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SEEDLING EMERGENCE AND GROWTH RESPONSE OF SPINACH TO SOIL AMENDED WITH DIFFERENT SPENT OYSTER MUSHROOM SUBSTRATES

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ABSTRACT

Soil health is one of the most important factors that influence plant productivity. Incorporation of soil into plant residues that are high in organic matter has been reported to improve soil's physical and chemical properties which enhance plant growth and development. Therefore, the objective of this study was to investigate the effect of soils amended with different spent oyster mushroom substrates on seedling emergence and growth of spinach (Spinacia oleracea). The respective soil samples were amended with different Spent Mushroom Substrates (SMS) at ratio 60:40 (soil/substrate). Treatments were: T1= *U. panicoides* + soil, T2= *Z. mays* + soil, T3= *D. stramonium* + soil, T4= Substrate mix [60% soil: *U. panicoides* (13.33%), *Z. mays* (13.33%) and *D. stramonium* (13.33%)] and T5= un-amended soil (100%). The respective treatments were filled into 25cm diameter pots and arranged in a Complete Randomized Design (CRD) in a temperature-controlled glasshouse. In each respective pot, three S. oleracea seeds were planted at a depth of 2cm and watered 3 times a week. S. oleracea growth response was determined by assessing the following variables: emergence rate, plant height, number of leaves/plant and chlorophyll content over a period of 12 weeks. Total dry biomass was assessed at harvest by oven drying the plants at 60ºC for 72 hrs. On the first assessment, seedlings raised in *Z. mays* SMS had significantly higher emergence percentage than seedlings raised in D. stramonium, Substrate mix, *U. panicoides* SMS ($p<0.05$). Seedlings raised in *Z. mays* SMS attained 100% emergence 7 days after planting (DAP), and un-amended soils had 91.75% 9 DAP. Seedlings raised in *Z. mays* and *D. stramonium* SMS had significantly higher plant height than seedlings raised in *U. panicoides* SMS and un-amended soils ($p<0.05$). Seedlings raised in *Z. mays* SMS attained 26.27cm height compared to *U. panicoides* SMS and un-amended soils which attained 12.67cm and 14cm height, respectively. Findings of this study revealed that *Z. mays* and *D. stramonium* SMS amended soils have inherent properties that positively influenced the seedling emergence, establishment, and growth of S. oleracea. This would, therefore, have influence on the leaves which are the main agronomic yield of the crop.

Key words: Spent mushroom substrates (SMS), Oyster mushroom, *Spinacia oleracea*, *Urochloa panicoides*, *Datura stramonium, Zea mays,* Seedling emergence, Germination

INTRODUCTION

The global increase in the use of inorganic chemical fertilizers has negatively affected soil structure, decreased fertility and contributed to air and water pollution. These fertilizers have been reported to contribute to the release of greenhouse gases into the atmosphere [1]. Agrochemicals are, therefore, of great concern to human health and environment [2]. Unlike inorganic chemical fertilizers, organic fertilizers are made from organic sources which make them environmentally friendly [3], provide plant nutritional requirements, suppress plant pest populations and increase yield and quality of crop in ways comparable to inorganic chemical fertilizers [4]. Extensive research has, therefore, been directed towards identifying various natural waste materials including among many others, use of spent mushroom substrates (SMS). Spent mushroom substrate refers to the residual substrate that remains after several cycles of mushroom fruiting bodies have been harvested [5]. Utilization of SMS as an organic manure and soil compost in horticulture has received considerable attention by several researchers [6,7,8]. The results have, however, been variable, thus indicating a need for further research [9,10]. This study sought to analyze the effect of different Pleurotus ostreatus SMS's on the seedling emergence, establishment and growth of Spinacia oleracea.

MATERIALS AND METHODS

Description of experimental site

The study was conducted at Molelwane, North-West University Research farm located 6.44km away from the Mafikeng University campus at a geographical location of 25' 47' 54'' south, 25' 32' 52'' east with temperatures ranging between 28-32 °C.

Description of laboratory analysis

Soil preparation and sterilization

Ninety kilograms (90 kg) of Hutton soil was collected from the farm's arable land and autoclaved for 30 minutes at 121°C under 15 psi (pounds per square inch). Spent Mushroom Substrates (SMS) were not autoclaved to maintain the bio-active chemical compounds which are reported to be produced during the growth of P. ostreatus. After autoclaving, a proportion of 60% soil: 40% of respective substrate ratio (v/v) were measured and mixed prior to filling forty (40) 25 cm diameter pots. The control (un-amended) was filled with 100% soil.

