



Application of *Pleurotus ostreatus* Spent Extracts on Enhancement of Faba Bean Production

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Authors' contributions

This work was carried out in collaboration between both authors. Author AG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author IEA managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Microbial extracts are eco-friendly and reduce threats effects of chemical fertilizers in the agricultural field. Rice straw used for of *Pleurotus ostreatus* (an edible fungus) production. The residual spent mushroom (SM) was extracted in alkaline medium (with 0.5 M Potassium hydroxide) and the obtained extract was assayed for carbohydrates, reducing sugar, organic acids and NPK-content. In vitro, the ability of these extracts to support the growth of beneficial microorganisms (*Azospirillium* sp, *Azotobacer, Bacillus subtilis, Enterobacter cloacae* and *Trichoderma* sp.) were evaluated. Spent mushroom extracts (SME) used for improving faba bean production in this proposal. Faba bean cultivars Sakha 4 and Misr 3 were conducted during two winter seasons; pots (2017-2018) and field experiment (2018-2019) at Agricultural Research Center, Sakha Agriculture Research Station, Kafrelsheikh, Egypt. The SME was applied with three methods (Spry, soil amendments and mixed of spry + soil amendment). The results revealed that the 10% w/v SME is rich with polysaccharide, reducing sugar, organic acids (Humic acid, Oxalic acid, Citric acid, Lactic acid, Ascorbic acid, Maleic acid, Formic acid, Salsylic acid) in addition NPK and microelements. It was also able to support the logarithmic growth of the previous beneficial microorganisms with (4.7, 3.8, 5.4 and 3.85 cfu ml⁻¹), respectively while *Azotobacter* was not able to grow in this media.



The experiments results shown that SME enhanced the vegetative growth (plant length, number of branches, number and dry weight of nodules, grain yield, and weight of 100 grain) and chemical composition (NPK) of treated plants compared with untreated ones.



Keywords: Faba bean; spent mushroom extract; organic acid; polysaccharides; HPLC.

1. INTRODUCTION

Fertilizers and additives have become a way of plant life, creating their nutrient request. But from the point of environmental view, it is necessary to demonstrate the risks of mineral fertilizers and find out new possibilities for a good sensorial aspect of other eco-friendly improvements. Mushrooms are moral sources of proteins, vitamins, and minerals, and create several bioactive materials like Lentinula edodes. Ganoderma lucidum and Cordyceps militaris, for example, polysaccharides [1,2,3]. The farming of mushrooms presence an efficient source of food (fruit bodies) and reduces environmental pollution by utilizing various waste products generated from agriculture, forestry and food processing, as a growth media [4]. Spent mushroom substrate (SMS) is the main byproduct of mushroom industry after sporocarp has been removed from the cultures.

Spent mushroom compost SMC is composed of fungal mycelia, extracellular enzymes created from mushrooms for deprivation of materials, and un-used lignocellulosic materials. SMC has been used in the production of value-added products such as biogas [5] and bulk enzymes [6] for bioconversion into organic fertilizer [7] and for use as animal feed supplements [8]. These procedures profit for mortal healthiness and the environs because of an agricultural unused practice the raw material for novel processes.

Currently, some spent substrates are applied as fertilizers, which serve as a source of organic nitrogen [9]. Numerous revisions have been

directed to recycle the spent substrates as constituents of materials used for the agronomy of mushrooms [10,11].

Recently, SMSs have been engaged for separating numerous beneficial metabolites, like polysaccharides and numerous enzymes [12,13,14]. However, despite these achievements, the best and most economically viable method for disposal of SMS is considered to be its utilization in successive mushroom cultivation.

Therefore, using biofertilizers of microbial origin is a demand for unpolluted and harmless natural. Synthetic fertilizers, particularly nitrogen, can extremely drain the nutritional content of foods. Nitrate, the final breakdown product of nitrogen fertilizers, collects in groundwater due to sudden increase using mineral nitrogen and thus can severely affect human health [15].

SMC was very effective and beneficiary for several cucumber growths such as total yield and fruit width obtained during the whole vegetation period were statistically significant in greenhouse harvesting [16].

The objective of our study was to evaluate the bioactive spent mushroom extracts application on faba bean by spraying individually or added to soil or in combination compared with 100% mineral fertilizer application.

2. MATERIALS AND METHODS

The Spent Mushroom Substrate (SMS) after three harvesting cycles of *P. ostreatus*, was

obtained from Microbiology Laboratory, Sakha Agriculture Research Station, Soils, Water and Environment Research Institute. Agriculture Research Center, Egypt. After drying at 70°C it was ground to a particle size of 250 μ m and stored in an airtight container at 4°C for further use. The dried SMS powder was soaked in 0.5 M KOH solution at room temperature for 24 hours then filtered using gauze.

2.1 Polysaccharide and Reducing Sugars Assay

The spent mushroom extract solution was concentrated in rotary evaporator at 40°C, then precipitated with cold ethanol 3:1 and centrifuged at 6000 rpm for 15 min. The precipitate was redissolved in distilled water and re-precipitated with 65% ethanol twice to remove salts, excess small sugars and other ethanol-soluble compounds. The final crude polysaccharide obtained by centrifugation was dried at 70°C. The total carbohydrate contented was assayed by the phenol sulfuric acid method using glucose as the standard [17]. The harvest of polysaccharide from dried SMS was considered by the next principle: Polysaccharide yield (%) = [(total sugar content × weight of extract)/dry weight of SMS] × 100 [13]. Reducing sugars were estimated using dinitrocalysilic acid method [18].

