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Application Efficiency of Spent Mushroom Compost Extract, Cyanobacteria and Bacteria on Green Fruit and Seed Yield of Squash under Drip Irrigation System

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ABSTRACT

Biofertilizers (fertilizers of a microbial origin) are eco-friendly and can reduce hazards effects caused by uncontrolled application of chemical inputs. Two experiments were conducted during winter seasons of 2012-2013 and 2013-2014 in productivity greenhouse at Kaha, Qalyubia Governorate, Horticulture Research Station, Egypt to investigate the effect of spent mushroom compost extract (SMCE), plant growth promoting rhizobacteria (PGPR) and cyanobacteria (individually and their mixture) as soil drench biofertilizer on squash (var. Eskandarani) growth, green fruit yield and seed yield as well as to compensate half reduction of mineral nitrogen under drip irrigation system. Data revealed that all biological inoculations with 50% of recommended mineral nitrogen enhanced the vegetative growth (stem length, number of leaves, leaf area, fresh and dry weights of plant), seed yield, weight of 100 seeds and NPK values of leaves with best performance by their mixture. The magnitude of the recorded data showed an inverse relation between vegetative growth and total green fruit yield i.e. individual inoculation recorded better yield than combined ones because excess nitrogen may orient plants to vegetative growth rather than yield. Early green fruit yield didn't affect much assuming that the microbial amendments at early stage didn't illustrate their full expression. Nitrate content of squash fruit recorded significant decrease, as compared with the control value, with the different applications. Results also revealed that total bacterial counts, CO_2 evolution and soil enzymes (dehydrogenase and nitrogenase activities) were conspicuously affected by cyanobacteria, bacteria and/or SMCE treatments comparing with control. Results revealed that plant growth promoting rhizobacteria (PGPR), cyanobacteria and spent mushroom compost extract (SMCE) could be used as a good tool to promote squash growth, green fruit yield and seed productivity as well as soil microorganism activities and could replace about 50% of mineral nitrogen dose under drip irrigation system.

Key words: Squash, cyanobacteria, PGPR-bacteria, spent mushroom extract

Introduction

Squash (*Cucurbita pepo* L.) is one of the most popular vegetables grown in Egypt. Squash fruit contains more than 95% water, is low in calories, sodium and fat and is a good source of vitamin C. Its extracts (from different parts of the plant) contain biologically active components which show antidiabetic, antibacterial, antioxidant, anticancer, immune modulatory, and other miscellaneous effects. In recent years, the phenolic compounds of seeds (as dietary antioxidants) represent potentially health-promoting substances (Malešević *et al*., 2011).

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 amendments. Consequently, there is a demand for clean and safe natural biofertilizers and this could apply by using biofertilizers of microbial origin. Synthetic fertilizers, especially nitrogen, can seriously deplete the nutritional content of foods. Nitrate, the final breakdown product of nitrogen fertilizers, accumulates in ground water due to steep increase using mineral nitrogen and thus can be severely affect human health. N_2-fixing cyanobacteria, blue-green algae (BGA), can be used as an alternative to synthetic nitrogen and make a major contribution to the soil fertility and assist crops by supplying plant growth substances (Shariatmadari *et al*., 2011). BGA have potential applications in agriculture areas, as nutrient supplements, as biofertilizer, plant growth promoting and as biocontrol agents (Gupta et al., 2013). Thus, it can reduce the hazard effects of using synthetic mineral fertilizer. In this concern, Osman *et al*. (2010) found that two cyanobacterial species (*Nostoc entophytum* and *Oscillatoria angustissima*) as biofertilization combined with half the recommended dose of the chemical fertilizer was usually more effective than the addition of the full rate of the chemical fertilizer, and this may allow saving 50% of the used chemical fertilizer. Another microorganism's amendments which have great potential for use as a biofertilizer, are

