1. However, the total porosity decreased after the mixing of rabbit manure from 69.2% in the growing media of T0 to the lowest value of 66.8% in the growing media of T45, except for T30 and T15, whose total porosity values were not significantly changed. The pH values of the five growing media increased significantly with increasing rabbit manure, from 6.2 in the growing media of T0 to 8.1 in the growing media of T60. Similarly, the EC values also increased with increasing rabbit manure, from 0.2 mS/cm in the growing media of T0 to 2.6 mS/cm in that of T60.

Table 3. Manufacture and characteristics of different growing media [1].

	T0	T15	T30	T45	T60
			Composition of growing media (based on dry weight)		
Composted rabbit manure [2]	0%	15%	30%	45%	60%
Peat	60%	45%	30%	15%	0%
Perlite	20%	20%	20%	20%	20%
Vermiculite	20%	20%	20%	20%	20%
			Characteristics of the growing media (n = 3)		
Bulk density (g/cm^3)	0.14 ± 0.01 a [3]	0.12 ± 0.00 a	0.12 ± 0.01 a	0.12 ± 0.01 a	0.13 ± 0.00 a
Air space (%)	15.7 ± 0.4 c	24.5 ± 0.8 b	28.7 ± 0.2 a	23.5 ± 1.7 b	24.7 ± 0.9 b
Total porosity (%)	69.2 ± 1.4 a	68.9 ± 0.9 a	68.8 ± 0.2 ab	66.8 ± 0.4 c	67.2 ± 0.9 bc
pH value [4]	6.2 ± 0.1 e	6.7 ± 0.2 d	7.2 ± 0.1 c	7.6 ± 0.1 b	8.1 ± 0.1 a
EC (mS/cm)	0.2 ± 0.0 e	0.8 ± 0.0 d	1.4 ± 0.0 c	1.9 ± 0.0 b	2.6 ± 0.0 a

[1] The means with different letters in a row differ significantly ($p < 0.05$). [2] Composted rabbit manure means the rabbit manure compost from early-fattening rabbits. [3] ±means standard deviation. [4] The pH and EC values were tested at a ratio of manure to distilled water of 1:10.

The increase in air space by mixing with composted rabbit manure may be due to the addition of lignocellulose from rabbit manure, just as the use of wood fibers may affect the results of particle size and shape [46,47]. Moreover, the increase in air space could allow the manufactured growing media to better meet the ideal range of air space, which is approximately 15–30%, in order to satisfy seedling root requirements for aeration performance and water permeability [48]. Furthermore, the total porosity of the growing media, which is always related to water-filled porosity, decreased after mixing the composted rabbit manure, but all the total porosities were in the ideal range of 50–90% [40]. The increased pH and EC values after the mixing of composted rabbit manure were attributed to the high concentrations of salt from the composted manure [49], which may result in salinity stress in green plants [50]. The same was found in the composting of rabbit and goat manure in the report of Paredes et al. [51], where the pH value obtained was 9.41 and the EC was 11.33 dS/m. Hence, the limited value of approximately 2.0 mS/cm means that the mixing ratio of composted rabbit manure should be no more than 45% based on these results [40].

3.4. Seedling Qualities

The seedling emergence percentages of cabbage in different growing media are shown in Figure 4. The emergence percentages of T0, T15, T30, and T45 were comparable and all were higher than 90% after day 10 after sowing. In the 20-day seeding assay, the growing media of T30, whose peat was replaced by rabbit manure at a ratio of 1:1, had the highest emergence percentages. However, in the growing media of T60, whose peat was completely replaced by rabbit manure, the emergence percentage was only 62.3% on day 10 but increased to 90.3% until day 20. The good performance in the growing media of T30 on the emergence percentage may be due to its highest air space and the suitable pH and EC values (Table 3). The worst performance observed in the growth medium at T60 should be attributed to the high pH and EC, which are not very suitable for seedlings.

