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Comparing the potential of spent mycelium substrate of *Pleurotus florida* with biofertilizers to enhance growth of *Capsicum annuum*

Padmavathi Tallapragada*, Ranjini Rajiv, Vedha Anjanappa, Sehaprabha Sardesai, Samrudhi Selvaraj and Shahid Khan

Department of Microbiology, Centre for PG Studies, Jain University, Bangalore, India

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ABSTRACT

To make a comparison of the potential of spent mycelium substrate (SMS) of Pleurotus florida as a soil conditioner compared to conventional biofertilizers in growth of Capsicum annuum, treatments used were SMS singly and with Azotobacter spp. and Glomus intraradices, to understand their interaction. Paddy straw, tea and sawdust were used as substrates in P.florida cultivation. Potted-plant experiments were conducted in triplicate, used randomized block design and standard garden soil was supplemented with 1% SMS, Azotobacter spp. and G.intraradices. Plants that used G.intraradices(T_4) as biofertilizer exhibited maximum height(28.13cm), flowers(8nos.), chlorophyll(31.34mg g^{-1}), shoot biomass(19.65gm) and uptake of leaf and fruit nitrogen (5.11 and 4.13%); plants grown on SMS+Azotobacter spp.(T_3) showed maximum auxiliary buds(11nos.), soil porosity(85%), root biomass(2.54gm) and uptake of leaf and fruit potassium (2.63%); SMS (T_2) used singly gave maximum soil phosphorus(0.1%), SMS+G.intraradices(T_6) used together gave maximum soil nitrogen(0.32%) and a combination of SMS+Azotobacter spp.+G.intraradices(T_5) exhibited highest soil carbon(1.7%) and uptake of leaf and fruit phosphorus (0.33%). Azotobacter spp.(T_1) used singly gave highest fruit biomass (4.9 gm). Carbon was the main factor influencing production of leaves, leaf pigments and flowers; indicated by positive correlation (0.48, 0.64, 0.65 and 0.78) between carbon and the above factors. Nitrogen had negative correlation with height (-0.76). SMS used singly resulted in increased soil phosphorus; used with Azotobacter spp. and G.intraradices, it enhanced leaves, auxiliary buds, flowers, root biomass, soil porosity, soil carbon and nitrogen. Hence, it is more beneficial when used as a supplement to conventional fertilizers compared to being used singly.

Keywords: *Azotobacter spp.*, Biofertilizer, *Glomus intraradices*, *Pleurotus florida*, Spent Mycelium Substrate.

INTRODUCTION

Environmental degradation is a major threat confronting the world, and the rampant use of chemical fertilizers contributes largely to the deterioration of the environment, loss of soil

fertility, less agricultural productivity and soil degradation [15]. Microorganisms are a vitally important component of soil. Bacteria and fungi mediate soil processes like decomposition, nutrient and water mobilization/ mineralization and storage/release, nitrogen fixation and denitrification. Fungi are adverse component of soil microbial communities, in which they function as decomposers, mycorrhizal mutualists and pathogens [21]. In the frame of agriculture, micro flora of soil is of great significance because it has both beneficial and detrimental influence upon mankind's ability to produce food [49]. Fungi play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bio remediation and many other ways [29].

Spent Mycelium Substrate (SMS) is a valuable by-product of edible mushroom cultivation. It consists of partially degraded paddy or wheat straw, coconut husk, bagasse or other agricultural waste. After a few cultivation cycles, it is bio chemically modified by fungal enzymes into a simpler form and enriched with protein. Fresh and aged SMS has been applied to propagation of fruits, vegetables, flower and foliage crops [34]. It is a rich 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 [6]. It also contains cations like K^+, Na^+, Ca^{2+} and Mg^{2+}, and anions like Cl^-, NO_3^- and SO_4^{2-}, all essential for optimal plant growth development.

