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Research Article

Effect of Spent Button Mushroom Substrate on Yield and Quality of Baby Spinach (*Spinacia oleracea*)

Forbes B. Muchena,[1] **Charity Pisa**,[2] **Moses Mutetwa**,[3] **Chido Govera**,[4] **and Wonder Ngezimana**[1]

[1]*Marondera University of Agricultural Sciences and Technology, Department of Horticulture, P. Bag 35, Marondera, Zimbabwe*
[2]*Marondera University of Agricultural Sciences and Technology, Department of Environmental Sciences and Technology, P. Bag 35, Marondera, Zimbabwe*
[3]*Midlands State University, Faculty of Natural Resource Management and Agriculture, Department of Agronomy and Horticulture, P. Bag 9055, Gweru, Zimbabwe*
[4]*Future of Hope Foundation, Christon Bank, Mazowe, Zimbabwe*

Correspondence should be addressed to Moses Mutetwa; mosleymtetwa@gmail.com

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Disposal of spent mushroom substrate (SMS) generated after mushroom cultivation is a subject of great concern. Unplanned disposal causes land, water, and air pollution together with the nuisance in the surroundings. During recent years, environmental legislation has forced mushroom growers to think about more amicable ways of SMS disposal. Hence, farmers in different corners of the country are using SMS as manure for various field crops and horticulture but without any support of the recommended rates from scientific data and therefore may not be getting the optimum benefits. This study sought to evaluate the effects of spent mushroom substrate on the yield and quality of baby spinach. The experiment was conducted in Mazowe, Zimbabwe, with 5 treatments ($10\,t\,ha^{-1}$, $20\,t\,ha^{-1}$, $30\,t\,ha^{-1}$ of SMS, and two controls) arranged in a CRBD and replicated 3 times. Biomass production (fresh mass, dry mass, root length, fresh root mass, and dry root mass) and sensory evaluation were determined 35 days after planting. Results showed that increasing rates of spent mushroom substrate significantly ($p < 0.05$) increased the fresh yield and quality of baby spinach. Application of spent mushroom substrate beyond $30\,t\,ha^{-1}$, however, increased the bitterness of baby spinach. The results of the study present the potential of the spent mushroom substrate as an organic fertilizer for the production of quality baby spinach.

1. Introduction

Vegetables which require marginal processing and are consumed fresh have gained importance and attention in the international vegetable market [1]. The increase in demand for baby leaf greens has resulted in many farmers gearing their production towards baby greens such as baby lettuce, baby spinach, and Swiss chard [2]. Baby spinach is recognized to have health benefits due to its distinctly high stages of bioactive compounds, antioxidants, and phytochemicals [3]. Its consumption is recognized to have wonderful outcomes on human fitness and has been linked to decreased risk of most degenerative illness associated with growing older [4], such as coronary heart ailment [5], cardiovascular disorder [6], Alzheimer's disorder [7], cataracts [8], and several forms of most cancers [9].

Production of baby spinach is still limited with low yields being characterized by poor agronomic practices including soil nutrient management options. Low yields of baby spinach and the problem of bitter taste caused by costly inorganic fertilizers thus require the intervention of using organic fertilizers such as spent mushroom substrate (SMS) which itself also has challenges with disposal. However, SMSs can be derived from many different mushroom substrates [10–15] with the potential for reuse in either mushroom production or in farming land as organic fertilizers. It is therefore critical to investigate and determine application rates that are beneficial and maximize yields while minimizing chemical fertilization.

