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CULTIVATION PRACTICES OF *CALOCYBE INDICA* (P & C) AND USE OF SPENT MUSHROOM SUBSTRATE FOR LEAFY VEGETABLES IN NORTH BENGAL

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ABSTRACT
In present decade Mushroom is considered as a crucial component for food safety and security. Micro-economy and community engagement in mushroom cultivation is helping rural economy of North Bengal. *Calocybe indica* an edible mushroom, native to India was to boost first described by Purkayastha and Chandra (1974). Its milky white colour and robust appearance is appealing to all and provided with easy, reliable, renewable method of growing, its popularity is bound to increase. Since this mushroom has not been cultivated in North Bengal region earlier, in the present study we report the attempts made to establish the cultivation here and hence popularize its commercial cultivation. Paddy straw was used as Substrate which was found to be suitable for growing *Calocybe indica*. The spent mushroom substrate (SMS) is the leftover of wastes after different flushes of mushrooms have been harvested. Spent mushroom substrate of milky mushroom was tested on the growth and development of some leafy spices and vegetables such as Coriander (*Coriendrum sativum),* Spinach (*Spinacia oleracea)* and *Amaranthus sp.* which are common and popularly grown in North Bengal. The results of these investigations showed that all the vegetables responded well to the spent mushroom substrate treatment. Each of the plant attained its less germination time and best growth and gave the highest number of leaves and quality aroma when planted on soil supplemented with 5kg of SMS in 6x7sq ft area. The biochemical constitutions of plant's part also increased in SMS treated plants. The results of these findings have been discussed in relation to the agro-usage of SMS as possible organic fertilizer for the improvement of growth of vegetables in North Bengal region of West Bengal.

KEY WORDS: Milky mushroom, Spent mushroom substrate, Soil conditioner, Organic fertilizer.

INTRODUCTION
One major problem and irony of our planet is there is concern for food safety and security on one hand and huge loss of various agricultural sources on the other. The agricultural waste constitute mainly of cellulose, hemicellulose and lignin. Lignin fraction which is generally considered as recalcitrant in nature, but in mushroom this fraction has remained as the material of choice as mushroom possesses the specific type of hydrolytic enzyme system with capacity of utilizing lignin for fruit body production (Bokaria *et al.,* 2014). Mushrooms have a long association with humankind and provide profound biological and economical impact. From ancient times, wild mushrooms have been consumed by man with delicacy probably, for their taste and pleasing flavor (Das, 2010). Mushroom is one of the cheapest sources of protein particularly for the vegetarian. They have rich nutritional value with high content of proteins, vitamins, minerals, fibers, trace elements and low/no calories and cholesterol (Agahar *et al*., 2005; Wani *et al*., 2010). In North Bengal area people collect mushrooms from forest and consume for nutrition but only the oyster mushroom (*Pleurotus sp.*) is the popularly known cultivated mushroom in the rural society of North Bengal. However milky mushroom (*Calocybe indica*) is being introduced relatively as a new addition among mushroom growers. This mushroom was first reported from West Bengal, India (Purakayasha, 1974). First attempts on the induction of fruit bodies of *Calocybe indica* in culture was made by Purkayastha and Chandha in 1976 (Purkayastha and Nayak, 1981); Chakravarty *et al*., 1981; Doshi *et al*., 1989). However, till date only limited success was achieved on its cultivation and exploitation (Purkayastha *et al.,* 1981, Purkayastha, 1982; Eswaran *et al*., 2003). The mushroom species is suitable for hot and humid environment and can be cultivated indoor in high temperature and high humidity area. It grows well at a temperature range of 26-38^{o} C and relative humidity more than 80%. For the first time milky mushroom was cultivated successfully in North Bengal in summer time (June-August) when the temperature and humidity is high which is suitable for milky mushroom cultivation. The cultivation process resembles that of oyster mushrooms but for the additional process of casing. The mushroom can be harvested from 30-34 days after spawning. The advantages of this mushroom over other mushrooms are easy method of cultivation, less investment, very attractive fruiting body, pleasing milk white color, long shelf life, more nutritious and less time to grow (Bokaria *et al.,* 2014). In rural areas of North Bengal, agro-waste and organic materials are available in plenty amount, which often end up in waste thus contributing to the pollution woes. Recycling is the need of modern times and

mushroom is an ideal candidate to harness its potentialities.

