



Article

Growth and Physiological Characteristics of Sour Jujube Seedlings in Different Substrate Formulations

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Abstract: The raising of container seedlings with light substrates has become an important method of seedling raising, without delaying the seedling period. In order to reduce reliance on non-renewable peat and to promote the reuse of organic waste, this study compared the growth of sour jujube seedlings in different substrate formulations (i.e., different proportions of vermicompost instead of peat), using a semi-subterranean placement of root control bags, and explored the application of vermicompost in the raising of sour jujube seedlings. The results showed that there were significant differences in the growth and the physiological and photosynthetic characteristics of sour jujube seedlings treated with different substrates, among which substrates A₂ (peat: vermicompost: vermiculite: garden soil = 0.5:0.5:1:1) and A₃ (peat: vermiculite: garden soil = 1:2:1) were suitable for sour jujube seedling raising. The seedling height, the seedling ground diameter, the number of secondary branches, the length of the longest secondary branch, the total fresh weight, the above-ground fresh weight, the total root length, the root projection area, and the root surface area were all significantly greater than those of jujube seedlings grown on other substrates. Especially in A₃, vermicompost can replace peat as the nursery substrate for sour jujube seedlings, removing dependence on non-renewable peat resources, reducing costs, and providing more prospects for application. The suitable substrate conditions for sour jujube seedlings were as follows: soil porosity 44.0–54.0%, electric conductivity (EC) value 0.2 mS/cm, organic matter 40.39–54.05 g·kg⁻¹, total nitrogen and total phosphorus of 1.67–1.91 g·kg⁻¹ and 0.95–1.20 g·kg⁻¹, respectively, alkali-hydrolyzed nitrogen 139.75–154.69 mg·kg⁻¹, and available phosphorus 137–224 mg·kg⁻¹.

Keywords: sour jujube (*Ziziphus acidojujuba* (Cheng et Liu)); container seedlings; vermicompost; enzymatic activity; photosynthetic parameters; chlorophyll content; seedling height; root



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1. Introduction

Chinese jujube (*Ziziphus jujuba* Mill.) is the most important cultivated species of the family Rhamnaceae. It is native to China, and it was one of the first deciduous fruit trees in the world to be cultivated. It has a long history of cultivation and a wide range of cultivation areas [1]. Chinese jujube has now been planted in 48 countries on five continents, and it is becoming more and more attractive to consumers, due to its high nutrition. Jujube is also a common clinical Chinese medicine in China; it is a natural resource with a long history of dual use as medicine and food. Jujube is rich in vitamin C and vitamin P, and has cholesterol-lowering, liver-protection, and other health functions [2]. Generally, Chinese jujube is propagated by grafting new cultivars on rootstock sour jujube (wild jujube, *Ziziphus acidojujuba* (Cheng et Liu)), which seriously influences the quality of Chinese jujube plantlets. Sour jujube seedlings are generally raised by directly sowing sour jujube seeds in the field. Bare root seedlings have many disadvantages, such as a less-developed absorbing root system, a lower survival rate, and a longer recovery period after transplanting.

Peat, perlite, rice husk, etc., are growing substrates that are widely used, with ideal effects on seedling raising [3]. Peat is an ideal container-seedling substrate, with the advantages of high porosity, good permeability, and fewer pests. However, peat is a non-renewable resource, with high costs and poor water retention [3–5]. Therefore, it is particularly urgent that natural lightweight substrates (e.g., vermicompost) be developed and utilized to cultivate seedlings.

Vermicompost is a decomposed organic fertilizer with good physical properties (porosity, water retention, etc.). It is rich in nutrients (nitrogen, phosphorus, potassium, and medium and microelements) and plant growth regulators, such as gibberellins and growth hormones [6,7], and it may be considered as a substrate to replace peat resources. The addition of vermicompost to peat substrate has influenced the biometric parameters of cucumber seedlings, the physiological parameters and the content of minerals in the leaves, and the early and total yield of the plants [8]. Messina et al. reported that supplements of vermicompost in peat and coir improved the nitrogen supply, which could benefit plant growth [9]. The addition of vermicompost to the culture media improved in-vitro-produced banana plants in a greenhouse. The use of vermicompost combined with vermiculite and sand (33.3% each) recorded the best results for most vegetative growth parameters. Vermicompost at 75%, with peat moss at 25%, provided the highest mineral content values in the two periods of the study.

In this study, we used different proportions of vermicompost, instead of peat, to prepare the seedling substrate by comparing the growth of sour jujube container seedlings and screening the best application rate of vermicompost to provide a theoretical basis for the application of vermicompost in sour jujube seedlings.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The experimental site was located in the Third Experimental Farm of Hebei Agricultural University in Lian Chi District, Baoding City, Hebei Province, China, with longitude 115°21′–115°30′ E and latitude 38°49′–38°56′ N (Figure 1). It is a warm temperate monsoon continental climate, with a dry and windy spring, a hot and rainy summer, a cool and less rainy autumn, and a cold and less snowy winter. The four seasons are distinct and the continental climate is obvious. The test was conducted outdoors in an open field. This study was conducted in 2022. It was a one-year experiment. In the test year (2022), Baoding, Hebei Province, had an annual average temperature of 20 °C, a maximum temperature of 41 °C, a minimum temperature of −13 °C, and an annual average rainfall of 659.5 mm. It had a calendar-year average temperature of 13.4 °C, a historical maximum temperature of 43 °C, a historical maximum low of −20 °C, calendar-year average sunshine hours of 2511 h, an annual average frost-free period of 211 days, and an annual average rainfall of 498.9 mm.

High-quality sour jujube seeds were purchased from Zanhuan County, Shijiazhuang City, Hebei Province, with high germination rates of about 95%. The matrix raw materials were peat (Wosong brand, produced by Dahanyuanjing Technology Co., Ltd., Shanghai, China, 250 L, weight 42.5 kg, CNY 160), vermiculite (7.5 kg per bag, CNY 15, produced by Kaicheng Mineral Products Co., Ltd., Lingshou, Shijiazhuang, Hebei Province, China), vermicompost (40 kg per bag, CNY 22, produced by Tengqiqianlong Agricultural Technology Co., Ltd., Jinan City, Shandong Province, China), garden soil (taken from the Third Experimental Farm of Hebei Agricultural University, with soil density 0.994 g·cm^{−3}, total porosity 46.5%, pH 8.23, EC 0.17 mS·cm^{−1}, organic matter 19.4 g·kg^{−1}, total nitrogen 1.20 g·kg^{−1}, total phosphorus 0.86 g·kg^{−1}, total potassium 17.94 g·kg^{−1}, alkali-hydrolyzed nitrogen 100.28 mg·kg^{−1}, available phosphorus 41.69 mg·kg^{−1}, and rapidly available potassium 315.63 mg·kg^{−1}), etc. The containers were bags made of new generation non-woven material (produced by Rino Plastic Co., Ltd., Anqing City, Anhui Province, China, 25 cm in diameter and 30 cm in height).