2.2 Humic acid Estimation

The method is based on a modification of the method described by [19]. 100 ml of SME was completed to 1 L by 0.1 N NaOH formerly varied for 2 h by magnetic stirrer. The alkaline mixed extract was centrifuged and the pH was then acidified to pH 2.0 using concentrated HCI and gone to stand overnight. The humic acidcontaining precipitate was obtained by centrifugation. The HA was then purified by repeated washing with dilute HCI and centrifugation [19] and dried in the same centrifuge tubes at 100°C for 24 h and weighing.

2.3 Extraction of Organic Acids from Spent Mushroom Substrate

Organic acids extraction was performed according to a described procedure [20]. 1 g sample was thoroughly mixed with methanol (20 mL), at 40°C. The methanolic extract was filtered, concentrated to dryness under reduced pressure (40°C), and redissolved in acid water (pH 2 with HCI). This aqueous extract was

evaporated to dryness under reduced pressure (40°C) and redissolved in 0.01 N sulfuric acid (1 mL),

2.4 Determination of Organic Acids by HPLC

The separation of organic acids was achieved with an analytical HPLC unit (mgl^{-1}) , using C18 column in conjunction with a column heating device at 30°C. Elution was carried out isocratically with 0.01 N sulfuric acid as the mobile phase, at a flow rate of 0.1 mL / min, for 120 min. Detection was performed with a UV detector set at 214 nm.

2.5 SME Evaluation in Media of Beneficial Microbe

Different concentrations of SME (2.5, 5 and 10%) in tab water were prepared, the pH was adjusted to 7.2 using HCl or KOH and the final solutions were distributed in 250 ml Erlenmeyer flasks then autoclaved at 121°C for 15 min. after cooling to room temperature, flasks were inoculated with *Azospirillum* sp., *Azotobacter* sp., *B. subtilis, E. cloacae* and *Trichoderma* sp.) in triplicates then incubated on orbital rotary shaker at 30°C and 150 rpm for 5 days. At the end of the incubation period, the microbial count was determined by plate count technique using nutrient agar for counting *Azospirillum* sp., *Azotobacter* sp., *B. subtilis, E. cloacae* and potato dextrose agar for *Trichoderma*.

2.6 Evaluation of SMS Application on Faba Bean Growth and Productivity

The experiments were conducted at the Agricultural Research Center, Sakha Agriculture Research Station, Kafrelsheikh, Egypt. Faba bean grains (Sakha 4 and Misr 3) were kindly supplied from Department of Cereals, Field Crop Research Institute, Agricultural Research Center, Sakha Agriculture Research Station, Kafrelsheikh, Egypt.

Treatments

- 1. Control treatment was with recommended minerals fertilizers.
- 2. Shoot system sprayed with spent mushroom extracts at 30 and 60 days of plant age.
- 3. Addition of spent mushroom to the rhizosphere of plants at 30 and 60 days of plant age.

4. Mixed application by spray and soil-applied of spent mushroom at 30 and 60 days of plant age.

Greenhouse and field experiments were carried out to investigate the effect of SME on two faba bean cultivars (Sakha 4 and Misr 3) production. The used pots were about 30 cm in diameter and 35 cm in height filled with 8.5 kg clayey soil obtained from the same location of the field experiment. Composite surface soil samples (0 -25 cm depth) were taken just before experimenting. The soil samples were air-dried, crushed and sieved through 2 mm sieve, and subjected to chemical characterizations according to [21]. The soil was slightly alkaline with pH 7.9, EC 2.78 dSm⁻¹, organic matter 1.72% and total carbonate 3%. The soil was clayey with clay 44% clay, silt 28.64 and sand 27.30. soluble cations recorded (7.22, 6.8, 13.0, 0.24) for (Ca⁺, Mg⁺⁺, Na⁺, K⁺), respectively where anions were (5.0, 13.44, 8.83) for (HCO3⁻, Cl⁻, SO4⁻⁻), respectively.

Fertilizer application: Greenhouse pots and field soils were fertilized with superphosphate at a rate of 240 kg ha⁻¹ during preparation and potassium Sulphate (at rate of 120 kg ha⁻¹) and an activating dose of urea 15 kg fed ⁻¹ was added at the beginning of seed sawing depending on the presence of native Rhizobia on the experimental soil, the pots were also fertilized by the equivalent quantity as in field experiment proportional to its size.

SME application: SME was diluted to 1:4 using tab water, and each pot was received 5 ml by one of the following methods, spraying on the shoot, amendment to the soil or spraying + amendment to the soil. The previous applications were started at the 20^{th} day of plant age and repeated at 40^{th} and 70^{th} day. The same procedure was used in field trial using $20Lfed^{-1}$ SME.

Plant growth analysis: At the harvesting, plants were harvested and subjected to the following analyses: After drying in an oven at 70°C until a constant weight; dry weight (g/plant), total number and dry of branches, kernels, nodules and grains/plant and grain dry weight (weight of 1000 grain) determined.