Soil analysis

Fifty (50)g was sub-sampled from the 90kg autoclaved soil and analyzed for macro and micronutrients. For Nitrogen, Phosphorus and Potassium analysis, a procedure described by Houba *et al.* [11] was followed. Micronutrients were analyzed using ICP- Mass spectrometry [12]. Soil organic matter was analyzed using the Warncke method [13].

Analysis of macro and micronutrients in substrates

Sub-samples of SMSs: U. panicoides, *Z. mays* and *D. stramonium* were analyzed for both macro and micronutrients. One (1) gram of substrate was placed into four (4) crucibles and subjected to dry ashing in a muffle furnace at 600°C for 8 hours. After ashing, the crucibles were cooled to room temperature and respective ash samples were dissolved in 8mL dilute nitric acid (HNO_3) and 2mL of hydrochloric acid (HCl) and microwaved for 45 min. Macro and micronutrients were measured using NexION 300Q ICP-Mass spectrometer. In addition, organic matter was measured using the Warncke method [13].

pH analysis

For all three respective SMS and un-amended soil, 20g of sub-sample was weighed and transferred into four 100ml beakers. A volume of 40ml distilled water was added, and the solutions were stirred and allowed to stand for 30 min. Thereafter, the solutions' pH values were read using a pH meter.

Experimental design and data collection

The three SMS types, that is, U. panicoides, *Z. mays* and *D. stramonium* harvested from the oyster North-West University WU mushrooms unit were used as experimental treatments. The Hutton soil was amended with respective SMS at a ratio of 60:40 (soil/substrate (v/v)). The treatments were as follows: T1= *U. panicoides* + soil; T2= *Z. mays* + soil; T3= *D. stramonium* + soil; T4= Substrate mix [60% soil: *U. panicoides* (13.33%), *Z. mays* (13.33%) and *D. stramonium* (13.33%)] and T5= un-amended soil (100%). These treatments were replicated eight times, making a total of 40 experimental units. The five (5) treatments, that is, the soil samples amended with respective SMS plus 100% soil were filled into forty 25cm diameter pots and arranged in a complete randomized design (CRD) in a temperature-controlled glasshouse. In each pot, three seeds of S. oleracea were directly planted at a depth of 2cm. The planted pots were irrigated at two-day interval with each pot receiving 500ml. Hand weeding was carried out whenever weeds emerged.

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Seedling emergence measurements were taken at 2-day intervals for 9 consecutive days, and emergence rate was calculated using the following formula:

$$\text{Emergence percentage} = \frac{\text{Number of emerged seedlings}}{\text{Number of planted seeds}} \times 100\%$$

Growth was assessed by measuring plant height, leaf number and chlorophyll content (CCI) at 6, 8, 10 and 12 weeks after planting (WAP). A measuring tape was used to measure the height of the plant, number of leaves was counted per plant and CCM-200 plus chlorophyll meter (Apogee instrument) was used to assess chlorophyll content index on a single leaf which was tagged with a red string rope on the petiole per treatment. Leaf chlorophyll content of plants grown in different growth media was tested to determine their respective photosynthetic potential. Fresh biomass of the shoots and roots was determined at harvest using Symmetry PR Precision Scale (Cole-Parmer, United States) and dry biomass was obtained after 72 hrs of oven drying at 60ºC.

Statistical analysis

The measured variables were subjected to one-way analysis of variance (ANOVA) using the GLM (General Linear Model) procedure of SAS software package, version 9.4 (SAS Institute, Inc. Cary. NC. USA, 2001-2011).

RESULTS AND DISCUSSION

Seedling emergence

Soils amended with *Z. mays* SMS resulted in significantly higher seedling emergence percentage than seedlings raised in soil amended with D. stramonium, Substrate mix, *U. panicoides* SMS ($p < 0.05$). This difference was, however, only noted for period 7 DAP and thereafter there was no significant difference in emergence percentage for all treatments. Seedlings raised in soils amended with *Z. mays* SMS reached 100% emergence at 7 days after planting (DAP). Seedlings raised in the other SMSs only reached a 100% emergence mark at 9 DAP with control reaching 91 % emergence (Fig.1).

