

*Original Research*

# Impact of Exogenous Addition of Sulphur Powder on the Effectiveness of Kitchen Waste Compost Seedling Substrates

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## Abstract

The feasibility of using sulphur powder (SP) to improve physicochemical properties and promote growth of cucumber seedlings in kitchen waste compost (KWC) was investigated using cavity trays in the greenhouse. The base substrate used consisted of KWC, peat, rice husk and perlite, in respective ratios of 15%:35%:40%:10% by volume. Treatments consisted of additions of SP at rates of 0 g (CK), 3 g (T1), 6 g (T2), 12 g (T3) and 24 g (T4) per liter of the seedling substrate (SS). The investigation showed that addition of SP significantly reduced pH of SS and increased its EC values ( $p < 0.05$ ). The germination index (GI) of cucumber seeds was significantly enhanced with SP addition at T1 ( $p < 0.05$ ). However, other growth indicators (shoot and belowground growth indices) showed maximum values at T2. Levels of plant nutrients (N, P, K) and organic matter in this SS reflected a trend of uptake by seedlings in the order  $T2 > T1 > CK > T3 > T4$ , while the levels of the growth substances in T3 and T4 were consistent with the deleterious effects of excess levels of SP. Fluorescence analysis of levels of *Fusarium* spp. in SS showed significant reductions in copy numbers of the fungi ( $p < 0.05$ ) as sulfur levels decrease in the order  $T4 > T3 > T2 > T1 > CK$ . For the SS composition used for this study, T2 gave the best results for the establishment of cucumber seedlings. Other combinations of substrate and seedlings must be evaluated to determine appropriate amounts of components to be used under specific circumstances.

**Keywords:** sulphur, kitchen waste compost, seedling substrate, cucumber

## Introduction

Over the last decade, Chinese people have experienced rapid improvement in quality of life, economic expansion and technological advancements [1, 2]. These positive developments are unavoidably accompanied by consumption practices that lead to generation of large amounts of waste that can pose serious threats to human and environmental health. One waste stream that has been attracting much attention recently in China is kitchen wastes (KW), which originate from family kitchens, restaurants, hotels and other food processing and associated industries [3]. According to one estimate, KW accounts for 20% to 45% of the total municipal solid waste in China [3, 4]. Traditional disposal options for such waste have typically involved incineration and burial, which have become increasingly unacceptable [5]. Relatively recently, resource conversion technologies have become prevalent not only for disposing of wastes but also for turning them into value-added products [6, 7]. For example, KW has been extracted for making biodiesel [8], producing biogas [9] and for building organic fertilizers [10].

Of the three examples cited above, the Chinese government has heavily supported conversions of KW into organic fertilizers (OFs), specifically, in the form of kitchen waste compost (KWC). For example, The Chinese government provides a range of subsidies to support the composting of organic waste, including KW [11]. However, characteristics such as high pH and high salt content (EC) of raw materials used in KWC-OFs have often presented difficulties for the everyday use of these products [12]; as a result, hardly any OFs manufactured from KWC have received fertilizer registration certificates in China [13]. Still, under certain conditions, KWC can exert a positive influence on crop growth and development [14]. Our group previously investigated the effects of KWC substrate on the growth of *Brassica chinensis* L. [15]. We found that when more than 10% of KWC was added to the substrate, the growth of *Brassica chinensis* L. was significantly inhibited. The nutrient content and basic physical properties of KWC-OFs were within the normal range and were not responsible for the inhibition of *Brassica chinensis* L. growth. Rather, we found that the pH and EC values of KWC-OFs were the main factors limiting its extensive use in the substrate. Therefore, it is necessary to overcome the high pH and EC that can make the physical and chemical environment of KWC-OFs seedling substrates' unfavorable for the growth of young plants [12, 16].

One solution for overcoming high matrix pHs of substrates may be found in the use of sulfur powder (SP) for lowering pH of alkaline soil [17]. The same attribute can make SP an excellent candidate for the development of KWC-derived substrates for sustainable growth of

seedlings in nursery crop production. Sulfur powder offers additional positive features to SS production; its fungicidal and insect repellent properties have frequently been exploited in the manufacture of pesticides [18]. Furthermore, it can serve as a micronutrient source for plant growth. The multi-purpose features of SP make it a most suitable agent for incorporation into SS schemes in the nursery industry. The investigation reported here is an assessment of the use of SP for improving the chemical properties of KWC-SS, while simultaneously enhancing growth of cucumber seedlings.