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beneficial to plants and have various physiological activities are plant growth promoting rizobacteria (PGPR). Inoculation of some bacteria strains as biofertilizers could significantly increase the production of vegetable crops by means of sustainable and organic agricultural system. The authors (Venkadesaperumal *et al*., 2014) isolated twenty four bacterial strains from the soil to use them as inoculant in chili and tomato fields. Some strains have an excellent plant growth promoting properties such as IAA production. They also indicated that co-inoculation gave a more pronounced effects on seedling emergence, secondary root numbers, primary root length and stem length, while inoculation by alone isolate showed a lower effect. Furthermore, PGPR inoculations provide a means of improving growth and quality of cauliflower transplant under greenhouse conditions by increased the macro and micro nutrient content (Ekinci *et al*., 2014). Different bacterial inoculations increased cauliflower plant growth parameters such as fresh shoot weight, dry shoot weight, plant height, stem diameter, leaf area and chlorophyll contents. In addition, Elbeshehy *et al*. (2015) stated that PGPR strains (*Bacillus subtilis*, *Bacillus pumilus*) individually and in combination significantly enhanced the plant height and fresh weight of pumpkin plants under greenhouse experiments. Spent mushroom compost (SMC) which is a waste product of mushroom processing was very effective and beneficiary for cucumber growth in greenhouse production (Polat *et al*., 2009). The effects of SMC on several cucumber yield related characteristics, such as total yield and fruit width obtained during the whole vegetation period were statistically significant.

The objective of this study was to evaluate the effect of cyanobacteria PGPR-bacteria and spent mushroom compost extract (SMCE) application either individually or in combination on green fruit and seed yield of squash to compensate half reduction of mineral nitrogen under drip irrigation system.

Materials and Methods

Two plastic house field experiments were conducted during winter seasons of 2012/2013 and 2013/2014 at Kaha (Kalyiobia govemorate) Horticulture Research Station, Egypt. Seeds of squash (*Cucurbita pepo*) variety Eskandrani were obtained from Vegetable Crop Seed Production and Technology Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. Seeds were allowed to germinate at first week of November in a plastic house using seedling trays of 84 cells. The trays were filled with a commercial plastic house transplanting mixture [1 peat moss: 1 vermiculite (v/v)] amended with macro-and micro-nutrients. Sowing was carried out so that each cell of the tray had one seed covered with 0.5 cm of the sowing mixture. After 15 days, seedlings were transplanted to a plastic house of an area 540 m^2 (60 m long x 9 m width x 3 m height) and the experimental plot was 7.5 m^2. The experiments were laid out in a randomized complete block design with three replications. The soil texture of the experimental was clay loamy as represented in Table (1). Seedlings were planted on the two sides of each ridge (zigzag pattern) at 50 cm apart. Other agricultural practices as harrowing, pests and diseases control were carried out as commonly practiced for the conventional squash planting.

Table 1: The physiochemical properties of the plastic house soil used for squash planting.

Soil texture	Organic matter (%)	pH	E.C. (dS m^{-1})	Available N (ppm)	Available P (ppm)	Available K (ppm)
Clayey	1.2	8.22	0.446	42.5	7.65	337

Plants under plastic house were irrigated by drip irrigation system and fertilized according to the flowing application:

Treatments:

1. Control (100% of the recommended dose of mineral nitrogen fertilizer)
2. 50 % of the recommended dose of mineral nitrogen fertilizer
3. Cyanobacteria
4. Rhizobacteria
5. Spent mushroom compost extract (SMCE)
6. Cyanobacteria + Rhizobacteria
7. Cyanobacteria+ SMCE
8. Rhizobacteria + SMCE
9. Cyanobacteria+ Rhizobacteria + SMCE

*Treatments from 3 to 9 received 50% of the recommended dose of mineral nitrogen fertilizer

Cyanobacteria inoculum at the rate of 30 $l.fed^{-1}$, PGPR-bacteria inoculum at the rate of 20 $l.fed^{-1}$ and spent mushroom compost extract (SMCE) at the rate of 200 $l.fed^{-1}$ have been added as soil drench application in two equal portions; the first was after one month from transplanting and the second was after two months from transplanting. All treatments were received 50 % of the recommended dose of mineral nitrogen fertilizer expect the control was received 100%N (the recommended dose of mineral nitrogen fertilizer for squash plant). Phosphorus, potassium and other macro or micro elements were added as recommended.