Chemical analyses: For determination of N, K contents expressed as plant samples or grains were dried and 0.2 g were incubated in 5 ml H_2SO_4 and 1 ml perchloric acid in a conical flask

for 24 h as described by Chapman and Parker [22]. The digested materials were completed to 50 ml H₂O and then distilled by a micro-Kieldahl method and the nitrogen concentration of distillate was determined by titration against 0.02 normal H₂SO₄ according to Black et al. [23]. Phosphorus concentration of samples was determined calorimetrically according to the methods described by Snell and Snell [24]. N, P, K and Na contents were calculated according to Black et al. [23]. Element content = element % x dry weight/ 100. Total chlorophyll was determined by a Minolta chlorophyll meter SPAD-502 for plant in the field at 60 days after sowing.

Micronutrients: In the digested solution, micronutrients (Zn, Fe and Cu) were measured using atomic adsorption spectrophotometer (Perkin Elmer 3300) according to Cottenie et al. [25].

2.7 Statistical Analysis

The collected data were subjected to statistical analysis, using the analysis of variance (ANOVA). LSD range tests were used to compare differences between the means [26].

3. RESULTS AND DISCUSSION

The chemical composition of spent mushroom extract was reported in Table 1. It was alkaline with pH 9.6 and EC 14.3 dSm⁻¹. Polysaccharides were 10.2 gl⁻¹, reducing sugar was 0.8 gl⁻¹ and organic acids (Humic acid, Oxalic acid, Citric acid, Lactic acid, Ascorbic acid, Maleic acid, Formic acid, Salsylic acid; 50, 40.8, 110.0, 29,6, 7,25, 79.4, 1217.7 and 46.8 mgl⁻¹, respectively). The SME also, contain a considerable concentration from NPK with 200, 0.3 and 102 ppm, respectively. This agreed with [27] who showed that spent mushroom compost contains about 1-2% nitrogen, 0.2% phosphorus and 1.3% potassium.

The data presented in Fig. 1 showed the nutrition value of SME for beneficial microbes. It was able to support the growth of *Azospirillum* sp., *B. subtilis*, *E. cloacae* and *Trichoderma* media containing 10% spent mushroom extract gave the highest microbial growth especially in case of *Trichoderma* and *E. cloacae*. It was noticed that, with decreasing the spent mushroom concentration in the media, the microbial growth decreased. This may be due to providing the main requirements for growth of microorganisms

like carbon, nitrogen, phosphorous and potassium in addition to microelements. Dakshayini and Mallesha, [28] showed that spent mushroom substrates richen with plant growthpromoting microorganisms conserved their survivability at different time intervals. These findings support the idea that SME can promote microbial activity in soil or introduce nutritive services to the microflora on plant surfaces when sprayed on shoot or root system [28].

Table 1. Chemical composition of 10% (W/V) extract of spent mushroom

Parameter	Value
рН	9.6
EC	14.3
Polysaccharides	10.2 gl⁻¹
Reducing sugars	0.8 gl ⁻¹
Organic carbon	1.25 %
Humic acid	0.5 gl⁻¹
Oxalic acid	40.8 mgl ⁻¹
Citric acid	110.0 mgl ⁻¹
Lactic acid	29.6 mgl⁻¹
Ascorbic acid	7.25 mgl⁻¹
Maleic acid	79.4 mgl⁻¹
Formic acid	1217.7 mgl⁻¹
Salsylic acid	46.8 mgl⁻¹
Ν	200 ppm
Р	0.3ppm
К	102 ppm
Cu	0.011 ppm
Fe	33.4 ppm
Zn	5.54 ppm

The potential of spent mushroom extract on two faba bean cultivars growth parameters was studied in the current experiments. In the pot experiment at 60 days of sowing Table 2, the results showed an effect of spent mushroom on nodule formation by the native rhizobia. It was found that spent mushroom increased the nodulation of the different cultivars compared with control. In the same time, the effect of dual application of soil+ spray treatment gave the best nodulation compared with the sporadic used of sprav or soil application. Misr 3 was more responsive to the treatment more than Sakha 4. The studied SME was rich in polysaccharide. reducing sugar, minerals and humic acid whish required to plant development as described by [29] that vitamins and polysaccharides are essential in a minimal quantity to keep usual development of plant and play an essential role in the regulation of metabolism as described by [30].

In the same experiment at 60 days of sowing Table 2, dry weight of nodules and plants were reported and the dual effect of spray + soil spent mushroom application gave the highest nodule dry weight. Misr 3 cultivar was more positively responded (0.35 g per plan) to SME than Sakha 4 cultivar. On the other hand, increasing in plant dry weight of treated plants may be attributed to organic content (humic substances, polysaccharides, sugars etc.) of spent mushroom and this agreed with [9] who reported that some spent substrates are used as fertilizers, which serve as a source of organic nitrogen. A humic acid application caused a marked increase in dry weight/plant [31]. Furthermore, [32] confirmed that humic acid was able to produce positive effects in improving dry biomass of faba bean and common bean plants.

In the pots experiment at 60 days of sowing, the plant height also was affected by SME application as shown in Table 2. All the treatments were increased compared with control except spray treatment of Misr 3 plants was the lowest with 67.0 cm per plant. Misr 3 and Sakha 4 plants treated with dual application of soil amendment + spray and spray only treatments were the pest with 82.6 and 80.0 cm per plant, respectively. This agreed with Dawood et al. [31] who showed that a humic acid application caused marked increases in shoot height, number of branches and leaves/plant, and fresh and dry weight/plant [31]. Also, the leaf area of faba bean plants increased with SME treatments compared with control. Misr 3 plants treated with soil amendment + spray gave the best leaf area (124.6 cm²). This agreed with [16] who showed SME was a significant increase of leaf area and fresh weight compared with control.

