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EFFECT OF SPENT MUSHROOM SUBSTRATE AND SULFUR ON GROWTH AND YIELD TRAITS OF BROCCOLI IN GYPSIFEROUS SOIL

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SUMMARY

The tendency to use natural organic wastes is one of the environmentally safe applications in crop production. Therefore, the presented study aimed to determine the effect of adding mushroom and sulfur residues to gypsiferous soil and their efficiency in improving the production of broccoli 'Balimo F1' (*Brassica oleracea* var. 'Italica'). The study set out in a randomized complete block design with three replications. The 10 combined treatments of the spent mushroom substrate (SMS) and sulfur residues consisted of 0% and 15% (SMS), and 0, 1000, 2000, 3000, and 4000 S kg ha^{-1}. The interaction of the spent mushroom substrate with sulfur showed significant differences for most growth and yield-related traits of broccoli, including leaf length, leaves per plant, roots per plant, head diameter, head weight, total yield, and harvest index, as compared with the control treatments. Among all the treatments in broccoli, the highest total yield (148,702 kg ha^{-1}) resulted in the treatment SMS - 15% + Sulfur - 3000 kg ha^{-1}, followed by the total yields of 111,608 and 105,663 kg ha^{-1} produced by the treatments SMS - 15% + Sulfur - 4000 kg ha^{-1} and SMS - 15% + Sulfur - 2000 kg ha^{-1}, respectively. However, the minimum total yield in broccoli (28,295 kg ha^{-1}) came from the treatment SMS - 0% + Sulfur - 3000 kg ha^{-1}, followed by a total yield of 33,793 kg ha^{-1} obtained in the treatment SMS - 0% + Sulfur - 4000 kg ha^{-1}. Overall, and compared with the control, a significant influence occurred due to the interaction of spent mushroom substrate and sulfur with levels of 15% and 3000 kg ha^{-1}, respectively.

Keywords: Broccoli, spent mushroom substrate (SMS), composting, sulfur, inorganic fertilizer, organic manure, gypsiferous soil

Key findings: For growth and yield-related traits of broccoli, a significant influence occurred due to the interaction of spent mushroom substrate (SMS) and sulfur at the levels of 15% and 3000 kg ha^{-1}, respectively.

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INTRODUCTION

Composting is the natural process of recycling organic matter, such as leaves and residual waste, into a valuable fertilizer for the soil that supplies the plant (Karbout *et al*. 2021). Composting is also a biological process through which microorganisms convert organic

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materials into beneficial end products, for use as soil conditioners and organic fertilizers (Buchanan and Gliessman, 1991; Stoffella and Kahn, 2001). Recycling food and other organic waste into compost provides environmental benefits, including improving soil health, reducing greenhouse gas emissions, recycling nutrients, and mitigating the impact of droughts. Composting is a sustainable strategy to convert organic wastes into organic additives as valuable as potting soil media (Barthod *et al*., 2018; Petrillo *et al*., 2020).

As only edible parts of the food fungi get consumed, the remaining substrate, known as 'spent mushroom substrate (SMS),' contains edible fungal residues with approximately 80% of unused nutrients (Moon *et al*., 2012). In general, for every kilogram of edible fungi harvested, 3.25 kg of SMS materialized (Zhao *et al*., 2012). The spent mushroom substrate is the soil-like material remaining after a crop of mushrooms (Owaid *et al*., 2017).

Mushroom compost comes from chopped hay, poultry manure, gypsum, and water. Upon harvest of the mushrooms, the spent compost can aid organic farming to improve soil water infiltration, water holding capacity, permeability, and aeration. The spent mushroom substrate contains a lot of salt and unstable organic matter, which requires about two years before application. It enables organic dissolved substances to leach out and decompose as organic substances. The mushroom compost used contains nitrogen (12%), phosphorus (0.2%), and potassium (1.3%). With 18 months of aging, the phosphorus and nitrogen do not change, but potassium can decrease (Uzun, 2004).

The study and use of SMS in large amounts as fertilizer has long started, as it possesses all the essential attributes of organic manure after recomposing (Dai *et al*., 2017; Rinker, 2017). The SMS has served as a substrate for culturing the same and other edible fungi (Ashrafi *et al*., 2014) and producing bacteria (Wu *et al*., 2014).