Biological application

Spent mushroom compost extract (SMCE):

To prepare grain master spawn: sorghum seeds were cleaned and then soaked in water overnight. Dead seeds were removed then boiled in water for 15 min. After cooling the seeds were transferred to a round bottle (⅔ of its volume) and mixed with calcium carbonate 2% w\w and calcium sulphate 1% w\w. Bottles were sterilized for 1 hour at 121 ^{o}C. After cooling, the bottles were inoculated with mycelia discs (5 mm) diameter of 6 days old culture of white root *Pleurotus ostreatus* (was obtained from Dep. Microbiology, Soils water and Environment Research Institute. Agricultural Research Center (ARC), Giza, Egypt) then, the inoculated bottles were incubated at 25 ^{0}C for 15-20 days. Rice straws were chopped to lengths of 2-3 cm, soaked in water (approximately three times the weight of the straw) and left for 24 hr. to allow the water to completely penetrate into the straws. The soaked rice straw were transferred to autoclavable plastic bags (100 Kg/pile), were sealed and were pasteurized (90 ^{0}C for 2 hr.), this process was followed by cooling to room temperature for 24 hr. After cooling, each bag was inoculated with 50 g of previously prepared spawn (culture) of *P. ostreatus*, immediately sealed. The inoculated rice straw was incubated at 30± 2 ◦C under relative humidity of 60-70% for not less than 30 days until it became well decomposed. The spent mushroom compost (SMC) and its extract chemical analysis (Table, 2) was done according to standard methods as described by Brunner and WaSMCEr (1978). Spent mushroom compost extract (SMCE) was prepared by soaking one m^3 from the previous compost in 500 L water for 48 hr., then was squeezed, filtered and the filtrate was used as spent mushroom compost extract (SMCE) according to the method described by Nasef *et al*. (2009). Rice straw and its extract analyses were done according to the standard methods as described by Brunner and WaSMCEr (1978). The conversion of rice straw into spent mushroom compost extract (SMCE) was done through five main steps as shown in Fig. 1.

Table 2: Chemical analysis of spent mushroom compost (SMC) and spent mushroom compost extract (SMCE).

Treatment	pH (1:2.5)	EC (dSm^{-1})	O.M* (%)	O.C* (%)	C/N ratio	Macronutrients (%)			Micronutrients ($mg.kg^{-1}$)		
						N	P	K	Fe	Mn	Zn
SMC	7.84	4.17	54	31.4	13.79	2.27	0.92	2.88	124	85	32
SMCE	7.77	2.94	65.72	38.12	19.54	1.95	0.43	3.19	32.7	65.1	25.77

**O.M : Organic matter *O.C: Organic carbon*

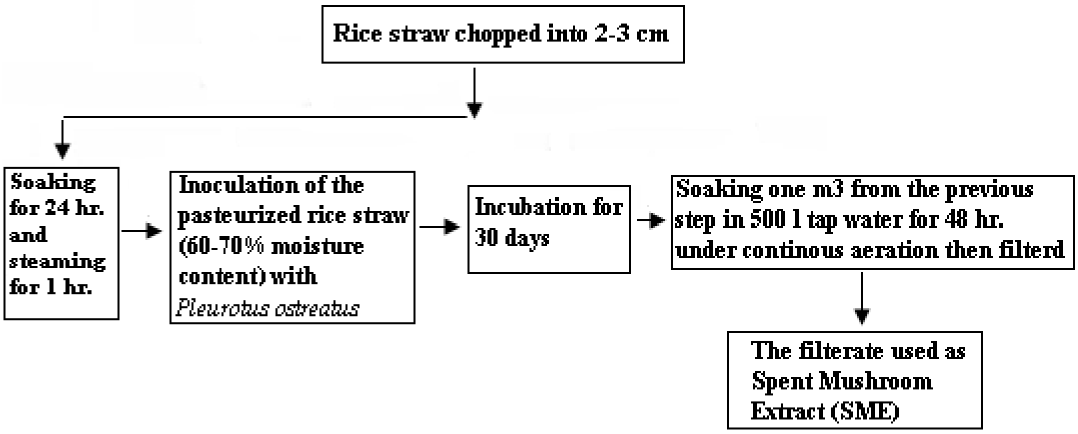

Fig. 1: Schematic diagram to produce spent mushroom compost extract (SMCE)

Cyanobacteria inoculum:

Cyanobacteria inoculum is composed of a mixture of N_2-fixing strains (*Nostoc muscorum* and *Anabaena oryzae)* and non N_2-fixing strain (*Spirulina platensis)*. N_2-fixing strains were grown separately on BG11 medium (Rippka*et et al*., 1979), while, non N_2-fixing (*Spirulina platensis*) was grown on Zarrouk medium (Zarrouk, 1966). Culture growth parameters and extracellular growth regulators in culture filtrates were determined, (Table, 3). PH values and algal dry weight were estimated according to Vonshak (1986). Cultures concentration was determined as optical density (OD) by spectrophotometer at 560 nm (Leduy and Therien, 1977). Chlorophyll-a was determined spectrophotometrically after extraction by absolute methanol as reported by Vonshak and Richmond (1988). Electric conductivity (EC) of algal culture filtrates was measured by using glass electrode conductivity meter Model Jenway 4310. Growth regulation substances (Indole acetic, Gibberellic and Abscisic acids) were fractioned according to Shindy and Smith, (1975) and quantified by HPLC apparatus (Hewlett-Pakard 1050) as described by Kowalczyk and Sandberg (2001). Cyanobacteria inoculum was prepared by mixing equal aliquots of the algal culture suspension at the log phase at the rate of 30 L.fed^{-1}.

Table 3: Algal cultures growth parameters and extracellular growth regulators analyses.

Algal strains	Algal cultures				Cultures filtrate			
	Optical density at 560 nm	Dry weight	Ch-a*	pH	EC	Indole acetic acid	Gibbrillic acid	Abscisic acid
		mgl^{-1}			(dSm^{-1})	mgl^{-1}		
Nostoc muscorum	1.198	800	5.76	8.16	0.65	1823.1	2859.0	5230.5
Spirulina platensis	2.998	2706	12.23	10.27	21.08	3500.0	7674.0	0.000
Anabaena oryzae	0.733	520	1.23	6.77	0.22	1118.3	8839.6	174.9

**Ch-a= Chlorophyll-a*

PGPR-bacteria inoculum:

PGPR rhizobacteria strains (*Bacillus subtilis and Pseudomonas fluorescens*) were grown on King's medium (King *et al*., 1954). Equal aliquots of *Pseudomonas fluorescens* and *Bacillus subtilis* pure cultures at log phase (10^7 CFU ml^{-1}) were used in mixture. Indole acetic acid contents (mg.l^{-1}) for *Pseudomonas fluorescens* and *Bacillus subtilis* were 163.0 and 112.0 mg.l^{-1}, respectively while, respective Gibberellic acid contents were 509.0 and 427.0 mg.l^{-1}, respectively.

Recorded data:

Soil biological activity:

Soil biological activity was evaluated in terms of total microbial counts (Allen 1959), CO_2 evolution (Gaur *et al*., 1971), dehydrogenase activity (Casida *et al*., 1964) and nitrogenase activity (Hardy *et al*.,1973) two months from planting.

Plant measurements:

Vegetative growth:

The following growth attributes were measured at the end of flowering stage, using three random plants from each experimental unit: plant stem length (cm), number of leaves/plant, leaf area/plant (cm^2), plant fresh weight (g) and plant dry weight (g).

Green fruits yield and its quality:

Yield and its components were determined as follows: early yield (g/plant) [the fruit yield of the first three harvests] and the total yield (g/ plant) throughout the entire harvesting season. The first harvest was taken after 60 days from planting then regular harvest twice a week. Fruit measurements were: green fruit weight (g), diameter (cm), length (cm) and dry matter %.

Seed yield and its components:

At harvest, samples of five random plants from each replicates were used to record the following characters: seed weight/plant (g), seed yield/ plot and then total seed yield (kg/plastic house) can be calculated. Almost one fruit per plant could be mature for seed while other fruits became atrophied.

Chemical contents:

Minerals (N, P and K) were determined as % in dry leaves according to (AOAC 2003). At the end of flowering stage, top fifth leaf from 3 random plants in each of the three replicates were picked up for determination of chlorophyll using Minolta chlorophyll meter SPAD- 502 as SPAD units. Nitrate was calorimetrically determined (mg/kg fresh weight) in green fruits by Cataldo *et al*. (1975).