Chlorophyll is a green pigment found in plants. Chlorophyll is the molecule that absorbs sunlight and uses its energy to produce carbohydrates from CO₂ and water. In this study chlorophyll a, b ant total chlorophyll evaluated as one of healthy important plant parameter and directly affected with spent mushroom effect. Regarding to chlorophyll content, spraying SME on plant shoot of Misr 3 cultivar was the best compared with the other treatments as reported in Table 3. All the treatment gave a positive response to the spent mushroom application compared with control. In this context, Polat et al. [16] reported that the product of mushroom processing was very effective and beneficiary for cucumber growth in greenhouse production [16]. Moreover, humic acid, polysaccharides motivate plant enzymes/ hormones and improve soil fertility in an

ecologically and environmentally benign manner [33,34]. As reported in Table 3 spray treatments of the two faba bean cultivars gave the highest chlorophyll A, B and total chlorophyll content

compared with other treatments this agreed with [16] who showed that SME causes a significant increase in leaf area and cholorophyll content compared with control.

 Table 2. Effect of spent mushroom extract on faba bean growth parameter at 60 days of sowing in the pot experiments

Treatments	No.	Nodules dry Plant DW. (g)		Leaf area	Plant height (cm)	
	nodules/plant	weight. Plant (g)		(cm²)		
Мс	23. 7	0.17	14.4	63.8	67.3	
M1	65.6	0.22	15.1	77.1	67.0	
M2	77.0	0.28	18.1	122.2	71.3	
M3	44.3	0.35	16.2	114.6	82.6	
Sc	24.0	0.20	15.9	74.2	62.6	
S1	33.6	0.22	16.7	73.1	69.6	
S2	32.4	0.22	18.2	86.1	76.0	
S3	31.0	0.23	18.5	103.6	80.0	
LSD 0.05	6.23**	0.056**	1.77**	19.11**	6.78**	

C: Control 1: Spray 2: (Spray+ Soil application) 3: Soil application S: Sakha 4 M: Misr 3 DW: dry weight

 Table 3. Effect of spent mushroom on faba bean chemical composition (NPK and chlorophyll)

 at 60 days of sowing in the pot experiments

Treatments	N% plant ⁻¹	P% plant ⁻¹	K% plant ⁻¹	Chl A	Chl B	Chl A+B
Мс	1.15	0.22	1.37	13.60	10.57	24.17
M1	1.38	0.25	1.82	13.95	12.31	26.26
M2	1.44	0.32	2.55	15.47	13.31	28.78
M3	1.27	0.28	2.25	14.48	12.42	26.90
Sc	1.08	0.19	1.34	13.72	10.69	24.42
S1	1.28	0.23	1.80	15.73	11.28	27.00
S2	1.33	0.29	2.52	16.01	12.08	28.09
S3	1.21	0.25	2.21	15.80	13.46	29.26
LSD 0.05	0.064**	0.041**	0.113**	0.93**	1.14**	1.26**

C: Control 1: Spray 2: (Spray+ Soil application) 3: Soil application S: Sakha 4 M: Misr 3 DW: dry weight



Fig. 1. Evaluation of spent mushroom as nutrient sources some microbial growth

Chemical composition of plants was studied in the pots experiment at 60 days of sowing Table 4. It was found that all treatments of spent mushroom recorded the best NPK% compared with control. Furthermore, soil treatment with spent mushroom of Misr 3 plants gave the best treatment NPK% compared with other treatments. Sakha 4 cultivar was positive responses to soil treatment more than other treatments. Increased in the NPK% compared with control may be due to organic acids, polysaccharide, mineral and humic acid. Also, the effectiveness of spent mushroom compost (SMC) as cucumber and common bean in a greenhouse soil application was recorded by [16,32].

In the field experiment, the data collected at two different times 70 and 128 day of sowing. The evaluation of the spent mushroom effect on two faba bean cultivars was carried out in the Sakha agriculture research station at season 2019. The data in Table 5 showed the effect of the previous treatments on faba bean cultivars growth parameters (No. and dry weight of nodules plant⁻¹g, plant dry weight (g) and plant height (cm) at 70 day of sowing.

Spent mushroom extraction increased nodulation process (nodule and nodules dry weight plant⁻¹). Dual application effect of soil + spray treatment gave the greatest nodulation number with Misr 3 (78.67 plant⁻¹). While soil treatment gave the best nodules dry weight plant⁻¹ and it was 0.38 g plant⁻¹ with Misr 3. On the other hand, soil amendment with SME increased plant length of Sakha 4 compared with other treatments and recording 81.33 cm. This may due to the rich minerals, reducing sugar, polysaccharides and humic acid which found in the SME. In these contacts, [35,36,37] showed that foliar spray of HA and polysaccharides improved plant growth, harvest, and quality because of its action on dissimilar physiological and metabolic progressions. HA increased nutrient absorption, cell separation, photosynthesis [38], exhalation, biosynthesis of nucleic acid and enzyme, and overall, dry weight of the plant [37].