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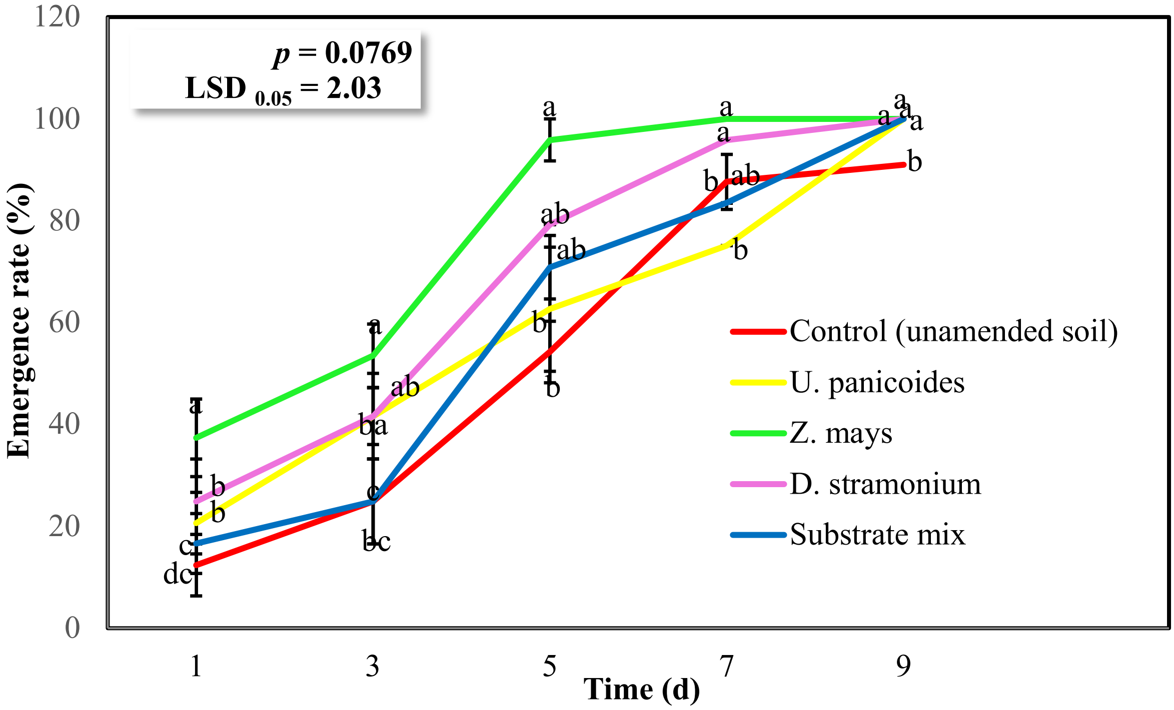

Figure 1: Effect of different SMS on the emergence percentage of *S. oleracea* 9 DAP. Superscript

***Standard Error (SE) Bars with different superscript letters (a-d) within each column denote significant ($p < 0.05$) differences between groups

The rapid increase on emergence rate in soils amended with *Z. mays* SMS could probably be attributed to improved soil structure and moisture retention. Good soil structure of *Z. mays* amended SMS, which is attained by high organic content (68.60%), could have enhanced soil moisture retention [14]. Adequate moisture in the soil supposedly triggered enzymatic processes in the seed cotyledon. These enzymatic activities promote production of plant growth gibberellin acid and cytokines that stimulate embryonic cell mitotic cell division and thereby triggering the germination. Variability of the soil moisture content and availability in the various SMS could have influenced the differing seedling emergence rate in the respective growth media. Seedling emergence has also been reported to be influenced by several soil factors, amongst these is soil structure [15]. Soil structure influences seedling emergence and growth rather indirectly. The pores are the controlling factor influencing water retention. Furthermore, soil structure influences soil, air, and temperature which in turn affect all factors cumulatively influencing seedling germination processes [16,17]. Garcia-Orenes *et al.* [14] highlighted that soils mixed with maize straw substrate not only improved soil organic carbon content but improved the seedling emergence of spinach. Alami *et al.* [15] and Zhang *et al.* [16] also reported similar findings. In this study, high organic matter content was recorded in *Z. mays* SMS (68.60%). The high organic

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matter content noted in the SMS (Table 1) could have probably enhanced the rate of emergence in seedlings planted in the substrate by improving the soil bulk density, porosity and particle size which promote aeration and water retention that enhance seed germination and subsequent seedling emergence. In addition to the influence on seedling emergence, the study highlighted that SMS also had an influence on the subsequent growth of seedlings.

