**Integrated Effects of NPK Fertilizer and Spent Mushroom Substrate on Lettuce Growth, Yield and Antioxidant Enzyme Activity**

**ABSTRACT**

The study investigates the effects of NPK fertilizer and spent mushroom substrate (SMS) on lettuce *(Lactuca sativa*) growth, yield, and antioxidant enzyme activity. SMS, a by-product of mushroom cultivation, is a sustainable organic fertilizer rich in organic matter and beneficial microbes, while NPK provides essential macronutrients for plant growth. A greenhouse experiment was conducted using four treatments: control (no amendment), SMS, NPK, and a combination of SMS and NPK. Key growth parameters, including plant height, number of leaves, and fresh and dry weights of leaves and roots, were measured, alongside oxidative stress markers and antioxidant enzyme activities. The results revealed that SMS and NPK independently improved lettuce growth and physiological responses, but their combined application had the most significant effects. The SMS+NPK treatment increased plant height, leaf and root biomass, and antioxidant enzyme activities while reducing oxidative stress markers like MDA and H₂O₂. This synergistic effect underlines the potential of integrating organic and inorganic fertilizers to enhance crop productivity sustainably. The findings highlight the benefits of SMS as an environmentally friendly amendment, cost-effectiveness, and its potential to complement NPK for sustainable agricultural practices. SMS enhances soil microbial diversity and organic matter content more effectively than inorganic fertilizers, leading to improved nutrient cycling and long-term soil fertility. This research offers a novel application of SMS as a low-cost substitute for fertilizers that pose environmental risks.

**Keywords:** Spent mushroom substrate, NPK fertilizer, Antioxidant enzyme activity, Lettuce growth, Sustainable agriculture

# 1. INTRODUCTION

Agriculture's usage of both organic and inorganic fertilizers has drawn a lot of attention because of its effects on crop sustainability and production (Rahman and Zhang, 2018). Spent mushroom substrate (SMS), is a by-product of mushroom farming and a possible organic alternative (Mwangi et al., 2024). Plant development and soil health can be enhanced by SMS's abundance of organic matter, nutrients, and helpful bacteria. The popular inorganic alternative, NPK fertilizer, on the other hand, supplies nitrogen, phosphorous, and potassium in precise ratios designed to promote quick plant development (Wang et al., 2024; Qaswar et al., 2020).

A spent mushroom substrate is the biomass left over after commercial mushroom businesses harvest their mushrooms (Mohd Hanafi et al., 2018). There is a lot of concern about how to dispose of SMS produced during mushroom farming. Worldwide, it is anticipated that between 10 and 50 million metric tons of discarded mushroom compost will be produced each year (Jayaraman et al., 2024; Rahi et al., 2023). SMS is bulky, which makes disposal more difficult. In addition to annoying the surrounding area, uncontrolled SMS disposal pollutes the land, water, and air (Othman et al., 2020). The fungic yield of *Agaricus bisporus* spent substrate has been examined for textile dyes by Toptas et al. (2014) and for acid mine drainage using the system developed by Grembi et al. (2016). Many *Pleurotus* species’ residual substrate has been used to adsorb pesticides from fruit packing industry wastes (Papazlatani et al., 2019), antibiotics from swine wastewater (Chang et al., 2014), and water and copper with nickel content respectively (Kamarudzaman et al., 2022).Certain mushroom species have been researched for their ability to remove sulfa antibiotics (Mayans et al., 2021), methylene blue from waste industrial fluids (Dey et al., 2017), and fluoride from drinking water (Sadhu et al., 2021).

The annual plant lettuce (*Lactuca sativa* L. var. eden) is a member of the Asteraceae family of sunflowers (Amedor, 2014). Lettuce is a popular leafy vegetable that is ideal for studying the effects of different fertilizers due to its short growing season and nutritional content (Martínez-Ispizua et al., 2022). Despite the increasing demand for sustainable agriculture practices, achieving high crop yields while maintaining soil health remains a challenge for many farmers. Lettuce, as a high-demand vegetable crop, requires adequate and balanced nutrient inputs to achieve optimal growth and yield. Conventional farming methods often rely heavily on synthetic fertilizers such as NPK to meet crop nutritional needs. However, excessive reliance on inorganic fertilizers can degrade soil quality over time, disrupt microbial ecosystems, and contribute to environmental pollution. As such, there is a growing need to explore alternative and sustainable fertilization methods that support high crop productivity without compromising environmental health. Spent mushroom substrate (SMS) presents a potential solution to this challenge, offering a sustainable, organic alternative to synthetic fertilizers. Rich in organic matter and beneficial microbes, SMS has shown promise in improving soil structure, enhancing nutrient retention, and fostering plant growth (Huang et al., 2022; Rajavat et al., 2022). However, SMS alone may not meet all the nutritional needs of lettuce, as it can vary in nutrient composition depending on the type of mushroom grown. NPK fertilizer, with its precisely balanced nutrient formulation, has the potential to supplement SMS by providing essential nutrients in a readily available form. Research is limited on how SMS and NPK interact and influence the growth and yield of lettuce, particularly in terms of finding the optimal ratio that maximises crop output while supporting sustainable practices. Therefore, this research seeks to evaluate the effects of NPK fertilizer and spent mushroom substrate (SMS) on lettuce growth, yield, and antioxidant enzyme activity.