Table 4. Effect of spent mushroom extract on faba bean growth parameters the field
experiments at 70 days

Treatments	No. nodules/plant	Nodules dry weight Plant (g)	Plant dry weight. (g)	Plant height (cm)
Мс	24.67	0.21	15.60	69.00
M1	67.67	0.24	16.99	68.00
M2	78.67	0.30	18.30	73.67
M3	46.00	0.38	16.93	84.33
Sc	25.67	0.22	16.77	65.33
S1	35.33	0.24	17.62	73.00
S2	34.33	0.25	19.23	76.00
S3	32.67	0.24	18.73	81.33
LSD 0.05	5.13**	0.042**	n.s	5.82**

C: Control 1: Spray 2: (Spray+ Soil application) 3: Soil application S: Sakha4 M: Misr 3 DW: dry weight

 Table 5. Effect of spent mushroom on faba bean chemical composition (NPK and chlorophyll)

 at 70 days of sowing in the field experiments

Treatments	N% plant ⁻¹	P% plant ⁻¹	K% plant ⁻¹	Chl A	Chl B	Chl A+B
Мс	1.16	0.25	1.39	13.92	10.96	24.89
M1	1.39	0.27	1.84	14.09	12.47	26.57
M2	1.46	0.34	2.58	15.74	13.48	29.22
M3	1.3	0.3	2.29	14.66	12.69	27.35
Sc	1.09	0.2	1.35	14.10	11.42	25.52
S1	1.30	0.25	1.82	16.22	11.61	27.84
S2	1.34	0.30	2.55	16.29	12.30	28.60
S3	1.23	0.27	2.29	15.98	13.85	29.82
LSD 0.05	0.067**	0.038**	0.091**	0.83**	0.58**	0.94**

C: Control 1: Spray 2: (Spray+ Soil application) 3: Soil application S: Sakha 4 M: Misr 3

The data of Table 6 showed the effect of SME on NPK%, chlorophyll a, chlorophyll b and total chlorophyll of faba bean cultivars at 70 day of sowing in the field experiment. Application of SME on Misr 3 increased NPK% compared with other treatments. All in all, SME application gave the best NPK% results and compared with control. Increased of NPK% with SME application may be due to its minerals (NPK), organic carbon and polysaccharides (carbon source) content compared with control treatment. Nitrogen content varies from 0.4-13.7% and also contains cations like K^+ , Na⁺, Ca₂⁺ and Mg₂⁺, and anions like Cl⁻, NO₃⁻ and SO₄²⁻, all essential for optimal plant growth development as [39] recorded.

The dual effect of spray + soil treatment of SME gave the best chlorophyll a with Sakha 4, while soil treatment with SME alone gave the best chlorophyll b and total chlorophyll content with Sakha 4 (16.29, 12.30 and 28.6), respectively. [40] showed that SMC humic acid had a positive effect on growth parameters and photosynthetic pigments. The positive effect of humic acid and polysaccharides on photosynthetic pigments could be attributed to an increased in CO_2 assimilation and photosynthetic rate [41].

These increases might be due to the role of humic acid in increasing rubisco enzyme activity and then increased the photosynthetic activity of plants and its yield [42].

Effect of SME on faba bean growth parameters (plant dry weight g plant⁻¹, plant height (cm), dry weight of g plant⁻¹, number of pods plant⁻¹, seed yield g and dry weight of 100-seed g) at harvesting after 128 day of sowing in the field experiments were reported in Table 6. Treatment of Misr 3 with soil+ spray treatment SME gave the highest plant length and dry weight compared with other treatments. All treatments with SME gave a good plant length and dry weight compared with control. A number of pods and dry weight recorded in Table 6 in the field experiment at harvest. Soil treatment of Misr 3 gave the greatest pods number and dry weigh compared with other treatments (13.7 pod plant⁻¹ and 37.7 g plant⁻¹, respectively). Control treatment gave the lowest pods number with 3.1 pod plant⁻¹. It may be due to SME which contain minerals, organic carbon, polysaccharides, reducing sugar and organic humic acid. [43,44] reported that application of SMC as an organic material source caused statistically important effects on dry

 Table 6. Effect of spent mushroom extract on faba bean growth parameters at harvesting of the field experiments 128 days of sowing

Treatments	Plant DW g/plant	Plant height cm	Pods DW g/plant	Number of pods/plant	Seed yield g/ plant	100- seed g
Мс	32.9	115.17	20.64	3.11	17.04	51.30
M1	37	135.83	24.63	8.67	20.41	62.46
M2	45.57	145.67	37.69	13.56	26.24	72.88
M3	41.07	136.50	25.64	7.00	21.62	56.00
Sc	30.73	129.27	19.64	3.22	16.07	52.03
S1	36.2	130.33	25.74	8.22	22.32	64.90
S2	42	142.00	24.99	10.44	25.86	50.17
S3	34.6	131.23	23.63	6.22	20.74	58.93
LSD 0.05	3.65**	13.71**	n.s	1.94**	2.59**	n.s

C: Control 1: Spray 2: (Spray+ Soil application) 3: Soil application S: Sakha G: Giza DW: dry weight

 Table 7. Effect of spent mushroom extract on faba bean chemical composition at harvesting of the field experiments 128 days of sowing