Spent mushroom substrate (SMS) is defined as leftover of biomass generated by commercial mushroom industries after harvesting period of mushroom [16]. Disposal of SMS generated after mushroom cultivation is a subject of great concern. An estimated 10–50 million metric tonnes of spent mushroom compost are expected to be generated annually worldwide [17–22]. The bulkiness of SMS makes disposal more laborious. Unregulated disposal of SMS causes land, water, and air pollution together with the nuisance in the surroundings [23]. Spent substrate from *Agaricus bisporus* has been evaluated for treatment of acid mine drainage [24] and textile dyes [25]. The spent substrate of various *Pleurotus* species has been investigated for the removal of nickel-contaminated water [26] and copper [27] and antibiotics in waste water from swine [28], pesticides in effluents from fruit packing industry [29], and textile dyes [30]. Some mushroom species have been investigated for fluoride removal from drinking water [31], methylene blue from waste industrial waters [32, 33], and sulfa antibiotics [34].

Pesticide removal or degradation has been explored using *Agaricus bisporus* spent substrate [35–39], *Pleurotus* spent substrate [36, 38, 40], and the spent substrate of *Lentinula edodes* [36, 39]. Spent substrate from other species has been explored to degrade pesticides, for example, *Agaricus blazei* (*A. brasiliensis* and *A. subrufrescens*) [41].

One of the natural destinations for the large volumes of SMS is on land for agricultural crops [40] with much advantage over chemical fertilizers since it releases nutrients slowly that will not burn crops. After suitable pretreatment, spent mushroom compost can completely or partially substitute the growing media for the cultivation of different economically important horticultural crops [42, 43]. Incorporation of composted SMS also neutralizes the acidity of soils and facilitates cultivation even in problem soils [44]. SMS can be a good source for plant nutrients since it is generally rich in nutrient content, slow mineralization, and high cation exchange capacity. Characteristically, however, they differ aided to the differences in the initial components. The quantity of mineral elements constitutes for 66–78%. SMC is a good source of general nutrients such as N (1.3–4.2), P (0.1–0.4%), K (0.5–1.8%), Na (0.05–0.2%), Mg (0.2–0.4%), and several trace elements such as Fe, Cu, Zn, Mn, Mo, and B (values in ppm: 1000–2500; 4–12; 50–200; 100–300; 1–2; 6–15) and soluble salts and no pests or weed seeds [42]. Studies have been done to explore the use of spent substrate of *Agaricus bisporus* for the production of various vegetable crops [45–47]. *Pleurotus* spp. spent substrate has also been evaluated in interplantings with cabbage and eggplant [48], for cultivation of lettuce [1, 45], and *Flammulina velutipes* spent substrate has been evaluated for production of honey dew melon seedlings [49]. On the other hand, the SMC as an addition to the casing or as a casing soil in the production of mushrooms is much more often described [50, 51]. Spent mushroom substrate also possesses appropriate biomanipulate endeavor towards foliar and soilborne diseases [52].

The main reason for the use of SMSs as a fertilizer is because they are rich in calcium, nitrogen, ash, and protein [53, 54]. Thus, the reuse of SMSs for soil conditioning has turn out to be the focus of consideration as it is also abundant in nitrogen. Besides, it is rich in vital nutrients for plant growth and organic materials that make it a suitable material for soil mulching and improvement. SMS is rich in phosphorus and is always applied to agriculture land to enhance soil organic matter and nutrient contents [55]. In regards to the concern of disposal of SMS, this study seeks to evaluate the potential of its reuse in vegetable production. However, there is no readily available published work that has been done using SMS in baby spinach production hence the basis upon which this investigation is done. The study evaluates the influence of SMS on yield and quality of baby spinach. The appropriate levels of application rates of SMS will also be determined in this study.

2. Materials and Methods

2.1. Experimental Site. The trial was conducted at The Future of Hope Foundation, located outside Harare, Christon Bank (Mazowe district), altitude range: 1200–1400 m.a.s.l. Climate is classified as warm with an average temperature of 18°C per year. The area receives rainfall during the summer season which lasts 4 months from mid-November to mid-March. Precipitation averages 900 mm per year. Soil samples were collected at the experimental site and analyzed for pH, conductivity, and resistance as well as macro- and micronutrients (Table 1) at laboratories of the Faculty of Agricultural Sciences and Technology of Marondera University of Agricultural Sciences and Technology using standard procedures. The mineral composition of the SMS was as follows: 4.85 (Ca), 0.8% (K), 0.36% (Mg), 6 290 ppm (Fe), 233 ppm (Mn), and 75 ppm (Zn).