Azotobacter spp. is a free-living N_2 fixing bacterium, fixing 3-10 kg N per hectare [35]. They stimulate plant growth by synthesizing secondary-growth-promoting-compounds and are known as plant growth promoting rhizobacteria (PGPR), in addition to phosphate solubilization and enhancement of nutrient uptake [34].

Roots of most plants also support diverse fungal communities which colonize roots intra and intercellularly and known as arbuscular mycorrhizal fungi (AMF) [46]. There are also ectomycorrhizal and ectotrophic associations between fungi and plants [8]. AMF have been shown to improve soil structure, improve plant growth through efficient nutrient uptake, alleviate nitrogen deficiencies, improve drought tolerance, overcome the detrimental effects of salinity and enhance tolerance to pollution [45]. There are other fungi which promote plant growth upon root colonization and functionally designated as plant growth promoting fungi (PGPF) [13]. They are beneficial to several crop plants not only by promoting growth but also by protecting them from diseases [39].

The relevance of this study is that mushroom production is the biggest solid-state fermentation industry in the world, with cultivation of *Pleurotus spp.* being ranked second or third in the world [23]. For every kilogram of mushroom produced, 5 kg SMS is generated [38]. SMS has traditionally been considered as a part of solid waste. The industry has been facing pressure from regulatory agencies to dispose or use SMS generated in a more environmental friendly manner (viz.) using it as biofertilizer, than simply burning it, as is being presently done. Production of horticultural crops has been enhanced through spent shiitake substrate being palletized into organic fertilizer, applied to tomato [18] or sugarcane production [26].

The main objective of this study was to compare the potential of the SMS of *P.florida* as a soil conditioner with that of conventional biofertilizers, in enhancing physicochemical soil characteristics and growth parameters of *Capsicum annuum.*

MATERIALS AND METHODS

Experimental Design

P.florida spawn was sourced from Indian Institute of Horticultural Research (IIHR), Bangalore, and cultivated on paddy straw, supplemented with varying quantities of tea and saw dust. After a few cultivation cycles were completed, the SMS was dried completely, separated out into fibre and used. *C.annuum* seeds were procured from Lalbagh Garden Nursery, Bangalore. One month old, uniform-height plants were transplanted for potted-plant experiments, conducted in triplicate using randomized block design and watered every alternate day. Treatments were T_1 (soil + *Azotobacter spp.*), T_2 (soil + SMS), T_3 (soil + *Azotobacter spp.* + SMS), T_4 (soil + *G.intraradices*), T_5 (soil + *Azotobacter spp.* + SMS + *G.intraradices*) and T_6 (soil + SMS + *G.intraradices*). Control was maintained with only soil. 1% (20 gm for 2 kg soil) *Azotobacter spp.,* SMS and *G.intraradices* were used. The selection of *G.intraradices* was based on soil pH. It is generally used in neutral to slightly alkaline pH and provides good growth response in a wide range of host plants [5].

Analytical Methods

Height of plant was measured using thread and graduated ruler, every ten days, from base of shoot to apical bud. Leaves, auxiliary buds and flowers were recorded by counting, also every ten days. Root, shoot and fruit biomass (wet) was estimated by weighing. Dry biomass was computed after drying plant parts in hot air oven at 50^oC for 24 hours.

Chlorophyll and carotenoid content of leaves was estimated spectrophotometrically at $A_{645,}$ A_{663} and A_{480}, A_{510} respectively, after extraction with 80% acetone and centrifugation at 1,500 rpm for four cycles [41].

Physical soil parameters analyzed were bulk density, particle density and percent pore space [36]. Bulk density (D_b) was estimated as weight of oven-dry soil sample / bulk volume of soil sample. Particle density (D_p) was weight of oven-dry soil sample / volume of particles in soil sample. Percent pore space was calculated as $100 – (D_b / D_p \times 100)$.

