

Research on preparing seedling substrates using edible mushroom waste and application

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ABSTRACT

To improve the utilisation of mushroom residue waste resources and identify a replaceable matrix material for peat, 10 different formula substrates and three commercially available substrates were examined to determine their physicochemical properties. Furthermore, the correlation and influence of these physicochemical properties on the substrates were explored. Pot experiments were conducted using *Brassica campestris* L. ssp. *chinensis* Makino var. *communis* Tsen et Lee (cabbage), *Brassica chinensis* L. (pakchoi), *Cucumis sativus* L. (cucumber), and *Cucurbita moschata* Duch. ex-Poiret (pumpkin). The results showed that the matrix was most significantly affected by water-holding porosity, aeration porosity, air-water ratio, total porosity, pH, electrical conductivity (EC), available phosphorous (AP), and available potassium (AK). The random forest (RFF) model indicated that pH and total nitrogen (TN) had the strongest influence on the plant height and stem diameter of the cabbage. Moreover, pH, water-holding porosity, and total porosity most significantly influenced the plant height, stem diameter, and seedling vigour index. AP and air-water ratio substantially affected the root length and root-to-crown ratio of the cucumbers, while EC, air-water ratio, and AP influenced the stem diameter, root length, and seedling vigour index of the pumpkin most. The biological characteristics of the four vegetables during the pot experiment indicated that the overall effect of the 10 substrates supplemented with perlite and vermiculite was better than in the basic group. Of these, T2 (mushroom waste: sawdust: catalyst: vermiculite = 8:2:5:5) displayed the best result and could be used as an alternative for peat seedling.

Keywords: mushroom waste, physicochemical properties, seedling substrate, vegetables

INTRODUCTION

China is responsible for more than 70% of the global edible mushroom production and export (Mleczek et al., 2018a). The edible mushroom industry has an important impact on poverty alleviation programmes in China, with returns reaching more than 10 times that of rice and corn (Li and Xu, 2022). It has been reported that the production of every 1 kg of edible mushrooms generates about 5 kg of mushroom waste (Grimm and Wösten, 2018; Syguła et al., 2019; Naim et al., 2020; Zied et al., 2020), while 200 million tons of edible mushroom waste

were generated in China alone in 2020 (China Edible Fungi Association, 2022). Since edible mushroom waste contains significant amounts of fungal proteins, metabolites and nutrients, it poses a substantial risk to the edible mushroom industry if not treated properly, damaging the environment and waste resources (Wang et al., 2015; Sardar et al., 2017; Qin et al., 2023).

With the rapid development of facility agriculture, the demand for seedling substrates has surged, with peat remaining as one of the most widely used and

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best-quality materials (Sangaré et al., 2013). Since peat is a non-renewable, expensive resource that is difficult to extract and can irreversibly damage the ecological environment, various studies have focussed on finding alternative seedling substrate materials (Giménez et al., 2019; Huang and Gu, 2019; Yu et al., 2022a). Hwang et al. (2015) presented the possibility of mixing 75% sawdust with 25% waxberry twigs as a substrate for apricot mushroom cultivation, providing a new market for edible mushroom cultivation substrates. Getachew (2017) used coffee fruit shells as a seedling substrate and significantly improved lettuce seed germination and yield, providing a novel way for selecting vegetable seedlingsubstratematerials.Bozzoloetal.(2012)partially substituted peat using ‘Nature’s Natural’ (a processed dairy manure product) as a substrate for vegetable and flower seedlings. This substrate performed better than peat, verifying the feasibility of partial replacement by agricultural waste as a seedling substrate. Meng et al. (2018) used mushroom and biogas waste to replace peat as a substrate. This mixture positively impacted the growth of tomato and pepper seedlings, exhibiting optimal growth at a 20%–50% substitution. Using mushroom waste as a complete or partial substitute for peat as a seedling substrate improves its total utilisation value and reduces agricultural production costs, alleviating the challenge presented by peat resource shortages while protecting the ecological environment and promoting the development of the edible mushroom and facility cultivation industries. However, minimal studies are available involving peat replacement with mushroom waste as a seedling substrate, while specific formulations and production technology standards are also lacking.

Verifying the feasibility of using edible mushroom waste as a substrate can provide an effective way for the resource utilisation of agricultural waste. This study prepares a composite seedling substrate using the waste of *Pleurotus eryngii* (king oyster mushroom) while keeping the addition of auxiliary materials to a minimum. It also analyses the physicochemical properties of the substrate to investigate their influence on the substrate, combining them with vegetable applications to verify the best seedling substrate formula.

MATERIALS AND METHODS

Experimental material

The discarded waste of king oyster mushrooms (provided by Yunnan Lu Liang Cuanxiang Lvyuan Mushroom Company Limited, Lu Liang County, Qu Jing City, Yunnan Province, China) was selected as the substrate, while chicken manure, urea and sawdust represented the auxiliary materials. The particle sizes of the vermiculite and perlite in the substrate formulation of the advanced group were <5 mm and contained no nutrients. The control group substrate 1 (CK1), mainly containing peat and coconut coir, was imported from Denmark

(Pindstrup Mosebrug A/S). The control group substrate 2 (CK2) was a conventional commercial substrate (Stanley Agricultural Group Co.), primarily containing peat and perlite. Control group substrate 3 (CK3) consisted of a self-mixed peat substrate with a specific composition ratio of peat:perlite:vermiculite = 3:1:1. The four vegetables selected for the seedling test included the nodular cabbage, Cuiying 252 pakchoi, Green Baby fruit cucumber and Mimoto pumpkin varieties.