Broccoli is an edible green plant in the cabbage family (Brassicaceae, genus *Brassica*). The mass of flower heads has leaves surrounding them. Broccoli resembles cauliflower, a different but closely related cultivar group of the same *Brassica* species. Broccoli is eaten either raw or cooked and is a rich source of vitamin C and vitamin K (CABI, 2022).

The growing media and ecological conditions are most necessary to cultivate healthy plantings of broccoli. Therefore, the search and use of better quality crop substrates are crucial (Sterrett, 2001). Several types of materials provide as seed planting mediums (Moldes *et al*., 2007), and the main properties needed in the growing media include being easily found, cheap, and abundant (Demir *et al*., 2010). Peat is an essential medium widely used as the chief substrate component for seedling production in containers for a long time, yet, it is more expensive (Ribeiro *et al*., 1999, 2007).

Seeking alternatives to traditional peat has long begun, mainly focusing on reusable and recyclable materials which do not come from non-renewable sources, such as peat bogs (Handar *et al*., 1985; Verdock, 1988). Several studies indicated organic residues, such as urban solid wastes, sewage sludge, compost, spent mushroom, pruning, and green spoils, after proper composting, proved effective, with excellent results as growth media instead of peat (García-Gómez and Bernal-Roig, 2002; Morra, 2019). However, the use of compost as a substrate component can cause some problems like high salt content (Castillo *et al*., 2004), unsuitable physical properties (Raviv, 1998; Ribeiro *et al*., 1999), and variable quality and composition (Hicklenton *et al*., 2001). Therefore, the spent mushroom substrate consists of a composted mixture of cereal straw and manure, calcium sulfate, residues of inorganic nutrients and pesticides, and soil, which is an equal alternative as sowing material for vegetable seedlings (Medina *et al*., 2009; Gupta *et al*., 2016; Sood *et al*., 2016).

Past studies reported that a mixture of spent mushroom compost (75%) with peat could help seed germination of vegetable species, and adequate spent mushroom compost substrates aided the better growth of courgette and peppers (Medina *et al*., 2009). Individually and the mixture of spent mushroom compost and commercial peat can serve as a seedling medium for kale and broccoli (Peksen and Uzun, 2008). Islam's *et al*. (2014) findings revealed that the enhanced plant height, number of leaves, leaf length and breadth, crown length, and yield resulted in broccoli with the spent mushroom substrate. Marques et al. (2014) and Ahmad (2018) researched the growth of lettuce and arugula, found top-quality and quantity by adding spent mushroom substrate. The said substrates also served as growing media, with specially aged spent mushroom compost provided as an alternative to the peat for the cultivation of eggplant seedlings (Sönmez, 2017). Given the above circumstances, the presented study aimed to investigate the performance of spent mushroom substrate and sulfur powder on the growth and yield of broccoli.

MATERIALS AND METHODS

The experimentation set out at the College of Agriculture, Tikrit University, Salah Aldeen, Iraq. A broccoli hybrid 'Balimo F1' served as the plant material. The seeds sown in the seedling trays with growing media included the peat moss and soil (with a ratio of 1:1) on 18 October 2016 and placed in the lath house to provide a suitable environment for germination of seeds and the formation of sturdy seedlings for further cultivation in the open field. The field soil prepared with good tillage proceeded its leveling and smoothing. After the seedlings reached the planting size (~15 cm height), the adaptation of the seedlings followed for 10 days on 29 November 2016. The seedling process took place in the pre-prepared soil divided according to a randomized complete block design (RCBD) with three replications to evaluate the various parameters of the study.

The sulfur powder came from commercial product marketing, with the aged spent mushroom substrate (ASMS) used in the experiment procured from the Tikrit region, Salah Aldeen, Iraq. The ASMS storage ensued in an open field without use in rainy conditions for more than one year. The physical and chemical properties of experimental soil and spent mushroom substrate used in the study appear in Tables 1 and 2. The experiment design used the RCBD in three blocks, with each block comprising 10 treatments resulting from adding fertilizers of the SMS at the levels 0% and 15% of the soil size and the addition of sulfur at five levels, i.e., 0, 1000, 2000, 3000, and 4000 kg ha^{-1} (Table 3). The broccoli study ended on 21 March 2020, with the plants evaluated in terms of leaf length (cm), leaves plant^{-1}, root length (cm), roots plant^{-1}, head diameter (cm), head weight (g), total yield (kg ha^{-1}), and harvest index.