The spent mushroom substrate is the leftover of wastes after different flushes of mushrooms have been harvested. These growing substrates may be composed from different wastes materials such as sawdust, rice straw, bedded horse manure, cotton wastes, paper wastes, cocoa shells, wheat straw, maize husks and various other wastes (Jonathan *et al.,* 2002). Therefore, the demand of milky mushroom usually filled up by oyster mushroom. After the cultivated mushroom has exhausted the nutrients within the substrates, and there are no more fruit bodies to harvest, the so called remains, regarded as 'the useless material' is known as spent mushroom substrate (Fasidi *et al.*, 2008). Spent mushroom substrate is believed to be a source of humus formation and humus is known to provide plants with micro-nutrients which improve soil aeration, soil–water holding capacity and contributes to maintenance of soil structure. SMS have been reported to contain nutrients which could be used for the growth of useful plants. These materials are generally non-toxic to cultivated plants; therefore, it could be employed as soil amendment for different crops (Muritala *et al.,* 2011). In the case of SMS, it has been revealed that application of SMS which consists of degraded cellulose and lignin is considered to be important for the improvement of soil and is safe for human consumption. In addition to providing a balanced nitrogen and carbon source for plant growth, the SMS will be further degraded in the soil humus which is very important to maintain soil structure, good aeration, water holding capacity, and also relevant to maximizing fruit crop productivity (Olutayo *et al.,* 2013). In North Bengal, green leafy vegetables coriander, spinach and amaranthus could serve as major component of food ingredients in winter season. Especially coriander could be used as condiments and food additives. In present decade, many of our agricultural lands have been over utilized by inadequate farming practice and use of inorganic fertilizers, thereby resulting in nutrient depletion of soils. There is a need to look for an alternative source of organic fertilizers which will boost the growth and production of leafy vegetables by the local farmers. Addition of spent compost to agricultural or garden soil has been found to be an effective soil manure and conditioner and has been found to increase considerably the yield of some leafy vegetables crops (Kadiri *et al.,* 2010). Chang *et al.,* (1981) and Iwase *et al.,* (2000) observed that spent compost of *Volvariella volvacea* on addition to soil increased the yield of tomatoes 7 fold and the yields of soybean, lettuce and radish 2 fold each. Furthermore, they observed that addition of *Agaricus bisporus* spent compost to the soil produced greater yields of cabbage, cauliflower, beans and celery compared to addition of poultry manure to soil. The present study was carried out to evaluate the effect of spent substrate of milky mushroom on yields of some leafy vegetables namely *Coriendrum sativum, Spinacia oleracea* and *Amaranthus sp*. which are common leafy vegetables from the North Bengal.

MATERIALS & METHODS

Selection and Preparation of substrate

Paddy straw (6 month old) was soaked in cold tap water for 8 hrs. After draining excess water, the straw was treated in hot water (80^0C) for 40 mints and air dried in a closed shade. After cooling, the dry straw was used for cylinder preparation.

Mushroom bed preparation and incubation

Black Polyethylene bags of 60 x 30 cm size and 100 gauge thickness were used and cylindrical mushroom beds were prepared following layer methods of spawning. Beds were made in which each bed contained 1.5 kg of paddy straw (wet weight basis). A moisture content of about 60% was allotted in the wetted substrate prior to spawning. The wheat grain spawn of *Calocybe indica* was used at 5% level to the wet weight of the substrate and the beds were spawned following layer method of spawning (Baskaran *et al*., 1978). The beds were incubated in semi dark spawn running rooms at about 30 to 32°C. After 12-14 days, when the beds were fully colonized by the vegetative mycelium of mushroom fungus, the upper surface of each bag was opened and the surface was applied with casing soil to a height of 2-3 cm over the spawn run substrate. The beds were uniformly and regularly sprayed with water to keep the surface of substrate moist. Watering was done after and before casin layer placement. After 2 weeks, the primordial initiation was observed. Within one week the pin head becomes mushroom fruit body and mature mushrooms were harvested. From one bed twice or thrice mushrooms were harvested in 9±3 day's interval. No remnants of harvested sporophores were allowed to remain in the substrate. After each harvest, the casing soil was slightly ruffled. Finally the spent mushroom substrates were collected and processed further for use as soil conditioner.

Cultivation chamber

Cultivation chambers were constructed in a shaded place, chamber lined with sky blue colored high density polyethylene sheet coated with paddy straw as roofing material. Beds after casing were incubated over racks in the chamber. The side walls were lined with paddy straw and plastic sheet. Sufficient ventilation was provided by windows present. The inside temperature range was 30 to 35°C and the relative humidity was more than 85 per cent. During day time approximately fluorescence light was available at least for 5 hrs inside the chamber.