Experimental scheme

The mushroom waste seedling substrate formula preparation

The experiment involved 13 substrate formulations, which were divided into the CK (CK1, CK2 and CK3), basic (T1, T3, T5, T7 and T9), and advanced groups (T2, T4, T6, T8 and T10). The parameters of the basic substrate group included a fermentation time of 37 days, an initial carbon-nitrogen ratio of fermentation of 25:1 and a moisture content of 60%. The substrate of the advanced group was obtained by adding perlite and vermiculite to the basic group. The specific components and proportions of the 13 substrates are shown in Table 1.

Table 1. The 13 substrate formulations.

Group	Treatment	Substrate formulations	Formulation ratio
CK	CK1	Coconut coir:peat	2:1
	CK2	Peat:perlite	2:1
	CK3	Peat:perlite:vermiculite	3:1:1
Basic	T1	Mushroom waste:sawdust	4:1
	T3	Mushroom waste:chicken manure:sawdust	5:1:1
	T5	Mushroom waste:chicken manure:sawdust	3:1:1
	T7	Mushroom waste:chicken manure:sawdust	2:1:1
	T9	Mushroom waste:urea:sawdust	4:1:1
Advanced	T2	T1:perlite:vermiculite	2:1:1
	T4	T3:perlite:vermiculite	2:1:1
	T6	T5:perlite:vermiculite	2:1:1
	T8	T7:perlite:vermiculite	2:1:1
	T10	T9:perlite:vermiculite	2:1:1

CK1, control group substrate 1; CK2, control group substrate 2; CK3, control group substrate 3.

The mushroom waste substrate (T2) displaying the best biological vegetable characteristics during the pot experiment was selected to optimise the composition ratio. The mushroom waste, perlite and vermiculite proportions were adjusted. A comparison between the biological characteristics, specific components and proportions of the F1, F3, CK1 and CK2 optimised substrates is shown in Table 2.

Vegetable application validation

The experimental simulation was performed in a glass greenhouse and included a daily illumination time of about 12 h, a temperature between 20°C and 25°C and an illumination intensity of approximately 7000 lx. After soaking to germinate, the vegetable seeds were sown, covered with 0.5 cm of vermiculite and watered. No fertiliser was added during the seedling and cultivation processes. Other management methods were used for conventional factory nursery measurements. The dry seed weights during each treatment included 0.024 g cabbage, 0.033 g pakchoi, 0.32 g cucumber and 2 g pumpkin. Each treatment was repeated thrice.

Measurement and methods

Determination of the physical properties of the seedling substrates

A container with a mass of a known volume (V) was weighed as (W1), after which the air-dried composite substrates of different treatments were placed in the container and weighed as (W2). The container was sealed with gauze and placed in water for 24 h, after which the gauze was removed, and the mass was weighed as (W3). Then the container was turned upside down to drain the water, and the mass was weighed as (W4). The calculation formula is shown in Table 3.

Determination of the chemical traits of the seedling substrates

(1) The total nitrogen (TN) content was determined using the Kjeldahl method. (2) The total phosphorus (TP) was determined using the sodium hydroxide fusion-molybdenum antimony anti-colorimetric method. (3) The total potassium (TK) content was determined using the sodium hydroxide fusion-flame photometric method. (4) The alkali-hydrolysable nitrogen (AN) was determined using the reduction alkali diffusion method. (5) The available phosphorous (AP) was obtained via the sodium bicarbonate extraction-molybdenum antimony anti-colorimetric method. (6) The available potassium (AK) was determined via the ammonium acetate leaching-flame photometric method. (7) The organic matter (OM) was determined using the potassium dichromate-sulphuric acid oxidation method (Bao, 2000). (8) The pH was measured using a Starter3C pH metre (this device was manufactured by OHAUS Instrument Co.). (9) The electrical conductivity (EC) was measured using an ST3100C-type conductivity

Table 2. Optimisation of the mushroom waste percentage in the composite substrate formulation.

Treatment	Substrate formulations	Formulations ratio
F1	T1:perlite:vermiculite	3:1:1
F2 (T2)	T1:perlite:vermiculite	2:1:1
F3	T1:perlite:vermiculite	1:1:1
CK1	Coconut coir:peat	2:1
CK2	Peat:perlite	2:1

CK1, control group substrate 1; CK2, control group substrate 2.

Table 3. The physical property determination methods.

Physical properties	Calculation formula
Weight capacity ($\text{g} \cdot \text{cm}^{-3}$)	$(W2-W1)/V$
Total porosity (%)	$(W3-W2)/V \times 100\%$
Ventilation porosity (%)	$(W3-W4)/V \times 100\%$
Water-holding porosity (%)	$(W4-W2)/V \times 100\%$
Air-to-water ratio	Ventilation porosity (%) / Water-holding porosity (%)

metre (this device was manufactured by OHAUS Instrument Co.).

