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# Evaluation of Spent Mushroom Substrate as biofertilizer for growth improvement of *Capsicum annuum* L.

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**ABSTRACT**

Influence of spent mushroom substrate (SMS) of oyster mushroom and button mushroom on the improvement of health status of *Capsicum annuum* L. was investigated. Analysis of growth promotion in terms of height, no of branches, yield and no of leaf drop indicated that the use of the spent mushroom substrate of oyster mushroom and spent compost of button mushroom had a positive effect on the overall growth of the tested plants. SMS had a role in mobilizing the soil phosphate which was evident by a decrease in soil phosphate level and increase in root and leaf phosphate following treatment with SMS. Chlorophyll content of plants increased when treated with oyster mushroom fresh substrate and button mushroom leachate compost. Fruits of plants treated with button mushroom leachate compost and oyster mushroom fresh substrate showed an increase in protein content of about 2.5 times over control. Similarly, carotenoid contents of fruits also increased significantly in the treated plants, but increases in leaves were not significant. It is evident from the present study that the use of different form of spent mushroom substrate of oyster mushroom and spent compost of button mushroom led to the overall increase in growth of *Capsicum annuum* L.

## 1. INTRODUCTION

After mushroom cultivation, the partially degraded paddy or wheat straw and other agricultural waste, which form as valuable by-products of edible mushroom cultivation, have been termed as Spent Mushroom Substrate (SMS). This SMS, which contains simpler form of protein rich component formed by modification of agricultural materials by the fungus after few cycles of cultivation, can be used as very good soil conditioners for the cultivation of fruits, vegetables flower and foliage crops [1]. Spent mushroom substrate is a good source of carbon, nitrogen and other elements. Nitrogen content varies from 0.4-13.7% with a C: N ratio of 9 to 15: 1 [2] which enhances the growth of plants. The mushroom production is increasing day by day and about 10 million metric tons of spent mushroom compost, a by-product of *Agaricus bisporus* is produced per year [3, 4]. After the mushroom harvest, the substrates have to be removed as storage of this spent substrate may cause environmental contamination. Using the spent mushroom compost as organic manure is one of the solutions to utilize the spent compost in a better way. The rich organic matter, moderate nutrient load, near neutral pH and presence of beneficial microbial population make SMC as a suitable organic waste for its conversion into quality manure for crops. It has been observed that the SMS has potential to bioremediate several agricultural grade fungicides and pesticides [5, 6, 7]. After suitable pre-treatment, spent mushroom substrate can completely or partially substitute the growing media for cultivation of different economically important horticultural crops [8, 9]. There are several methods of using the spent mushroom compost and weathering is one of them. Spent substrates can be spread on the land and allowed to weather for one or more years which allow to reduce the salt and nitrate contents of the spent materials. However, weathering alone is not sufficient and leaching is a better method for reducing salinity of the spent mushroom substrate. Leached spent compost has been reported to have less salinity than the weathered compost and most of the essential elements as well as the microbial properties remain the same as normal spent compost [10, 11, 12]. Thus spent mushroom substrate is considered to be a good source of organic matter and rich in macro and micro elements for plants, which help to increase the soil biological activity [13, 14]. It is also known that roots of most plant supports a wide range of fungal communities which colonize roots intra and intercellularly. Such fungi are known as arbuscular mycorrhizal fungi (AMF) [15]; besides, ectomycorrhizal and ectotrophic associations between fungi and plants [16] are also common. Another group of fungi which are commonly found in soil help in growth promotion upon root colonization are known as plant growth promoting fungi (PGPF) [17].

These are beneficial to several crop plants in respective of growth promotion as well as disease suppression [18]. The present study has been undertaken to evaluate the effect of spent mushroom substrate of different edible mushroom as biofertilizer on the growth and biochemical changes of *Capsicum annuum* L.