Statistical Analysis:

All the collected data were tabulated and the treatments means were compared using the Duncan Multiple Range test as published by Duncan (1955).

Results and Discussion

Soil biological activity:

Results of squash rhizosphere biological activity under drip irrigation in season of 2012/ 2013 and 2013/2014 (Fig. 2) revealed that all the biological activities of squash rhizosphere that have been tested were conspicuously affected by cyanobacteria, bacteria and/or SMCE treatments combined with 50%N comparing to control (100% N). During the two cultivating seasons, the superiority of total bacterial counts, CO_2 evolution

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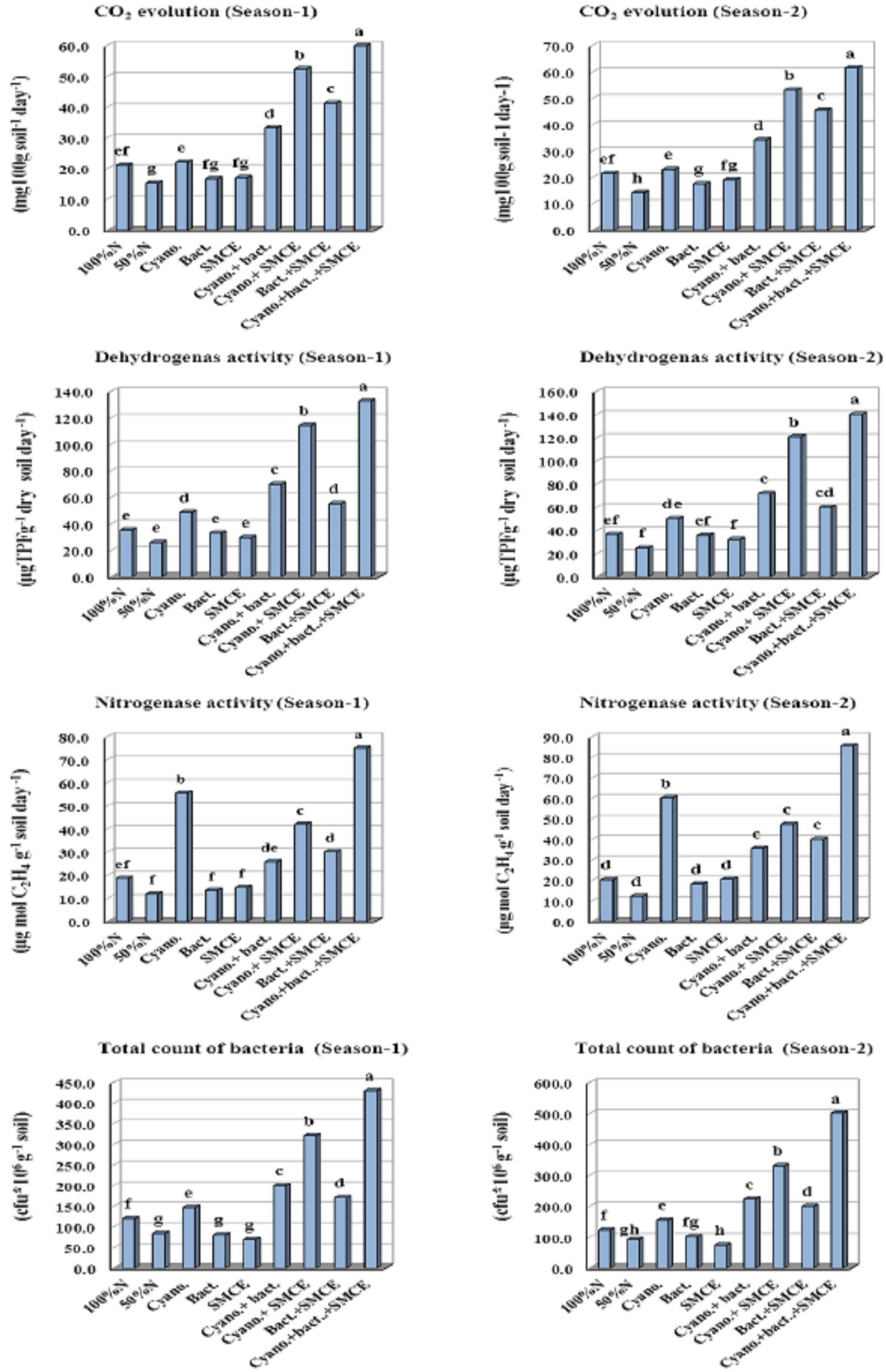