2.2. Experimental Design and Treatment Details. Baby spinach response to SMS was determined on-farm in a trial set-up in a completely randomized block design with 5 treatments (positive control (chemical fertilizer 7-14-7) 45 kg ha^{-1}, negative control 0 t ha^{-1}, spent mushroom substrate 10 t ha^{-1}, spent mushroom substrate 20 t ha^{-1}, and spent mushroom substrate 30 t ha^{-1}) replicated three times. The treatments were based on the fact that farmers are encouraged to apply organic amendments at the rate of 10–20 t ha^{-1}. Communal farmers apply organic matter depending on availability rather than on the recommendations [56]. The spent mushroom substrate was broadcast and incorporated in the soil using hoes. The treatments were assigned randomly to the subplots using random number tables. Baby spinach seedlings (cv. Ohio) were raised in trays and then transplanted 2 weeks after emergence when they had 4 leaves to plots that measured 2.2 × 2.2 m. The spinach was planted at an inter-row spacing of 20 cm, and intrarow spacing of 10 cm to make up 3 rows with 10 plants in each

Table 1: Site soil characterisation at The Future of Hope Foundation research site.

Color	Texture	pH (calcium chloride)	Free carbonate	Conductivity micromhos	Mineral nitrogen parts/ ammonia + nitrate, N	Available phosphorus resin extract (p/m)	Exchangeable cation mg equivalents (100 *g*)		
							P	C	Mg
Brown	Loam	6.8	Moderate	209	37	220	1.98	17.9	2.59

row. After planting, irrigation was done 3 times per week skipping one day based on the soil moisture condition.

2.3. Data Collection. Data were collected from the (middle row) at 35 days after planting. Parameters recorded included fresh and dry above-ground biomass production, fresh and dry root biomass, and root length. At harvest, the roots were dismembered and washed to remove soil particles and then length, fresh biomass, and thereafter dry matter were determined. The samples were oven-dried at 65°C for 72 hours and then weighed to determine the dry biomass (dry matter).

A sensory evaluation test was conducted on the fresh leaves of the spinach. Fresh leaves were chopped and boiled in 500 ml of water for 5 minutes. Samples were served in small bowls, prelabeled using letters, and arranged randomly on a table. A total of 15 female panelists between the ages of 20–30 were trained and asked to score the sample patterning to the taste of the leaves. Women are predisposed to have a better sense of taste and smell than men [57].

The panelists were allocated 15 minutes to evaluate the samples. The panelists rinsed their palate using water after testing each sample and also were to take a mouth wash-down with clear water. Score sheets were used to taste the vegetables. The scoring range was 0–10 with 0 being not bitter and 10 extremely bitter.

2.4. Statistical Analysis. The data were transformed for normality and thereafter analyzed using a one-way analysis of variance (ANOVA) at $\alpha = 0.05$ significance level using the Minitab Statistical Package version 2017. Mean separation was done using Fisher's least significant difference (LSD) test at $\alpha = 0.05$. Trends were shown using graphs drawn in Microsoft Excel.

3. Results

3.1. Above-Ground Fresh and Dry Mass. The fresh and dry weight of baby spinach was recorded after harvest. Fresh mass significantly ($p < 0.05$) increased with increasing rates of SMS (Figure 1). The positive control with the NPK amendment recorded the highest in fresh mass (9.01 g/plant) yield followed by 30 t ha^{-1} which had 8.29 g/plant. The lowest fresh mass was obtained from the negative control treatment with 1.15 g/plant. The average fresh mass recorded was 5.27 g/plant.