Determination of the morphological and biological traits of the vegetable seedlings

(1) The plant height referred to the distance from the rootstock to the growing point, measured with a digital Vernier calliper. (2) The stem thickness was denoted by the lateral distance of the rootstock, measured using a digital Vernier calliper. (3) The root length signified the distance from the growing point to the end of the root, measured with a straightedge. (4) The dry weight of the vegetable seedlings above and below the ground was determined. Fresh plant samples were placed in an oven at 105°C for 15 min to deactivate the enzymes, after which the temperature was adjusted to 80°C for drying and weighing. (5) The vegetable seedling vigour index = (stem thickness/plant height + lower ground dry weight/dry weight of the upper part of the ground) \times whole plant dry weight. (6) The root-to-crown ratio of the vegetable seedlings = lower ground dry weight/dry weight of the upper part of the ground (Zhao and Zhang, 2023).

Statistical analysis

Redundancy analysis (RDA) was used for the correlation assessment of the physicochemical properties of substrates. The data were statistically analysed using Excel and SPSS software, and the graphs were prepared using the Paisano Gene Cloud data analysis platform (<https://www.genescloud.cn>; this website was developed by Shanghai Personalbio Co.). The blue arrows in Figure 1 and Figure 2 represent different influencing factors, while the angle between these factors denote

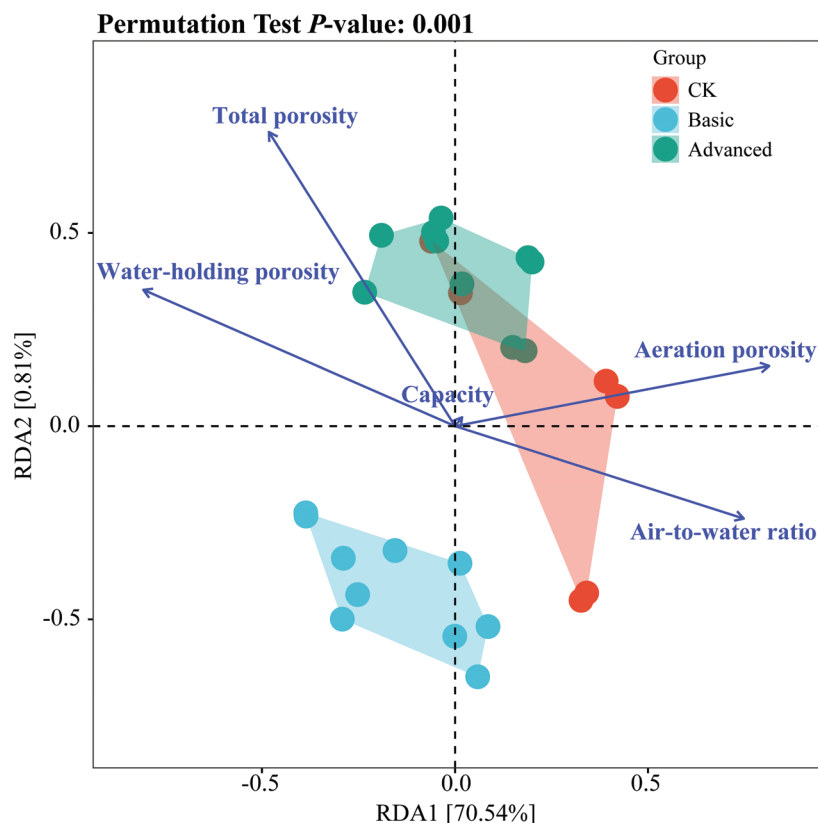


Figure 1. The RDA of the different substrate formulations and physical properties. RDA, redundancy analysis.

the magnitude of the correlation between them. An acute angle between two factors indicates that they are positively correlated, a right angle denotes the absence of a correlation and an obtuse angle signifies a negative correlation. A longer ray indicates a more significant influence of the factor on the background index. The P -value at the top of the ranking graph represents the P -value obtained using the random permutation substitution nonparametric test. A lower P -value denotes a more significant effect by the influencing factor on the background indicators. The percentages in the axes in parentheses represent the proportion of variation in the original data that could be explained by the corresponding axes. A RFF model was used to analyse the degree of influence of the physicochemical substrate properties on the biological traits of the four vegetable seedlings.

RESULTS

The correlation analysis of the physicochemical properties of the mushroom waste seedling substrates

The correlation analysis of the physical properties of the different formulated substrates

The RDA used the chemical traits of the different substrate formulations to determine their physical properties (Figure 1). The results revealed that the basic group was significantly separated from the advanced and

CK groups in the RDA score plot, indicating significant differences between these groups ($P < 0.001$). The CK and advanced groups were crossed, demonstrating that the advanced substrate was closer to the CK group. On the horizontal and vertical axes, RDA1 and RDA2 were 70.54% and 0.81%, respectively, accounting for 71.35% of the differences and showing that the RDA explained most of the differences between the substrates of the different formulations. Of these physical factors, the water-holding porosity ($R^2 = 0.5411$, $P = 0.001$), aeration porosity ($R^2 = 0.4904$, $P = 0.001$), air-to-water ratio ($R^2 = 0.4472$, $P = 0.001$), and total porosity ($R^2 = 0.3397$, $P = 0.004$) significantly affected the substrates, while capacity ($R^2 = 0.0003$, $P = 0.994$) had a negligible effect. The water-holding porosity was positively correlated with the total porosity and the aeration porosity with the air–water ratio, while the water-holding porosity and total porosity were negatively correlated with the aeration porosity and air–water ratio, respectively.