Treatments	N% straw	P% straw	K% in straw	N% seed	P% seed	K% in seed
Мс	0.33	0.10	0.26	2.60	0.23	1.13
M1	0.46	0.13	0.46	3.47	0.33	1.25
M2	1.58	0.23	1.32	4.57	0.50	1.53
M3	1.45	6.11	1.11	4.33	0.39	1.38
Sc	0.33	0.10	0.23	2.45	0.27	1.13
S1	0.43	0.12	0.47	3.33	0.32	1.21
S2	1.55	0.21	1.00	4.53	0.50	1.47
S3	1.41	0.16	0.50	4.24	0.37	1.36
LSD 0.05	0.21**	n.s	0.18**	0.36**	0.064**	0.056**

C: Control 1: Spray 2: (Spray+ Soil application) 3: Soil application S: Sakha 4 M: Misr 3

matter contents in the pepper plant grown in greenhouse soil. These increased might be due to the role of humic acid and polysaccharides in increasing rubisco enzyme activity and then increased the photosynthetic activity of plants and its yield [42]. Also, soybean dry and fresh weight, fruit fresh weight, pod no, plant height, leaf number and leaf area, have also been significantly influenced by SMS at (P< 0.05) [45].

The harvest index production of faba bean was reported in Table 6 and showed decreased in seeds per plant and 100 seeds dry weight of control compared with other treatments. Application of SME on faba bean plants with soil + spray treatment increased production. Spent mushroom humic compounds may have various positive biochemical effects at cell wall and in the cytoplasm (including photosynthesis, respiration rates, enzymatic activities, protein synthesis) this agreed with [46] and plant hormone-like activity [47]. These increases might be due to the role of humic acid, polysaccharides and menials in swelling rubisco enzyme activity and its yield [42]. Alternatively, increasing harvest and 100 seed dry weight may be as a result of the potassium's role of stimulating the enzyme doings and enhancing the translocation of integrates and protein production as affirmed by [48,49]. SMC humic acid had a helpful effect on photosynthetic pigments, seed harvest, and harvest components, as well as some biochemical constituents of the, yielded faba bean seeds [40]. These increases might be because of the role of humic acid in swelling rubisco enzyme motion and then improved the photosynthetic commotion of plants and its profit [42].

These outcomes endorse those of [50] who exposed amended harvest attributable to potassium higher close application. Similarly, these outcomes are in covenant with the finding of [51] who emphasized higher grain incomes weight with bigger potassium rates and [52].

Oppositely, the chemical structure of straw, plants and seeds were noticed in Table 7. Application of SME treatment significantly increased all NPK% in compared with control and dual effect of soil + spray was the most promising usage this can be attributed to SME rich formula. These believed with [53] they showed that use of spent mushroom compost in growing agricultural yields improved maintainable agriculture or creation of food yields. Afagh et al. [40] showed that absorption of K improved pointedly by collective of SMC percentage in

growing media but there was no significant difference in N and P uptake in SMC usages [40]. [54] showed that humic acid, polysaccharides, organic matter and menials in were practical to sandy soils, it adds vital organic substances necessary for water retention, thus improving the root growth and enhancing the sandy soil ability to retain and not leach out vital plant nutrients. Low-molecular-weight organic acids are considered to be effective in the release of inorganic phosphorus (P) [55]. HA application produced observable growths of the pod and seeds number/pod, dry weight of 100 seeds, seed yield plant⁻¹, and seed yield feddan⁻¹ relative to control [31].

4. CONCLUSION

Spent mushroom extracts are a good source of mineral, organic carbon and are working as ecofriendly fertilizers. It has an appositive consequence on faba been growth parameters and harvest. SME was the affirmative effect on native microorganisms as nitrogen-fixing bacteria and this positive reflected on plant health and growth parameters.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Mizuno T, Wang J, Zhang H Kawagishi T, Nishitoba, Li JX. Reishi, Ganoderma lucidum and Ganoderma tsugae: Bioactive substances and medicinal effects. Food Rev. Int. 1995;11:151-166.
- Wasser SP, Weis AL. Medicinal properties of substances occurring in higher basidiomycetes mushrooms: Current perspectives (review). Int. J. Med. Mushr. 1999;1:31-62.
- Wang M, Meng XY, Yang RL, Qin T, Wang XY, Zhang KY, Fei CZ, Li Y, Hu YL, Xue, FQ. Cordyceps militaris polysaccharides can enhance the immunity and antioxidant activity in immunosuppressed mice. Carbohyd. Polym. 2012;89:461-466.
- 4. Sanchez C. Cultivation of *Pleurotus* ostreatus and other edible mushroom.

Appl. Microbiol. Biotechnol. 2010;85:1321-1337.