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## 2. MATERIALS AND METHODS

### 2.1. Experimental design

Cultivation of *Pleurotus ostreatus* was done using the chopped paddy straw spawn and *Agaricus bisporus* on the pasteurized compost consisting of chopped paddy straw, wheat bran, poultry manure and gypsum. After a few cycles of production were completed, the spent mushroom substrate cylinders and the compost of button mushroom were dried completely under sunlight and used for experiment. In this experiment, six treatments were done using the spent mushroom substrate of oyster mushroom and button mushroom, and there was a control without any treatment. The treatments were: Control(only soil), T1=Soil + Oyster mushroom leachate (100ml/kg soil), T2=Soil + oyster mushroom substrate fresh (250gm/kg soil), T3= Soil + Button mushroom leachate (100ml/kg soil), T=4 Soil + Button mushroom spent compost fresh (250gm/kg soil), T5= Soil + Weathered Spent oyster mushroom substrate (250gm/kg soil), T6= Soil + Weathered Button mushroom compost (250gm/kg soil), T7= Soil + Fresh oyster mushroom substrate + Fresh button mushroom spent compost (250gm/kg soil). The experiment was designed in a complete randomized design which was set up in 3 replicates. Pots were placed in open field condition and no other organic or inorganic fertilizer used in the growing media.Growth promotion was recorded in every 7 days intervals afterthe transfer of plants in terms of plant height, number of branches, leaf drop ratio and yield in comparison to the control sets.

### 2.2. Plant material

Hybrid seed of *Capsicum annuum* L. (Brand name Bullet) was collected from the local market, germinated in the germination tray at 25-27$^0$ C and the seedlings were allowed to grow in the tray for 15 days. The 15-day- old seedlings were then transferred to the pre-treated pots.

### 2.3. Extraction and quantification of phosphate from soil, roots and leaves

Soil samples (1g, air dried) or plant materials (1g, oven dried) were suspended in 25 ml of the extracting solution (0.025N $H_2SO_4$, 0.05N HCl) and activated charcoal (0.01 g) was also added. The extracted samples were shaken for 30 min in a rotary shaker and filtered through Whatman No. 2 filter paper. Quantitative estimation was carried out following ammonium molybdate-ascorbic acid method as described by Knudsen and Beegle [18].

### 2.4. Extraction and estimation of Chlorophyll content

For extraction of chlorophyll, 1g of leaf sample was ground using 10 ml of 80% acetone, it was filtered through Whatman No 1 filter paper. The absorbance was taken spectrophotometrically at 663 nm and 645 nm. Calculation of Chlorophyll a, Chlorophyll b and Total Chlorophyll was done following the methodology as described by Arnon [19].

### 2.5. Extraction and estimation of Carotenoid

1gm of fruit was grinded in dark using 10ml methanol and then it was filtrated by Whatman No1 paper and used as crude sample for estimation. The absorbance was taken at 480 nm, 645nm and 663nm. Carotenoid content was estimated by the following formula

Total carotenoid = $[A_{480} - (0.114 \times A_{664}) - (0.638 \times A_{645})]$ µg/gm tissue

### 2.5. Determination of total protein

Protein was extracted from the plant materials using Phosphate buffer (pH7.2) and protein content was determined following the methods as described by Lowry *et al.*, (1951) using BSA as standard.

## 3. RESULTS AND DISCUSSION

Spent mushroom substrate of Oyster mushroom and Compost of Button mushroom were tested for their effect on growth promotion of *Capsicum annuum* L. in potted conditions. Spent mushroom substrates were used directly as well as in leached form and weathered compost was also applied either singly or in combination. After this, growth promotion in terms of height, number of branches and root-shoot biomass were evaluated at several intervals. Final yield was also estimated by harvesting the capsicum according to their treatment. The results revealed that all the treated plants showed significant increase of height after 35days out of which, those treated with spent substrate of fresh oyster mushroom, button mushroom leachate and weathered compost of button mushroom showed highest increment in growth (Fig 1 & 2A). On the other hand, it was observed that the number of branches significantly increased in oyster mushroom leachate, button mushroom weathered and button mushroom fresh compost (Fig 2B). In case of yield highest yield was obtained by treatment with SMS of oyster mushroom leachate followed by oyster mushroom weathered SMS (Table 1).