Table 4 shows the growth, physiological status and comprehensive evaluation of cabbage seedlings after 28-day seeding in the five growing media. Compared to the growing media of T0 without rabbit manure, the growing media mix with a ratio of composted rabbit manure less than 30% (i.e., T15 and T30) has better seedling quality, and the seedling height was significantly improved. In the growing media with a rabbit manure ratio of 45% (i.e., T45), the chlorophyll was significantly improved but the stem length was significantly decreased. The worst performance was still observed in the growing media of T60, whose peat was completely replaced by composted rabbit manure, including significant decreases in stem diameter, stem length, seedling height, and aboveground fresh weight compared to T0. Additionally, the seedling quality was comprehensively evaluated by a comprehensive score calculated using the membership function (as described in Section 2.4) and it is shown in Table 4. The highest score of 0.85 was obtained in the growing media of T30, which was higher than the 0.67 and 0.58 in the growing media of T45 and T15, and higher than the 0.52 in the growing media of T0. The lowest score was only 0.14, observed in the growing media of T60.

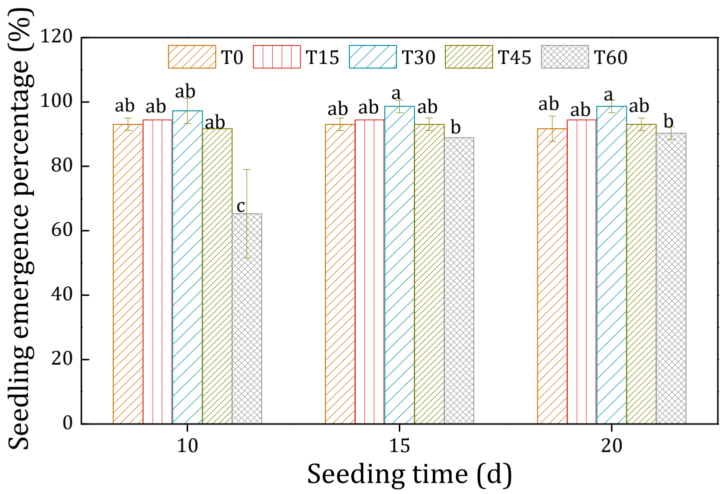


Figure 4. Changes in the seedling emergence percentage of different growing media (T0, T15, T30, T45, and T60). Note: The means with different superscript letters for a bar differ significantly ($p < 0.05$).

Table 4. Growth, physiological status, and comprehensive evaluation of cabbage seedlings after 28-day sowing in different growing media (T0, T15, T30, T45, and T60) [1].

	Unit	T0	T15	T30	T45	T60
Stem diameter	mm	1.2 ± 0.1 a	1.2 ± 0.1 a	1.3 ± 0.1 a	1.2 ± 0.1 a	0.9 ± 0.0 b
Stem length	mm	75.3 ± 2.7 a	75.7 ± 7.5 a	77.0 ± 2.1 a	69.5 ± 2.0 b	55.6 ± 2.4 c
Root length	mm	65.0 ± 11.6 b	62.6 ± 14.9 b	114.3 ± 57.2 a	62.7 ± 15.8 b	45.9 ± 12.9 b
Seedling Height	mm	70.3 ± 5.8 b	84.5 ± 6.7 a	74.6 ± 2.9 b	70.5 ± 2.9 b	54.8 ± 8.1 c
Aboveground fresh weight	g	0.410 ± 0.071 ab	0.445 ± 0.063 a	0.454 ± 0.058 a	0.372 ± 0.033 b	0.301 ± 0.025 c
Belowground fresh weight	g	0.019 ± 0.011 ab	0.017 ± 0.005 b	0.021 ± 0.007 ab	0.028 ± 0.008 a	0.020 ± 0.003 ab
Aboveground dry weigh	g	0.019 ± 0.004 ab	0.019 ± 0.003 ab	0.022 ± 0.003 a	0.018 ± 0.002 b	0.016 ± 0.001 b
Belowground dry weight	g	0.002 ± 0.001 a	0.002 ± 0.001 a	0.002 ± 0.000 a	0.002 ± 0.001 a	0.002 ± 0.000 a
Chlorophyll	mg/dm^2	0.53 ± 0.03 b	0.96 ± 0.06 ab	0.92 ± 0.20 ab	1.15 ± 0.30 a	0.99 ± 0.16 ab
Root–shoot ratio	/	0.10 ± 0.03 ab	0.08 ± 0.03 b	0.10 ± 0.02 ab	0.13 ± 0.03 a	0.10 ± 0.01 ab
Seedling vigor index	/	0.003 ± 0.001 a	0.002 ± 0.001 a	0.003 ± 0.001 a	0.003 ± 0.001 a	0.002 ± 0.000 a
Comprehensive score	/	0.52	0.58	0.85	0.67	0.14