## Experimental

### Materials

The experiment was carried out at the Institute of Organic Recycling (Suzhou), China Agricultural University, at the Artificial Climate and Plant Culture Centre. The KWC was obtained from the Taihu Lake Organic Waste Treatment and Utilization Demonstration Centre by aerobic fermentation of Taihu Lake region KW. KWC contained 2.02 g·kg<sup>-1</sup> of ammonium nitrogen, 0.99 g·kg<sup>-1</sup> of effective phosphorus (phosphorus available for plant uptake), 2.98 g·kg<sup>-1</sup> of fast-acting potassium (potassium that is rapidly absorbed by plants) and 35.21% organic matter. SS for the trial actually was made from KWC, peat, rice husk and perlite in the following proportions: 15%:35%:40%:10% by volume. SS had a pH of 7.67, an electrical conductivity of 1.12 mS·cm<sup>-1</sup>, 1.22 g·kg<sup>-1</sup> of ammonium nitrogen, 1.14 g·kg<sup>-1</sup> of effective phosphorus, 1.96 g·kg<sup>-1</sup> of fast-acting potassium. The basic physicochemical properties of the raw materials required to prepare the substrate are detailed in reference [15].

### Methods

The experiment consisted of five treatments designated as CK, T1, T2, T3 and T4; where CK (control) represented 0 SP plus KWC-SS and T1-T4 represented additions of 3 g, 6 g, 12 g, and 24 g of SP per liter of SS respectively. Each treatment was replicated three times. The SP was combined with the KWC-SS according to each treatment's dosage, and the seedlings were planted in 50-hole seedling trays with one seed per hole, sowed at 0.5 cm from the surface of the substrate. For 15 days, the seedlings were nurtured in an artificial climate chamber at 25°C and 80% humidity. Precision drip irrigation was used to water the treatments, with 10 mL of water injected into each hole every tray and replaced with 10 mL of water every 48 hours, keeping each hole wet and free of water droplets. For testing, each treatment was sampled in 100 g by quadruplicate.

## Measurement Items

### Determination of Key Indicators for Seedling Substrates

The pH and EC values and germination index (GI) of the seedling substrates were determined using the method specified in reference [19], and the fresh weight of the cucumber seed germ was determined. The Germination Index is an indicator used to measure the germination capability of plant seeds. It is typically employed to assess the health status and germination rate of seeds. The Germination Index is one of the crucial parameters in seed biology and holds significant importance in fields such as agriculture, horticulture, and ecology. The formula for calculating GI is shown below:

$$GI = \frac{A_T \times B_T}{A_C \times B_C}$$

Where,  $A_T$  represents the germination rate of seeds in the treatment group;  $B_T$  represents seed root length of treatment group;  $A_C$  represents the germination rate of seeds of CK treatment;  $B_C$  represents seed root length of CK treatment.

### Growth Indicators for Young Cucumber Seedlings

The number of cucumber seedlings that emerged 10 days after seeding was recorded and the seedling emergence rate were estimated. 15 cucumber seedlings were taken from each hole tray 15 days after seeding and their height was measured with a straightedge and stem thickness was assessed with a vernier caliper. The cucumber seedlings' leaf area was calculated using the length and width coefficient method; the cucumber seedlings' completely expanded leaves were measured using a chlorophyll content meter, and the chlorophyll content was reported as SPAD values. Using a brush, the seedling substrates adhered to the 15 cucumber seedlings were removed and stored in a sealed container at 4°C for testing. A root scanner (WinRHIZO) was used to measure the root length and root surface area of cucumber seedlings. The above-ground and below-ground weights of cucumber seedlings were measured using an electronic balance. Subsequently,

they were killed at 90°C and dried at 60° to a constant weight. The strong seedling index was calculated using plant height, stem thickness and plant weight, which provides a relatively comprehensive picture of the overall quality of cucumber seedlings. The formula is shown below:

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

### Nutrient Content of Seedling Substrates

The cucumber SS was air-dried, ground, and passed through a 2 mm sieve, and the changes in nutrient content were measured using the method specified in the reference [10] for the determination of alkaline nitrogen, effective phosphorus, fast-acting potassium, and organic matter.

### Fluorescence Quantification of *Fusarium* spp.

The sample for analysis was the seedling substrate to which the cucumber seedlings were attached, and the genomic DNA of the sample was extracted using the CTAB technique and utilized as a template for qPCR. DNA was eluted in 40 L of Tris-HCl buffer (pH 8.0) and kept at -20°C until testing. *Fusarium*-specific primers ITS-Fu-f and ITS-Fu-r were used in real-time qPCR analysis. Meng et al were consulted for the amplification protocol [20].