Chemical soil parameters analyzed were pH [24] by pH meter and electrical conductivity [31] by electronic conductimeter. Water holding capacity was estimated using the formula: % Moisture = weight of wet soil – weight of dry soil/ weight of dry soil [12]. Total organic content was estimated by rapid titrimetric oxidation technique [48].

Soil and plant nutrients analyzed were nitrogen, phosphorus, potassium and carbon. Nitrogen was estimated by Kjeldahl's method, in which soil was digested with concentrated sulphuric acid, the catalyst mixture was raised to the boiling temperature, promoting conversion from organic-N to ammonium-N. Ammonium-N from the digest was obtained by steam distillation, using excess NaOH to raise pH. The distillate was collected in saturated H_3BO_3 and then titrated with dilute H_2SO_4 to pH 5.0 [4]. Total phosphorus [25] was estimated by digestion with a strong acid for dissolution of all insoluble inorganic matter and measured spectrophotometrically at A_{410}. Potassium was estimated using flame photometer [31]. Organic carbon was estimated by rapid titrimetric oxidation technique [48]. The procedure consisted of oxidization with a hot mixture of potassium dichromate and concentrated sulphuric acid, the excess of chromate remaining was titrated against ammonium ferrous sulphate and the amount of reduced chromate during reaction with soil was equivalent to the amount of organic carbon.

Statistical Analysis

The data was subjected to 2 –way ANOVA and correlation using using SPSS (18 package) and MS Excel software. In all cases, the significant differences (P<0.05) between the experimental values were determined using Duncan's New Multiple Range (DMR) test.

RESULTS AND DISCUSSION

Maximum height of 28.13 cm was observed when *G.intraradices* (T_4) was used as biofertilizer, followed by a combination of SMS + *Azotobacter spp.* + *G.intraradices* (T_5 – 18.53 cm) and SMS + *Azotobacter spp.* (T_3 – 16.31 cm); control showed least height of 10.8 cm. Significant difference existed between control and all treatments [Table 2], and the increase in height occurred on days 25, 45, 55, 65, 75 and 85 [Table 1]. Maximum no. of leaves was recorded again when *G.intraradices* (T_4 – 26 nos.) was the biofertilizer, followed by a combination of SMS + *Azotobacter spp.* + *G.intraradices* (T_5 – 24 nos.) and SMS + *Azotobacter spp.* (T_3 – 19 nos.); control had least with 10 nos. [Table 2]. Increase occurred on days 15, 45, 55, 65, 75 and 85 [Table 1].

Table 1. Effect of 1% *Azotobacter spp.*, *G.intraradices* and SMS of *P. florida* on height and leaves of *C. annuum* (day-wise)

S. No	Day of growth	Height of plant (cm)	No. of leaves
1	0	4.93**a**	4.90**a**
2	5	4.93a	4.89a
3	15	5.62a	5.57**b**
4	25	6.83**b**	6.32b
5	35	7.25b	7.12b
6	45	7.94b	9.02**c**
7	55	9.06**c**	10.08**d**
8	65	10.83**d**	13.52**e**
9	75	12.64**e**	14.81**f**
10	85	13.83**f**	15.99**g**
11	95	13.91f	14.45e
12	105	14.10f	15.48f
13	115	14.13f	15.29f
14	125	14.51f	15.29f

Values in each column followed by the same letter are not significantly different (P > 0.05) from each other according to DMR test.

Table 2. Effect of 1% *Azotobacter spp.*, *G.intraradices* and SMS of *P. florida* on height and leaves of *C. annuum* (treatment-wise)