**Table 1:** Effect of different treatments of spent mushroom substrates on yield of *Capsicum annuum.*

| Treatments | Yield in first harvest (g/ plant) |
|---|---|
| Control | 49.67±1.37 |
| Fresh oyster SMS[1] | 57.67±0.67 |
| Oyster SMS leachate | 67.33± 1.65 |
| Oyster Weathered SMS | 59.33±3.57 |
| Button SMC[2] leachate | 54.67±4.78 |
| Button weathered Compost | 58.00±2.35 |
| Fresh SMC of Button | 50.75±1.76 |
| Oyster SMS + Button SMC | 53.50±2.20 |

[1]SMS= Spent Mushroom Substrate (oyster mushroom), [2]SMC=Spent Mushroom Compost (Button mushroom)

It has been reported that the PGPR also stimulate the beneficial plant fungal symbiosis involving both AM fungi and ectomycorrhizae [20]. Results revealed that the spent oyster mushroom leachate, fresh oyster mushroom substrate and button mushroom leachate showed better yield. It was also reported that the ectomycorrhizal treatment influences the growth of plants.

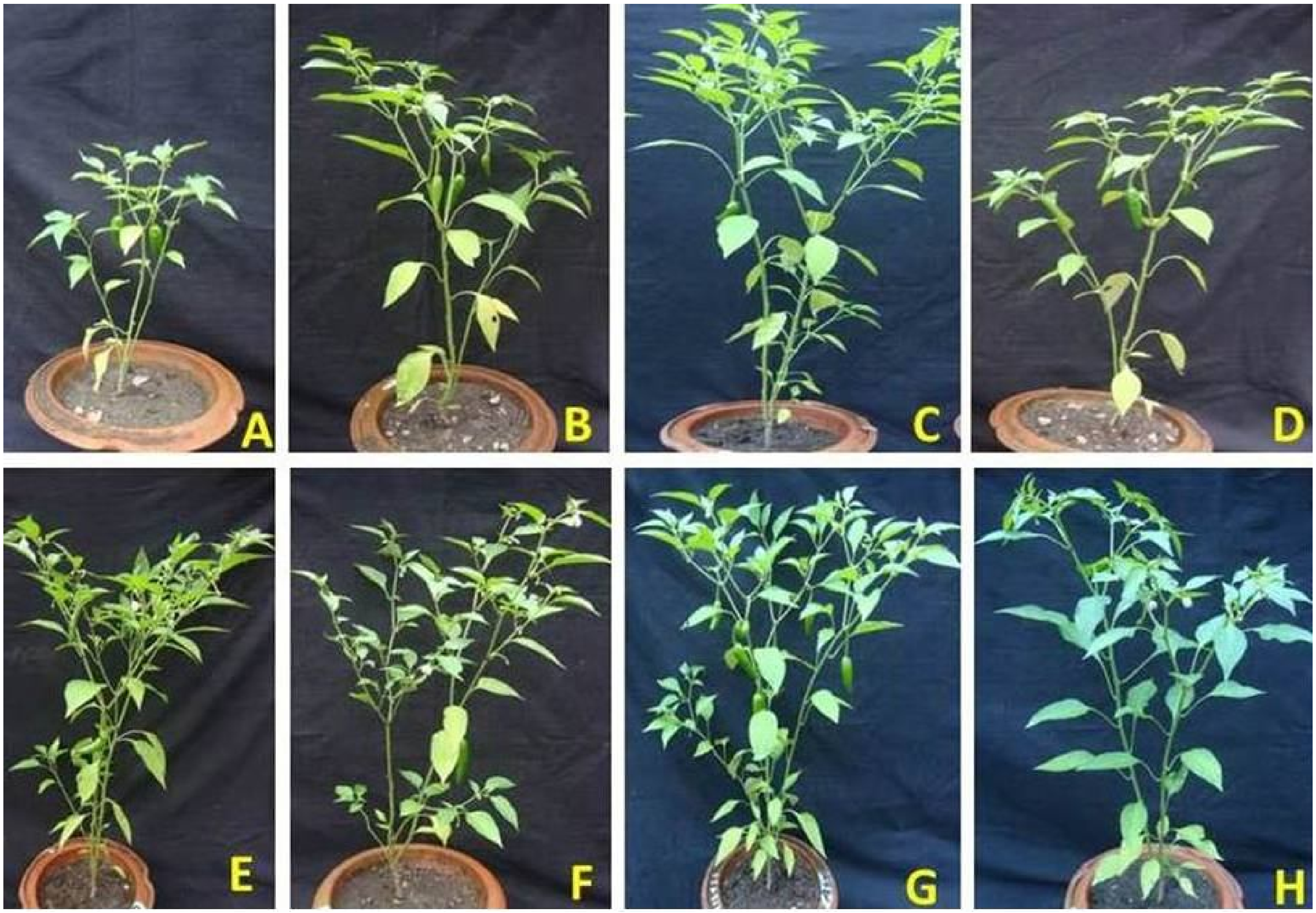


**Fig. 1:** Effect of Spent mushroom substrates on the growth of *Capsicum annuum* L. (A)= Control; (B)= Fresh spent oyster mushroom substrate; (C)= spent oyster mushroom substrate leachate; (D)= Spent weathered oyster mushroom substrate; (E)=Fresh button mushroom compost; (F)= Spent button mushroom compost leachate; (G)= Spent weathered button mushroom compost; (H)= Combined treatment of Spent oyster mushroom and button mushroom substrate

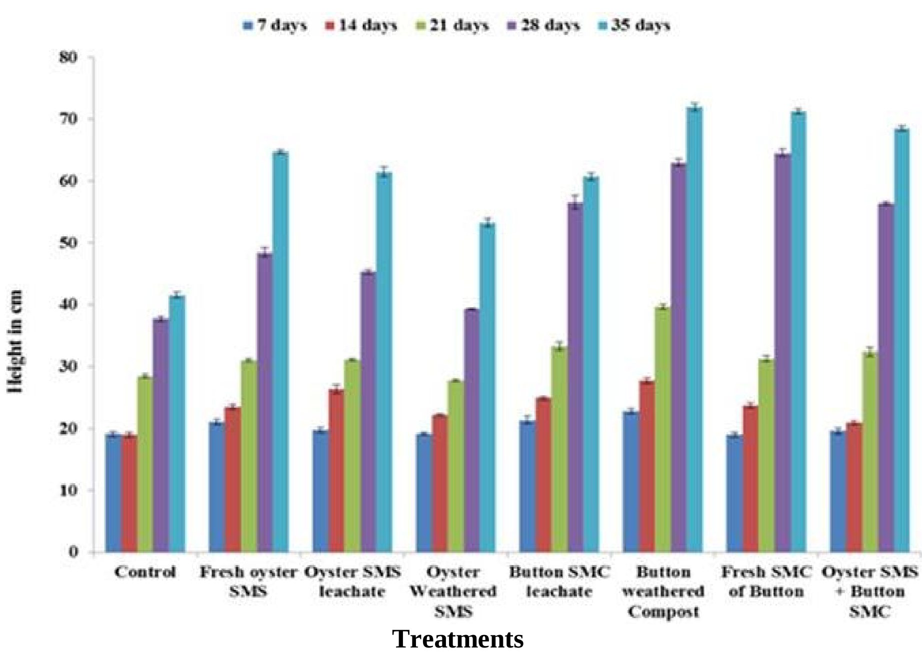


**Fig. 2a:**

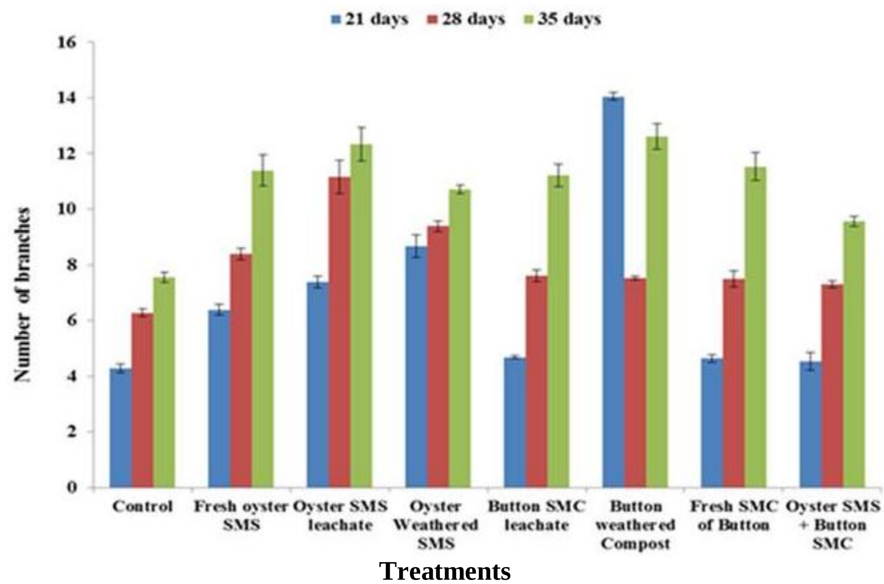


**Fig: 2 (B)**

**Fig. 2:** Effect of Spent mushroom substrate on the growth of *Capsicum annuum* L. showing the rate of increase in height (A) and number of branches (B).