- Bisaria R, Vasudevan P, Bisaria VS. Utilization of spent agroresidues from mushroom cultivation for biogas production. Appl. Microbiol. Biotechnol. 1990;33:607-9.
- Kumaran S, Sastry CA, Vikineswary S. Laccase, cellulase and xylanase activities during growth of Pleurotus sajorcaju on sago hampas. World J. Microbiol Biotechnol. 1997;13:43-9.
- Soechtig H, Grabbe K. The production and utilization of organic-mineral fertilizer from spent mushroom compost. Mushroom Sci. 1995;2:907-16.
- Vikineswary S, Kumaran KS, Ling SK, Dinesh N, Shim YL. Solid substrate fermentation of agroresidues for value added products: The Malaysian experience. In: Wise DL, editor. Global environmental biotechnology. Dordrecht: Springer. 1997;301-5.
- Hackett R. Spent mushroom compost as a nitrogen source for spring barley. Nutr. Cycl. Agroecosyst. 2015;102:253-263.
- Royse DJ. Recycling of spent shiitake substrate for production of the oyster mushroom, Pleurotus sajorcaju. Appl. Microbiol. Biotechnol. 1992;38:179-182.
- González Mature R, Figlas D, Curvetto N. Agaricus blazei production on noncomposted substrates based on sunflower seed hulls and spent oyster mushroom substrate. World J. Microbiol. Bio-technol. 2011;27:1331-1339.
- 12. Singh AD, Abdullah N, Vikineswary S. Optimization of extraction of bulk enzymes from spent mushroom compost. J. Chem. Technol. Biotechnol. 2003;78:743-752.
- He P, Li F, Huang L, Xue D, Liu W, Xu C. Chemical characterization and antioxidant activity of polysaccharide extract from spent mushroom substrate of Pleurotus eryngii. Journal of the Taiwan Institute of Chemical Engineers. 2016;69:48–53. Available:http://dx.doi.org/10.1016/j.jtice.20 16.10.017
- Zhang JJ, Meng GY, Zhai GY, Yang YH, Zhao HJ. Extraction, characterization and antioxidant activity of polysaccharides of spent mushroom compost of Ganoderma lucidum. Int. J. Biol. Macromol. 2016;82: 432-439.
- 15. Gupta R, Yadav A, Garg VK. Influence of vermicompost application in potting media on growth and flowering of marigold crop.

– International Journal of Recycling of Organic Waste in Agriculture. 2014;3-47.

- Polat E, Uzun HI, Topçuoglu B, Onal K, Onus AN, Karaca M. Effects of spent mushroom compost on quality and productivity of cucumber (Cucumis sativus L.) grown in greenhouses. Africa Journal of Biotechnology. 2009;8(2):176-180.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956;28:350–6.
- 18. Miller GL. Anal. Chem. 1972;31:426.
- 19. Schnitzer Μ. Organic matter characterization. In Methods of soil analysis, part 2: Chemical and microbiological properties, ed. Page AL, Miller RH, and DR Keeny, Madison, Wisc .: American Society of Agronomy. 1982;581 594.
- Silva BM, Andrade PB, Mendes GC, Seabra RM, Ferreira MA. Study of the organic acids composition of quince (Cydonia oblonga Miller) fruit and jam. J. Agric. Food Chem. 2002;50:2313-2317.
- Richards LA. (ed.) Diagnosis and improvement of saline and alkaline soils. U.S.D.A. Agric, Handbook No. 1954;60.
- Chapman HD, Parker FP. Methods of analysis for soils, plants and waters. Univ. California. Div. of Agric. Sci. 1963;941– 947.
- Black AC, Evans DD, White JL, Ensminyer EL, Clark EF. Methods of soil analysis. Amer. Soc. Agro. Inc. Mad. Wise U. S. A; 1965.
- 24. Snell FD, Snell CT. Colorimetric methods of analysis. D. Von. Nastrand company, Inc. 1967;551-552.
- 25. Cottenie A, Verloo M, Velglie G, Kiekens L, Camerlynck R. Biological and analytical aspects of soil pollution. Lap of Analytical Agro. State Univ. Ghent-Belglum. 1982; 218.
- 26. Steel RGD, Torrie JH. Principles and procedures of statistics 2nd Ed. McGraw Hill, Newyork; 1980.
- Uzun I. Use of spent mmushroom compost in sustainable fruit production. J Fruit Ornam Plant Res. 2004;12:157–165.
- Dakshayini G, Mallesha BC. Survivability of Plant Growth-Promoting Microorganisms on Spent Mushroom Substrate. Int. J. Curr. Microbiol. App. Sci. 2018;7(11):1880-1891. DOI:https://doi.org/10.20546/ijcmas.2018.7 11.213

- Bassouny FM, Hassanein RA, Baraka DM, Khalil RR. Physiological effects of nicotinamide and ascorbic acid on Zea mays plant grown under salinity stress. Il-Changes in nitrogen constituents, protein profiles, protease enzymes and certain inorganic cations. Aust. J. Basic Appl. Sci. 2008;2(3):350–359.
- Bronzetti G, Cini M, Andreoli E, Caltavuturo L, Panunzio M, Croce CD. Protective effects of vitamins and selenium compounds in yeast. Mutation Research. 2001;496:105–15.
- Dawood MG, Abdel-Baky YR, El-Awadi MES. Enhancement quality and quantity of faba bean plants grown under sandy soil conditions by nicotinamide and/or humic acid application. Bull. Natl. Res. Cent. 2019;43:28. Available:https://doi.org/10.1186/s42269-019-0067-0
- Meganid AS, Al-Zahrani HS, EL-Metwally MS. Effect of humic acid application on growth and chlorophyll contents of common bean plants (Phaseolus vulgaris L.)under salinity stress conditions. Int. J. Inn. Res. Sci. Eng. Technol. 2015; 4(5):2651–2660.
- Sarir MS, Sharif M, Ahmed Z, Akhlaq M. Influence of different levels of humic acid application by various methods on the yield and yield components of maize. Sarhad. J. Agric. 2005;21(1):75–81.
- Mart I. Fertilizers, organic fertilizers, plant and agricultural fertilizers. Agro and Food Business Newsletter. 2007;i-iv.
- Karakurt Y, Unlu H, Padem H. The influence of foliar and soil fertilization of humic acid on yield and quality of pepper. Acta. Agric. Scand. Sect B Plant Soil Sci. 2009;59(3):233–237.
- EI-Nemr MA, EI-Desuki M, EI-Bassiony, AM, Fawzi ZF. Response of growth and yield of cucumber plants (CucumissativusL.) to different foliar application of humic acid and biostimulators. Aust. J. Basic. Appl. Sci. 2012; 6(3):630–637.
- Said-AlAhl HAH, El Gendy AG, Omer EA. Humic acid and indole acetic acid affect yield and essential oil of dill grown under two different locations in Egypt. J. Pharm. Sci. Res. 2016;8(8):146–157.
- 38. Atiyeh RM, Edwards CA, Metzger JD, Lee S, Arancon NQ. The influence of humic acids derived from earth warm-processed