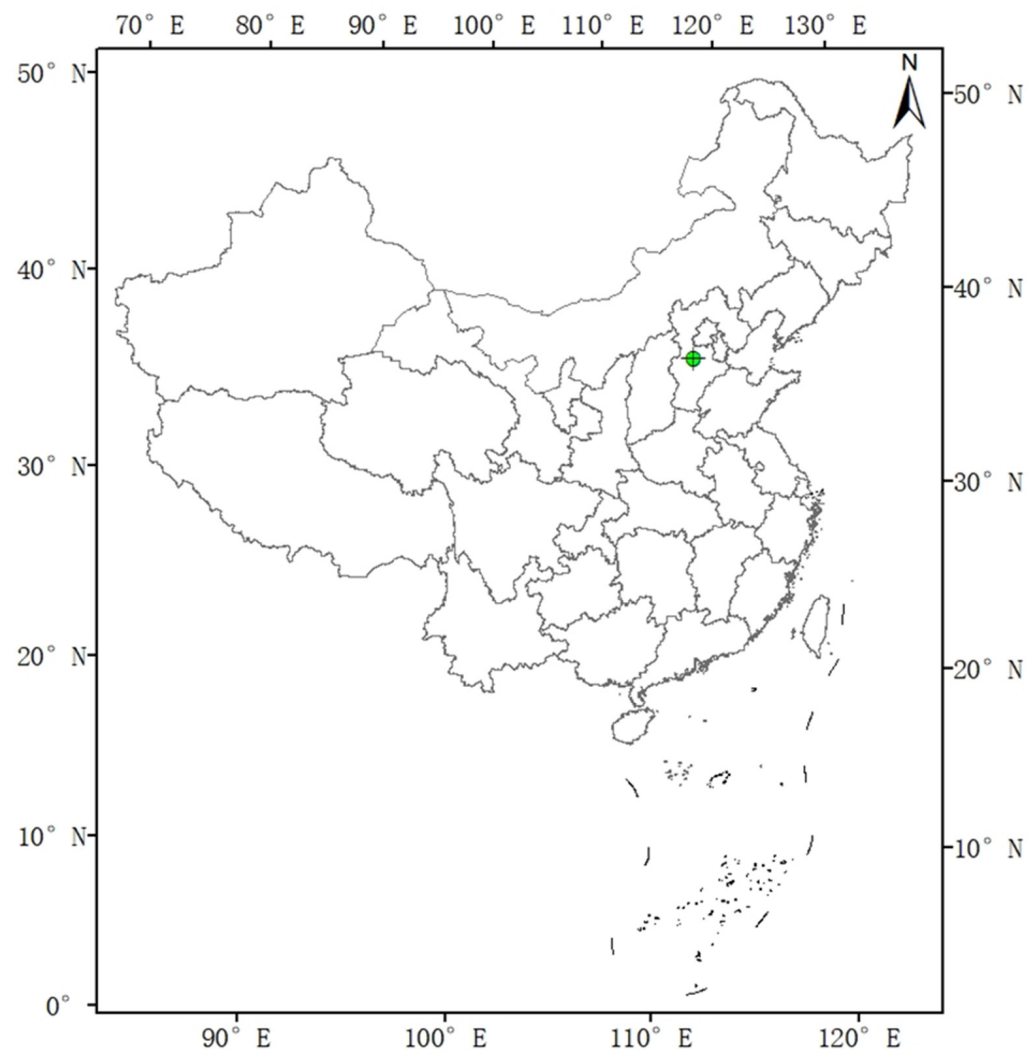


Figure 1. Location of the study site. The green dot in the figure is the test sites for this study. It was located in the Third Experimental Farm of Hebei Agricultural University in Lian Chi District, Baoding City, Hebei Province, China, with longitude $115^{\circ}21'–115^{\circ}30'$ E and latitude $38^{\circ}49'–38^{\circ}56'$ N.

Vermicompost was made as follows. Firstly, the straw was finely crushed. Secondly, cow dung was added, mixed, and rotted with straw. Thirdly, earthworms were added to the mixture and the moisture of the mixture was maintained for the growth earthworms. The earthworms ate straw, absorbed the nutrients of straw, and excreted them. Half a month later, the mixture had been fully fermented and decomposed to make organic fertilizer, namely vermicompost.

Using a single-factor randomized block design, the experiment was designed with five treatments (Table 1), and each treatment was divided into three plots with 15 bags each, for a total of 45 bags. Sowing of sour jujube seeds was carried out on 18 April 2022 by sowing 2–3 well-developed sour jujube seeds in a non-woven bag containing substrate, and, after 1 month, one robust seedling was selected and retained. The bags were placed fully underground, and irrigation was carried out to keep the substrate moisture not less than 60% of the maximum field water holding capacity, and other daily management methods were consistent. Considering that rootstock diameter is of greater concern in rootstock seedling (sour jujube seedling) raising, tip removal was performed on 29 July 2022 to promote thickening growth of sour jujube seedlings. The above-ground growth of sour jujube seedlings was investigated at 100 days (27 July 2022), 130 days (26 August 2022), 160 days (25 September 2022), and 300 days (12 February 2023) after sowing, respectively.

The root determination were conducted 300 days (12 February 2023) after sowing to compare the effects of different substrates on the growth of container seedlings.

Table 1. Different substrates treatments.

Substrates	Treatments
A ₀	peat: vermiculite = 2:1
A ₁	peat: vermicompost: vermiculite: garden soil = 0.75:0.25:1:1
A ₂	peat: vermicompost: vermiculite: garden soil = 0.5:0.5:1:1
A ₃	vermicompost: vermiculite: garden soil = 1:2:1
A ₄	vermicompost: vermiculite: decomposed sheep manure = 1:2:1

2.2. Determination of Physical Properties of Matrix

The determination of physical and chemical indicators of the rhizosphere substrates was carried out on 27 July 2022. The indicators included the substrate density, total porosity, pH, EC value, organic matter, humic acid, total nitrogen, total phosphorus, total potassium, alkali-hydrolyzed nitrogen, available phosphorus, and rapidly available potassium [10].

2.3. Determination of Enzymatic Activity of Matrix

The enzyme activity of the rhizosphere substrate was measured on 26 August 2022. Soil urease activity was determined by the phenol-sodium hypochlorite colorimetric method. The soil phosphatase activity was determined by colorimetric method using sodium benzene phosphate. The activity of sucrase was determined by a colorimetric method using 3,5-dinitrosalicylic acid and catalase activity was determined by potassium permanganate titration method [11].

2.4. Determination of Growth Characters, Biomass and Root Parameters

We measured above-ground growth characteristics (27 July 2022; 26 August 2022; 25 September 2022; 12 February 2023), biomass, and root parameters (12 February 2023) of sour jujube seedlings. Seedlings were measured for plant height, stem diameter, number of secondary branches, length of secondary branches, above-ground fresh weight, above-ground dry weight, root fresh weight, root dry weight, total root length, primary root length, root surface area, root volume, root diameter, and root projected area. Seedling height and longest secondary branch length were measured by steel tape measure. Stem diameter was measured by vernier caliper. The fresh weight was conducted as follows: dig out the jujube seedlings from the non-woven bags, clear the matrix from the roots, separate the above-ground and roots of the jujube seedlings with pruning shears, and weigh the fresh weight of above-ground and roots separately. Total root length, root surface area, root volume, root diameter, and root projected area of seedlings were determined by scanning root images using a (Microtek) Scan Maker i800Plus scanner and obtaining root morphological indices using the Wanshen LA-S Series Plant Image Analysis System [12].

2.5. Determination of Photosynthetic Parameters

On 25 September 2022, the net photosynthetic rate (P_n), transpiration rate (Tr), stomatal conductance (G_s), and intercellular CO₂ concentration (C_i) of healthy leaves were measured by LI-6800 portable photosynthetic instrument. Five vibrant and healthy leaves on the longest secondary branch of sour jujube seedlings were selected for each treatment. The determination time was 9:00 a.m.~11:30 a.m. in sunny weather [13].

2.6. Determination of Chlorophyll Content

At 160 days after sowing, 10 healthy leaves were randomly picked from the longest secondary branch of each treatment, and immediately bagged and taken back to the laboratory, and rinsed with ddH₂O three times. After rinsing, 2 g of cut leaves was weighed, 10 mL 95% ethanol was added, the extract was left in a dark place at room temperature overnight, and then the absorbance values were measured. The content of chlorophyll a, chlorophyll b, and total chlorophyll of the leaves were determined and calculated three times [14].

2.7. Data Processing and Statistical Analysis

Excel 2010 was used for data collation and SPSS 25.0 (International Business Machines Corporation, Armonk, NY, USA) was used for one-way analysis of variance (ANOVA) and Duncan's multiple comparisons.