### Data Statistics and Analysis

Using DPS 18.1 software [21], the trial's raw data were submitted to one-way ANOVA after being tallied using Excel 2019.

## Results

### Key Indicators for Seedling Substrates

Table 1 shows that increasing additions of SP from CK (0) to T4 (24g/L) significantly ( $p < 0.05$ ) lowered

Table 1. Key indexes of different seedling substrates.

	pH	EC (/mS·cm <sup>-1</sup> )	Germination /%	Germ fresh weight /g	Root length /cm	GI
CK	7.67±0.02a	1.12±0.01d	76.67±6.67a	0.17±0.01a	91.17±9.58a	1.15±0.04b
T1	7.00±0.01b	1.88±0.02c	86.67±3.33a	0.17±0.03a	100.83±6.99a	1.45±0.06a
T2	6.96±0.01c	1.91±0.02bc	86.67±6.67a	0.15±0.01ab	77.07±2.27b	1.10±0.21b
T3	6.68±0.01d	2.00±0.01b	83.33±6.67a	0.14±0.01ab	68.67±8.39bc	0.98±0.11b
T4	6.13±0.01e	2.16±0.08a	76.67±8.82a	0.12±0.01b	54.83±1.12c	0.82±0.17b

Note: Significant differences between treatments are indicated in the same column by different letters ( $p < 0.05$ ), same below.

pH of SS. At the same time, EC values in SS increased significantly ( $p < 0.05$ ) with increasing addition of SP. Germination of cucumber seedlings was not affected by progressive additions of SP; however, the fresh weight of cucumber embryos was significantly inhibited under T4. Furthermore, addition of  $SP \leq 6$  g/l (T2) significantly inhibited root growth of cucumber seedlings. Table 1 also shows that the germination index (GI), a reflection of SS conditions prior to cucumber germination, was significantly higher under T1 than other treatments.

### Cucumber Seedling Growth Indicators

Table 2 shows that there were no significant differences in heights of cucumber seedlings that were grown under T1 and T2 treatments or those left untreated (CK). Furthermore, there were no significant differences between CK and T3 treatments; however, the T4 treatment caused significant depression/inhibition ( $p < 0.05$ ) in height of the cucumber seedlings. Similarly, stem thickness of the seedlings showed no significant differences among CK, T1, T2 and T3 treatments. However, at the T4 treatment, thickness of stems was significantly ( $p < 0.05$ ) reduced compared to the other treatments.

Significant differences ( $p < 0.05$ ) were found among chlorophyll content, ground fresh weight, dry weight as well as leaf area of cucumber seedlings; however, the highest values of the measures were consistently observed under the T2 treatment. On the one hand, results CK and T1 appeared to reflect lack or insufficient amounts of SP in SS; on the other hand, results of T3 and T4 were consistent with harmful effects of excess SP.

Belowground growth responses of cucumber seedlings under SP incorporation were measured in terms of root length, root area, root weight (fresh and dry) and strong seedling index are shown in Table 3. The data show that strong seedling index was significantly higher ( $p < 0.05$ ) at 6g/L SP additions to SS compared to control and other treatments. Root length, area and weights were generally not significantly different from the control and T1 treatments. However, the measures showed significant declines with higher levels of SP additions to SS.

### Variation in Nutrient Content of Seedling Substrates

The dynamics of plant nutrients, nitrogen (N), phosphorus (P) and potassium (K) as well as organic matter (OM) during the growth of cucumber seedlings in SS with or without SP is shown in Table 4. Levels of all these growth substances were significantly lower ( $p < 0.05$ ) T2 than CK. This was consistent with the highest uptake of the nutrients and OM in T2 than CK (in a closed system). Table 4 also showed that uptake of the growth substances was lowest at T4, which was consistent with some limitation to uptake by seedlings. For T1 and T3 uptake of the growth substances were mixed.

### Fluorescence Quantification of *Fusarium* spp.

For this experiment, we used fluorescence analysis to quantify the *Fusarium* spp. present in the SS, with or without SP additions. Fig. 1 shows that the copy number of *Fusarium* spp. per unit of seedling substrate was

Table 2. The shoot growth indexes of cucumber seedlings with different treatments.