The correlation analysis of the chemical properties of the different substrate formulations

The RDA utilised the physical properties of the different formulation matrices to determine the extent of the influence of the chemical factors (Figure 2). The results showed that the CK group was not significantly separated from the basic and advanced groups in the RDA score plot, while all three groups were crossed, indicating no significant differences, and the basic and advanced group matrices were closer to the CK group.

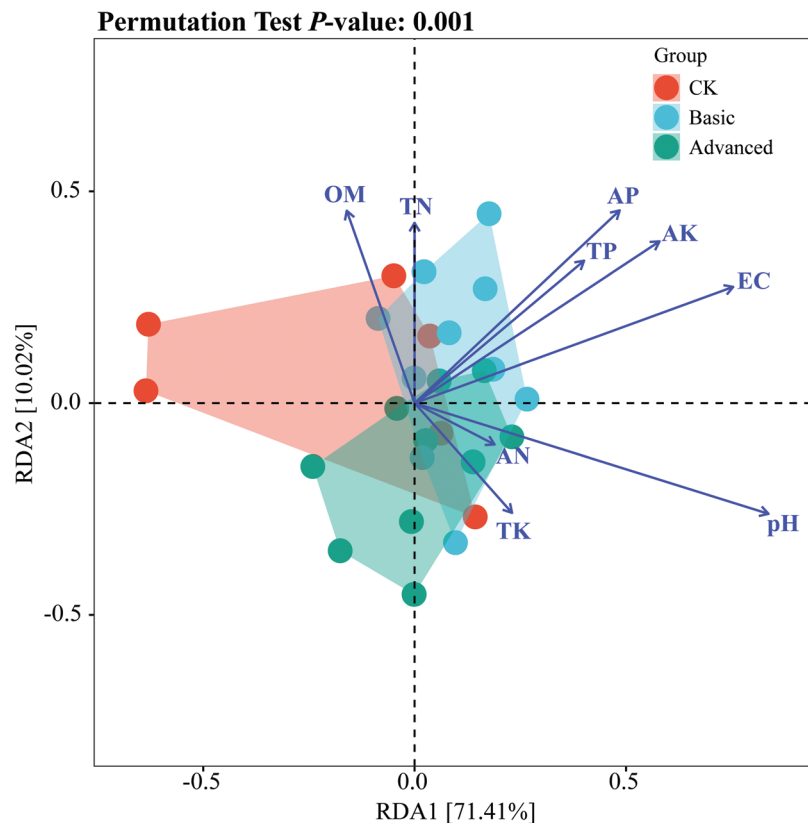


Figure 2. The RDA of the different substrate formulations and chemical properties. RDA, redundancy analysis.

RDA1 and RDA2 were 71.41% and 10.02% on the horizontal and vertical axes, respectively, accounting for 81.43% of the differences. Therefore, the RDA explained most of the differences between the substrates of the different formulations. Of the chemical factors, pH ($R^2 = 0.6327$, $P = 0.002$), EC ($R^2 = 0.5292$, $P = 0.001$), AK ($R^2 = 0.3925$, $P = 0.003$) and AP ($R^2 = 0.3581$, $P = 0.006$) significantly affected the substrates, while TP ($R^2 = 0.2219$, $P = 0.051$), OM ($R^2 = 0.1839$, $P = 0.095$), TN ($R^2 = 0.1428$, $P = 0.145$), TK ($R^2 = 0.0967$, $P = 0.326$) and AN ($R^2 = 0.0387$, $P = 0.651$) displayed a negligible impact. OM was positively correlated with TN, AP, TP and AK and negatively associated with pH, AN and TK. Furthermore, OM showed a lower association with the EC values, which were positively correlated with all the chemical factors except OM. In conclusion, AP, AK, EC and pH had the most significant effect on the quality of the different formulated substrates and were the main factors influencing the quality changes in the vegetable seedling substrates.

The effect of the mushroom waste seedling substrate on the vegetable seedlings

The effect of the different substrate formulations on the biological traits of the four vegetable seedlings

Four vegetable varieties, including cabbage, pakchoi, cucumbers and pumpkin, were selected, and the

seedlings were cultivated in 13 substrates, after which five biological traits, namely plant height, stem thickness, root length, seedling vigour index and root-to-crown ratio, were measured and calculated. The results are shown in Figure 3.

The plant height values are shown in Figure 3A. The overall effect of the substrates in the basic and advanced groups was close to that of the control group, where T7 and T2 were more successful in promoting the height of the four vegetables, second only to CK2. The stem thickness values are shown in Figure 3B. The overall impact of the substrates in the basic and advanced groups was close to that of the control group, where T1, T2, T4 and T10 were superior in enhancing the stem thicknesses of the four vegetables, second only to CK3. The root length values are shown in Figure 3C. The overall effect of the substrates in the basic and advanced groups was higher than that in the control group, while that of the advanced group exceeded the basic group and was close to the control group. T2, T4 and T10 more successfully promoted the root lengths of the four vegetables, while the impact of T2 exceeded that of the control group. The seedling vigour index values are shown in Figure 3D, indicating that the overall effect of the substrates in the basic and advanced groups was lower than in the control group. T2 and T10 were superior at promoting the seedling vigour index of the four vegetables, while the impact of T2 was higher than CK2 and lower than CK3, and T10 was second only to CK1. The root-to-crown ratios are shown in Figure 3E.