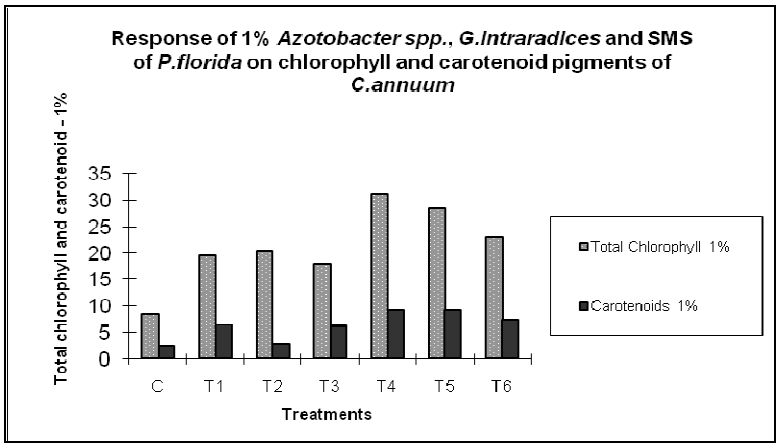
S. No	Treatment	Height of plant (cm)	No. of leaves
1	Control	3.95**a**	4.24**a**
2	T1	8.27**b**	8.91**b**
3	T2	10.01**c**	11.40**c**
4	T3	11.28**d**	13.18**d**
5	T4	17.95**e**	15.46**e**
6	T5	9.62c	11.07c
7	T6	9.17c	12.11c

Values in each column followed by the same letter are not significantly different (P > 0.05) from each other according to DMR test.

Chlorophyll content was maximum when *G.intraradices* (T_4 - 31.34 mg g^{-1} wt) was used as biofertilizer, followed by a combination of SMS + *Azotobacter spp.* + *G.intraradices* (T_5 – 28.53 mg g^{-1} wt) and usage of SMS + *G.intraradices* (T_6 – 23.13 mg g^{-1} wt) together, control showed

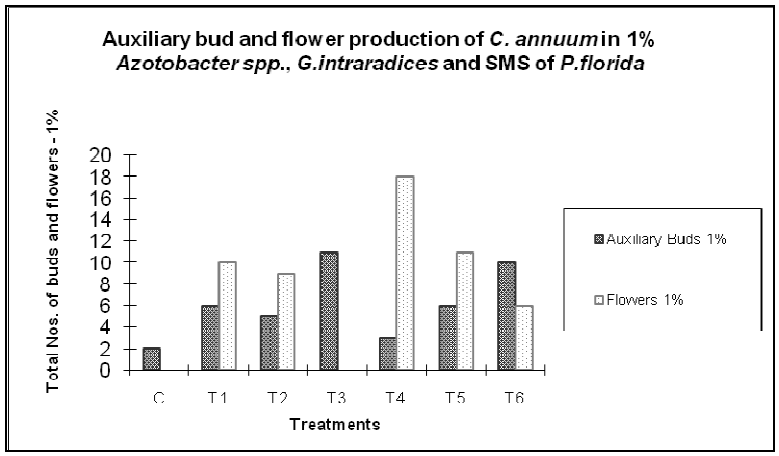
least value of 8.5 mg g^{-1} wt [Figure 1]. Carotenoid content was also maximum in T_4 and T_5 – 9.2 mg g^{-1} wt, control had least value of 2.4 mg g^{-1} wt [Figure 1].

Figure 1. Response of 1% *Azotobacter spp., G.intraradices* and SMS of *P.florida* to foliar pigments of *C. Annuum*



Maximum auxiliary buds was seen when SMS + *Azotobacter spp.* (T_3 -11 nos.) was used together as biofertilizer, followed by a combination of SMS + *G.intraradices* (T_6 – 10 nos.); control had least with 2 nos (Figure 2). Maximum flowers was in T_4 -18 nos., followed by T_5 – 11 nos. and when *Azotobacter spp.* (T_1 – 10 nos.) was used singly - Figure 2.

Figure 2. Auxiliary bud and flower production in 1% *Azotobacter spp., G.intraradices* and SMS of *P. florida*



SMS + *Azotobacter spp.* (T_3 – 85%) used together as biofertilizer and a combination of SMS + *Azotobacter spp.* + *G.intraradices* (T_5 – 90%) exhibited high porosity - Table 3.