Statistical analysis

The Genstat packet program (12th ed.) determined the statistical differences among the various treatments, with Tukey's test ($P_{0.05}$) also used for comparison and separation of means (Buysse *et al.,* 2004).

Table 1. Chemical and physical properties of applied soil in the experiment.

pH	EC (mS cm^{-1})	N (ppm)	P (ppm)	K (ppm)	O.M. (%)	Sand (%)	Silt (%)	Clay (%)	Textural class
8.15	2.64	8.1	9.1	53.7	1.14	48	29	23	Loam

Table 2. Chemical characteristics of the SMS used in the experiment.

pH	O.C. (%)	O.M. (%)	N (%)	P (%)	C:N Ratio	K (%)	EC (mS cm^{-1})
6.56	22.23	38.33	1.96	0.94	11.34	1.96	11.75

Table 3. The treatments used in the experiment.

Treatments	Spent mushroom substrate (V/V - %)	Sulfur (kg ha^{-1})
T_1	0	0
T_2	0	1000
T_3	0	2000
T_4	0	3000
T_5	0	4000
T_6	15	0
T_7	15	1000
T_8	15	2000
T_9	15	3000
T_{10}	15	4000

RESULTS

Leaf length

The application of spent mushroom substrate (SMS) with sulfur caused a significant influence on the leaf length (Figure 1). The measured longer leaf (35.67 cm) occurred in the treatment T9 (SMS - 15% + Sulfur - 3000 kg ha^{-1}), and the said treatment was at par with five other treatments in leaf length, i.e., T10, T7, T6, T8, and T3, ranging from 24.00 to 33.33 cm (Table 3). However, the recorded shortest leaf (17.67 cm) showed in treatment T4 (SMS - 0% + Sulfur - 3000 kg ha^{-1}).

The number of leaves per plant

In broccoli, applying various levels of the SMS with sulfur displayed significant differences in the number of leaves per plant (Figure 2). The maximum number of leaves per plant showed 19.67, as observed in the treatment T9 (SMS - 15% + Sulfur - 3000 kg ha^{-1}), showing the same with four other treatments having different levels of SMS and Sulfur, i.e., T10, T8, T6, and T7 ranging from 17.33 to 18.33 leaves per plant (Table 3). However, the minimum number of leaves per plant (13.67) came from the treatment T4 (SMS - 0% + Sulfur - 3000 kg ha^{-1}).

Root length

Various levels of SMS with sulfur exhibited nonsignificant effects on root length in broccoli plants (Figure 3).

Roots per plant

Applying SMS with sulfur resulted in a significant influence on the roots per plant (Figure 4). Evaluating the various treatment of SMS and sulfur with respect to root number, the higher number of roots per plant (16) appeared in the treatment T6 (SMS - 15% + Sulfur - 0 kg ha^{-1}), followed by two other treatments T9 (SMS - 15% + Sulfur - 3000 kg ha^{-1}) and T10 (SMS - 15% + Sulfur - 4000 kg ha^{-1}) with 14.67 and 14.33 roots per plant, respectively (Table 3). However, the treatment T4 (SMS - 0% + Sulfur - 3000 kg ha^{-1}) showed the least number of roots per plant (8.33), followed by T5 (SMS - 0% + Sulfur - 4000 kg ha^{-1}) and T1 (SMS - 0% + Sulfur - 0 kg ha^{-1}) at 9.33 and 9.00 roots per plant, respectively.

Head diameter

The spent mushroom substrate with sulfur's various application levels also triggered significant differences in head diameter (Figure 5). The highest value (27.67 cm) for head diameter surfaced in the treatment T9 (SMS - 15% + Sulfur - 3000 kg ha^{-1}), followed by a 25.00 cm score obtained in the treatment T10 (SMS - 15% + Sulfur - 4000 kg ha^{-1}) (Table 3). Conversely, the lowest head diameter (12.34 cm) occurred in the treatment T4 (SMS - 0% + Sulfur - 3000 kg ha^{-1}), followed by T5 (SMS - 0% + Sulfur - 4000 kg ha^{-1}) at 13.67 cm.

Head weight

Various application levels of SMS with sulfur effectively impacted the head weight in broccoli plants (Figure 6). Among all the treatments, the maximum head weight measured 1338.3 g in the treatment T9 (SMS - 15% + Sulfur - 3000 kg ha^{-1}), followed by the head weight of 1004.5 g obtained in treatment T10 (SMS - 15% + Sulfur - 4000 kg ha^{-1}) (Table 3). In contrast, the minimum head weight (254.7 g) recorded showed in the treatment T4 (SMS - 0% + Sulfur - 3000 kg ha^{-1}).