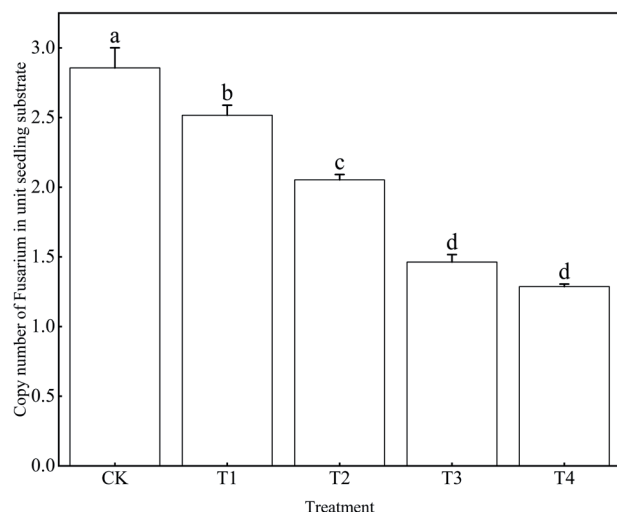
	Height /cm	Stem thickness/mm	Chlorophyll content/SPAD	Ground fresh weight/g	Ground dry weight/mg	Leaf area/cm <sup>2</sup>
CK	3.97±0.24ab	2.27±0.06a	50.97±1.89bc	1.04±0.06b	132.33±1.45b	16.42±0.08c
T1	4.17±0.07a	2.30±0.12a	53.30±2.91b	1.19±0.03ab	128.27±1.80b	27.91±0.72b
T2	4.27±0.27a	2.50±0.21a	59.37±2.08a	1.47±0.09a	142.47±1.85a	37.43±1.23a
T3	3.50±0.06bc	2.47±0.18a	47.60±1.91bc	0.62±0.12c	75.73±3.77c	16.77±0.39c
T4	3.17±0.19c	1.70±0.10b	45.77±1.29c	0.47±0.05c	54.30±2.68d	16.76±0.36c

Table 3. The underground growth indexes of cucumber seedlings with different treatments.

	Length /m	Root area /cm <sup>2</sup>	Root fresh weight /g	Root dry weight /mg	Strong seedling index
CK	4.13±0.47ab	42.29±5.69a	0.51±0.11b	12.03±0.81b	1.31±0.01b
T1	4.81±0.39b	42.78±3.65a	0.64±0.08b	26.07±2.34a	1.15±0.02c
T2	4.87±0.27ab	47.74±1.16a	0.81±0.07a	32.53±5.91a	1.42±0.03a
T3	2.06±0.03c	39.15±3.03b	0.66±0.12b	30.63±1.39a	0.75±0.04d
T4	1.08±0.06d	20.57±0.43b	0.26±0.03c	9.53±0.48b	0.47±0.07d

Table 4. Changes of nutrient content of seedling substrate in different treatments.

	Available N/(g·kg <sup>-1</sup> )	Available P/(g·kg <sup>-1</sup> )	Available K/(g·kg <sup>-1</sup> )	Organic matter/%
CK	1.05±0.01b	0.61±0.01a	1.80±0.01c	20.11±0.04b
T1	0.94±0.01c	0.23±0.01b	1.74±0.02c	18.57±0.22c
T2	0.84±0.01d	0.17±0.01c	1.55±0.02d	16.81±0.02d
T3	1.02±0.02b	0.50±0.01d	1.91±0.02b	18.13±0.06c
T4	1.62±0.02a	0.56±0.02e	2.09±0.01a	32.92±0.42a

Fig. 1. Fluorescence quantitative analysis of *Fusarium* spp. in different seedling substrates.

Note: Significant differences between treatments are indicated by different letters ( $p < 0.05$ )

reduced significantly ( $p < 0.05$ ) with the addition of SP at a concentration of 0-12 g/L. This suggests that adding SP to the SS can reduce the emergence of belowground diseases in cucumber seedlings.

## Discussion

Beneficial uses of KWs have been receiving increasing attention recently in the face of needs for waste disposals as well as the prevalence of technologies for waste conversions into value-added products. One of the most common uses of KWs in China is in the production of organic fertilizer (OFs) [12, 16, 22]. In preliminary investigations, our group found that KW-based OF (KWC) may partially replace peat in the production of seedling substrate (SS) in agriculture [15]. Peat-based substrates have long dominated the market share of SS production worldwide [23]. However, this practice has become acutely unsustainable. Peat is a C-rich, non-renewable resource, whose excessive extractions have increasingly led to serious global environmental degradation, including releases of sequestered C back into the atmosphere [24] as well

as the destruction of biodiversity [25]. In view of this, our investigation of KWC as an alternative component to peat in the production of SS is highly desirable from agricultural and environmental standpoints. Not only can our results contribute to reducing dependence on peat in SS production but also, they can contribute to environmental sustainability by diverting wastes into useful products. Despite such benefits, our findings also point out the need for caution in the routine use of KWC. In our specific instance, KWC required amendment with specified levels of Sulphur powder (SP) to greatly reduce pH to allow growth of cucumber seedlings.