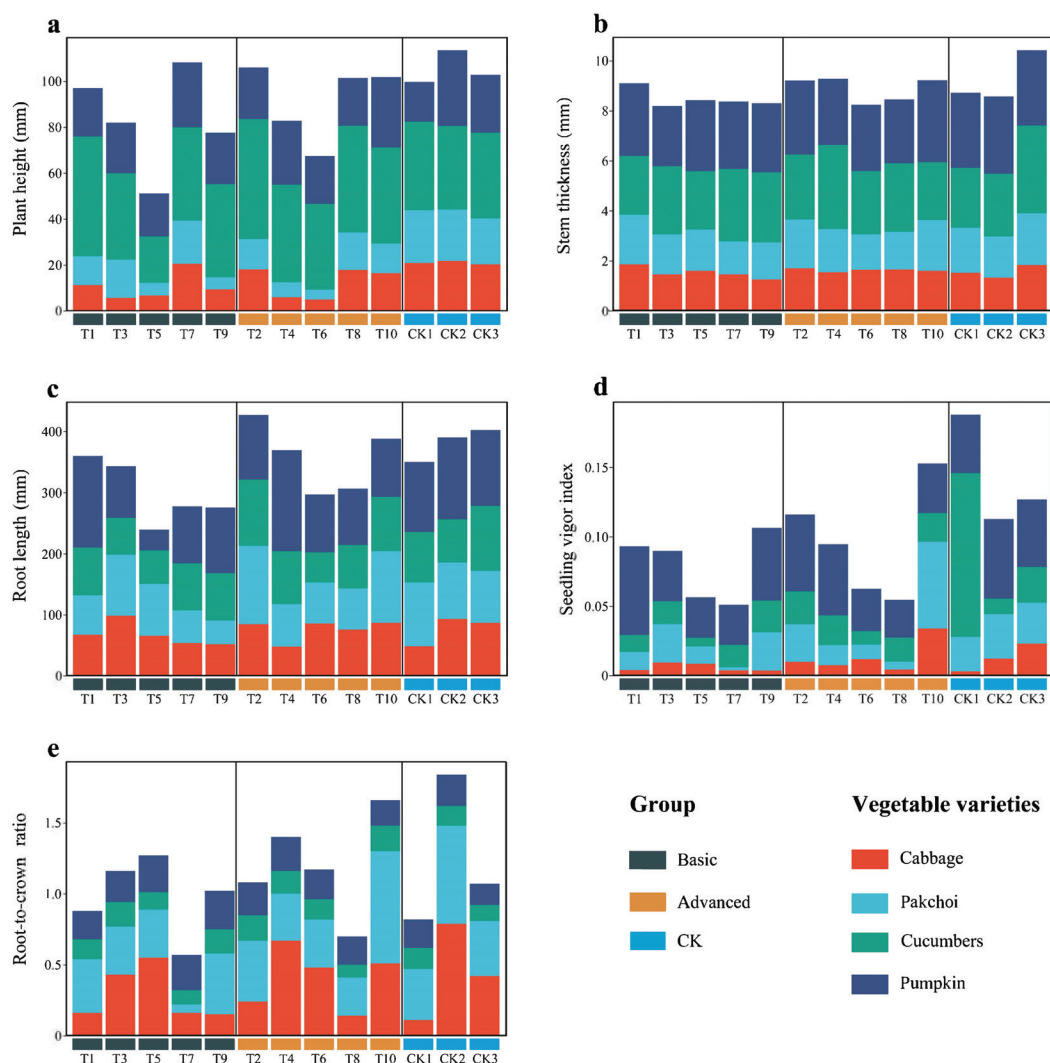


Figure 3. The effect of the different substrate formulations on the biological traits of the four vegetable seedlings: (A) plant height; (B) stem thickness; (C) root length; (D) seedling vigour index; and (E) root-to-crown ratios.

The overall effect of the substrate in the advanced group was higher than in the basic group and close to that in the control group. T3, T5, T2, T4, T6 and T10 were more successful in enhancing the root-to-crown ratios of the four vegetables, second only to CK2.

In conclusion, in potting conditions, the substrate formulation of the advanced group containing perlite and vermiculite more successfully impacted the biological traits of the vegetable seedlings than that of the basic group, with T2 displaying the best effect, while the nursery influence was close to the control group.

The effect of the physicochemical substrate properties on the biological traits of the seedlings of the four vegetable species

An RFF model was used to investigate the influence of the physicochemical substrate properties on the biological traits of the vegetable seedlings (Breiman, 2001; Svetnik et al., 2003; Probst et al., 2018). This included the regression of 14 physicochemical substrate properties of the plant height, stem thickness, root