3. Results

3.1. Analysis of Physical Properties of Mixed Substrates

The physicochemical properties differed with substrates. As shown in Table 2, the addition of vermicompost to the substrate significantly increased the density of the substrate, and the addition of garden soil increased the density of the substrate by about two times. The total porosity all reduced by different degrees compared with the control (peat: vermiculite = 2:1), especially that in the A₁ (peat: vermicompost: vermiculite: garden soil = 0.75:0.25:1:1) substrate, which reached the minimum total porosity. The pH values of different substrates varied from 7.64 to 7.97, all of which were greater than 7 and were weakly alkaline, and the pH values of all treatments increased compared to the control. The EC values of different substrates varied from 0.17 to 0.51 mS/cm, with the A₄ treatment (vermicompost: vermiculite: decomposed sheep manure = 1:2:1) having the largest EC values. From the different substrate components, the organic matter and humic acid content of peat and decomposed sheep manure were higher, and the organic matter and humic acid contents of vermicompost were lower than those of peat and decomposed sheep manure. Therefore, after adding different proportions of vermicompost to the substrate instead of peat, the organic matter, humic acid, and total nitrogen content of the substrate were significantly decreased, especially after adding garden soil, which increased the soil density and decreased the total porosity, organic matter, humic acid, total nitrogen, and alkaline nitrogen content, with decreases of 16.92~32.31%, 80.37~89.64%, 85.41~92.36%, and 56.49~61.96%, respectively. However, the addition of vermicompost significantly increased the total phosphorus, total potassium, effective phosphorus, and fast-acting potassium content of the substrate by 39.29~262.5%, 49.45~55.85%, 53.91~1227.66% and 6.72~32.47% for total phosphorus, total potassium, available phosphorus, and rapidly available potassium, respectively.

Table 2. Physicochemical properties of different substrates.

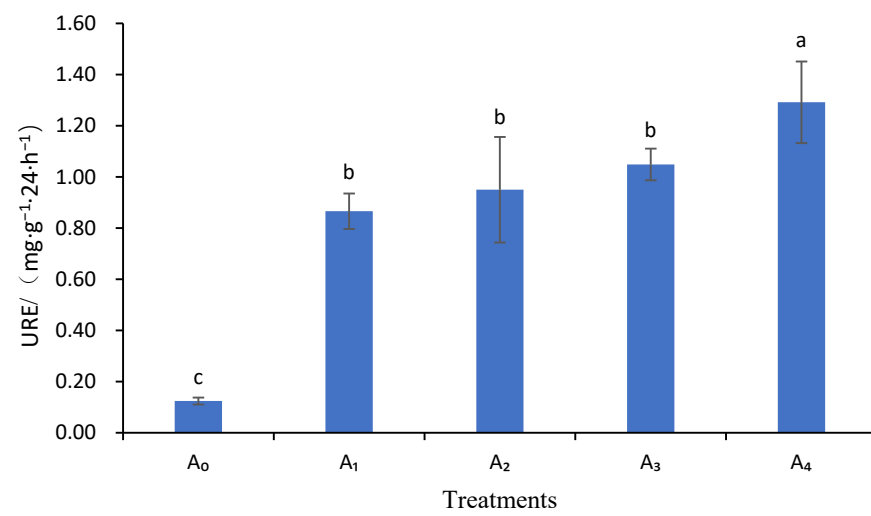
Treatments	Density (g·cm ⁻³)	Total Porosity (%)	pH	EC Value (mS·cm ⁻¹)	Organic Matter (g·kg ⁻¹)	Humic Acid (%)
A ₀	0.18 ± 0.01 c	65.0 ± 4.0 a	7.64 ± 0.05 c	0.17 ± 0.01 c	389.91 ± 22.48 a	20.42 ± 1.5 a
A ₁	0.57 ± 0.04 a	44.0 ± 6.0 d	7.96 ± 0.05 a	0.2 ± 0.004 b	76.53 ± 2.52 c	2.98 ± 0.61 c
A ₂	0.53 ± 0.05 a	51.0 ± 3.0 bc	7.97 ± 0.06 a	0.2 ± 0.01 b	54.05 ± 3.33 d	1.56 ± 1.21 c
A ₃	0.54 ± 0.04 a	47.0 ± 1.0 cd	7.95 ± 0.05 a	0.2 ± 0.002 b	40.39 ± 0.41 d	2.12 ± 0.27 c
A ₄	0.33 ± 0.01 b	54.0 ± 1.0 b	7.73 ± 0.03 b	0.51 ± 0.01 a	109.52 ± 4.00 b	7.72 ± 2.36 b

Treatments	Total Nitrogen (g·kg ⁻¹)	Total Phosphorus (g·kg ⁻¹)	Total Potassium (g·kg ⁻¹)	Alkali-Hydrolyzed Nitrogen (mg·kg ⁻¹)	Available Phosphorus (mg·kg ⁻¹)	Rapidly Available Potassium (mg·kg ⁻¹)
A ₀	4.39 ± 0.13 a	0.56 ± 0.01 c	12.82 ± 0.01 c	368.64 ± 26.95 b	71.08 ± 4.92 d	270.23 ± 13.79 d
A ₁	1.85 ± 0.1 cd	0.78 ± 0.12 bc	19.16 ± 0.31 b	149.89 ± 4.03 c	109.4 ± 10.68 c	291.44 ± 5.24 c
A ₂	1.67 ± 0.12 d	0.95 ± 0.01 bc	19.67 ± 0.17 a	139.75 ± 1.67 c	137.79 ± 5.74 c	288.41 ± 10.48 c
A ₃	1.91 ± 0.06 c	1.20 ± 0.06 b	19.75 ± 0.11 a	154.69 ± 4.62 c	224.42 ± 9.38 b	309.59 ± 5.24 b
A ₄	3.16 ± 0.1 b	2.03 ± 0.68 a	19.98 ± 0.13 a	453.69 ± 49.30 a	943.7 ± 34.16 a	357.98 ± 0.00 a

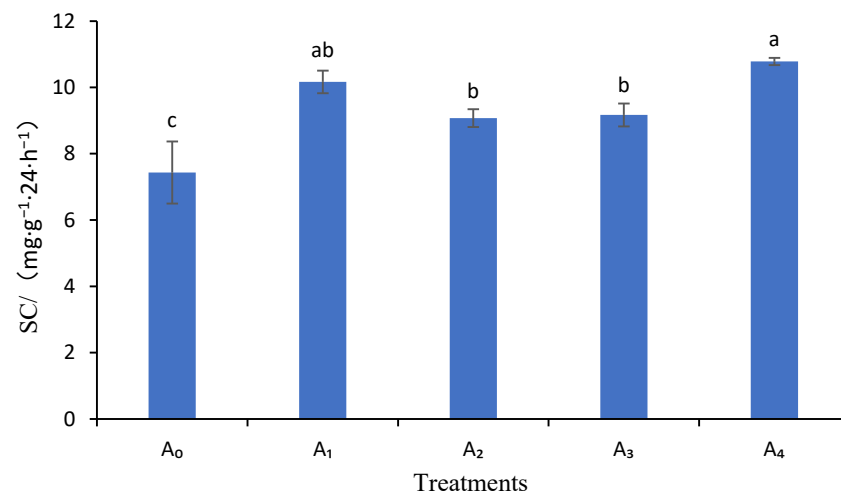
Note: A₀ (Peat: vermiculite = 2:1), A₁ (peat: vermicompost: vermiculite: garden soil = 0.75:0.25:1:1), A₂ (peat: vermicompost: vermiculite: garden soil = 0.5:0.5:1:1), A₃ (vermicompost: vermiculite: garden soil = 1:2:1), A₄ (vermicompost: vermiculite: decomposed sheep manure = 1:2:1), the same below. Significant differences between treatments are indicated by different lowercase letters (Duncan's test; $p < 0.05$; mean ± SD, $n = 3$).