Fig. 2: Effect of various amendments on dehydrogenase activity, CO_2 evolution, nitrogenase activity and total bacteria count of squash rhizosphere under drip irrigation in winter season of 2012/ 2013 and 2013/2014.

and soil enzymes (dehydrogenase and nitrogenase activities) were achieved by the combination of cyanobacteria (cyano.), bacteria and spent mushroom compost extract (SMCE) with half dose of recommended mineral nitrogen which positively reflected on all the vegetative growth, green fruit yield and seed yield. Also, these treatments increased the chlorophyll content of leaves and NPK% of leaves while, reduced the nitrate content of squash fruit (Tables 5-7). These findings are agreed with Mostafa *et al*. (2015) who found that the integration between cyanobacteria, PGPR-bacteria and rice straw broth medium (RSBM) led to the highest superiority of CO_2 evolution, total count bacteria, total count cyanobacteria, nitrogenase and dehydrogenasas activities in rhizosphere of rice resulting in enhancement in plant growth and yield productivity in spite of decreasing the recommended dose of nitrogen fertilizer by 50%. The influence of plant growth promoting bacteria (PGPB) and cyanobacteria, alone and in combination, was investigated on micronutrient enrichment and yield in rice–wheat sequence, over a period of two years (Rana *et al*., 2015). On the basis of our previous studies (Mostafa *et al.*, 2011; Mostafa *et al.*, 2013; Mostafa *et al.*, 2015) they indicated that all the treatments receiving cyanobacteria/bacteria co-inoculation, are known to be PGPR and nitrogen fixers, therefore the dose of N fertilizer applied was reduced in many crops. This is reflective of the efficiency of the micro-organisms in promoting plant growth, biomass and thereby yields due to effective nutrient mobilization from soil to plant. It can therefore be surmised that the integration effect PGPR-bacteria/N2-fixing cyanobacteria co-inoculation a traits of the micro-organisms used and their colonization in the rhizosphere lead to the enhanced yields and nutrient quality of grains.

Vegetative growth:

Squash vegetative growth criteria represented in Table (4) indicated that the three amendments combination of cyanobacteria (cyano.) or bacteria (bact.) or spent mushroom compost extract (SMCE) with half dose of recommended mineral nitrogen showed significant increase than control or in some cases have more or less similar values in number of leaves, leaf area and fresh and dry weight in both seasons. In case of stem length, individual application of bacteria gave the best results. Generally it can be notice that the enhancement in growth criteria was superior when using the three combined application followed by the double application then individual amendments. These microbial origin additives provided squash plants with excess nitrogen which reflected on leaf area, fresh and dry weight resulted in vigorous vegetative growth (Fig.3).

Table 4: Effect of various amendments on vegetative growth of squash in winter season 2012/ 2013 and 2013/2014.

Treatments	Plant stem length (cm)	No. of leaves/ plant	Leaf area/ plant (m^2)	Fresh weight / plant (g)	Dry weight/ plant (g)
First season					
100%N	17.10de	23.50c	0.721c	1123cd	71.11b
50%N	15.70e	22.25cd	0.704c	1070d	63.04c
Cyano.	19.58abc	23.25c	0.856a	1242bc	71.90ab
Bact.	20.25a	22.50cd	0.894a	1337ab	72.43ab
SMCE	17.88bcd	20.33d	0.856a	1320ab	74.03ab
Cyano.+ bact.	17.70cd	28.50b	0.819ab	1183bcd	73.55ab
Cyano.+ SMCE	20.23a	28.50b	0.756bc	1207bcd	75.33ab
Bact.+SMCE	19.75ab	28.80b	0.853ab	1254abc	75.45ab
Cyano.+bact.+SMCE	18.15bcd	34.25a	0.897a	1407a	76.03a
Second season					
100%N	19.20bcd	21.65bc	0.762e	1244b	78.17ab
50%N	14.28e	16.50d	0.652f	925c	54.21c
Cyano.	18.00d	21.50bc	0.855cd	1373a	77.39b
Bact.	22.00a	23.00bc	0.813de	1363ab	78.34ab
SMCE	19.25bcd	21.00c	0.812de	1343ab	77.77ab
Cyano.+ bact.	18.50cd	22.00bc	0.913ab	1366ab	79.99ab
Cyano.+ SMCE	19.25bcd	22.15bc	0.806de	1272ab	79.43ab
Bact.+SMCE	20.40b	23.30b	0.880bc	1338ab	78.27ab
Cyano.+bact.+SMCE	19.65bc	26.00a	0.938a	1389a	82.30a