SMS (T_2 – 0.1%) and *Azotobacter spp.* (T_1 - 0.09%), each used singly, showed higher phosphorus content, while higher nitrogen content was found when SMS + *G.intraradices* (T_6 - 0.32%) was used together. Higher carbon content of 1.7% was observed in a combination of SMS + *Azotobacter spp.* + *G.intraradices* (T_5) - Figure 3.

Figure 3. Nutrient estimation of *C.annuum* in 1% *Azotobacter spp., G.intraradices* and SMS of *P. florida*

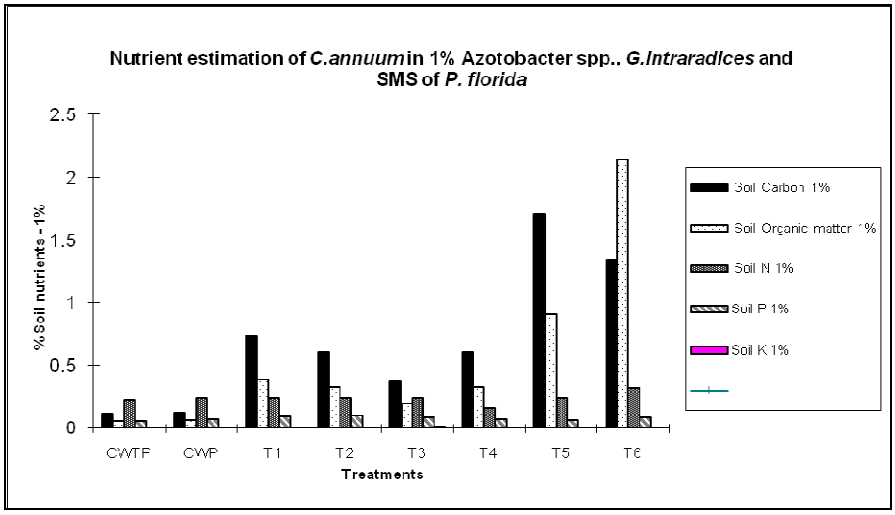
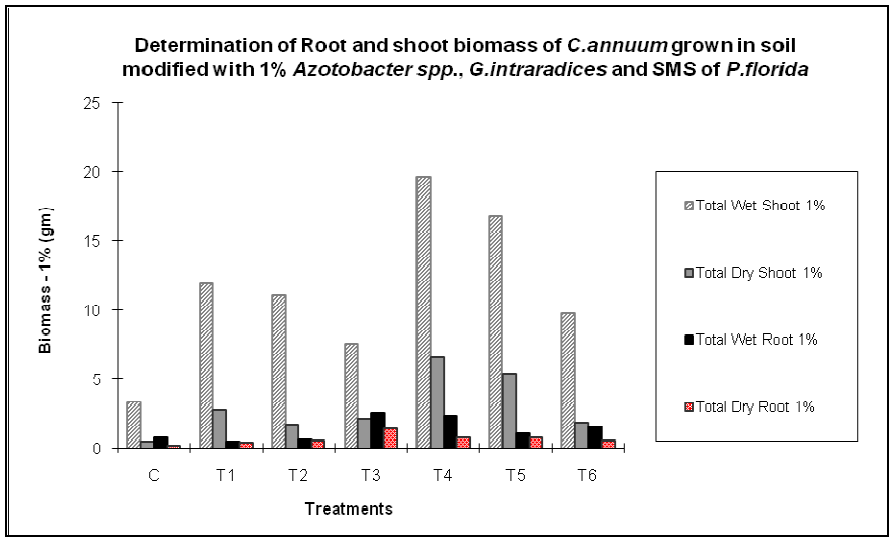


Table 3. Effect of 1% *Azotobacter spp., G.intraradices* (AMF) and SMS of *P.florida* on physico-chemical soil characteristics