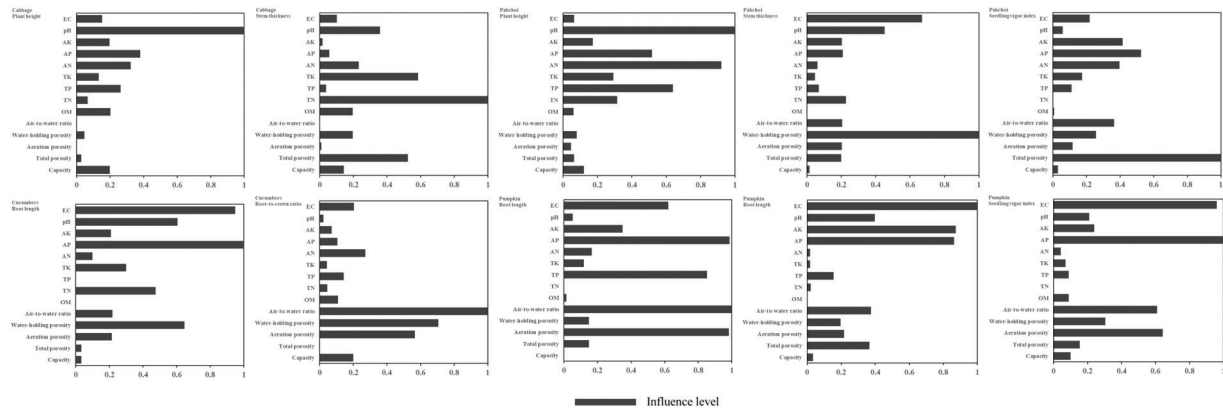
length, seedling vigour index and root-to-crown ratio of each of the four vegetables. The model variable $%Varexplained$ is shown in Table 4. The explanatory variables ranged from 0% to 100%. The reliability of the operational model results increased the closer the explanatory variables were to 100% (Cutler et al., 2012). The results showed that the plant height and stem thickness of the cabbage; the plant height, stem thickness and seedling vigour index of the pakchoi; the root length and root-to-crown ratio of the cucumber; and the stem thickness, root length and seedling vigour index of the pumpkin were significantly correlated ($%Varexplained > 0$) with the physicochemical properties of the substrate.

As shown in Figure 4, the normalised results displayed a significant correlation after RFF model analysis, illustrating the effect of each influencing factor on the dependent variable in terms of 0–1. The dependent variables with the most significant impact on the plant height and stem thickness of the cabbage included pH and TN, while pH, water-holding porosity and total porosity had the strongest influence on the pakchoi

Table 4. The explanatory variables obtained via RFF analysis.

<i>The explanatory degree of model variables var% (% var explained).</i>					
Types	Plant height (mm)	Stem thickness (mm)	Root length (mm)	Seedling vigour index	Root-to-crown ratio
Cabbage	27.57	4.25	−72.22	−43.45	−45.83
Pakchoi	14.20	21.75	−17.84	4.56	−2.12
Cucumber	−27.13	−76.81	10.27	−36.96	7.31
Pumpkin	−43.86	0.56	7.22	16.97	−10.69

RFF, random forest.

**Figure 4.** The correlation between the biological traits of the vegetable seedlings and each influencing factor. The correlation between the biological traits of the vegetable seedlings and each influencing factor.

plant height, stem thickness and seedling vigour index, respectively. The most influential dependent variables on the cucumber root length and root-to-crown ratio included AP and air-water ratio, respectively, while those impacting the stem thickness, root length and seedling vigour index of the pumpkin were EC, air-water ratio and AP, respectively.

Analysis of the physicochemical substrate properties to optimise the mushroom waste percentage

Analysis of the biological characteristics of four kinds of vegetables during the seedling period revealed that the T2 in the mushroom waste substrate had the optimal impact. The mushroom waste content in T2 was further optimised to obtain F1 (T1:perlite:vermiculite = 3:1:1), F2 (T2, T1:perlite:vermiculite = 2:1:1) and F3 (T1:perlite:vermiculite = 1:1:1). The physicochemical matrix properties of F1, F2, F3, CK1 and CK2 are shown in Table 5. The variance analysis results showed significant differences between the physicochemical properties of the different matrices ($P < 0.05$).

As shown in Table 5, the bulk density, total porosity, aeration porosity, water-holding porosity, air–water ratio, OM, pH, EC and other physicochemical property values of F1, F2 and F3 were between those of CK1 and CK2. The bulk density, total porosity, water-holding porosity, pH and EC values ranged between $0.13 \text{ g} \cdot \text{cm}^{-3}$ and $0.15 \text{ g} \cdot \text{cm}^{-3}$, 68.27% and 69.42%,

46.15% and 56.75%, 6.83 and 6.88, and $0.099 \text{ mS} \cdot \text{cm}^{-1}$ and $0.120 \text{ mS} \cdot \text{cm}^{-1}$, respectively, which were higher than CK1 and lower than CK2. The aeration porosity, air–water ratio and OM values ranged between 21.30% and 24.58%, 0.39 and 0.50, and 26.86% and 37.62%, respectively, which exceeded those of CK2 and were lower than CK1. The TN, TP and TK of F1, F2 and F3 were $5.20 \text{ g} \cdot \text{kg}^{-1}$ and $6.19 \text{ g} \cdot \text{kg}^{-1}$, $4.00 \text{ g} \cdot \text{kg}^{-1}$ and $6.28 \text{ g} \cdot \text{kg}^{-1}$, and $16.47 \text{ g} \cdot \text{kg}^{-1}$ and $26.78 \text{ g} \cdot \text{kg}^{-1}$, respectively, which were higher than CK1 and CK2. In summary, the OM, pH, and EC of the matrix declined as the mushroom waste content decreased and the perlite and vermiculite levels increased, while the TP showed an overall upward trend, and TN and TK values were initially higher, followed by a decrease.