Plants that were grown in the positive control (NPK) treatment recorded the highest dry mass (6.11 g/plant), followed by 30 t ha^{-1} which had 4.97 g/plant. The negative control recorded the lowest dry mass (0.24 g/plant), followed by the 10 t ha^{-1} treatment which recorded a dry mass of 2.08 g/plant. The average dry mass recorded was 3.46 g/plant.

3.2. Root Length. Data pertaining to the effect of spent mushroom substrate on root length are shown in Figure 2. The data recorded show that the spent mushroom substrate exerted a statistically significant ($p < 0.05$) influence on the root length of baby spinach. Root length decreased with the increasing SMS application rate. The longest root length (8.04 cm) among the SMS treatments was recorded in the 10 t ha^{-1} treatment while the 30 t ha^{-1} treatment recorded the shortest length (6.42 cm). Negative control had the longest root length of 8.12 cm. The average root length recorded was 7.42 cm.

3.3. Below-Ground Fresh and Dry Mass. Data regarding the below-ground fresh and dry biomass of baby spinach recorded at harvest are presented in Figure 3. Both fresh and dry mass of roots differed significantly ($p < 0.05$) between treatment means. Among all the treatments applied, baby spinach produced the highest root fresh mass (0.93 g/plant) when no amendment was applied (negative control). However, the least root fresh mass (0.36 g/plant) was recorded from treatment with 20 t ha^{-1} spent mushroom substrate. The average root fresh mass recorded was 0.59 g/plant.

Root dry mass was lowest (0.07 g/plant) for baby spinach grown in soils amended with 20 t ha^{-1} SMS and highest in the negative control. The root dry mass of baby spinach decreased with an increase in SMS applied. The average root dry mass recorded was 0.20 g/plant.

3.4. Sensory Evaluation. Figure 4 shows results of the sensory evaluation that was done to determine the taste of boiled leaves in terms of bitterness. Positive control treatment scored the highest extremely bitter taste followed by 30 t ha^{-1}. The negative control and 10 t ha^{-1} SMS treatment recorded zero scores on extreme bitter taste. The mild taste was present in all treatments and was highest in the negative control treatment and lowest in the 30 t ha^{-1}. The bitter taste score was present in all treatments and was highest in the 30 t ha^{-1} SMS treatment and lowest in the negative control treatment.

4. Discussion

The effect of SMS on plant growth and yield was evaluated in comparison to mineral fertilization in baby spinach. Several

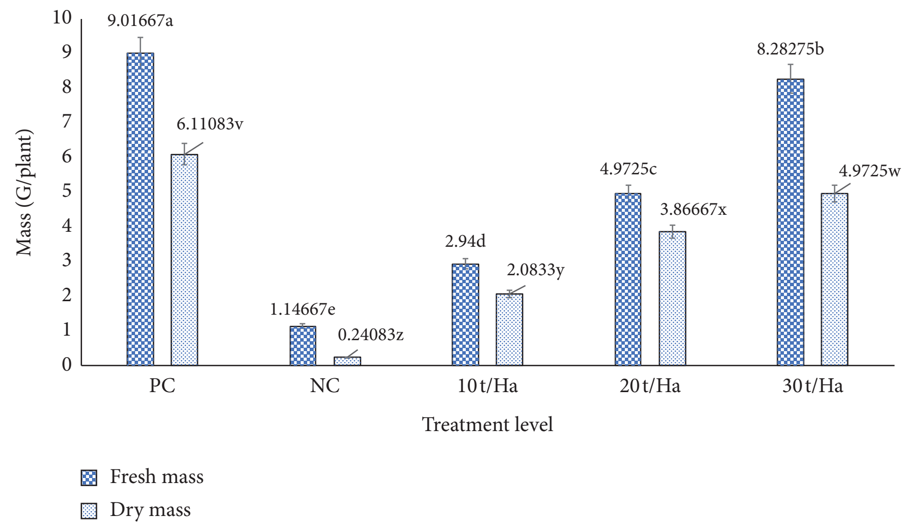


Figure 1: Above-ground fresh mass and dry mass. *Means with different letters are significantly different at $p < 0.05$. PC = positive control. NC = negative control. t/Ha = spent mushroom rate per hectare.