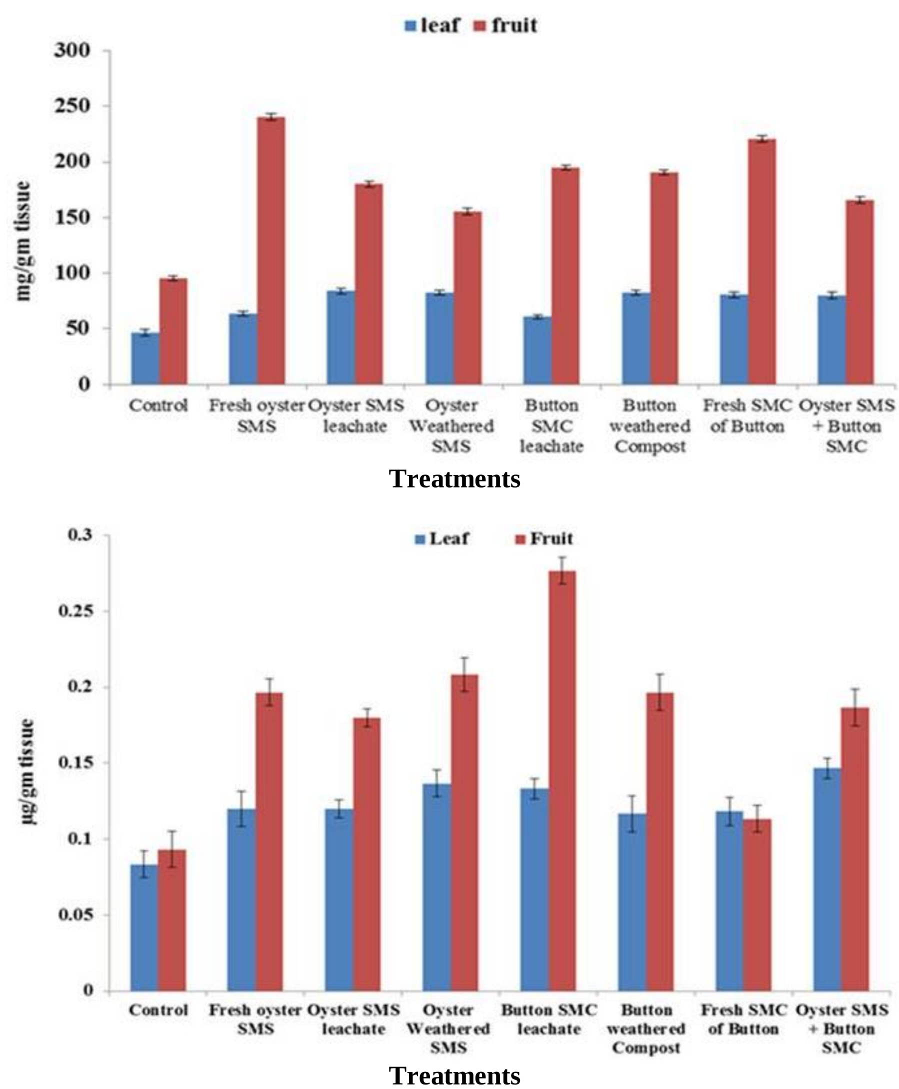


**Fig. 3:** Total protein content (A) and carotenoid content (B) in leaves and fruits of *Capsicum annuum* L. plants treated with spent mushroom substrates of oyster mushroom and button mushroom.

Apart from this, mobilization of soil phosphate by these treatments were evaluated in terms of total phosphate content in soil, roots and leaves of the treated plants in comparison to the untreated control sets [21]. The uptake of phosphate content was significantly increased in fresh oyster mushroom substrate and in button mushroom weathered compost (Table 2).