Preparation of casing soil

Garden soil and sand were sieved and dry and finally mixed in 4:1 ratio, autoclaved for 20 mints. After 12 hrs when the soil was cool, it was used for casing. This soil was uniformly spread over the spawn run beds. Regular spraying on the surface of the casing soil was done to maintain moisture on the bed surface.

Spent mushroom substrate preparation and application

The spent mushroom substrates collected above were covered with polythene nylon and further fermented for 40 days. Thereafter, the fermented spent substrate was sun dried and used in the experiment field. The experimental field area of 6x7sq ft. was supplemented with 5kg of SMS (wet weight) of *Calocybe indica.*

Seed materials collection

The seeds of the three leafy vegetables such as Spinach (*Spinacia oleracea*), Coriander (*Coriendrum sativum)* and Amaranthus *(Amaranthus sp*), were obtained from local markets in North Bengal. The SMS used for the cultivation of green leafy vegetables were collected from mushroom farm of mushroom group North Bengal University, West Bengal, India.

Growing bed Preparation

The experimental design was a randomized complete block design. The seeds of three green leafy vegetables local variety were collected and sowing was done in February at the sandy land of 6ft X 7ft area. 5 kg SMS was applied on each stand. These treatments were applied at the depth of 6 inch and mixing properly. SMS was applied and incorporated into the soil manually 2 days before of sowing the seed.

Data Collection

Data on germination period and growth parameters; plant height, number of leaves, and length of leaves, diameter of leaves were recorded. Data was also collected during the flowering period.

Statistical analysis

Significant differences between means of growth parameters of treatment and untreated plants for the selected three different leafy vegetables were determined using T-test at >5% probability.

Extraction and quantification of phosphate from soil, roots and leaves:

One gram of oven dried Soil samples and plant materials was used for extraction and quantitative estimation of phosphate following ammonium-molybdate-ascorbic acid method as described by Knudsen and Beegle (1988), where dried samples were suspended in 25 ml of the extracting solution made up of 0.025 N H_2SO_4, and 0.05 N HCL to which activated charcoal .01gm was added, shaken well for 30 mints on a shaker and filtered through watchman no. 2 filter paper (Mehlich, 1984).

Extraction and quantification of total Chlorophyll:

One gram of leaves sample of each vegetables was extracted in 80% acetone using mortar and pestle and extracted homogenate was filtered through watchman no. 1 filter paper and the volume will be made up to 10 ml. absorbance of the filtrate was taken at 645nm and 663 nm spectrophotometrically as described by Harborne (1973).

Carotenoid content:

One gm of sample of each vegetable was extracted in methanol and filtered using watchman no. 2 filter paper and the absorbance were measured at 480nm, 645nm and 663nm spectrophotometrically following the method given by Litchten thaler (1987).

RESULTS & DISCUSSION

The substrate took lowest 12 days for fully colonization and after placing casing soil it took 8 days for pin head formation. The maximum size of sporophore has been recorded as 16.2 cm in length and 4.5cm in diameter and the pileus diameter was recorded as 12.5 cm. The mean length of sporophores was 14.49±0.61 cm and mean diameter of pileus was recorded as 5.7±.41 cm and the highest weight of a single spirophore was recorded as 240 gm. The fist harvested fruit bodies were more healthy and heavy than the 2^{nd} and 3^{rd} flushes. Bacterial contamination on fruit body was recorded in 3rd flush. Mean number of sporophores/bed have been recorded as 7±.52 during first flush. The mean number of pin head/bed appeared as 23.7±1.82 in 1^{st} flush. The highest yield (580±28.15 gm) recorded during 1^{st} flush and the yield gradually decreased in successive flushes. Sandy soil mixed with spent mushroom substrate (SMS) of milky mushroom show significantly faster germination, greater plant height, greater aroma in coriander plant, number of leaves and total leaf area than untreated only sandy soil for three leafy vegetables.

Effect of SMS on seed germination

SMS of milky mushroom showed the positive effect on seed germination of leafy green leafy vegetables. Germination period of seed for *Spinacia oleracea*, *Coriendrum sativum* and *Amaranthus sp* has been recorded as lesser (5, 9, 13 days respectively) in SMS treated field in compared to that of untreated field (i.e.7, 10, 16 days respectively). Among the selected leafy vegetables Spinach has the lowest germination period (5 days).