The effect of optimising the substrate ratio of the mushroom waste on the biological traits of the pakchoi seedlings

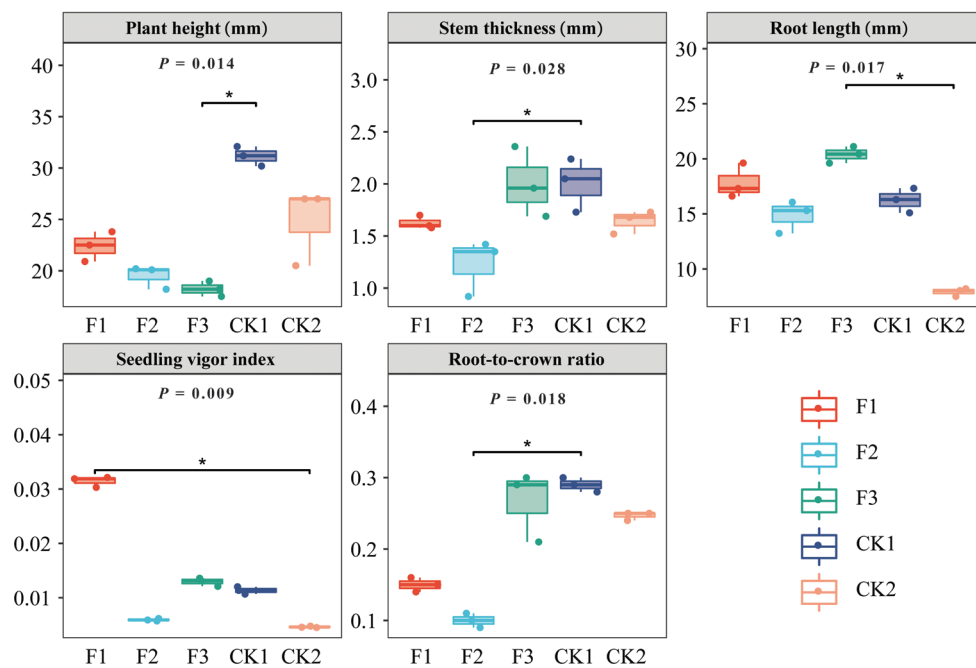
As shown in Figure 5, the impacts of the different substrates on the biological traits of the pakchoi seedlings differed. The highest average plant height of CK1 was 31.17 mm, which was 1.4, 1.6 and 1.7 times that of F1, F2 and F3, respectively, with a significant difference between CK1 and F3 ($P = 0.014$). The average stem thickness of CK1 was highest at 2.01 mm, followed by F3 at 2.00 mm. The CK1 stem thickness was 1.2 and 1.6 times higher than F1 and F2, respectively, and differed significantly from F2 ($P = 0.028$). F3 displayed

Table 5. The physicochemical substrate properties used to optimise the mushroom waste percentage.

Physicochemical properties		F1	F2	F3	CK1	CK2
Physical properties	Bulk weights ($\text{g} \cdot \text{cm}^{-3}$)	0.27 ± 0.03 a	0.15 ± 0.02 b	0.13 ± 0.02 b	0.11 ± 0.01 b	0.28 ± 0.03 a
	Total porosity (%)	68.27 ± 0.48 b	69.09 ± 2.07 b	69.42 ± 0.02 ab	55.76 ± 1.34 c	74.45 ± 2.23 a
	Aeration porosity (%)	21.30 ± 0.00 d	22.94 ± 0.53 c	24.58 ± 0.00 b	26.54 ± 0.61 a	15.81 ± 0.36 e
	Water-holding porosity (%)	56.75 ± 9.26 a	46.15 ± 1.15 ab	55.56 ± 10.74 a	29.22 ± 0.56 b	58.64 ± 1.47 a
	Air-water ratio	0.39 ± 0.07 bc	0.50 ± 0.01 b	0.46 ± 0.09 b	0.91 ± 0.03 a	0.27 ± 0.00 c
Chemical properties	OM (%)	37.62 ± 1.71 b	35.19 ± 1.09 b	26.86 ± 1.22 c	55.76 ± 0.32 a	26.66 ± 2.15 c
	TN ($\text{g} \cdot \text{kg}^{-1}$)	5.20 ± 0.70 a	6.19 ± 0.19 a	6.15 ± 0.08 a	3.74 ± 0.04 b	3.18 ± 0.14 b
	TP ($\text{g} \cdot \text{kg}^{-1}$)	4.00 ± 0.00 a	4.56 ± 0.04 a	6.28 ± 1.55 a	1.23 ± 0.01 b	0.59 ± 0.07 b
	TK ($\text{g} \cdot \text{kg}^{-1}$)	16.47 ± 0.72 c	26.78 ± 1.60 a	23.70 ± 0.47 b	9.79 ± 0.02 d	9.06 ± 0.24 d
	pH	6.88 ± 0.16 a	6.85 ± 0.00 a	6.83 ± 0.09 a	4.50 ± 0.01 b	7.08 ± 0.03 a
EC ($\text{mS} \cdot \text{cm}^{-1}$)		0.120 ± 0.001 b	0.110 ± 0.002 bc	0.099 ± 0.002 c	0.072 ± 0.002 d	0.171 ± 0.010 a

The data in the table are expressed as the means (\pm SD) of three biological replicates. Different lowercase letters after peer data represent significant differences between the treatments at the $P < 0.05$ level.

CK1, control group substrate 1; CK2, control group substrate 2; EC, electrical conductivity; OM, organic matter; TK, total potassium; TN, total nitrogen; TP, total phosphorus.