**Table 2:** Effect of spent mushroom substrate on total phosphate content of soil, root and leaf of *Capsicum annuum* L. after 15days of seedling transfer.

| Treatment | Total Phosphate Content [µg/gm ] | | |
|---|---|---|---|
| | **Soil** | **Root** | **Leaf** |
| Control | 53.47±4.73 | 11.42±1.73 | 7.67±0.83 |
| Fresh SMS of oyster | 43.50±3.45 | 17.45±1.44 | 10.33±1.76 |
| Oyster mushroom SMS leachate | 47.51±3.33 | 13.3±1.12 | 10.65±1.32 |
| Weathered SMS of oyster | 39.50±3.21 | 11.42±1.39 | 9.05±1.11 |
| SMC leachate of Button | 41.1±3.02 | 12.51±1.12 | 9.65±0.91 |
| SMC weathered of Button | 48.39±4.12 | 13.70±1.83 | 10.25±0.93 |
| Fresh SMC of Button | 44.56±3.31 | 12.15±1.19 | 10.70±1.12 |
| SMS[1] + SMC[2] | 41.35±4.17 | 11.95±1.90 | 10.25±0.93 |

[1]SMS= Spent Mushroom Substrate (oyster mushroom), [2]SMC=Spent Mushroom Compost (Button mushroom)

SMS improved soil quality by having a direct influence on soil aggregation and thus, aeration and water movements in addition to increasing availability of insoluble sources of phosphorus [22, 23]. Chlorophyll is the main photosynthetic pigment and it was observed that the use of these spent mushroom substrates enhanced the total chlorophyll content of the leaves of treated plants. Chlorophyll content including chlorophyll a and chlorophyll b was significantly increased in both oyster mushroom and button mushroom leachate, and in fresh oyster mushroom substrate. The dual treatment of both the substrate also showed significant amount of chlorophyll content (Table 3).

**Table 3:** Effect of spent mushroom substrate on chlorophyll content of *Capsicum annuum* L.

| Treatments | Chlotophyll a | Chlorophyll b | Total Chlorophyll |
|---|---|---|---|
| Control | 8.38 | 6.38 | 14.76 |
| Fresh SMS of oyster | 12.92 | 4.72 | 17.64 |
| Oyster mushroom SMS leachate | 13.51 | 5.30 | 18.81 |
| Weathered SMS of oyster | 11.40 | 3.5 | 14.90 |
| SMC leachate of Button | 12.76 | 4.81 | 17.57 |
| SMC weathered of Button | 11.24 | 4.35 | 15.59 |
| Fresh SMC of Button | 10.32 | 3.37 | 13.69 |
| SMS[1] + SMC[2] | 12.40 | 3.41 | 15.81 |

[1]SMS= Spent Mushroom Substrate (oyster mushroom), [2]SMC=Spent Mushroom Compost (Button mushroom)

Total protein content was also evaluated in leaves and fruits. The results revealed that the total protein content was maximum in the fruits of fresh oyster mushroom substrate and fresh button mushroom compost treated plants ranging between 200-250 µg/gm tissue while it was lower in case of treatment with oyster mushroom weathered substrate and the dual treatment of button mushroom and oyster mushroom substrate. Higher leaf protein was also observed in oyster mushroom leachate, button mushroom weathered compost treatment as well as dual application of both the substrates. Among the pigments, carotenoid, being an antioxidant compound is also important and hence carotenoid content was estimated in the study. Carotenoid was estimated in the leaf as well as in fruits of *Capsicum annuum* L. and button mushroom leachate treated plants showed a high range of carotenoid compound (0.25-0.30 µg/gm tissue) followed by the oyster mushroom fresh substrate and button mushroom weathered compost treatment (0.15-0.20 µg/gm tissue). Capsicums were also rich in carotenoids, which is an antioxidant as well as anticarcinogenic compound. It was also observed that immature or mature fruits also contain a high concentration of phenolic compounds [24, 25]. From the above study, it can be concluded that spent mushroom substrate of oyster mushroom and button mushroom compost are good sources of biofertilizer as they influence the growth of *Capsicum annuum* positively. These not only affect the growth but also affect the physiochemical properties. Thus the management of spent mushroom substrate as soil conditioner in the agricultural field can be very effective for crop improvement.

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