Effect of SMC application on vegetable height

Table.2 shows the mean heights of the selected leafy vegetables in response to SMS treatments. The highest height in all the leafy vegetables was attained with SMS treatment at 20^{th} and 40th days of sowing. In treatment field for each leafy vegetables at 20^{th} day *Spinacia oleracea* has the mean height of 6.69 cm and while these values significantly (p >0.05) increased to 22.83 cm at 40 days. *Coriendrum sativum* has the highest mean height of 4.62 cm which increased to 21.05 cm at 40days. *Amaranthus sp* has the highest mean height of 1.96 cm at 10days and at 40 days it attained 5.43 cm, while in untreated plot at 20^{th} day, *Spinacia oleracea* has the mean height of 4cm and while it increased to15.6 cm at 40^{th} day, *Coriendrum sativum* has the mean height of 3.70 cm which increased to 15.72 cm at 40^{th} day, *Amaranthus sp* has the highest mean height of 1.2 cm at 20^{th} day and at 40^{th} day it attained 2.6 cm. It could be clearly seen that the untreated experiment had least stimulatory effect on the height of leafy vegetables compared with the SMS treated soil which promoted the plant to development. This showed that the introduction of SMS into the soil enhanced growth significantly (p>0.05) of leafy vegetables.

Influence of SMS on leaves of vegetables

The effect of SMS on number of leaves of leafy vegetables maintained at 20^{th} day and 40^{th} day of sowing was represented on Table 2. Leaves are very important in green vegetables, as they help in the manufacturing of food during the process of photosynthesis. Sandy soil, supplemented with 5kg of SMS, gave the highest mean number of leaves, while the least mean number were observed in the untreated experiments. At 40^{th} day, SMS treated field showed the highest number of leaves for each leafy vegetables. In *Spinacia oleracea*, the mean number of leaves is 10 while mean number of leaves is 7.5 in untreated plot. In coriander mean number of leaves is 6.83 in treated plot while it is 5.17 in untreated plot. Increased number of leaves also found in *Amaranthus sp.* (5.83) in SMS treatment while in untreated mean number of leaves found as 3.83. Table 2 shows the leaf area (cm^2) of the vegetables was increase significantly in SMS treated field. All the leafy vegetables under this study had their highest number of dropped leaves in the untreated field. This may be due to the fact that the depleted garden soil alone lacks the essential mineral elements which vegetables needed to retain leaves. It could also be seen that these nutrients were available in the SMS applied field.

TABLE 1. Yield performance of *Calocybe indica* in paddy straw

Harvesting time	Number of pin head appeared	Number of sporophores /bed	Length of sporophores (cm)	Diameter of stipe (cm)	Diameter of pileus (cm)	Yield/bed (gm)
1st harvest	23.7±1.82	7±.52	14.49±.61	3.14±.128	5.7±.41	580±28.15
2nd harvest	14.4±.89	4±.45	12.27±.52	2.85±.117	4.99±.40	410±14.05
3rd harvest	9.9±.62	2.5±.34	9.87±.64	2.2±.15	3.76±.21	271±19.53

* Values are average of the 3 replicate experiments. '±' Standard Error. Difference between untreated and treated plants significant at p> 0.05 in all the experiment set up as determined by Student's t-test.

TABLE 2. Effect of SMS of *Calocybe indica* on growth parameters of three different leafy-vegetables

Plants	Field condition	Growth Parameters					
		Growth (cm)		Number of leaves		Leaf-area (cm^2)	
		Days interval		Days interval		Days interval	
		20th day	40^{th} day	20^{th} day	40^{th} day	20^{th} day	40^{th} day
Spinach oleracea	Untreated	4±.10	15.6±.68	3.83±.16	7.5±.42	2.08±.031	8.88±.030
	Treated	6.69±.19	22.83±.47	5.67±.21	10±.45	8.52±.039	22.94±.42
Coriendrum sativum	Untreated	3.70±.17	14.3±.42	3.33±.21	5.17±.30	1.83±.028	6.05±.020
	Treated	4.63±.18	21.05±.56	4.17±.30	6.83±.31	3.37±.036	9.41±.033
Amaranthus sp	Untreated	1.2±.17	2.6±.21	2.6±.21	3.83±.30	1.45±.021	5.94±.026
	Treated	2.01±.25	5.43±.36	3.83±.31	5.83±.47	3.35±.022	7.99±.034

* Values are average of the 15replicate experiments. ± Standard Error. Difference between untreated and treated plants significant at p> 0.05 in all the experiment set up as determined by Student's t-test.