**Figure 5.** The box plot of the differences between the biological traits of the pakchoi seedlings.

the highest average root length of 20.39 mm, which was 1.1 and 1.4 times higher than F1 and F2, respectively, and differed substantially from CK2 ($P = 0.017$). F1 exhibited the highest average seedling vigour index of 0.0314, which was 5.3 and 2.4 times that of F2 and F3, respectively, and showed significant differences from CK2 ($P = 0.009$). CK1 presented the highest mean root-to-crown ratio of 0.29. F3 was second only to CK1 at 0.27, while CK1 was 1.9 and 2.9 times that of F1 and F2, respectively, and differed substantially from F2 ($P = 0.018$). In conclusion, during the pakchoi nursery

trial, F1 performed better than F2 after increasing the mushroom waste percentage.

DISCUSSION

Physicochemical properties form the basis for assessing the efficacy of seedling substrates, indirectly determining seedling growth and quality, and are a prerequisite for cultivating strong seedlings and obtaining good yields. Related studies showed that seedling substrates should have properties such as a

total porosity between 50% and 80%, a bulk weight between $0.1 \text{ g} \cdot \text{cm}^{-3}$ and $0.8 \text{ g} \cdot \text{cm}^{-3}$ and an OM content $\geq 35\%$, which were all achieved in this experiment (Lonardo et al., 2021). With the gradual increase in the mushroom waste content in the optimised formulations, the pH gradually became neutral while the plant height also increased, further verifying the possibility of mushroom waste as a seedling substrate. However, since fermented mushroom waste usually has a high EC value, it may be toxic to salt-sensitive plants (Massa et al., 2018a, 2018b).

During actual production, substrates are generally used for seedlings or cultivating valuable crops, such as horticulture and herbs. Considering the cost, the types of materials that can be used as substrates for seedlings or cultivation are limited. The most widely used is peat, which is a non-renewable resource due to its original coal state. The total amount is limited, and over-exploitation can severely damage the ecological environment. Therefore, developing alternatives to peat as cultivation substrates has attracted considerable research attention (Hultberg et al., 2022). Mushroom waste is rich in OM, mineral elements and several bacteriophagic proteins (Mleczek et al., 2018b; Collela et al., 2019) while also presenting advantages such as high yield and cost-efficiency. Therefore, using mushroom waste for the complete or partial replacement of peat can provide a new research direction and is highly significant for conserving non-renewable resources. Studies have shown that mushroom waste reduces the growth of plant- and soil-borne pathogens and improves plant resistance to diseases (Eudoxie and Alexander, 2011; Zhang et al., 2012; Moraes et al., 2020). Medina et al. (2009) selected three vegetables to verify the effect of mushroom waste and peat cultivation, revealing that vegetables grown in a 75% mushroom waste +25% peat substrate outperformed the peat substrate in terms of biomass and nutrients. Yu et al. (2022a,b) used the membership function to comprehensively evaluate the indexes of each substrate combination, revealing that substrates mixed with coconut bran, perlite and vermiculite scored the highest. Strong aboveground and underground growth were evident, while the quality exceeded other peat substrates. Afagh et al. (2019) showed that using mushroom waste for German chamomile cultivation significantly increased the plant growth, flower yield, essential macronutrient uptake, sodium concentration, proline and soluble sugar content, and essential oil percentages.

Therefore, more extensive research on the resource utilisation of mushroom waste can realise the reuse of agricultural waste, turn waste into a valuable commodity and reduce environmental pollution while promoting the development of a sustainable edible mushroom industry. It can also help combine the edible mushroom, planting and breeding industries to create a

new edible mushroom field and promote the sustainable development of the agricultural economy.

CONCLUSIONS

In the experimental conditions, the substrate properties mainly depend on water-holding porosity, aeration porosity, air-water ratio, total porosity, pH, EC, AP and AK. The RFF model showed that pH has the most significant influence on the plant height of cabbage and pakchoi, while the root development of cucumber and pumpkin is substantially impacted by AP and air–water ratio. The pot experiment indicates that adding perlite and vermiculite to the mushroom waste substrate has a better effect on the biological characteristics of the vegetables than direct fermentation. T2 (mushroom waste:sawdust:zeolite:vermiculite = 8:2:5:5) displays the strongest influence, with a seedling-raising effect closest to the peat substrate. Furthermore, the impact on pakchoi seedling raising can be improved by appropriately increasing the mushroom residue content. This study uses waste mushroom residue instead of peat as a seedling substrate. On the one hand, it provides a theoretical basis for improving the resource utilisation of waste mushroom residue. On the other hand, it can promote the green development of the facility cultivation industry while reducing the cost of factory seedlings.

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AUTHOR CONTRIBUTIONS

Q.L., S.W. and N.Z. – conceptualisation. Q.L., S.W. and N.Z. – methodology. Q.L., S.W., N.Z. and L.B. – validation. Q.L. and S.W. – formal analysis. Q.L., S.W. and N.Z. – investigation. Q.L., J.Z. and X.Z. – resources. Q.L., J.Z. and W.C. – data curation. Q.L. – writing: original draft preparation. Q.L., S.W. and N.Z. – writing: review and editing. Q.L., S.W. and T.H. – visualisation. N.Z. – supervision. N.Z. – project administration. N.Z. – funding acquisition. L.Q. and W.S. contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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