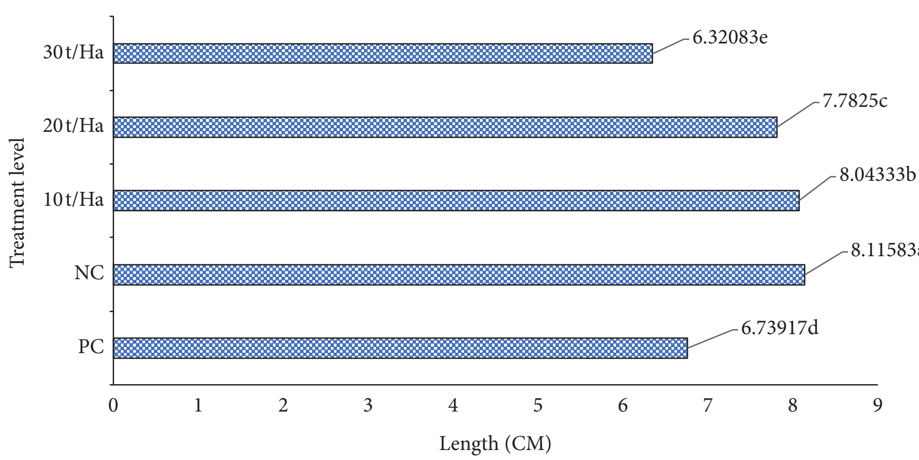


Figure 2: Root length. *Means with different letters are significantly different at $p < 0.05$. PC = positive control. NC = negative control. t/Ha = spent mushroom rate per hectare.

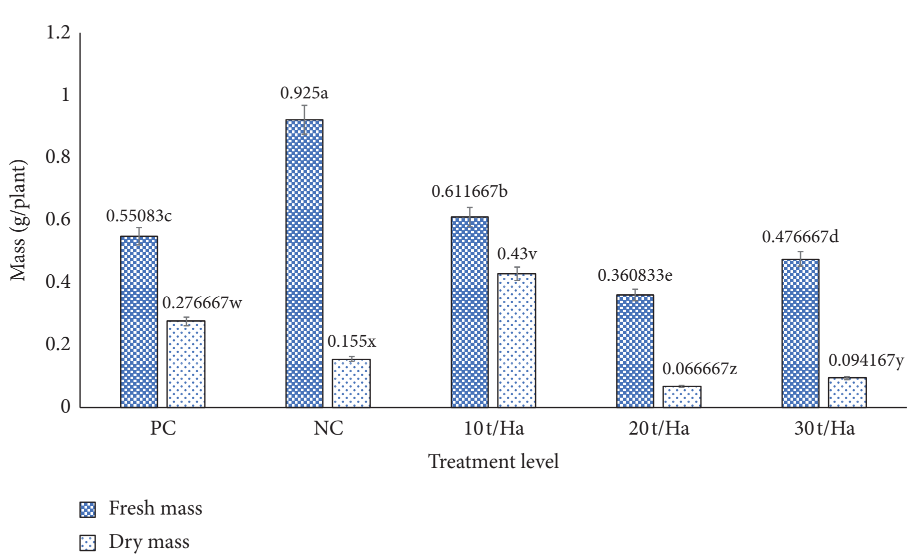
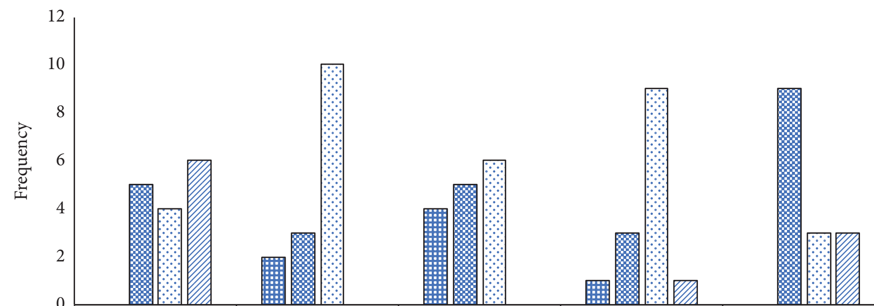


Figure 3: Below-ground fresh and dry mass. *Means with different letters are significantly different at $p < 0.05$. PC = positive control. NC = negative control. t/Ha = spent mushroom rate per hectare.