3.2. Effects of Different Formulations on Matrix Enzyme Activity

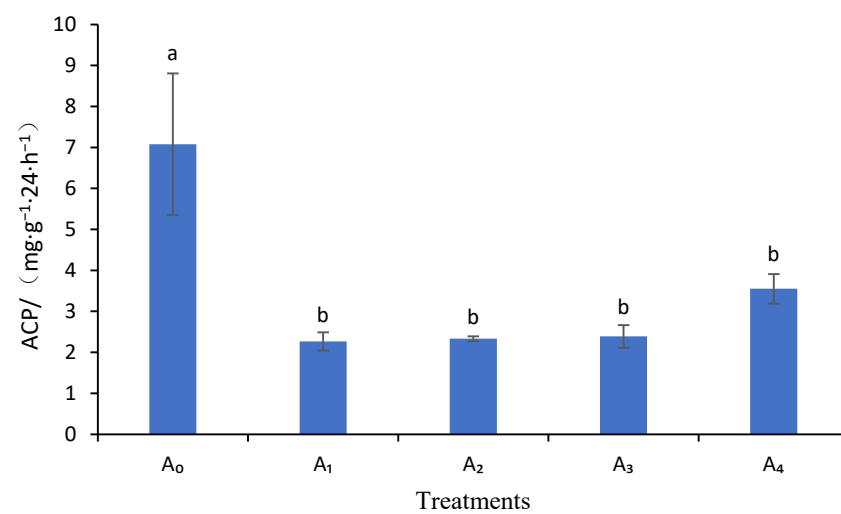
The enzyme activity of rhizosphere substrate, closely related to the growth and development of seedlings, has the function of accelerating soil biochemical reactions, and plays an important role in the ecosystem. Its activity is closely related to soil physical and chemical properties and environmental conditions, etc., and is regarded as an early warning indicator of changes in soil quality [15]. The role of urease is, specifically, to catalyze the breakdown of urea to ammonium and carbonic acid [16], with significant differences in enzyme activity between substrates (Figure 2). The addition of vermicompost to the substrate significantly increased the urease activity, especially in the A₄ treatment (vermicompost: vermiculite: decomposed sheep manure = 1:2:1), which had the highest urease activity of 1.29 mg·g⁻¹·24·h⁻¹. A₀ (peat: vermiculite = 2:1) had the lowest urease activity of 0.12 mg·g⁻¹·24·h⁻¹, and the three substrates with garden soil had no significant difference in urease activity. Sucrase is widely found in plants, animals, and microorganisms and its role is to catalyze the hydrolysis of sucrose into fructose and glucose [17]. The sucrase activity was significantly higher in all substrates with the addition of vermicompost than that in the control, and it was highest in the A₄ substrate (vermicompost: vermiculite: decomposed sheep manure = 1:2:1), followed by the A₁ substrate, and the difference in sucrase activity was not significant between the A₂ and A₃ substrates. Acid phosphatases are a class of enzymes that catalyze the mineralization of soil organic phosphorus compounds into inorganic phosphorus, and their activity level directly affects the decomposition and conversion of organic phosphorus in soil and its biological effectiveness [18]. Its content was highest in the A₀ (peat: vermiculite = 2:1) substrate, and there were no significant differences among the remaining four treatments. Alkaline phosphatase directly affects the mineralization of organic phosphorus and the nutrient status of plant phosphorus [19], and its content was lowest in the A₀ (peat: vermiculite = 2:1) substrate and highest in A₄ (vermicompost: vermiculite: decomposed sheep manure = 1:2:1), with significant differences between them and both reaching significant levels with the remaining three substrates, respectively. Catalase, which acts to break down hydrogen peroxide produced by biological respiration in the soil and reduces the toxicity of hydrogen peroxide to the soil [19], was highest in A₃ (vermicompost: vermiculite: garden soil = 1:2:1) and lowest in A₀ (peat: vermiculite = 2:1), with significant differences between them.



(a)

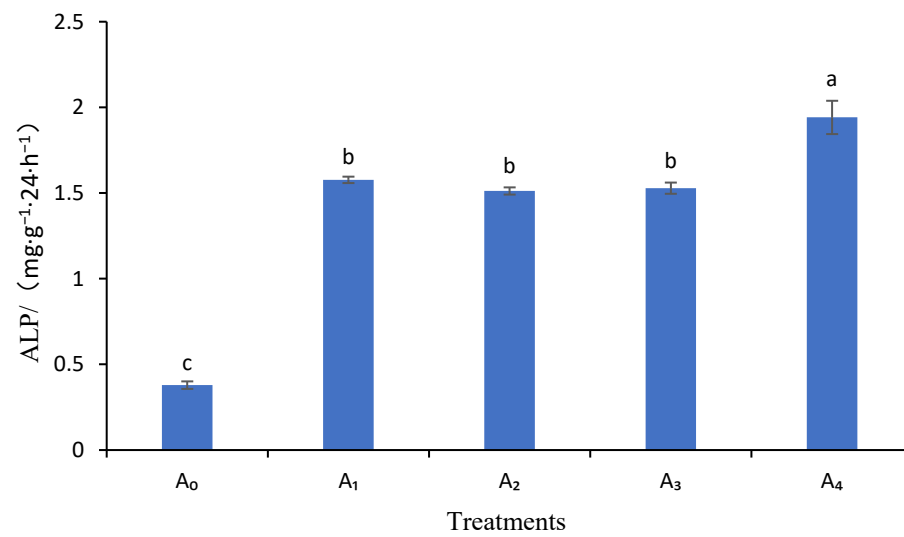


(b)

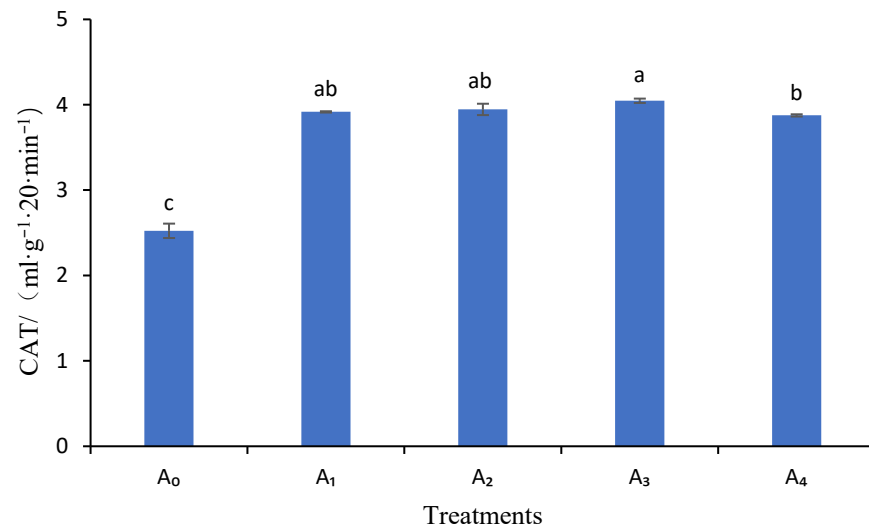


(c)

Figure 2. Cont.



(d)



(e)

Figure 2. Enzyme activity of different substrates: (a) urease activity (URE, mg·g⁻¹·24·h⁻¹); (b) sucrase activity (SC, mg·g⁻¹·24·h⁻¹); (c) acid phosphatase activity (ACP, mg·g⁻¹·24·h⁻¹); (d) alkaline phosphatase (ALP, mg·g⁻¹·24·h⁻¹); and (e) catalase activity (CAT, mg·g⁻¹·20·min⁻¹). Significant differences between treatments are indicated by different lowercase letters (Duncan's test; $p < 0.05$; mean \pm SD, $n = 3$).