Soil Para	CWTP	T1	T2	T3	T4	T5	T6
BD	0.044	0.04	0.04	0.04	0.04	0.05	0.04
PD	0.087	0.1	0.09	**0.26**	0.13	**0.53**	0.13
Porosity	49.65	59.9	49.7	**85.2**	66.6	**90**	66.6
CP	27.5	**48.3**	38.6	**46.4**	35.6	30.9	34.8
FP	64.3	51.7	61.4	53.6	64.5	69.1	65.2
pH	5.67	6.7	7	6.9	7	7	6.7
EC	58.9	66.7	**72**	**71.4**	58.9	**114**	**122**
WHC	66	**62**	34	48	**60**	56	54

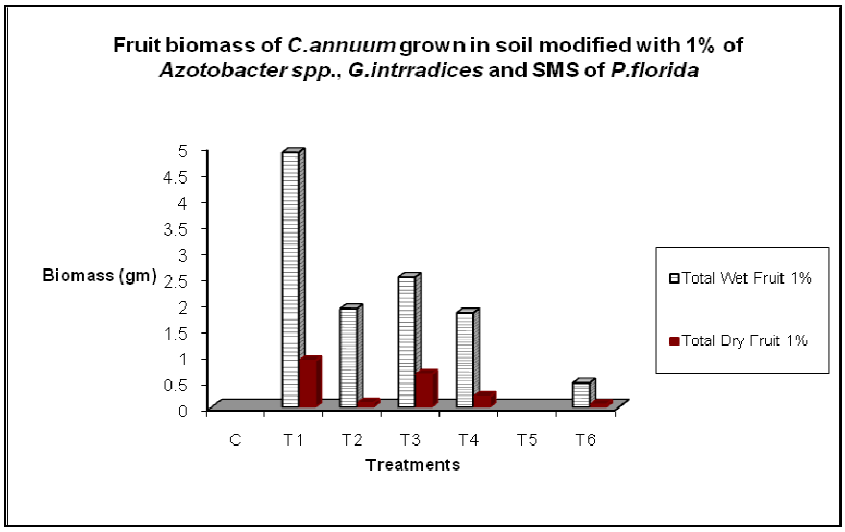
BD = Bulk density (gm/cm3)
PD = Particle density (gm/cm3)
CP = Coarse particles (%)
FP = Fine particles (%)
EC = Electrical conductivity (mS/cm)
WHC = Water holding capacity (%)
CWTP = Control soil before planting

Figure 4. Determination of Root and Shoot Biomass of *C.annuum* grown in soil modified with 1% *Azotobacter spp., G.intraradices* and SMS of *P. florida*



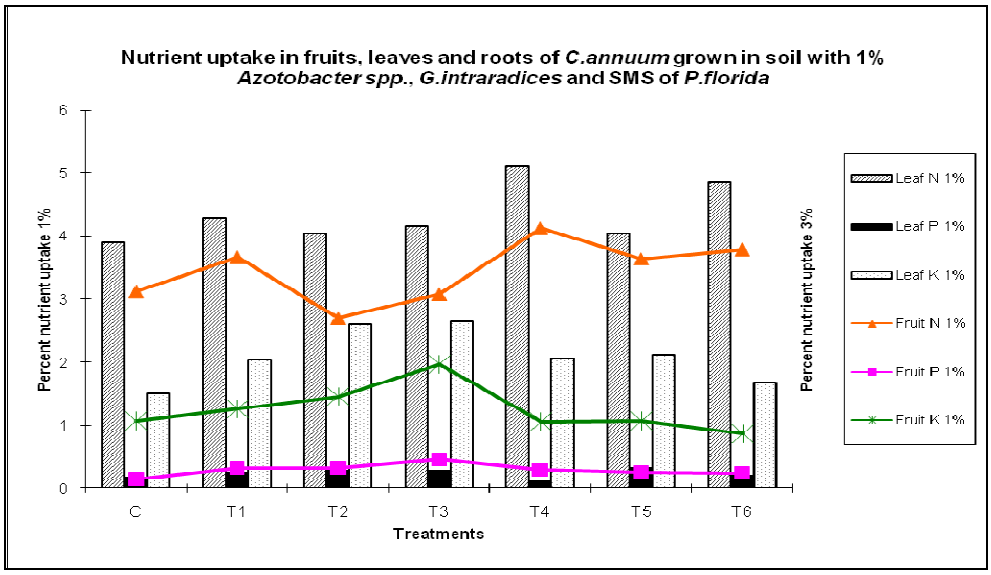
Use of *G.intraradices* (T_4) as biofertilizer exhibited highest shoot biomass (19.65 gm), followed by a combination of SMS + *Azotobacter spp.* + *G.intraradices* (T_5 - 16.78 gm). When SMS + *Azotobacter spp.* (T_3) was used, highest root biomass (2.54 gm) was produced, followed by *G.intraradices* used singly (T_4 - 2.37 gm) - Figure 4. *Azotobacter spp.* (T_1) used singly had highest fruit biomass (4.9 gm), followed by a combination of SMS + *Azotobacter spp.* (T_3 - 2.5 gm) - Figure 5.