Seedling height

Seedlings raised in Z. mays-SMS and D. stramonium-SMS amended soils had a statistically significant higher growth rate than seedlings in SMS mix, U. panicoides-SMS amended soils and the 100% soil ($p < 0.05$) [Table 2]. Seedlings raised in *Z. mays* SMS attained 26.27cm height, and seedlings raised in *D. stramonium* SMS attained average height of 25.08cm 12 weeks after planting (WAP). The comparatively higher growth in seedlings grown in the two SMSs was probably because of the availability of certain macro and micronutrients in the SMSs, which may have promoted the early rapid growth. Table 1 indicates that *Z. mays* SMS has the highest macro and micro-nutrients levels: [N (57.24 mg/100g), P (67.05 mg/100g), K (41.07 mg/100g) and Mg (27.89 mg/100g)], followed by *D. stramonium* SMS [Nitrogen (45.66 mg/100g), P (63.05 mg/100g), Potassium (31.29 mg/100g) and Magnesium (18.69 mg/100g)]. The lowest nutrient levels recorded was in the control [N (24.96 mg/100g), P (37.16 mg/100g), K (24 mg/100g) and Mg (0.98 mg/100g)]. Nutrients such as N, P and K play a significant role in plant growth and development. Nitrogen plays a vital role in the biochemical and physiological functions of the plant, that is, increasing photosynthetic processes, leaf production, leaf area, root development as well as net assimilation rate [17]. This is evident in this study where *Z. mays* and *D. stramonium* SMSs which had the highest N levels had a correspondingly high level of chlorophyll content index and leaf production per seedling. Seedlings raised in Z. mays-SMS amended soils had CCI reading of 14.54 µg/cm^2 and leaf count of 11 leaves/plant after 12 weeks of growth. Similarly, seedlings raised in *D. stramonium* amended soils had a correspondingly high leaf CCI attained 13 µg/cm^2 and leaf count was 9 leaves/plant (Table 2). The high N level in *Z. mays* SMS was probably because maize is often fertilized with N and P therefore its SMS may be rich with N and P which are the common nutrients that are added to soils in maize production systems. The residual content of these nutrients in respective SMSs consequently promoted chlorophyll synthesis and plant growth.

Importance of N in plant growth is highlighted by various researchers [17,18] while significance of P has also been established by several researchers [19]. Phosphorus plays a major role in energy availability through photosynthesis and

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respiration. It is required in large quantities by young cells, such as shoots and roots where the metabolism is high and cell division is rapid [20]. In this study, high P levels was noted in *Z. mays* and *D. stramonium* SMS. The high P levels in the SMS may have promoted the growth and development of shoots and roots in seedlings raised in these respective substrates. As shown in Table 1, *U. panicoides* SMS had low levels of P, a macronutrient which is known to be an important element that limits shoots and roots growth and development particularly in the seedling stage. In addition to N and P nutrients, K is another macro element that is important for plant growth. Potassium (K) is associated with the movement of water, nutrients and carbohydrates in plant tissues. If K is not supplied in adequate amount, plants become stunted [21]. Potassium was particularly low in *U. panicoides* SMS amended soils, that is 36.93 mg/100g and in un-amended soil 24 mg/100g [Table 1]. As shown in Table 2, the lowest plant heights measured was 14.71cm in seedlings raised in *U. panicoides* SMS amended soils, and 16.53 cm in un-amended soils. The poor growth was probably associated with inadequate supply of the macro nutrients N, P and K by the growth media.

Number of leaves and Chlorophyll Content Index

Furthermore, results also highlighted that seedling raised in soils amended with *U. panicoides* SMS and control had the lowest leaf count and CCI readings. Leaf count was between 4 and 7 leaves/plant and CCI readings ranged between 6.2 and 12.4 μg/cm^2 (Fig. 2).