	PC	NC	10 t/Ha	20 t/Ha	30 t/Ha
Not bitter	0	2	4	1	0
Bitter	5	3	5	3	9
Mild	4	10	6	9	3
Extreme bitter	6	0	0	1	3

Figure 4: Sensory evaluation. *Total score per treatment = 15. PC = positive control. NC = negative control. t/ha = spent mushroom rate per hectare.

researches on SMS reveal the influence of SMC from oyster and button mushroom on plant growth yield [43, 58–62].

In this current study, the increase in the fresh mass of baby spinach with an increase in spent mushroom substrate applied could be attributed to improved nutrient availability and improved soil structure and release of nutrients. The average pH of the spent mushroom substrate is 6.6 (6.0 to 7.0); hence, it is ideal for most plants. The amount of carbon to nitrogen ratio is a significant indicator of nitrogen availability for plant growth, and the ideal compost should have a ratio of 30 : 1 or less. The spent mushroom substrate has an excellent 14 : 1 ratio, indicating outstanding nutrient availability. The increase in fresh weight could be ascribed to the fact that several living organisms are activated in the soil with addition of organic matter and these organisms promote the absorption of nutrients from the soil and stimulate plant growth as a result of released phytohormones in the soil. Jonathan et al. [63] reported that SMS compost of *Pleurotus pulmonarius* mixed with depleted garden soil generally enhanced all the variables of growth considered when compared with control. Similarly, [64] their findings revealed that composted spent mushroom substrate mixed with loamy soil produced greater vegetative growth and yields of both vegetables than loamy soil (controls). Studies by Ogbonna et al. [65] demonstrated that SMC could be used to improve growth and yield of maize (*Zea mays*).

Other researchers have proven that SMS can be used with useful effects in crop production. Peksen and Uzun [66] have proved that SMS is rich in organic matter and constitutes macro- and micronutrients for crops and microorganisms; hence, it increases the soil microflora and enhances soil enzymatic activities. SMS additionally carries calcium carbonate which provides a buffering effect and elevates soil pH [18, 67]. Polat et al. [68] reported that there were statistically significant differences among different levels of spent mushroom compost applications in terms of total yield in lettuce growing as two and four tons/ha spent mushroom compost applications gave the best result in terms of total and marketable yield.

Idowu and Kadiri [69] reported that SMS supplied sufficient mineral elements to plants such as okra which resulted in a higher number of leaves with more leaf area. Similar findings have been previously reported by Eudoxie and Alexander [70] in the use of spent mushroom substrate as a transplant media replacement for commercial peat in tomato production. Wiafe-Kwagyan and Odamtten [71] reported that SMS contained dry matter, crude protein, cellulose, lignin, NDF, and ADF (calcium, potassium, nitrogen, and sodium) which could influence the dry matter of test plants.

From the present research, spent mushroom substrate exerted a significant influence on root length of baby spinach (Figure 2). These results are similar to those presented by Sendi et al. [14] who reported that spent mushroom waste (SMW) exerted a significant influence on root length of Kai-lan (Chinese broccoli) at harvest. Addition of suitable organic manure improves the soil physical and chemical properties which encourage better root development, increased nutrient uptake, and water holding capacity which leads higher fruit yield and better fruit quality [72].