Figure 5. Fruit Biomass of *C.annuum* grown in soil modified with 1% *Azotobacter spp., G.intraradices* and SMS of *P.florida*



Uptake of leaf and fruit nitrogen was maximum in T_4 - 5.11 and 4.13%. Leaf phosphorus was highest in T_5 – 0.33%, fruit phosphorus in T_1 and T_2 - 0.33%. T_3 exhibited maximum uptake of leaf and fruit potassium uptake - 2.65 and 1.97% – Figure 6.

Figure 6. Nutrient uptake in fruits, leaves and roots of *C.annuum* grown in soil with 1% *Azotobacter spp., G.intraradices* and SMS of *P. florida*



There was positive correlation in between height of plant and leaves (0.86), leaf production and foliar pigments (viz.) chlorophyll and carotenoids (0.91; 0.85), foliar pigments and soil carbon

(0.645), leaves and soil carbon content (0.49), flower production and soil carbon (0.78). There was negative correlation in between plant height and soil nitrogen content (-0.76); flower production and soil phosphorus, potassium (-0.59) - Table 4.

Table 4. Correlation between physicochemical characteristics of soil modified with 1% *Azotobacter spp.*, *G.intraradices* and SMS of *P.florida* and growth parameters of *C.annuum*

Correlation between height of plant and leaves	**0.8685**
Leaves and chlorophyll content	**0.9172**
Leaves and carotenoid content	**0.8565**
Height of plant and soil nitrogen content	**-0.767**
Leaves and soil carbon content	0.4888
Chlorophyll content of leaves and soil carbon	**0.6492**
Carotenoid content of leaves and soil carbon	**0.6552**
Flower production and soil carbon content	**0.7859**
Flower production and soil potassium content	-0.595

Soil carbon improved the physical properties of soil. It increased the cation exchange capacity (CEC) and water-holding capacity of sandy soil and contributed to the structural stability of clay soils by helping to bind particles into aggregates Soil organic matter, of which carbon is a major part, holds a great proportion of nutrients, cations and trace elements that are of importance to plant growth [17].

The main interpretation was that addition of *G.intraradices* to soil (T_4) helped it to colonize the roots and enhanced most growth parameters by better absorption of nutrients from soil. This was an expected outcome, as *G.intraradices* is a well studied AMF, known to modify plant physiological processes by altering nutrient balance, partitioning carbon, changing phytohormone production and preventing disease [7]. AM fungi colonization was found to be positively correlated with nitrogen [44].