Total yield

Using various levels of the spent mushroom substrate (SMS) with sulfur revealed significant variations in total yield (Figure 7). Overall, in all treatments of SMS + sulfur, the highest total yield (148,702 kg ha^{-1}) arose in the treatment T9 (SMS - 15% + Sulfur - 3000 kg ha^{-1}), followed by 111,608 and 105,663 kg ha^{-1} total yields produced by the treatments T10 (SMS - 15% + Sulfur - 4000 kg ha^{-1}) and T8 (SMS - 15% + Sulfur - 2000 kg ha^{-1}), respectively (Table 3). Inversely, the minimum total yield (28,295 kg ha^{-1}) came from treatment T4 (SMS - 0% + Sulfur - 3000 kg ha^{-1}).

Harvest index

The harvest index of broccoli plants revealed good results with the various levels of SMS and sulfur applications (Figure 8). The highest and at par value for harvest index (0.4766) emerged in two treatments, i.e., T8 (SMS - 15% + Sulfur - 2000 kg ha^{-1}) and T9 (SMS - 15% + Sulfur - 3000 kg ha^{-1}) (Table 3), whereas, the lowest harvest index (0.2403) in treatment T7 (SMS - 15% + Sulfur - 1000 kg ha^{-1}).

Figure 1. Effect of spent mushroom compost and sulfur on leaf length of broccoli.
Different letters indicate significant differences among the treatments after Tukey's test ($P_{0.05}$).

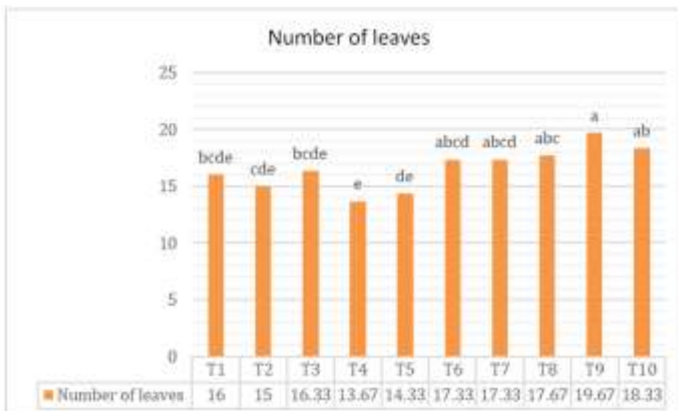

Figure 2. Effect of spent mushroom compost and sulfur on the number of leaves of broccoli.
Different letters indicate significant differences among the treatments after Tukey's test ($P_{0.05}$).

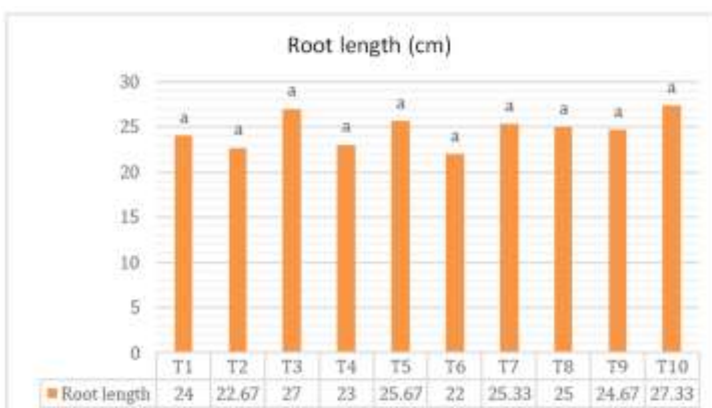

Figure 3. Effect of spent mushroom compost and sulfur on root length of broccoli.
Different letters indicate significant differences among the treatments after Tukey's test ($P_{0.05}$).