3.3. Effects of Different Formulas of Vermicompost Substrate on the Growth of Sour Jujube Seedlings

3.3.1. Weight, Cost, and Jujube Seedling Survival Rate in Different Substrates

As can be seen from Table 3, in terms of substrate weight, A₀ (peat: vermiculite = 2:1) was topped at 2.77 kg/bag; A₁, A₂, and A₃, with garden soil in the substrate, reached 7.07~8.49 kg/bag, and all three differed significantly and were significantly higher than A₀. A₄ (vermicompost: vermiculite: decomposed sheep manure = 1:2:1) had sheep manure instead of garden soil and weighed less, at 6.20 kg/bag, which was significantly lower than A₁, A₂, and A₃. In terms of cost, A₀ (peat: vermiculite = 2:1) was the highest at CNY 8.65 per bag. The addition of vermicompost reduced the cost to different degrees, and the cost gradually decreased as the ratio of vermicompost increased, with A₃ treatment

(vermicompost: vermiculite: garden soil = 1:2:1) costing 38.15% less than A₀ (peat: vermiculite = 2:1), reducing the cost by CNY 3.3 per bag. As far as the survival rate of sour jujube seedlings was concerned, the survival rate of sour jujube seedlings in A₁, A₂, and A₃ substrates was the highest and significantly higher than that of A₀ (peat: vermiculite = 2:1). In case of 25% sheep manure being added to the substrate, the survival rate of seedlings was only 60.62%. Although the contents of total phosphorus, total potassium, alkaline decomposed nitrogen, effective phosphorus, and rapidly available potassium were the highest in this treatment, indicating that the higher EC values did not benefit the growth of sour jujube seedlings. Therefore, the A₃ treatment (vermicompost: vermiculite: garden soil = 1:2:1) was not only low-cost but also suitable for the growing of sour jujube seedlings.

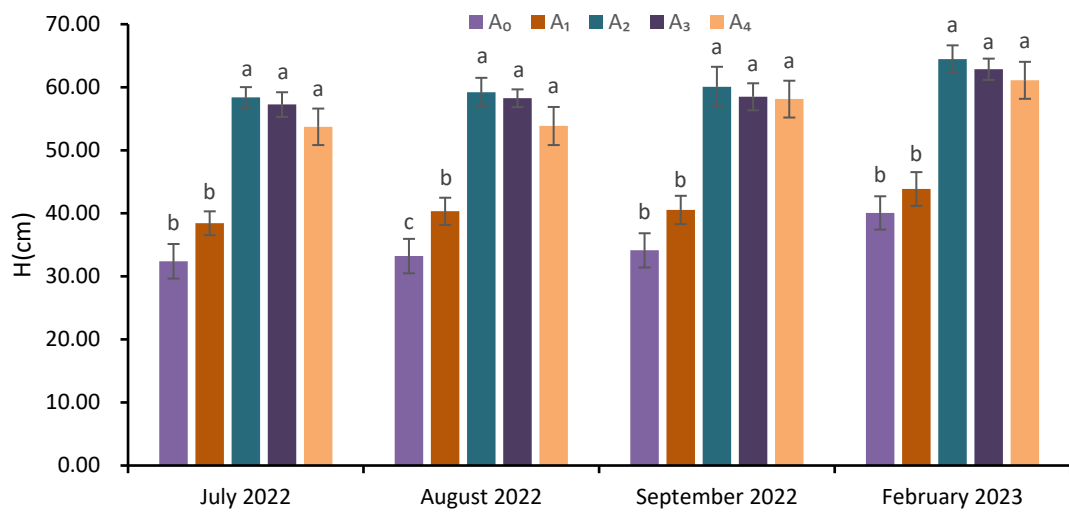
Table 3. Comparison among weight, cost of different matrixes, and survival of sour jujube seedlings.

Treatments	Weight (kg/bag)	Cost (CNY/bag)	Survival Rate of Seedlings (%)
A ₀	2.77 ± 0.05 e	8.65 ± 0.05 a	82.93 ± 3.30 b
A ₁	7.07 ± 0.04 c	7.40 ± 0.06 b	91.73 ± 8.34 a
A ₂	8.30 ± 0.11 b	6.77 ± 0.05 c	93.40 ± 4.13 a
A ₃	8.49 ± 0.03 a	5.35 ± 0.05 d	94.57 ± 4.44 a
A ₄	6.20 ± 0.10 d	7.35 ± 0.05 b	60.62 ± 13.03 c

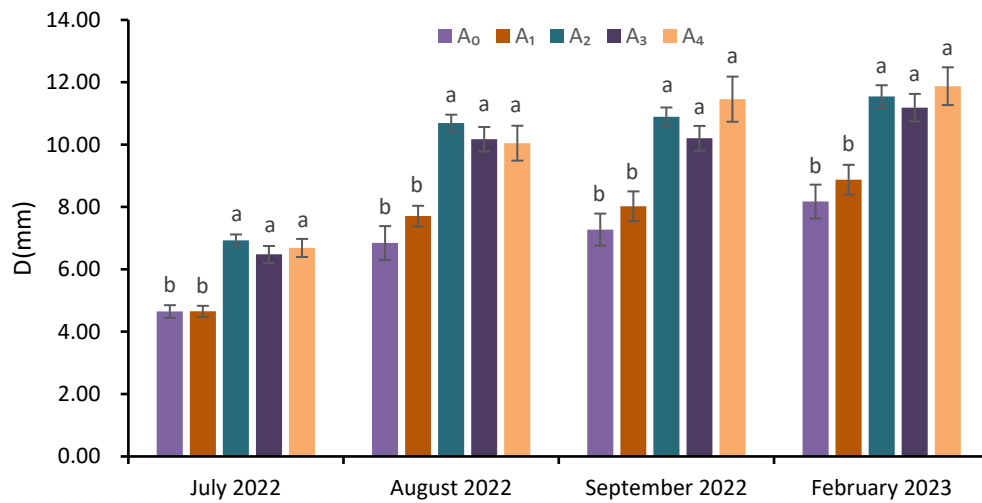
Note: Significant differences between treatments are indicated by different lowercase letters (Duncan's test; $p < 0.05$; mean ± SD, $n = 10$).

3.3.2. Growth of Sour Jujube Container Seedlings in Different Substrates

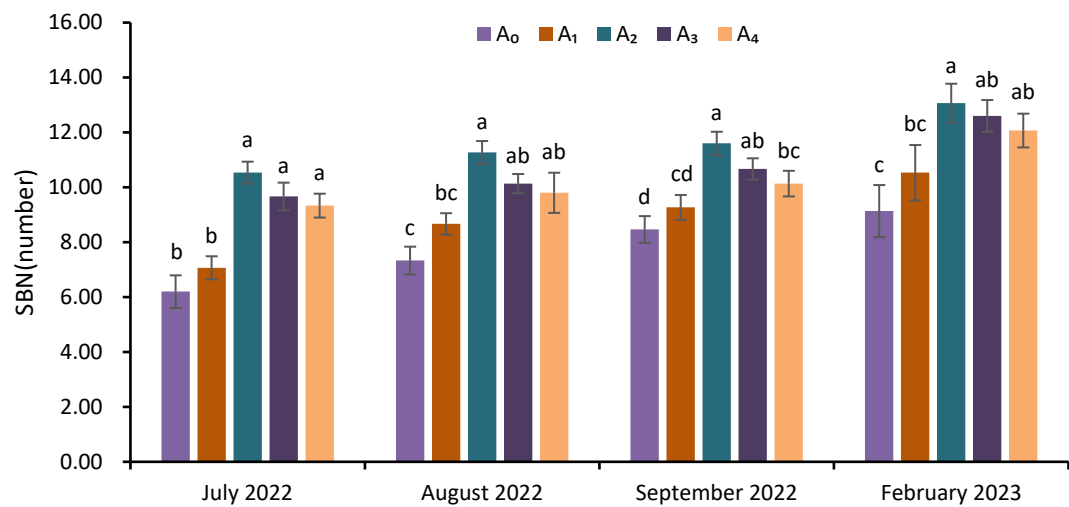
Seedling height and stem diameter are important indicators of seedling growth and directly reflect the quality of seedlings, while the number of secondary branches and the length of the longest secondary branch can also reflect the strength of plant growth. From Figure 3a–d, it can be seen that seedling height changed slowly after July, indicating that before July was a critical period for plant height increase. The differences in seedling height among A₂, A₃, and A₄ in the same period were not significant, but they were all higher than that of A₁ (peat: vermicompost: vermiculite: garden soil = 0.75:0.25:1:1) and A₀ (peat: vermiculite = 2:1). Regarding the stem diameter, it changed slowly after August, indicating that before August was a critical period for seedling thickening. The differences of stem diameter among A₂, A₃, and A₄ were not significant in the same period, but they were all higher than those in A₁ and A₀. The number of secondary branches showed the same pattern as the seedling height. The length of the longest secondary branch showed the same pattern as the stem diameter. It can be seen that before July is a critical period for plant height and secondary branch growth, and before August is a critical period for stem thickening and secondary branch elongation. The A₂ treatment (peat: vermicompost: vermiculite: garden soil = 0.5:0.5:1:1) had the highest number of secondary branches, reaching 13.07 in the February of the following year, which was significantly different from the A₀ (peat: vermiculite = 2:1) and A₁ (peat: vermicompost: vermiculite: garden soil = 0.75:0.25:1:1) treatments, and not significantly different from the rest. Therefore, the A₂, A₃, and A₄ substrates were all favorable for the aboveground growth of sour jujube seedlings.