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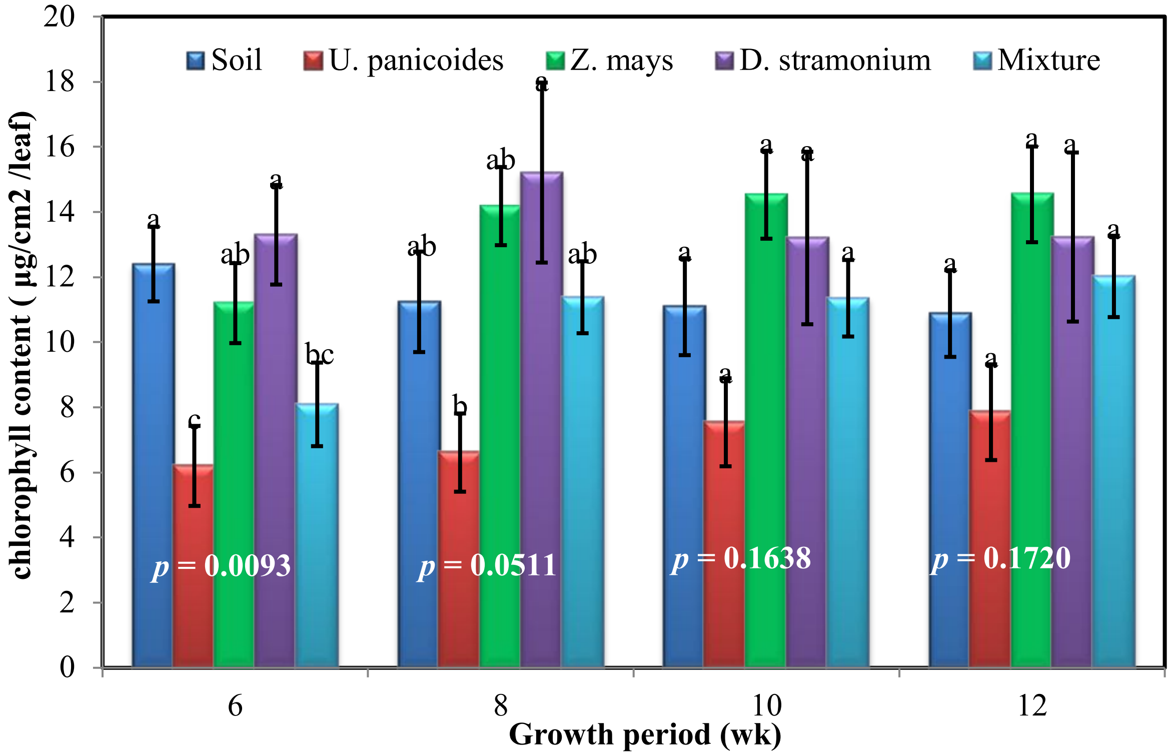

Figure 2: Effect of different SMS on the total chlorophyll content of *S. oleracea* 12 WAP

***Standard Error (SE) Bars with different superscript letters (a-d) within each column denote significant ($p < 0.05$) differences between groups

The poor leaf production and CCI measurements noted in seedlings raised in these growth media was probably because of low levels of N (24.96 mg/100g), Mg (0.98 mg/100g) and other trace elements such as Cu (0.07 mg/100g), Fe (1.76 mg/100g) and Zn (0.18 mg/100g) which play a major role in chlorophyll production (Table 1). Early yellowing of leaves was observed in seedlings raised in 100% soil, which also indicated low levels of both macro and micronutrients (Table 1).

Apart from macronutrients, plant growth is also influenced by the uptake and supply of micronutrients. Micronutrient analysis results of the various growth media indicated that they were variable. Micronutrients such as Mg, Cu, Fe, Mn, and Zn play a vital role in plant nutrition. These nutrients are, however, required in small amounts. Lawlor *et al.* [22] reported that the small quantities of such micro elements are fundamental for growth and development by acting as constituents of cell walls and membranes, enzyme activators and photosynthesis. The good growth rate of seedlings grown in Z. mays, D. stramonium, and substrate mix amended soils could also have been the result of available micronutrients which were noted to be relatively higher in these SMSs. These micro-nutrients are known

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to be important for various plant physiological processes. For instance, Mg is known to play a major role in plant photosynthesis. In the absence of Mg, chlorophyll cannot capture sun energy needed for photosynthesis which in turn affects the green colour on the leaves [18]. Copper is known to activate certain enzymes in plants which are involved in lignin synthesis and is key to the formation of chlorophyll [23]. Iron (Fe) is involved in the synthesis of chlorophyll and is essential for the maintenance of chloroplast structure and function [20]. Zinc (Zn) participates in chlorophyll synthesis and protects chlorophyll from decomposition, and it also influences nitrogen assimilation [24]. Soil analysis results also indicated that 100% soil (control) had the lowest trace element levels, whilst SMSs such as *Z. mays* and *D. stramonium* had relatively high levels of trace elements. The high level of these trace nutrients in the SMSs may have contributed to the increase in CCI levels observed in seedlings raised in these respective SMSs. As availability and uptake of both macro and micronutrient by plants is known to be influenced by soil pH, this study also analysed pH for various growth media.