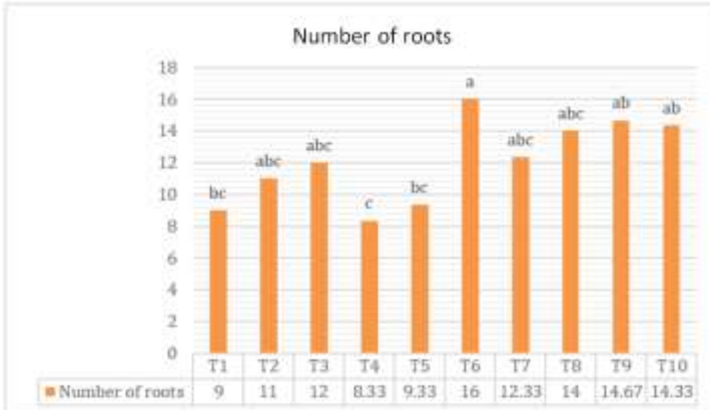

Figure 4. Effect of spent mushroom compost and sulfur on the number of roots of broccoli. Different letters indicate significant differences among the treatments after Tukey's test ($P_{0.05}$).

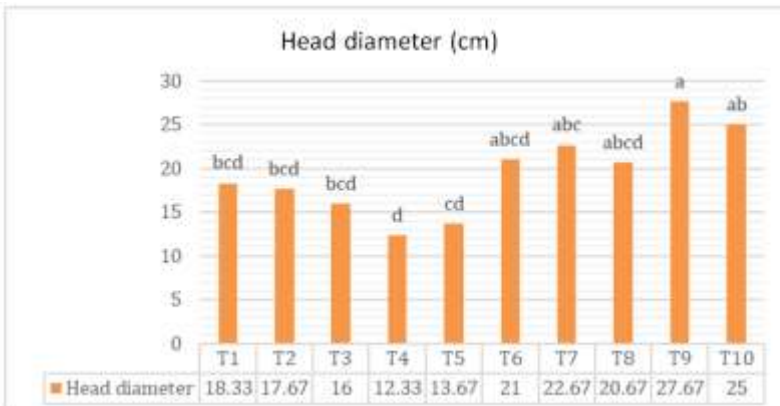

Figure 5. Effect of spent mushroom compost and sulfur on the head diameter of broccoli. Different letters indicate significant differences among the treatments after Tukey's test ($P_{0.05}$).

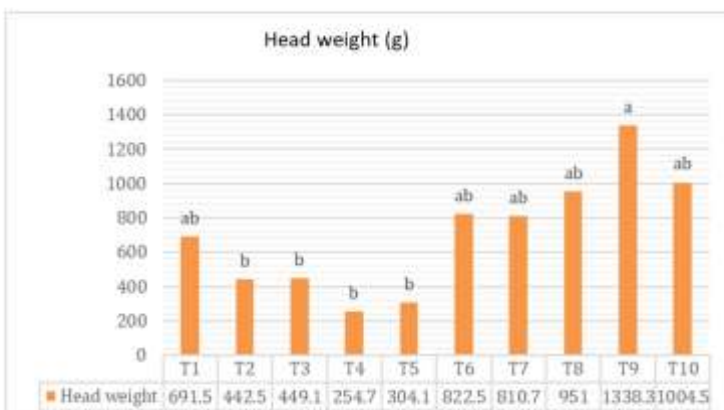

Figure 6. Effect of spent mushroom compost and sulfur on the head weight of broccoli. Different letters indicate significant differences among the treatments after Tukey's test ($P_{0.05}$).

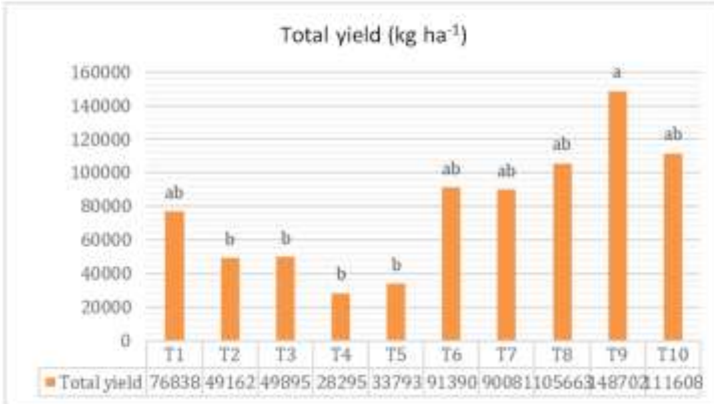

Figure 7. Effect of spent mushroom compost and sulfur on the total yield of broccoli.
Different letters indicate significant differences among the treatments after Tukey's test ($P_{0.05}$).