(a)



(b)



(c)

Figure 3. Cont.

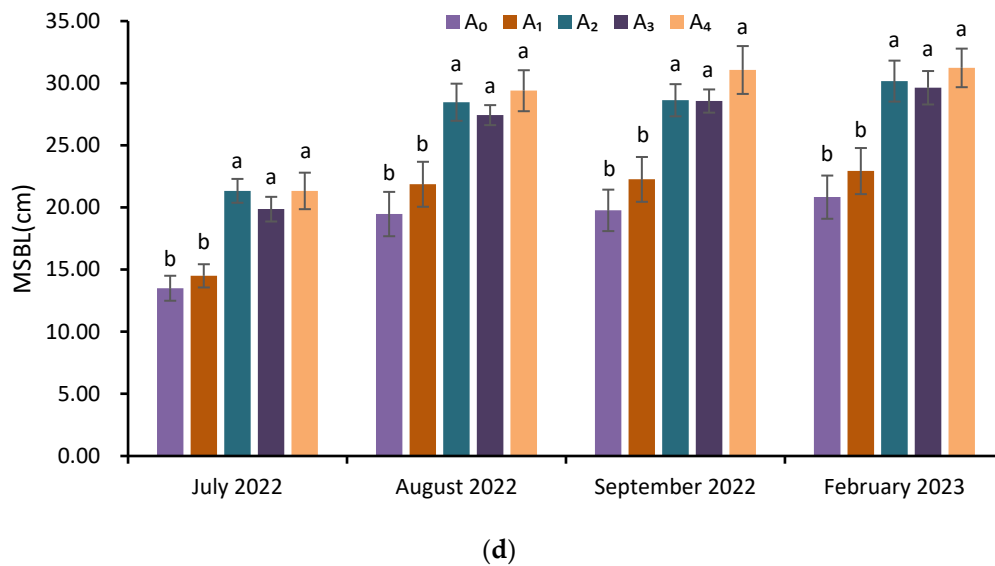


Figure 3. Growth characteristics of sour jube seedlings with different mixed substrates: (a) height (H, cm), (b) stem diameter (D, mm), (c) secondary branch number (SBN/number), and (d) maximum secondary branch length (MSBL/cm). Significant differences between treatments are indicated by different lowercase letters (Duncan’s test; $p < 0.05$; mean \pm SD, $n = 10$).

3.3.3. Photosynthetic Parameters of Leaves of Sour Jube Container Seedlings in Different Substrates

The photosynthetic capacity of plants can be expressed in terms of net photosynthetic rate, stomatal conductance, intercellular CO₂ concentration, and transpiration rate. From Figure 4a–d, the net photosynthetic rates of the seedlings of the A₂, A₃, and A₄ treatments were significantly higher than those of A₀ and A₁. For intercellular CO₂ concentration, the seedlings of treatment A₀ (peat: vermiculite = 2:1) showed the highest intercellular CO₂ concentration (315.51 $\mu\text{mol}\cdot\text{mol}^{-1}$), while treatment A₄ (vermicompost: vermiculite: decomposed sheep manure = 1:2:1) showed the lowest (297.54 $\mu\text{mol}\cdot\text{mol}^{-1}$). In terms of transpiration rate and stomatal conductance, the seedlings of A₂, A₃, and A₄ were significantly higher than that of A₀. Therefore, the seedlings of the A₂, A₃, and A₄ treatments had better photosynthetic performance.

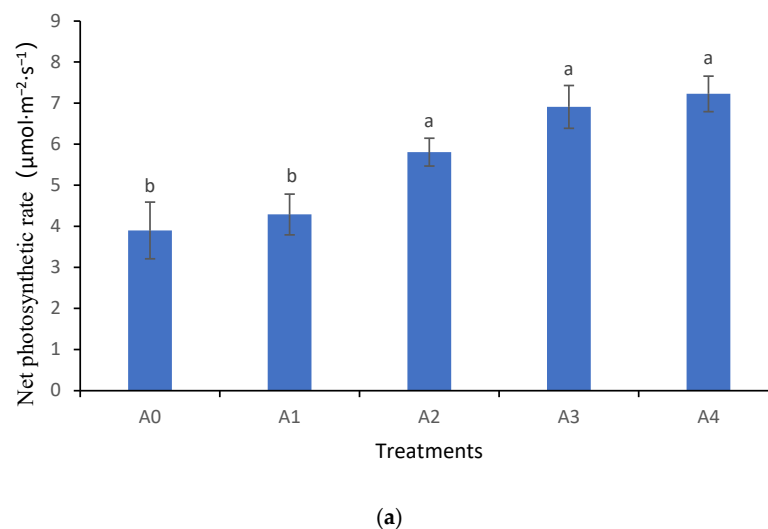
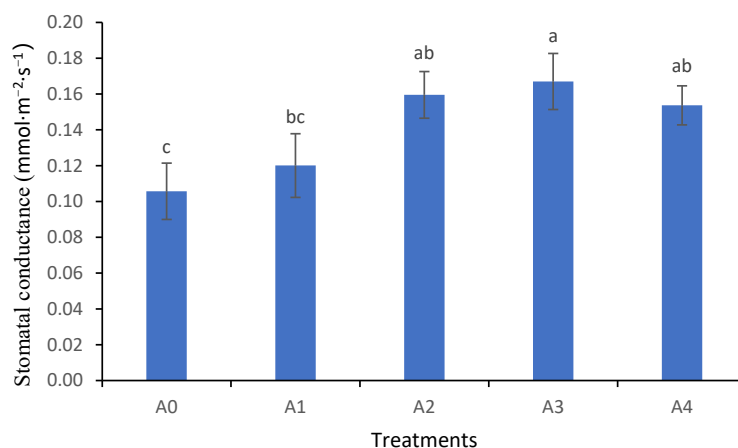
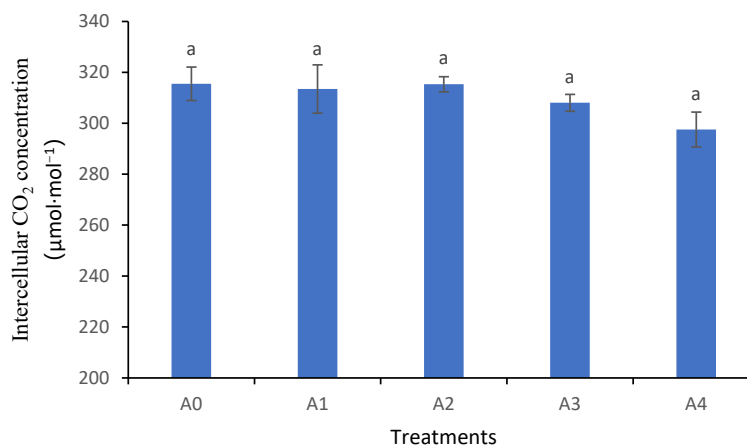


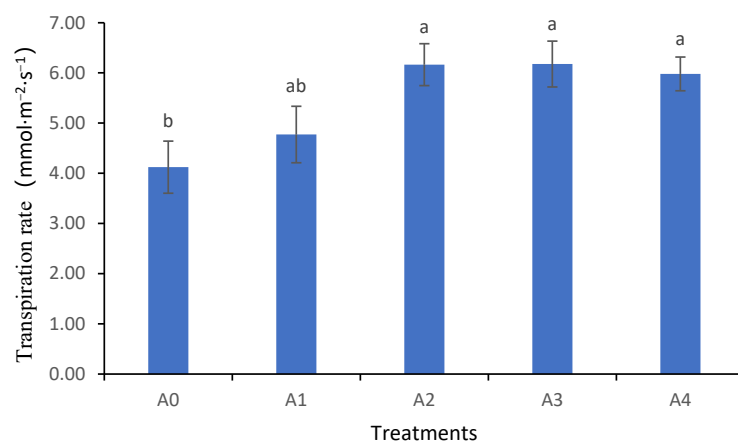
Figure 4. Cont.