FIGURE 1. Developmental Successive stages of pin head to mature fruit body of *Calocybe indica*, A. Spawn run or fully colonized substrate. B. Casing soil placement on the colonized substrate; C-E. Primordial initiation, F. Young pin heads, G. Young fruit bodies, H. Mature fruit bodies; I. Over mature fruit bodies

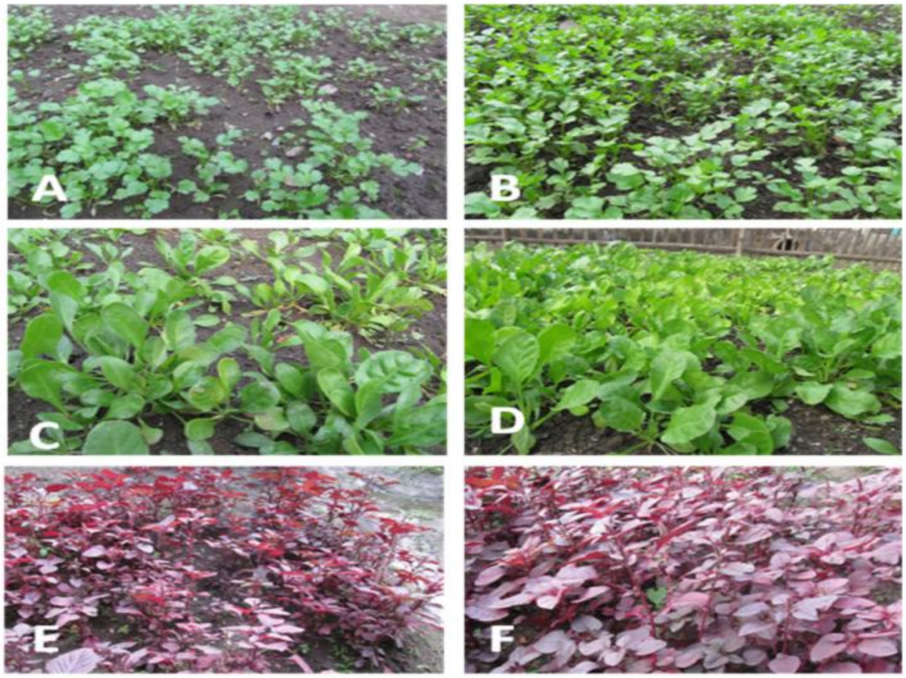

FIGURE 2. Effect of SMS on growth parameters; A. Untreated; *Coriendrum sativum*, B. SMS treated; *Coriendrum sativum,* C. Untreated; *Spinacia oleracea,* D. SMS treated; *Spinacia oleracea*, E. Untreated; *Amaranthus sp*. and F. SMS treated; *Amaranthus sp.*

Effects of SMS on Biochemical constituents

The biochemical and nutritional constituents of each leafy vegetable were analyzed; results are presented in figure 3-6. The result showed nutritional constituents of each leafy vegetable significantly ($p>0.05$) increased when it grow in the SMS amended soil. Extraction and quantitative estimation of chlorophylls has done following the method described by Harborne (1973). Every leafy vegetables grow in sms amended soil showed significant increased in total Chlorophyll content (Fig. 3). The maximum chlorophyll content was observed in *Coriendrum sativum* (2.97 mg gt^{-1}) grown in SMS amended field followed by *Amaranthus sp* (2.38 mg gt^{-1}) and *Spinacia oleracea* (2.19 mg gt^{-1}) as compared to control of each. Carotenoid content (mg gt^{-1}) on leaves of the vegetables was estimated as high in three vegetables grown in SMS amended soil, following the method given by Litchten thaler (1987). Carotenoid content has been estimated as maximum in *Coriendrum sativum* treated with SMS among the vegetables (Fig. 4). Quantitative estimation of phosphate has been done following ammonium molybdate ascorbic acid method as described by Kundsen and Beegle (1988). The highest soluble phosphate content (mg gt^{-1}) observed in both root and leaves from the vegetables grown in SMS incorporated field in compared to untreated one (Fig. 5). The increased of the soluble phosphate content in SMS treated plant indicate that SMS of *Calocybe indica* increase the availability of soluble phosphate in soil that can be easily taken by vegetables. Figure 6 represents the ascorbic acid content of leaves in three vegetables. The maximum Ascorbic acid content (mg gt^{-1}) was observed in leaves of *Coriendrum sativum* in SMS treated field.