Cyano.= Cyanobacteria, Bac.= Bacteria, SMCE= spent mushroom-extract.
Values within the same column followed by the same letters are not significantly different at 5% level (Duncan's multiple range test).
All biological treatments were combined with 50% of the recommended dose of mineral nitrogen fertilizer.

In this concern, Elsharkawy *et al*. (2014) found that spray cucumber plants under greenhouse condition with suspension of some plant growth-promoting rhizobacteria (PGPR) strains (*Bacillus subtilis* and *Pseudomonas fluorescens*) can increase plant height, fresh and dry weight. The ameliorative effects of PGPR-application may be due to it increased plant cells water potential and decreased electrolytic leakage and also it reduce plant sodium ion concentration and increase salicylic acid and gibberellins (Kang *et al*., 2014). Also blue-green algae (BGA) could assist higher vegetable growth by supplying growth substances when used as inoculum in pot culture of cucumber, tomato and squash (Shariatmadari *et al*., 2011). The results revealed that the addition of BGA can enhance plant height, number of leaves, fresh and dry weight of root. Similar to our findings in this work, Osman *et al*. (2010) found that biofertilization for pea plant combined with half the recommended dose of the chemical fertilizer was usually more effective than the addition of the full rate of the chemical fertilizer, and this may allow saving 50% of the used chemical fertilizer. Thus, our findings demonstrated the potential of using fertilizers of microbial origin to save 50% N mineral fertilizer and also to induce plant growth parameters.

Fig. 3: Effect of cyanobacteria, bacteria and spent mushroom compost extract (SMCE) on the vegetative growth, green fruits yield and mature stage of squash in presence with 50% N under plastic house with drip irrigation system.

Green fruit yield:

Subsequent early and total yields of green fruits were followed, in the present work, to evaluate the effectiveness of the applied treatments. In this instance**,** early yield not affected in response to the different applied amendments and in most cases the increases or decreases compared with control weren't significant. This may be due to that the microbial amendments at early stage didn’t illustrate their full expression. On the other hand, total yield per plant or per unit area indicated that single applications showed better results than combined ones. The magnitude of the recorded data (Table 4 and 5) showed an inverse relation between vegetative growth and green fruit yield because excess nitrogen may orient plants to vegetative growth rather than yield. The highest significant increase of total plant green fruit weight over the control was recorded by using PGPR and it was 20.99% and 10.85% in first and second season, respectively. Cyanobacteria combined with spent mushroom compost extract induced the highest significant increase in the fruit diameter and green fruit weight, as compared with the control values. Generally, all applications recorded better results but not significant in terms of fruit quality i.e. length, diameter and fruit weight. The enhancement of total green fruit yield was assumed to the increase in fruit diameter and average fruit weight.

Fruit dry matter decrease with different applications because of the increment average fruit weight attributed to the increase in fruit water content. Organic material source and amendment like spent mushroom compost (SMC) of greenhouse soil application were very effective and beneficiary for cucumber growth and productivity (Polat *et al*., 2009). Positive effects of SMC have been notice on several yield related characteristics such as total yield, fruit width, fruit length, first quality fruit yield and nutrition content of the cucumber fruit.

Another bio amendment like blue-green algae showed an increase in biomasses of cabbage, pepper and Chinese cabbage by 9.96%, 19.28% and 13.51%, respectively (Liu *et al*., 2011). The combination of tow inoculation method: soaking seeds as well as drenching with *Bacillus subtilis* and *Bacillus cereus* promoted growth, increased yield and fruit nutrient contents of pepper (Zhou *et al*., 2014).