The results indicated that *Z. mays* (7.8), *D. stramonium* (8.7) and substrate mix (7.9) SMSs had slightly high pH levels (Table 1). Though these SMSs were noted to have relatively high pH levels, seedling growth in these growth media were, however, not affected. This was probably because some major nutrients which influence plant growth and development require slightly acidic to slightly alkaline pH levels to be available for uptake. Neumann and Römheld [25] highlighted that soil pH is one major factor influencing the availability of soil nutrients. McCauley *et al.* [26] highlighted that nutrients such as N, K, P, Ca, Mg and S are more available within a pH range of 6.5 to 8, whereas most of micronutrients (B, Mn, Zn, Fe, Cu and Zn) are available within a pH range of 5 to 7. This was probably the case with seedlings raised in the three respective substrates. Results further indicated that 100% soil was acidic with a pH level of 4.10. The low pH noted might have negatively influenced the availability of certain macro- and micronutrients and may thus have contributed to the reduction in seedling height, leaf production and rooting system in seedlings raised in un-amended soils. Thus, stunted growth with poor foliage and rooting system was noted in seedlings planted in un-amended soil. Lawlor *et al.* [22] reported similar findings where plants grown in soils with low pH had poor plant growth. It was further indicated that at low pH levels, nutrients such as Al, Fe, Zn and Mg become toxic to plants and are likely to injure the developing seedling roots. In cases where pH is low, liming soil with $CaCO_3$, CaO or $Ca(OH)_2$ has been recommended [26].

Importance of influence of both macro and micronutrients on seedling growth determined by shoot height and leaf number per seedling has been established in this study. In addition, these results further indicated that these nutrients have also influenced seedling biomass accumulation.

Shoots and roots biomass

Seedlings raised in *Z. mays* amended soils attained shoot dry weight of 14.94 g/plant and root dry weight of 8.51 g/plant. Similarly, seedlings raised in *D. stramonium* amended soils attained 14.56 g/plant for shoot dry weight and 4. 66 g/plant for root dry weight. Seedlings raised in *Z. mays* and *D. stramonium* amended soils attained significantly higher shoot and root dry weight than seedlings raised in *U. panicoides SMS* and un-amended soils ($p < 0.001$). Shoots harvested from *U. panicoides* amended SMS had total dry weight of 7.54 g/plant and root dry weight of 0.82 g/plant. On the other hand, seedlings harvested from the control (un-amended soils) had total shoot dry weight of 3.50 g/plant and dry root weight of 0.59 g/plant (Figs. 3 and 4).