Interaction of SMS with *Azotobacter spp.* (T_3) proved beneficial because it was observed that most white rot fungi exhibited low cellulolytic activity in soil and the presence of a nitrogen-fixing microbe may help to improve availability of nutrients, which in turn would have a positive influence on activity of cellulolytic enzymes of fungal mycelia [43]. SMS improved soil quality by having a direct influence on soil aggregation and thus, aeration and water movements [32], in addition to increasing availability of insoluble sources of phosphorus [40]. Inoculation of plants with mycorrhizal fungi during seedling stage and subsequently transplanting them in manured fields could substitute for chemical fertilizers, particularly phosphorus [1]. Indian soils are usually deficient in phosphorus and when applied to soils, quickly gets fixed and becomes unavailable to plants [24]. Transport of phosphate to roots via mycorrhizal hyphae could be nearly 1,000 times faster than through soil diffusion [3] and contribute upto 75% of total phosphorus absorbed by the plant [16]. Soil moisture affects phosphorus release and uptake by plants, as also growth of roots [22]. This is evident in treatment of SMS with *Azotobacter spp.* where phosphorus and soil porosity levels were maximum. Soil porosity increased movement of water through the soil layers and enhanced phosphorus release and root growth, essentially a hydration process [27].

There was also positive influence of SMS interaction with mixture of *Azotobacter spp.* and *G.intraradices* (T_5). PGPR possess a wide variety of other direct mechanisms to support mycorrhizal symbiosis. Their interaction with AMF, thus, produces positive effects which develop activities involved in plant growth promotion and plant protection [37]. Mycorrhizal

symbiosis has a significant effect on bacterial community composition of rhizosphere, where fungal interaction with bacteria is beneficial. Bacteria adhere superficially or intracellularly to fungal hyphae on roots and soil [28]. This is known as mycorrhizosphere effect [19]. PGPR are known to stimulate beneficial plant-fungal symbioses involving both AM fungi and ectomycorrhizae [2]. In T_1 and T_2, where *Azotobacter spp.* and SMS were used singly, the effect was less than T_3 or T_5.

However, these relationships were not always positive [9]. This may be because during mycorrhizal symbiosis, AM fungi competed with other fungi for resources [34]. They are obligate symbionts of the associated plant and alter the root exudates [11]. In a study with *Cucumis sativus* L. labeled with ^{14}C, nearly 20% of the photo-assimilated carbon was used up by the mycobiont or the AMF [14]. This could be the probable cause for reduction in growth of plants during interaction of SMS with *G.intraradices* (T_6).

CONCLUSION

Key findings of the study were that using SMS alone resulted in maximum increase of phosphorus in soil, while when it was used with *Azotobacter spp.* and *G.intraradices*; it enhanced biomass, leaf and auxiliary bud production, soil porosity, soil carbon and nitrogen. Hence, it would be more beneficial to use it as a supplement to conventional fertilizers rather than as a stand-alone soil conditioner. This is because SMS improved physical properties of soil by decreasing bulk density, increasing aggregate stability, reducing surface crust formation and diurnal temperature changes, increasing infiltration rate, aeration and water retaining capacity. It maintained high organic matter content in soil, contained higher percentage of three primary nutrients e.g. nitrogen, phosphorus and potassium and could be used as fertilizer [33].

It has been suggested that during growth on straw, *Pleurotus* released humic acid like fractions when added to soil, which increased its fertility [50]. Humic substances may affect plant biochemical process [47]. Addition of straw in soil caused an increase in the number of total bacteria, actinomycetes and fungi of rhizosphere [42]. Yield of green gram increased in plots previously supplied with mushroom spent rice straw [30]. *Pleurotus* waste was adequate to sustain the growth of *Salvia officinalis* by improving air porosity and mineral content of soil [20]. For improving the biological basis for long-term agricultural sustainability, greater emphasis needs to be placed on management of renewable resources within the crop production system. Microorganisms as biological control agents have high potential to control plant pathogens and no effect on the environment (or) other non-target organisms [10]. Use of spent mycelium substrate of *P.florida* is an alternative for promoting plant health and productivity in agricultural systems, thus resulting in decreased usage of pesticides, fertilizers and nutrients, leading to environmental and economic benefits.

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