# 2. MATERIALS AND METHODS

**2.1 Study site and source of materials**

This experiment was performed in the greenhouse at Gansu Agricultural University in Lanzhou, Gansu, China in 2023. The lettuce used in the study was Looseleaf (*Lactuca sativa* var. *crispa*).

**2.2 Experimental design and treatments**

Four treatments were included in the fully randomized experimental design: T1 (control) = No soil amendment, T2 = NPK (15:15:15), T3 = SMS (nutrient composition; nitrogen, phosphorus, potassium, calcium, magnesium (Othman et al., 2020)) and T4 = NPK+SMS. Table 1 shows the treatment description used in the field. A hand fork was used to disperse and integrate the SMS into the soil. Three replicates and a fully randomized design were used to position the pots. Following planting, watering was carried out every two days, and the temperature was kept at a steady 25 ± 0.5 °C with additional day/night illumination of 16/8 hours and 65% relative humidity. There were two separate tests conducted.

**Table 1: Description of treatment used in an experimental trial**

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Description** | **Rate** |
| T1 | Control | No Amendment  |
| T2 | Spent mushroom substrate (SMS)  | 10t/ha |
| T3 | NPK (15:15:15) | 200kg/ha |
| T4 | SMS+ NPK | 5t/ha + 140kg/ha  |

Note: The NPK rate was reduced by 30% when combined with 5 tons of SMS

**2.3 Data collection procedures**

At 28 days following transplanting and harvesting, information was gathered on the plant's height (cm), number of leaves per plant, fresh weight (g), dry weight (g), and length of roots (cm). To measure fresh weights, the roots of the plants that were sampled were cleaned, separated from the shoots, and weighed right away. To determine their dry weights, each sample was oven-dried at 80 °C until its weight was constant.

**2.4 Determination of oxidants and antioxidants in roots**

After being powdered in liquid nitrogen, a 0.5 g fresh root sample was submerged in 1 mL of acetone in an ice bath. Beijing Solarbio Science and Technology, Beijing, China, provided the MDA content assay kit (BC0025), which was used to determine the amount of malondialdehyde (MDA). The absorbance of each sample was assessed at 450, 532, and 600 nm. The hydrogen peroxide (H2O2) content was measured using the manufacturer's protocol/kit (H2O2 content assay kit; Beijing Solarbio Science and Technology) (BC3595). Each sample's absorbance was measured at 415 nm. The MDA and H2O2 values were expressed in μmol g-1 FW. The activities of catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7), and superoxide dismutase (SOD; EC 1.15.1.1) were assessed in compliance with the guidelines provided by the test kit (Solarbio Science and Technology Co., Ltd., Beijing, China). 0.5 g of fresh lettuce roots were ground in liquid nitrogen, 1 mL of extract solution was added, and the mixture was centrifuged at 8000 g for 10 minutes at 4 °C. The supernatant was collected, transferred to a separate centrifuge tube, and centrifuged at 8000 g for 10 minutes at 4 °C. The various chemicals were added following the manufacturer's instructions. POD, SOD, and CAT were measured using a spectrophotometer (EPOCH2 Plate Reader, BioTek, Winooski, VT, USA) at 470, 560, and 240 nm, respectively. U mg− 1 FW was used to express the activity.

**2.5 Statistical analysis**

After all statistical analyses were finished, a one-way ANOVA was performed using SPSS 20.0 software (SPSS, Chicago, Illinois, USA). The experimental data were expressed as the means (± SE) of two independent investigations with three repeats. At a 5% probability level, means were differentiated using the Duncan multiple range test.