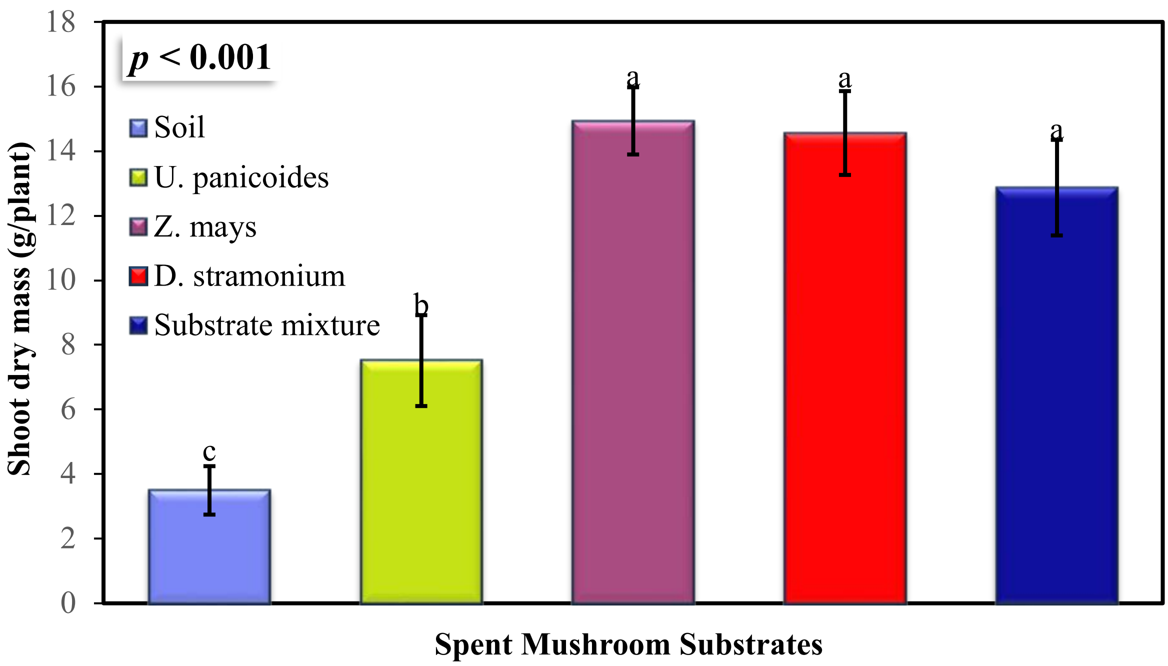

Figure 3: Effect of different SMS on shoot dry mass of *S. oleracea* at 12 WAP

***Standard Error (SE) Bars with different superscript letters (a-d) within each column denote significant ($p < 0.05$) differences between groups

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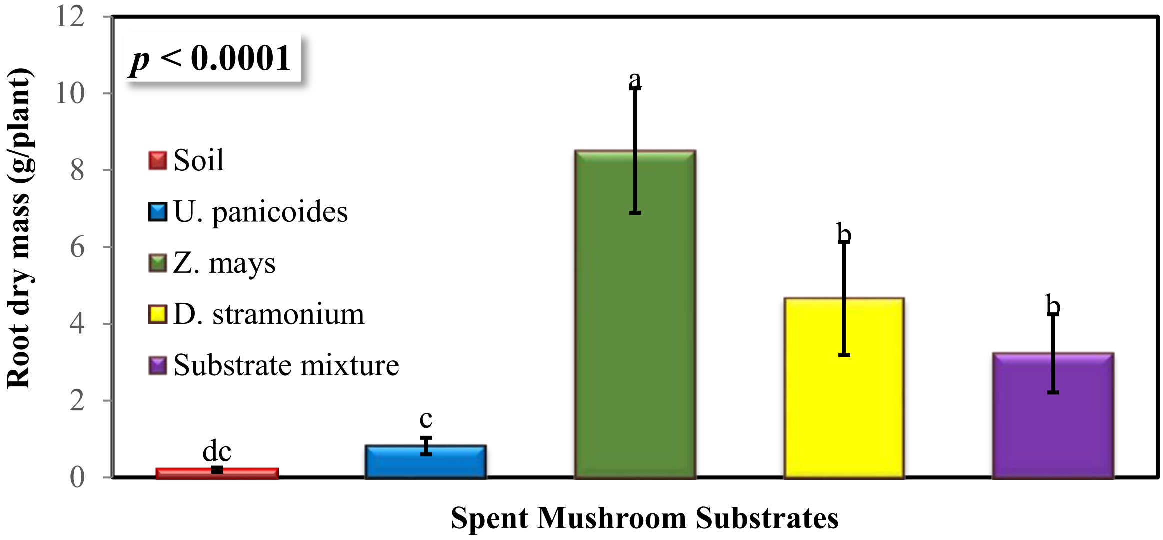

Figure 4: Effect of different SMS root dry mass of *S. oleracea* at 12 WAP
***Standard error (SE) Bars with different superscript letters (a-d) within each column denote significant ($p < 0.05$) differences between groups

The poor shoot and root dry weight from the two respective test growth media, that is, 100% soil and *U. panicoides* SMS was probably because of low level of available nutrients (Table 1).

CONCLUSION

In summary, findings of this study established that *Z. mays* and *D. stramonium* SMS have great potential for use as soil amendment in enhancement of seedling establishment and growth of spinach, and probably various other vegetables. This would be suitable particularly in organic vegetable production systems that require minimizing or eliminating the use of chemicals including inorganic fertilizers. These SMSs enhance vegetable production by improving the soil's physical and chemical properties. The improved spinach growth was noted to be associated with improved soil organic matter, fertility, and moisture. Although this study did not analyse the soil microbial populations and enzymatic content of the SMSs that would have been produced by P. ostreatus during the mycelial development and fruiting phase, P. ostreatus has been reported to produce to a wide range of enzymes that are known to be important for the biochemical processes involved in organic matter transformation and nutrient cycling in agro-production systems. This is an area that needs further investigation.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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Ethics statements

This study was submitted to North-West University Health Research Ethics Committee where it was established that it did not need ethical clearance as it did not involve human participants or animals and there was no possible negative environmental impact or risk to the researcher.