[1] The means with different letters in a row differ significantly ($p < 0.05$).

These results indicate that the seedling qualities were improved through manufactured growing media with mixed composted rabbit manure. The improvement may have been caused by three aspects. Firstly, the ventilation and drainage performance affected by the air space (Table 3) may have been enhanced [12]. Secondly, the development of seedlings may have benefited from nutritional content [52], such as the high levels of P, Ca, and Mg from the composted rabbit manure [13]. Finally, the stem and leaf developments could also have been positively affected by some agronomical-effective hormones from substances similar to rabbit manure and humus produced from the composting process, such as gibberellin [3], fulvic acids, and humic acids [53]. Similarly, Mahmoud et al. [11] also revealed the good performance of rabbit manure used as an organic fertilizer for organic tomato. Overall, these results indicate that it is a feasible strategy to use composted rabbit manure as an organic matrix replacing peat to manufacture growing media. The best seedling performance in this study was obtained in the growing media where the composted manure, peat, perlite, and vermiculite ratio was 3:3:2:2. However, the mixing ratio of rabbit manure and peat should be decided carefully due to the high pH and EC values in rabbit manure. However, these problems could be further modified by some methods such as leaching, adding amendments (such as phosphogypsum and gypsum [54]), or using these growing media for some salt-tolerant plants (such as calendula officinalis [55]). Moreover, the different sensitivities and requirements of specific crops for substrates need to be further studied, and more effort may be required to improve the quality of the seedlings.

4. Conclusions

This study proved and highlighted that composted rabbit manure could effectively replace peat to manufacture growing media. Composting could ensure the safe use of rabbit manure for seedling. The heavy metal contents and the characteristics of growing media mixing with composted rabbit manure could satisfy the seedlings' requirements, and the seedling qualities could be enhanced. Moreover, the best seedling performance was obtained in the growing media where the composted manure, peat, perlite, and vermiculite ratio was 3:3:2:2. The mixing ratio could be further optimized for the different requirements of specific crops. More effort may be required to improve the quality of the seedlings in further research.

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Appendix A

Table A1. Product characteristics of different rabbit feeds (Jinyu No. 573, Jinyu No. 518, Jinyu No. 572).

Rabbit Feed Label	Product Ingredients (%)								
	Crude Protein	Crude Fiber	Ash	Calcium	Total Phosphorus	Sodium Chloride	Lysine	Methionine	Moisture Content
Jinyu 573	≥17.0	≤20.0	≤12.0	0.60–1.80	≥0.55	0.30–1.20	≥0.65	/	≤14.0
Jinyu 518	≥14.5	≤20.0	≤12.0	0.60–1.80	≥0.50	0.30–1.20	/	0.3–0.9	≤14.0
Jinyu 572	≥16.0	≤20.0	≤12.0	0.60–1.80	≥0.55	0.30–1.20	≥0.65	/	≤14.0

Table A2. Hygienic indicators of different composted rabbit manures (R1, R2, and R3) [1].

	Mortality of Ascarid Egg (%)	Fecal Coliforms (Pcs/g)
R1	100	<3.0
R2	100	<3.0
R3	100	>1.1 × 1000
Threshold values [2]	≥95	≤100

[1] The manures from R1, R2, and R3 were collected from a pregnant doe room, an early-fattening rabbit (after weaning) room, and a late-fattening rabbit room, respectively. [2] Chinese standard: organic fertilizer (NY 525-2021).

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