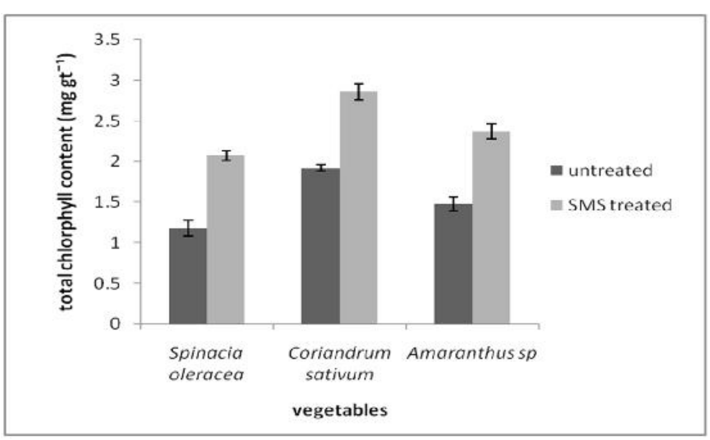

FIGURE 3. Effect of SMS on chlorophyll content (mg gt^{-1}) in three selected leafy vegetables

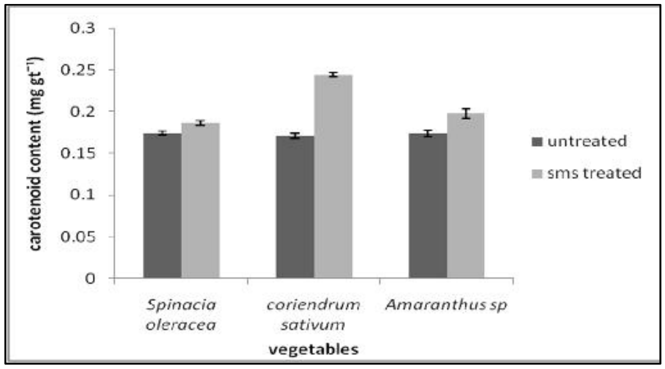

FIGURE 4. Effect of SMS from milky mushroom on Carotenoid content (mg gt^{-1}) in leafy vegetables

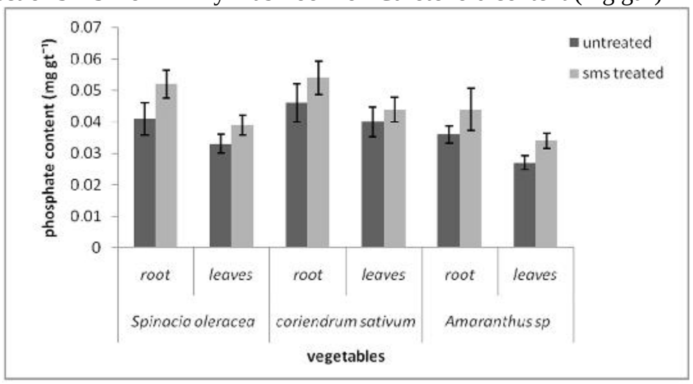

FIGURE 5. Effect of SMS from milky mushroom on soluble phosphate content (mg gt^{-1}) on root and leaves of leafy vegetables

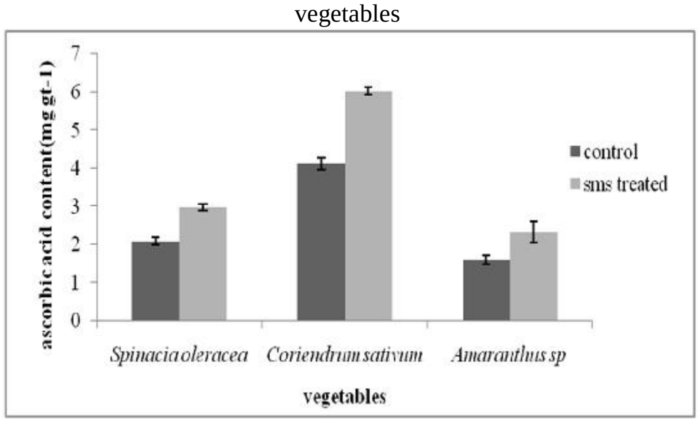

FIGURE 6. Effect of SMS of milky mushroom on Ascorbic acid content (Mg gt^{-1}) in leaves of leafy vegetables

CONCLUSION

From the present study it can be concluded that the spent substrate of *Calocybe indica* is a good soil amendment carrying the essential minerals and compounds that are very important for plant growth and development and have the potential to increase the supply of essential component for the plants.

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