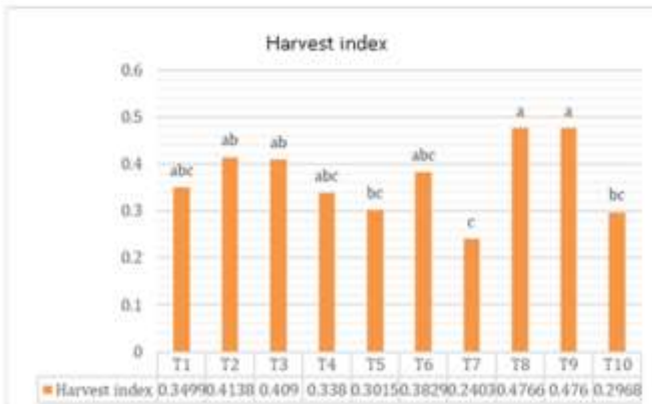

Figure 8. Effect of spent mushroom compost and sulfur on harvest index of broccoli.
Different letters indicate significant differences among the treatments after Tukey's test ($P_{0.05}$).

DISCUSSION

The evaluated effects of the spent mushroom substrate (SMS) alone and combined with sulfur at different levels on broccoli found a suitable ratio of these which could give an economic yield. Application of SMS (15%) with sulfur (3000 kg ha^{-1}) showed significantly higher yield as compared with the control (SMS 0% + sulfur 0 kg ha^{-1}), also over sulfur, the combination of SMS with sulfur, and SMS alone. The SMS (15%) with sulfur produced the highest head diameter and greater head weight per plant. Islam *et al*. (2014) also reported that the application of SMS increased broccoli growth and productivity, enhancing soil fertility status more than the recommended fertilizer alone.

The spent mushroom substrate possesses soil amendments of good quality for raising healthy vegetable crops like broccoli

Adding suitable organic manure improves the soil's physical and chemical properties, which enhances better root development and increases nutrient uptake and water-holding capacity for higher yield and better quality (Suge *et al*., 2011). Organic manure activates many species of living organisms that release phytohormones and may stimulate plant growth and absorption of nutrients (Arisha *et al*., 2003). Organic inputs alone will not meet the nutritional needs of crops because they contain a comparatively less quantity of nutrients than inorganic fertilizers; therefore, the need to integrate the two forms to achieve better crop yield. Organic manure alone cannot produce a higher economic return (Seran *et al*., 2010).

when applied with recommended doses of chemical fertilizers. Through the combined application of organic manure and chemical fertilizers, more nutrient uptake has occurred in the plant system with better plant biomass (Kavita and Subramanian, 2007). Jonathan *et al*. (2011) reported that SMS of *Pleurotus pulmonarius,* mixed with depleted garden soil, generally enhanced all the growth traits versus the control, and significantly promoted plant height, stem girth, number of leaves, flowers, and fruit production in the vegetables. Integrated use of organic manure and chemical fertilizers resulted in a higher onion yield than just the exclusive application of chemical fertilizers (Jeyathilake *et al*., 2006). Seran *et al*. (2010) also reported that combined organic and inorganic fertilizers could produce better yields than organic manure alone.

Sulfur is considered one critical element needed for all living things, including plants. This element is a component of amino acids, such as glutathione, methionine, and vitamins, and is necessary in chlorophyll, coenzyme A, and S-adenosyl-methionine functions (Scherer *et al*., 2008; Li *et al*., 2020; Nakai and Maruyama-Nakashita, 2020). Sulfur protects crop plants from potential damage caused by oxidation stresses (Leustek and Saito., 1999; Aarabi *et al*., 2020). The element also constituents many secondary metabolites, functioning in the physiological operation, growth stages, and plant development. The requirement for sulfur relies on the plant type and development stage. For example, its requirement is higher at seed germination and vegetative growth (Gohain *et al*., 2019). Several biological processes, such as photosynthesis, photoprotection, and energy generation, demand sulfur-involving compounds (Bashir *et al*., 2011; Jogawat *et al*., 2021; Yadav *et al*., 2021). The main source of sulfur for plants comes from inorganic sulfate (SO42−) (Leustek *et al*., 2000; Li *et al*., 2020). The transportation of SO42 − from source to sink occurs by multiple transporters. Although prominent assimilation of SO42− to cysteine occurs in chloroplasts of young leaves, a synthesis of methionine and cysteine can also occur in roots and seeds (Takahashi *et al*., 2011; Aarabi *et al*., 2020). In addition, sulfur involvement in plants manages the function of metabolism, signaling, and biotic and abiotic stress. Sulfur deficiency's impact on plant growth and development could generally summarize in poor quality and yield of crops (McGrath *et al*., 1996; Etienne *et al*., 2018). A moderate deficiency may cause a trivial effect on yield, but the quality is significantly affected. Soils with lower or limited sulfur lead to plants with poor protein storage (Hawkesford *et al*., 2016; Yu *et al*., 2021).