The use of SP to ameliorate high pH in our investigation is akin to the practice of using the material for lowering pH of alkaline soils [17]. Essentially, the mechanism involves the oxidation of S by S-oxidizing bacteria in the presence of air, first into sulphuric anhydride and then into sulphuric acid, thereby lowering the pH of a substrate [26, 27]. Because S is an essential element in plant growth, substrate amendment with SP will not only adjust the pH of the medium to minimize alkaline toxicity to seedlings, but also, it can boost seedling growth by supplementing S. However, excessive levels of SP can severely depress the germination of cucumber seeds, as evidenced by decreased GI with increasing SP beyond 3 g/L in our experiment. It is generally assumed that a high GI of the substrate after leaching solution treatment indicates that its chemical qualities are better in line with the crop's growth requirements [19]. Germination Index (GI) is often used as an important indicator for verifying whether an organic fertilizer (OF) is fully decomposed [28]. GI greater than 70%, is generally considered as a good indicator of maturity. Factors affecting GI, and therefore OF maturity, include pH, EC and level of  $\text{NH}_4^+$  as well as presence of exogenous substances. These factors must be optimized in OF-SS mixtures to avoid phytotoxic reactions to plant seedlings [28]. We introduced the GI concept into our substrate development, which allowed us to substantiate substrate conditions that enhanced seed germination and promoted the early growth of cucumber seedlings. In our case, GI exceeding 100% established the growth-promoting effect at T1 (optimum SP = 3 g/l). In contrast, GI lower than 100% indicated the presence of SP levels that inhibited seed germination, namely T3 and T4 (SP = 12 g/l and 24g/l respectively) (Table 1). Although

GI was reflective of substrate suitability for cucumber in our experiments, it must be emphasized that the predictive value of the index can often be quite limited, primarily due to the brief 48-hour period required for GI its determination [25, 28]. In practice, suitability of a substrate for crop growth still necessitates assessments based on seedling growth under actual prevailing conditions [29].

In our investigation, we found that addition of 6 g/L of SP to SS improved growth indices of cucumber seedlings. Specifically, chlorophyll content, leaf area and above ground biomass measures were all maximized at this level of SP. The same 6 g/L addition of SP to SS produced the highest level of nutrient uptake by cucumber seedlings, based on the residual nutrient content in the substrate after seedling establishment. The treatment with 6 g/L SP addition had the best root indices, including root length, root area, and root weight, in terms of cucumber seedling root development. However, the addition of too much SP inhibited the root development of cucumber seedlings. When combined with the strong seedling index, it is clear that the addition of 3-6 g/L SP had a considerable promotion impact on cucumber seedling growth.

Our research also covered the suppression of disease development by SP in SS. Although root diseases did not arise in cucumber seedlings during the experiments, information obtained indirectly about gene copies of *Fusarium* spp. in SS after seedling growth was still relevant in predicting what could happen in the event of infestation by the pathogen. Specifically, the gene copies of *Fusarium* spp. in the seedling substrate were dramatically reduced with the addition of SP. This would suggest that the addition of SP to SS holds significant potential for suppressing soilborne diseases caused by *Fusarium* species. This ensures the protection of cucumber plants from relevant diseases during the seedling growth stage. Taking all of the experimental findings into account, 6 g/L of SP will be the recommended amount to be added to the current formulation of SS for growth of cucumber. However, it must be emphasized that differences in crop type, substrate composition and environmental conditions exert profound influences on applicability of data generation in studies of the nature described here.

## Conclusions

In this study, a method for adjusting the physicochemical parameters of KWC-SS by adding SP was developed in order to enhance integrated seedling development. Excessive SP addition can also impede cucumber seedling growth, and the amount of addition should be controlled by the unique composition of the KWC-SS. A 6 g/L addition of SP to seedling substrates produced from KWC, peat, rice hulls, and perlite in a ratio of 15%:35%:40%:10% by volume will yield the best seedling results.

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## Conflict of Interest

The authors declare no conflict of interest.

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