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Table 1: Macro- and micronutrients composition of different spent mushroom substrates (mean percentage ± SD). Each data point derived from 3 replicates

SMS	N mg/100g Mean ± SD	P mg/100g Mean ± SD	K mg/100g Mean ± SD	Mg mg/100g Mean ± SD	Ca mg/100g Mean ± SD	S mg/100g Mean ± SD	Mn mg/100g Mean ± SD	Cu mg/100g Mean ± SD	Fe mg/100g Mean ± SD	Zn mg/100g Mean ± SD	OM	pH
U. p	41.06 ± 7.9^{b}	44.69 ± 19^{b}	36.93 ± 0.8^{b}	27.37 ± 5.5^{a}	0.39 ± 0.2^{c}	0.22 ± 0.0^{b}	0.16 ± 0.1^{a}	0.15 ± 0.0^{a}	2.53 ± 0.5^{a}	0.35 ± 0.5^{a}	36.1^{c}	7.0
Z. m	57.24 ± 5.9^{a}	67.05 ± 17^{a}	41.07 ± 1.8^{a}	27.89 ± 8.8^{a}	0. 51 ± 0.2^{b}	0.19 ± 0.1^{c}	0.19 ± 0.2^{a}	0.18 ± 0.1^{a}	2.29 ± 0.8^{a}	0.39 ± 0.1^{a}	68.6^{a}	7.8
D. s	45.66 ± 9.1^{b}	63.31 ± 82^{a}	31.29 ± 0.8^{b}	18.69 ± 14^{b}	0.69 ± 0.5^{a}	0.16 ± 0.0^{c}	0.13 ± 0^{ba}	0.17 ± 0.1^{a}	2.06 ± 0.6^{a}	0.25 ± 0.0^{b}	51.6^{b}	8.7
S.m	50.89 ± 8.4^{a}	60.74 ± 80^{a}	32.25 ± 1.4^{b}	24.65 ± 7.6^{a}	0.57 ± 0.4^{a}	0.28 ± 0.1^{b}	0.16 ± 0.1^{a}	0.16 ± 0.0^{a}	2.42 ± 0.5^{a}	0.22 ± 0.0^{b}	56.5^{b}	7.9
S	24.96 ± 0.6^{c}	37.16 ± 13^{b}	24.0 ± 3.9^{c}	0.98 ± 17^{c}	0.24 ± 0.0^{b}	0.32 ± 0.7^{a}	0.00 ± 0.0^{c}	0.07 ± 0.1^{b}	1.76 ± 0.5^{b}	0.18 ± 0.4^{b}	1.40^{d}	4.1

Note. U. p = Urochloa panicoides, Z. m = Zea mays, D. m = Datura stramonium, S. m = Substrate mix, S = Soil

*Mean values with different superscript letters (a-d) within each column denote significant ($p < 0.05$) differences between groups

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Table 2: Effect of different spent mushroom substrates on plant height and number of leaves of *S. oleracea*

SMS	Plant height (cm)/plant				SMS	No. of leaves/plant			
	6wk	8wk	10wk	12wk		6wk	8wk	10wk	12wk
Control	12.38±0.67^{b}	13.75±0.58^{c}	14.87±2.18^{c}	16.53±2.43^{b}	**Control**	4.12±0.23^{b}	4.75±0.25^{c}	5.63±0.23^{b}	5.88±0.93^{d}
U. panicoides	10.55±1.67^{b}	11.96±1.85^{cd}	14.71±2.20^{c}	13.83±3.12^{c}	**U. panicoides**	5.00±0.78^{b}	5.56±0.87^{b}	6.50±0.94^{b}	7.37±1.08^{c}
Z. mays	18.76±1.33^{a}	24.96±1.26^{a}	24.43±1.14^{a}	26.27±1.09^{a}	**Z. mays**	7.31±0.50^{a}	8.50±0.35^{a}	9.00±0.26^{a}	11.00±0.35^{a}
D. stramonium	18.23±1.11^{a}	20.05±1.21^{a}	23.20±1.03^{a}	25.08±1.25^{a}	**D. stramonium**	6.37±0.30^{a}	8.25±0.48^{a}	8.75±0.48^{a}	9.07±0.59^{ba}
Substrate mix	15.25±0.71^{ab}	17.18±0.67^{b}	19.81±0.80^{b}	21.20±0.86^{ab}	**Substrate mix**	5.62±0.26^{b}	6.50±0.37^{b}	6.88±0.35^{b}	8.13±0.35^{b}
p =	0.0034	0.0012	0.0155	0.0146	p =	0.0014	0.0010	0.0001	0.0150

*Mean values with different superscript letters (a-d) within each column denote significant ($p < 0.05$) differences between groups

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