# 3. RESULTS AND DISCUSSION

**3.1 Effect of SMS and NPK application on growth parameters**

The findings indicate that the different growth characteristics of lettuce are strongly (p<0.05) impacted by the application of SMS and NPK fertilizers. When SMS was applied alone, the plant's height, leaf number, fresh weight, and dry weight rose by 32.2%, 22.9%, 36.6%, and 63.9%, respectively, in comparison to the control (Figure 1A-1D). Once more, when NPK was applied alone, the plant's height, leaf number, fresh weight, and dry weight rose by 61.2%, 40.6%, 33.8%, and 52.7%, respectively, compared to the control. However, the SMS + NPK treatment increased plant height, leaf count, fresh weight, and dry weight by 89.9%, 71.4%, 51.5%, and 79.1%, respectively, compared to the control (Figure 1A-1D).



**Figure 1.** **Impact of NPK and SMS on lettuce's (A) plant height, (B) leaf count, (C) fresh weight, and (D) dry weight.**

The data is displayed as mean ± SE. According to Duncan's multiple range test, different letters denote a significant difference (p < 0.05). CK stands for control treatment in the absence of a soil amendment.

Additionally, after applying SMS and NPK fertilizers, there was a significant (p<0.05) increase in root length, fresh root weight, and dry root weight. Root length, fresh root weight, and dried root weight increased by 44.9%, 63.1%, and 55.7%, respectively, due to SMS treatment compared to the control (Figure 2A-2C). Additionally, the administration of NPK led to increases in root length, fresh root weight, and dried root weight of 21.3%, 40.7%, and 34.4%, respectively, in comparison to the control. Furthermore, the treatment of SMS and NPK in combination (SMS + NPK) increased root length, fresh root weight, and dried root weight by 61.3%, 84.6%, and 91.8%, respectively, in comparison to the control (Figure 2A-2C).

The application of SMS and NPK fertilizers increased all the growth parameters (height, and weights) measured in the study. These results are in line with those of Alzain et al. (2023), who found that applying biofertilizer and NPK increased the sunflower's plant height, root length, fresh mass, and dry mass. The Kundu (2021) study found that the application of cow dung, either by itself or in conjunction with inorganic fertilizer, increased plant height, leaf count, and the fresh and dry weight of the roots. According to Zaman et al. (2017), the height of *Stevia rebaudiana* plants was affected by cow dung treatment and rose with higher dosages of cow dung. According to Amendola et al. (2017), applying compost all at once boosted the height of the grapevine crops. Based on these observations, it can be concluded that the use of organic manure enhances the growth and development of lettuce and leads to the highest growth parameters than the control. Therefore, when viewed holistically, the study suggests that the likelihood of increasing the height of lettuce plants is realised where the direct application of organic fertilizers including spent mushroom substrate (SMS) is deployed strictly or in combination with inorganic fertilizers (NPK). Somya et al. (2024) found that increasing NPK levels positively impacted linseed growth parameters and yield attributes. The highest NPK application (60-60-50 kg ha-1) resulted in the most favourable outcomes regarding plant height, dry matter, and number of branches. Muchena et al. (2021) found that spent mushroom substrate-treated soil yielded higher yield and quality of baby spinach than untreated soil. This suggests that SMS had a beneficial impact on vegetable growth. A study by Eremrena and Iregbundah (2024) showed that the application of spent mushroom substrate significantly increased plant height, number of leaves, leaf length, leaf width and stem girth of maize. Shi et al. (2023) found that adding spent oyster mushroom substrates increased alfalfa yield.



**Figure 2.** **Impact of NPK and SMS on lettuce's (A) root length, (B) fresh weight, and (C) dry weight**.

The data is displayed as mean ± SE. According to Duncan's multiple range test, different letters denote a significant difference (p < 0.05). CK stands for control treatment in the absence of a soil amendment.

**3.2 Effect of SMS and NPK application on oxidants in roots of lettuce**

In response to SMS and NPK fertilizers application, lipid peroxidation (MDA) and hydrogen peroxide (H2O2) content in the lettuce roots significantly decreased. Spent mushroom substrate application reduced MDA and H2O2 content by 28.9% and 29.9%, respectively, compared to control (Figure 3). Again, applying NPK lowered MDA and H2O2 content by 11.1% and 12.8%, respectively, compared to control. However, the combined application of SMS and NPK (SMS + NPK) further declined MDA and H2O2 content by 39.6% and 42.6%, respectively, as compared to the control treatment (Figure 3A. B). The results show that MDA and H2O2 levels of lettuce root were higher in the control treatment than the soil amendments. Oxidants, also known as reactive oxygen species (ROS), include non-radical compounds like H2O2, free radicals like O₂⁻, and ROS (Ozougwu, 2016). These chemicals are produced as byproducts of metabolic processes including respiration and photosynthesis (Martemucci et al., 2022).