(b)



(c)



(d)

Figure 4. Photosynthetic parameters of sour jujube seedlings under different substrates: (a) net photosynthetic rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), (b) stomatal conductance ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), (c) intercellular CO₂ concentration ($\mu\text{mol}\cdot\text{mol}^{-1}$), and (d) transpiration rate ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). A₀ (Peat: vermiculite = 2:1), A₁ (peat: vermicompost: vermiculite: garden soil = 0.75:0.25:1:1), A₂ (peat: vermicompost: vermiculite: garden soil = 0.5:0.5:1:1), A₃ (vermicompost: vermiculite: garden soil = 1:2:1), and A₄ (vermicompost: vermiculite: decomposed sheep manure = 1:2:1). Significant differences between treatments are indicated by different lowercase letters (Duncan's test; $p < 0.05$; mean \pm SD, $n = 5$).

Chlorophyll is the carrier of photosynthesis and is an important indicator of photosynthetic capacity and growth stage of plant leaves [20]. Also, chlorophyll content was positively correlated with net photosynthetic rate within a certain range. As shown in Table 4, the chlorophyll a content was significantly higher in the seedlings of treatments A₂ (peat: vermicompost: vermiculite: garden soil = 0.5:0.5:1:1) and A₄ (vermicompost: vermiculite: decomposed sheep manure = 1:2:1) than in those in the other treatments. For chlorophyll b, seedlings of the A₄ treatment (vermicompost: vermiculite: decomposed sheep manure = 1:2:1) had a higher content than in the other treatments. The total chlorophyll content was significantly higher in seedlings of treatments of A₂ (peat: vermicompost: vermiculite: garden soil = 0.5:0.5:1:1) and A₄ (vermicompost: vermiculite: decomposed sheep manure = 1:2:1) than those in the other treatments, with a total content of 2.77 (mg/g) and 2.80 (mg/g), respectively. Chlorophyll a/b represents the plant's utilization of light, and this was lower in sour jujube seedlings of the A₃ substrate (vermicompost: vermiculite: garden soil = 1:2:1) than those in the other treatments, indicating a low utilization of light. There was no significant difference in the carotenoid contents of the sour jujube seedlings in different substrates. Therefore, the photosynthetic capacity of sour jujube seedlings was higher under A₂ and A₄ substrates.

Table 4. Chlorophyll content of sour jujube seedlings under different substrates.

Treatments	Chlorophyll a (mg·g ⁻¹)	Chlorophyll b (mg·g ⁻¹)	Total Chlorophyll (mg·g ⁻¹)	Chlorophyll a/b	Carotenoid (mg·g ⁻¹)
A ₀	1.41 ± 0.31 b	0.47 ± 0.12 c	1.89 ± 0.43 b	3.01 ± 0.19 a	0.22 ± 0.03 a
A ₁	1.65 ± 0.13 b	0.57 ± 0.05 bc	2.22 ± 0.17 b	2.88 ± 0.12 ab	0.22 ± 0.01 a
A ₂	2.08 ± 0.09 a	0.69 ± 0.04 ab	2.77 ± 0.13 a	3.01 ± 0.07 a	0.26 ± 0.01 a
A ₃	1.66 ± 0.13 b	0.61 ± 0.04 ab	2.27 ± 0.17 b	2.72 ± 0.18 b	0.21 ± 0.05 a
A ₄	2.07 ± 0.06 a	0.73 ± 0.02 a	2.80 ± 0.08 a	2.84 ± 0.02 ab	0.26 ± 0.02 a

Note: Significant differences between treatments are indicated by different lowercase letters (Duncan's test; $p < 0.05$; mean ± SE, $n = 3$).

3.3.4. Effects of Different Substrates on the Root Indices of Sour Jujube Seedlings

Ten representative sampled plants were selected for each treatment, and they were scanned and analyzed by the root scanning instrument. It was found that the growth of seedlings' root systems under different substrate conditions varied (Table 5). The root growth of sour jujube in substrates A₂, A₃, and A₄ were generally better than in other substrates, as shown by having larger total root length, root projected area, root surface area, and root volume, but the average root diameter did not vary significantly among different substrates. This also showed that the addition of vermicompost to the substrate promoted the elongation of the root system. Moreover, from Figure 5, it can be seen that the growth of the root system was significantly improved in comparison to that of the above-ground part, which showed that the non-woven bag mainly promoted the growth of the root system of the one-year-old sour jujube seedlings. As far as the sour jujube seedlings in different substrates were concerned, the above-ground and root growth of container seedlings in A₂, A₃, and A₄ were significantly increased. The A₂, A₃, and A₄ treatments had the highest whole plant fresh weight of 109.21 g, 100.03 g, and 102.52 g, respectively, while the fresh weight of the root system reached 62.62 g, 56.44 g, and 63.08 g, respectively.

Table 5. Sour jujube root morphology under different substrates.

Treatments	Root Length (cm)	Projected Area (cm ²)	Surface Area (cm ²)	Root Diameter (cm)	Root Volume (cm ³)
A ₀	784.89 ± 218.80 b	127.91 ± 43.46 b	401.83 ± 136.53 b	1.63 ± 0.28 a	16.76 ± 7.17 a
A ₁	840.23 ± 280.22 ab	139.78 ± 45.77 b	439.14 ± 143.78 b	1.68 ± 0.36 a	18.89 ± 7.04 bc
A ₂	1235.05 ± 242.14 a	207.50 ± 45.63 a	651.87 ± 143.78 a	2.21 ± 0.92 a	27.69 ± 7.53 ab
A ₃	929.58 ± 342.44 ab	160.59 ± 53.47 ab	504.52 ± 167.99 ab	1.89 ± 0.36 a	22.32 ± 8.63 abc
A ₄	1203.54 ± 757.44 a	206.96 ± 119.05 a	650.20 ± 374.00 a	2.23 ± 1.45 a	28.80 ± 16.29 a

Note: Significant differences between treatments are indicated by different lowercase letters (Duncan's test; $p < 0.05$; mean ± SE, $n = 10$).

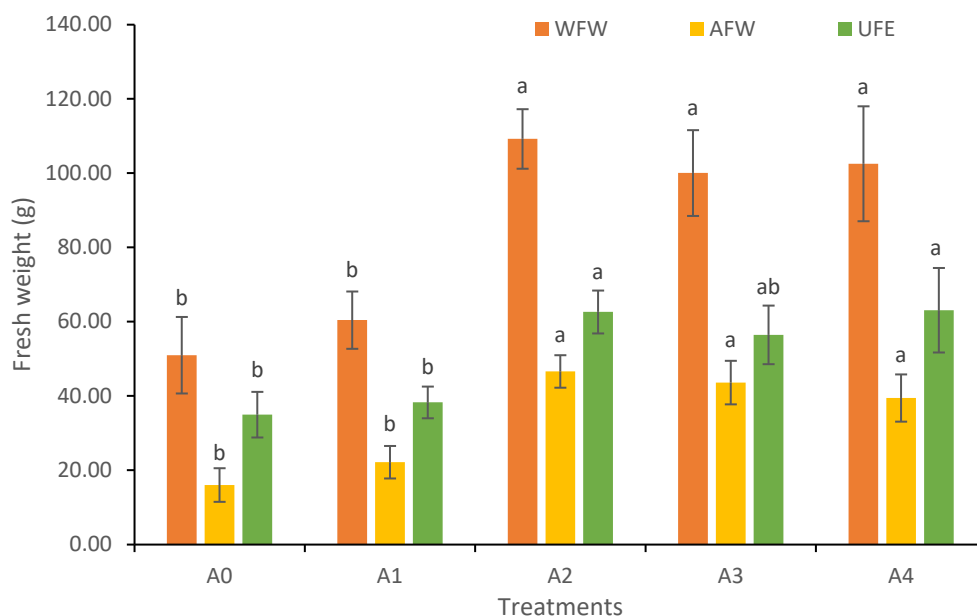


Figure 5. Effects of different vermicompost substrates on aboveground, underground, and total fresh weight of sour jujube seedlings (mean ± SE, $n = 10$). Note: WFW—total fresh weight; AFW—aboveground fresh weight; UFW—underground fresh weight. Significant differences between treatments are indicated by different lowercase letters (Duncan's test; $p < 0.05$; mean ± SD, $n = 10$).