organic wastes on plant growth. Bioresour. Technol. 2002;84:7–14.

- Chorover J, Fox RH, Hatcher PG, Romaine CP. The nature, chemistry, and environmental impact of weathering spent mushroom substrate. June 15th Final Research Project Report. Penn. State: Mushroom Industry Farmer-based Applied Research Program (MIFBAR). 2000;87.
- 40. Afagh HV, Sara S, Hossein R, Khavari-Nejad RA. Influence of Spent Mushroom Compost (SMC) as an Organic Fertilizer on Nutrient, Growth, Yield, and Essential Oil Composition of German Chamomile (Matricaria Recutita L.), Communications in Soil Science and Plant Analysis. 2019; 50(5):538-548.

DOI:10.1080/00103624.2019.1568450

41. Ameri A, Tehranifar A. Effect of humic acid on nutrient uptake and physiological characteristic Fragaria x AnanassaVar: Camarosa. J. Biol. Env. Sci. 2012;6:77–79.

- 42. Delfine S, Tognett R, Desiderio E, Alvino A. Effect of foliar application of N and humic acids on growth and yield of durum wheat. Agron. Sustain. Dev. 2005;25:183-191.
- 43. Onal MK, Topcuoğlu B. The Effect of spent mushroom compost on the dry matter and mineral content of pepper (Piper nigrum) grown in greenhouse. The annual Conference on Tropical and Subtropical Agricultural and Natural Resource Management (Tropentag). Witzenhausen, Germany; 2007.
- 44. Rahman MS, Rahman MH, Chowdhary MFN, Sultana MS, Ahmed KU. Effect of spent mushroom substrate and cowdung on growth, yield and proximate composition of brinjal. International Journal of Scientific and Research Publications. 2016;6(10).
- 45. Jonathan SG, Lawal MM, Oyetunji OJ. Effect of spent mushroom compost of Pleurotus pulmonarius on growth performance of four Nigerian vegetables. Mycobiology. 2011;3:2833.
- Nardi S, Pizzeghello D, Muscolo A, Vianello A. Physiological effects of humic substances in plant growth. Soil Biol Biochemistry. 2002;34(11):1527–1536.
- Chen Y, Aviad T. Effects of humic substances on plant growth. In: Mac. Carthy P, Clapp CE, Malcolm RL, Bloom PR, editors. Humic Substances in Soil and Crop Science: Selected Readings. Madison, WI: American Society of

Agronomy Inc., Soil Science of America, Inc. 1990;161–186.

- Devlin RM, Witham FH. Plant physiology. 4thEd. CBS publishers and distributors 485, Jan Bhawan, Shadhara, Delhi, 110032 (India); 1986.
- Ibrahim HA, EI-Behairy UA, EL-Desuki M, Bakry MO, Abou–Hadid AF. Response of Green Bean to Fertilization with Potassium and Magnesium. Research Journal of Agriculture and Biological Sciences. 2010;6(6):834-839.
- 50. Abou Hadid A. Response of green bean to fertilization with potassium and magnesium. Research Journal of Agriculture and Biological Sciences. 2010; 6(6):834.
- Kanaujia SP, Raj Narayan, Sumati Narayan. Effect of phosphorus and potassium on growth, yield and quality of French bean (Phaseolus vulgaris L.) cv. Contender. Vegetable Science. 1999; 26(1):91-92.

- 52. Kwizera Chantal, Basil T. Iro Ongor, Kaboneka Salvator, Nkeshimana Fulgence, Ahiboneye Norbert. Effects of potassium fertilizer on bean growth and yield parameters. International Journal of Advances in Scientific Research and Engineering (ijasre). 2019V;5(1).
- Olfati JA, Khasmakhi-Sabet SA, Shabani H, Peyvast G. Alternative organic fertilizer to cow manure for French dwarf bean production. International Journal of Vegetable Science. 2012;18(2):190-198.
- 54. Khaled H, Fawy HA. Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. Soil Water Resour. 2011;6(1):21–29.
- 55. Lili W, Chengrong C, Zhihong X. Citric acid enhances the mobilization of organic phosphorus in subtropical and tropical forest soils. Biol Fertil Soils. 2010;46:765– 769

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