Longest root length among the SMS treatments was recorded in the treatment with 10 t/ha. Shortest length (6.42 cm) was recorded in the 30 t/ha treatment amongst all other treatments. Regarding root growth, the higher levels of SMS applied might have started to exhibit a phytotoxicity effect, hence the reduced root length. This could also be attributed to that higher concentrations of elements may limit or inhibit growth beyond a threshold concentration. Salts accumulation around the root zone could also be a possible explanation to the smaller roots development at higher SMS application. However, Sendi et al. [14] found that growth media containing 100% SM or SMW and PM mixtures in 1 : 1 or 2 : 1 ratio without NPK amendment resulted in the lowest root length (ranging from 6.8 to 8.7 cm). Media amended with NPK produced a numerically longer root than media not amended with NPK, but no significant influence of NPK on root length of Kai-lan was evident. With regards to reduced root fresh and dry weight at higher application levels of SMS, others studies have reported that composts can also show limiting factors for horticultural use, such as the presence of heavy metals, poor physical properties, and excess in salts or nutrients that result in high electrical conductivity [73].

It was observed that the treatments with a higher biostimulant effect on the above-ground biomass tended to produce a smaller amount of roots. Baby spinach produced the highest root fresh mass (0.93 g/plant) when grown in negative control treatment and the lowest one when grown in a treatment containing 20 t ha^{-1} of SMS. These results are in accordance with those presented by Canellas and Olivares [74], which show that plants grown in optimal nutritional conditions allocate less energy to growing roots.

Root dry mass was lowest (0.07 g/plant) when baby spinach was grown using 20 t ha^{-1}. The positive control (45 kg ha^{-1} compound D) treatment was found beneficial for root growth of baby spinach since it had the highest dry mass (0.28 g/plant). Beyond application rates of 10 t ha^{-1}, the root dry mass decreased probably ascribed to higher level of soil conditioner application might be linked to salinity of the soil, thus affecting root growth. Contrary to findings by Baldotto [75], treatments containing fertilizers and biostimulants were superior to other treatments. Plants from the control treatment need to adjust their morphology to compensate for the nutritional limitations, thus the proportion of roots in relation to increase in leaf mass since nutrient availability is a limiting factor in the soil.

The sensory evaluation was done to determine the taste in terms of bitterness [76]. Positive control treatment scored the highest with extreme bitter taste followed by 30 t ha^{-1}. Negative control and 10 t ha^{-1} SMS treatment recorded zero scores on extreme bitter taste.

Spinach contains oxalic acid, which causes a lingering bitter taste that can overpower an otherwise tasty dish. The treatment with recommended fertilizer 45 kg ha^{-1}, a compound D, and 30 t ha^{-1} and 20 t ha^{-1} from the scoring tested extreme bitter. Nitrogen fertilization has been identified as the major factors that influence nitrate content in vegetables. In particular, light intensity and nitrate content in soil before or at harvest are known to be critical factors in determining nitrate levels in spinach or other leafy vegetables.

The bitterness of the taste of treatments contributed to by the amount of nitrogen that had accumulated on the plant leaves. These treatments had the highest percentage of leaf nitrogen (Figure 4). These results are supported by Santamaria [77] who noted that nitrate accumulation in leaves due to high levels of nitrogen application results in a bitter taste. The bitterness in taste decreased as the amount of SMS applied decreased in this experiment. The bitterness as a result of higher application levels of the SMS could have been due to high salinity around the root area as well.

5. Conclusion

Yield of baby spinach increased as application rate of spent mushroom substrate increased. Spent mushroom substrate improves soil health and productivity. It is feasible to use in the production of baby spinach. Use of spent mushroom substrate in the production of baby spinach will certainly contribute to its disposal in an environment friendly way. This will reduce pollution and give profit to the farmer. However, despite the fact that application and use of spent mushroom substrate in cultivation of baby spinach is highly recommended, application of spent mushroom substrate beyond 30 t ha^{-1} tends to increase the bitterness of baby spinach in this study. In addition to the sensory evaluation, it would be interesting to consider for future works the performance of a proximate analysis of fresh leaves.

Data Availability

The raw data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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