The analysis showed that the survival rate of sour jujube seedlings was highest in substrates A₁, A₂, and A₃, while jujube seedlings of substrates A₂, A₃, and A₄ had the best aboveground growth potential, the greatest net photosynthetic rate, leaf transpiration rate, and stomatal conductance, and the greatest aboveground and root growth. Therefore, the A₂ (peat: vermicompost: vermiculite: garden soil = 0.5:0.5:1:1) and A₃ (vermicompost: vermiculite: garden soil = 1:2:1) substrates are suitable as substrates for sour jujube seedling cultivation, especially in A₃, where vermicompost completely replaces peat, which reduces the cost by CNY 1.42/bag and gets rid of the dependence on non-renewable resources of peat, making it more promising for application. In the A₂ and A₃ substrates, the total porosity is larger and the EC value is lower; except for the content of total K, other nutrients, such as organic matter, humic acid, total nitrogen, total phosphorus, alkali-hydrolyzed nitrogen, and available phosphorus, are at medium or even lower levels, while they are higher than those in garden soil. And rapidly available potassium is slightly lower than in garden soil; urease, sucrose acid phosphatase, alkaline phosphatase, and catalase are basically at medium levels.

4. Discussion

In this study, vermicompost was added to the substrate instead of peat for the first time in the production of sour jujube container seedlings. In traditional seedling production,

soil is used as the substrate, and the physical and chemical properties of soil (density, porosity, pH, etc.) are very inconsistent due to regional differences. Permeability, water retention, and mineral element supply capacity often do not meet the needs of seedling growth. Seedlings need suitable soil and substrates to grow well, and the physical and chemical properties of various substrates vary and affect the growth of seedlings differently. Sour jujube seedlings play an important role in the production of Chinese jujube. In this study, we replaced the peat with vermicompost, and the results showed that the agronomic traits of sour jujube seedlings cultivated with vermicompost substrate were significantly better. This shows that it is feasible to use vermicompost as an alternative substrate instead of peat for seedling cultivation. The results of this study have promoted the widespread use of vermicompost substrates in sour jujube container seedling cultivation.

4.1. Physicochemical Properties of Different Substrates

The physicochemical properties of different formulated substrates vary greatly and have different effects on the growth of seedling plants [8]. Our study showed that the emergence rate and the growth of sour jujube seedlings varied greatly among different substrates, which also indicated that the physicochemical properties of seedling substrates could determine the emergence and growth of sour jujube to different degrees. Among the five substrates prepared in this experiment, substrates A₂ (peat: vermicompost: vermiculite: garden soil = 0.5:0.5:1:1) and A₃ (vermicompost: vermiculite: garden soil = 1:2:1) have higher organic matter content, total nitrogen, total phosphorus, alkaline decomposed nitrogen, and effective phosphorus than garden soil, although they are not the highest, so the nutrients are sufficient for the healthy growth of seedlings. According to Wang X [21], an ideal growing media for plants should have a minimum total porosity of 85%, a water-holding capacity between 55~75%, and a porosity of 20~30%. The results of this study showed that the addition of vermicompost increased the substrate density to some extent and reduced the porosity of the substrate, which led to the enhancement of the aeration and water retention capacity of the substrate to some extent. The addition of vermicompost also significantly increased the content of nutrients such as total phosphorus, total potassium, effective phosphorus, and fast-acting potassium in the substrate, indicating that vermicompost can significantly increase the nutrients in the seedling substrate. Therefore, we believe that the improvement in growth of sour jujube seedlings was explained by the increased availability of plant nutrients in the matrix owing to vermicompost addition. In our study, the A₁ treatment had the highest porosity but it had the weakest growth, which may mean that sour jujube seedlings are not suitable for substrates with higher porosity. According to the results of this study, the suitable substrate conditions for the growth of sour jujube seedlings were: total porosity 44.0~54.0%, EC value 0.2 mS·cm⁻¹, organic matter 40.39~54.05 g·kg⁻¹, total nitrogen and total phosphorus of 1.67~1.91 g·kg⁻¹ and 0.95~1.20 g·kg⁻¹, respectively, alkali-hydrolyzed nitrogen 139.75~154.69 mg·kg⁻¹, and available phosphorus 137~224 mg·kg⁻¹.

4.2. Enzyme Activity of Different Substrates

Soil enzymes are one of the most active organic components of the soil and are directly involved in the metabolism and transformation of various substances and the release and fixation of nutrients in the soil. The enzymatic activity of soil is an important indicator for evaluating soil fertility and soil quality [22]. In this experiment, in addition to acid phosphatase, the application of vermicompost increased the activities of urease, sucrase, alkaline phosphatase, and catalase of the substrate. It indicates that the increase in soil enzyme activity can promote the release and transformation of fertility, reduce the accumulation of toxins in the soil, and improve the soil environment.

4.3. Effects of Substrate on the Growth of Sour Jujube Seedlings

The growth environment of different substrate materials is necessarily different, which affects the growth of plants to some extent. The addition of vermicompost into the seedling

matrix for nursery raising substantially increased the photosynthetic pigment contents, enhanced net photosynthetic, and substantially improved the growth of fragrant rice cultivars during nursery raising. The increased root length, surface area, mean diameter, root volume, and root tip number, as well as enhanced root activity of fragrant rice seedlings, were observed due to the vermicompost application [23]. Zhang et al. [24] showed that eucalyptus bark was the ideal peat-replacement choice for the cultivation of *H. chrysanthus* seedlings. To facilitate proportioning in future production, decomposed eucalyptus bark (75%) with coconut bran (25%) can be used as the seedling medium for the growth of *H. chrysanthus*. This natural alternative to peat not only protects the environment and saves costs, but also makes use of agricultural and forestry wastes. Through previous studies, it was found that environmentally friendly and low-cost cultivation substrates are the main measure to solve the problem of soil degradation in facilities and the key to innovation in soilless cultivation technology. To a certain extent, seedling growing indicators can reflect the robustness of seedlings and can visually judge the effect of different substrate formulations on seedling growth. The results of this study showed that sour jujube seedlings in the A₂ (peat: vermicompost: vermiculite: garden soil = 0.5:0.5:1:1) and A₃ substrates (vermicompost: vermiculite: garden soil = 1:2:1) had the best overall performance in terms of seedling survival, plant height, ground diameter, number of secondary branches, length of longest secondary branch, total root length, root projection area, root surface area, average root diameter, root volume, and fresh weight of root system. However, in the A₃ substrate, vermicompost completely replaces peat, which reduces the cost by CNY 1.42/bag and gets rid of the dependence on non-renewable resources of peat, making it more promising for application.

5. Conclusions

In this study, the formulation characteristics of seedling substrates of vermicompost as peat substitutes, and their effects on the growth, biomass, and photosynthesis of wild jujube seedlings, were analyzed. This work aimed to determine an economically and environmentally friendly seedling substrate with suitable efficiency, high accessibility, and a low cost in order to provide a scientific basis for cultivating high-quality container sour jujube seedlings. The results highlighted A₃ (vermicompost: vermiculite: garden soil = 1:2:1) as the ideal peat-replacement choice for the cultivation of sour jujube seedlings. This natural alternative to peat not only protects the environment and saves costs, but also makes use of agricultural wastes.

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