**Figure 3.** **Impact of NPK and SMS on lettuce roots' (A) MDA and (B) H2O2 content.**

The data is displayed as mean ± SE. According to Duncan's multiple range test, different letters denote a significant difference (p < 0.05). CK stands for control treatment in the absence of a soil amendment.

**3.3 Effect of SMS and NPK application on antioxidants in roots**

The application of SMS and NPK fertilizers improved the antioxidant activity of lettuce plants under stress and inhibited reactive oxygen species (ROS) by increasing enzymes like SOD, CAT, and POD (Figure 4). In comparison to the control, the activities of SOD, CAT, and POD rose by 31.4%, 18.3%, and 29.3%, respectively, under NPK fertilizer treatment. Again, SMS treatment enhanced the activities of SOD, CAT, and POD by 44.4%, 31.6%, and 53.4%, respectively, compared to the control. However, when SMS and NPK fertilizers were applied together, SOD, CAT, and POD increased by 58.6%, 44.8%, and 82.7%, respectively, in comparison to the control. This implies that applying SMS and NPK fertilizers together gives the strongest protection, as evidenced by the greatest levels of enzyme activity.

In the present research, the fertilization treatment raised the activities of CAT, POD, and SOD in comparison to the control. The application of SMS or NPK alone reduced MDA and H2O2 levels in lettuce leaves due to promoting antioxidant enzyme activity. However, the combined application of SMS and NPK fertilizers further reduced MDA and H2O2 levels in lettuce leaves by increasing SOD, POD, and CAT activities. Plant defense mechanisms against biotic and abiotic stressors rely heavily on antioxidant enzymes. According to Mittler (2017), increased antioxidant enzyme activity results in potential and targeted ROS scavenging under various stressors. Fertilizing Chinese cabbage with organic fertilizer resulted in a considerable increase in SOD, POD, and CAT activities as well as a decrease in MDA and H2O2 buildup (Qiu et al., 2013). Additionally, Alzain et al. (2023) found that the activities of CAT, POD, and SOD were increased by the application of organic fertilizer. In a similar study, Shahkolaie et al. (2020) discovered that adding both organic and inorganic fertilizers to the soil considerably raised the activities of SOD and CAT in maize. Three types of protective enzymes with anti-oxidative properties are found in plants: SOD, POD, and CAT (Rajput et al., 2021). The superoxide anion may be quickly catalyzed into H2O2 and O2 by SOD, a crucial enzyme in the oxygen-scavenging mechanism (Batool et al., 2022). The two main enzymes that eliminate peroxide are POD and CAT (Sharma et al., 2019).



**Figure 4.** **Impact of SMS and NPK on lettuce root (A) SOD and (B) CAT (C) activity**.

The data are shown as mean ± SE. According to Duncan's multiple range test, different letters denote a significant difference (p < 0.05). CK stands for control treatment in the absence of a soil amendment.

Inorganic fertilization through urea, NPK, and superphosphate chemicals has ruled modern agriculture because they provide instant nutrient quantities at high concentrations. Developments in fertilizer prices coupled with environmental pollution worries and soil deterioration problems have prompted researchers to search for economical organic methods. According to Gao et al. (2021), soil amendment with SMS produced results equivalent to NPK fertilization while cutting down operational costs since it needed fewer inputs. The slow nutrient discharge mechanism of SMS fertilizer reduces farmers' frequency of fertilizer applications thus cutting down both labour requirements and fertilizer purchasing costs. Zhang et al. (2020) discovered that while agricultural output remained comparable, SMS use resulted in a 30% decrease in reliance on synthetic fertilizers. According to Ma et al. (2023), applying organic fertilizers improved the soil's nutrient content and biological indicators more than using chemical fertilizers alone. Applying organic fertilizers also had a greater impact on the soil's overall enzyme activity than using chemical fertilizers alone.

# 4. CONCLUSION

From the experiment, it was shown that mean values of lettuce plant growth and physiological attributes were enhanced by using spent mushroom substrate (SMS) and NPK fertilizers either alone or in combination. When combined with the SMS and the NPK, the results were even better, proving that the use of organic and inorganic fertilizers supplement each other well. In addition, the present investigation also showed that there was an increase in oxidative stress markers and antioxidant enzyme activity due to the application of SMS and NPK fertilizers in lettuce. In detail, the combined application caused a synergistic effect in down-regulating MDA and H2O2, which can be attributed to the lowered oxidative stress. This implies that these fertilizers not only encourage growth but also the immunological system of the plant against, biotic and abiotic stressors.

**COMPETING INTERESTS**

The authors have declared that no competing interests exist

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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