Sulfate deficiency reduces the synthesis of the Rubisco enzyme, which influences CO2 assimilation rates. It eventually retards carbohydrate synthesis and causes young leaves chlorosis (Gilbert *et al*., 1997; Hawkesford *et al*., 2016; Jobe *et al*., 2019; Yu *et al*., 2021). Several studies suggested the impact of sulfur deficiency on the plant's nutritional value, biomass, and morphology. The scarcity caused changes in chlorophyll synthesis and biomass production of *Eruca sativa* L. (Houhou *et al*., 2018). Moreover, a study indicated improvements in vital traits of protein and wheat grain yield. Protein average and grain yield induction showed from 0.018 to 0.024 $kg\,m^{-2}$ yield and from 0.20 to 0.29 $kg\,m^{-2}$, respectively, in various 21 cultivars. Sulfur addition enhanced protein and grain yield in oilseed rape (Filipek-Mazur *et al*., 2019).

Additionally, sulfur insufficiency leads to decreased root hydraulic conductivity, as a response probably implicated with signaling nutrient starvation from root to shoot (Karmoker *et al*., 1991; Mitchell, 2021). Moreover, lacking sulfur results in the reduction of the internal sulfur pool and an increase in the soluble nitrogen pool together with amide and nitrate as a consequence of the ratio of nitrogen and sulfur imbalance (Karmoker *et al*., 1991; Carciochi *et al*., 2017). A summary of sulfur deficiency symptoms in economically vital plants exists. A report stated that between 1990 and 2011, the atmospheric concentration of SO2 decreased by 20 teragrams (Klimont *et al*., 2013). Further, the report said that soil factors also affect sulfur deficiency. Organic sulfur is the primary source of sulfur utilized by plants. Therefore, the soil's organic content is crucial; when low, it will lead to a sulfur shortage in plants. Organic sulfur becomes available to plants through mineralization carried out by microorganisms (Zublena *et al*., 1991; Santana *et al*., 2016).

This microbial activity is dependent on soil temperature and moisture content. Cold and excessively wet or dry conditions reduced microbial activity, decreasing sulfur availability from soil organic matter to the plants (Camberato and Casteel, 2010; Santana *et al*., 2016). The lack of sulfur could be highly varied at the field level because soil sulfur availability differs significantly from soil organic matter and texture. Sulfur deficiency frequently materializes in sandy soil, lower organic matter, and higher elevation areas of a field. However, high organic matter, lower-lying, and heavier textured lands typically have sufficient sulfur (Sawyer *et al*., 2012).

The SMS compost application at 15% plus sulfur produced a higher head weight in broccoli. These results gained support from Ahlawat and Sagar (2007), who reported that mixing soil with composted SMS enhanced the fruit weight with better quality in tomatoes. The composted SMS is an excellent growing medium for vegetables and field crops and has shown multifaceted utility in improving crop yield. Organic manure supplemented with chemical fertilizers exhibited better results than the crop plants treated separately with different fertilizers (Chanda *et al*., 2011). Integrated use of organic and inorganic nutrient sources is advantageous over inorganic fertilizer alone.

Combining organic and inorganic nutrient sources results in synergy and improved conservation and synchronization of nutrient release and crop demand, leading to increased fertilizer efficiency and higher yields (Vanlauwe *et al*., 2002). The use of organics could enhance the efficiency of chemical fertilizers (Dulal and Roy, 1995). Integrated organic and inorganic nutrient inputs may serve as an alternative option versus only inorganic fertilizer to increase fertilizer use efficiency and maintain a more balanced nutrient supply.

CONCLUSIONS

Combining spent mushroom substrate (SMS) with sulfur fertilizer was more effective in giving better results for the broccoli growth and yield traits. Compared with the control, the combined application of SMS plus sulfur impacted an enhanced leaf length, leaves per plant, head diameter and weight, and total yield in broccoli. The presented findings authenticated the spent mushroom substrate use with sulfur, proving more successful in supporting vegetative growth and broccoli yield.

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