Seed yield and seed quality:

Using amendments with microbial origin: of cyanobacteria (cyano.) or bacteria (bact.) or spent mushroom compost extract (SMCE) had positive effect on squash seed yield (Table 6). Data stated that the increment of seed yield/plant and consequently seed yield/ plastic house (540 m^2) was significantly over the control. It was evident that individual application showed an increase but with a lower magnitude compared with double application. The best seed yield per plant or per unit area (plastic house) was record by using the three combination of cyano. +bact. + SMCE with a percentage increase of 12.8% and 6.0% in first and second season, respectively. Also seed index (weight of 100) was obtained by using same treatment. In this respect, Elsherif *et al*. (2013) found that inoculation of faba with Cyanobactiria and /or Azolla as dry or foliar treatments recorded significant increases in faba bean yield. Also cyanobacteria as seed inoculation or soil drench increased seed yield and weight of 100 seeds of common bean (Hegazi *et al*., 2010). The quality of obtained seed expressed with germination percentage showed significant enhancement in first season over the corresponding control by using the three combinations. This enhancement wasn't significant in case of either individual or combined of any two applications. Mean germination time i.e. the period required for the seed to indicate obvious germination recorded lower values (seeds required fewer days to germinate) by using different application.

Table 5: Effect of various amendments on green fruits yield and quality of squash in winter season 2012/ 2013 and 2013/2014

Treatments	Early yield (g/plant)	Total yield (g/plant)	Total yield/ plastic house (ton)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Fruit dry matter (%)
First season							
100%	547ab	2120d	2.205d	12.28ab	2.68c	72.65bc	7.53a
50%	503bc	1952e	2.030e	11.53b	2.73bc	68.15c	7.10ab
Cyano.	524abc	2300bc	2.392bc	12.40a	2.88a	76.40ab	7.13ab
Bac.	573a	2565a	2.668a	12.08ab	2.88a	78.13ab	7.03ab
SMCE	497bc	2415b	2.512b	12.75a	2.90a	77.33ab	7.08ab
Cyano.+ bac.	508bc	2129d	2.214d	12.58a	2.85ab	81.23a	7.13ab
Cyano.+ SMCE	523abc	2297bc	2.388bc	12.68a	2.90a	81.33a	7.13ab
Bac.+SMCE	492c	2214cd	2.303cd	12.38a	2.78abc	75.95abc	7.08ab
Cyano.+bac.+SMCE	518bc	2222cd	2.311cd	12.18ab	2.83ab	73.58abc	6.95b
Second season							
100%	618a	2350bc	2.444bc	12.98ab	2.57d	75.10cd	8.03a
50%	443e	1726d	1.795d	12.45ab	2.65bcd	71.20e	7.38ab
Cyano.	512cd	2530abc	2.631abc	12.23ab	2.60cd	74.50d	7.05b
Bac.	581abc	2605a	2.709a	12.03b	2.58d	75.20cd	7.00b
SMCE	495de	2407abc	2.503abc	12.18ab	2.65bcd	74.95cd	7.30b
Cyano.+ bac.	608ab	2537ab	2.638ab	12.55ab	2.78ab	77.90bc	7.15b
Cyano.+ SMCE	555abcd	2434abc	2.531abc	13.20a	2.90a	81.03a	7.30b
Bac.+SMCE	514cd	2339bc	2.433bc	13.15a	2.65bcd	80.33ab	7.03b
Cyano.+bac.+SMCE	543bcd	2317c	2.410c	12.45ab	2.75abc	74.70d	6.98b

Cyano.= Cyanobacteria, Bac.= Bacteria, SMCE= spent mushroom-extract.
Values within the same column followed by the same letters are not significantly different at 5% level (Duncan's multiple range test)
All biological treatments were combined with 50% of the recommended dose of mineral nitrogen fertilizer.

Table 6: Effect of various amendments on seed yield and seed quality of squash in winter season 2012/ 2013

Treatments	Seed yield/ plant(g)	Wt. of 100 seeds (g)	Seed yield/ plastic house (kg)	Germination %	Mean germination time
First season					
100%N	28.9f	19.29d	30.06f	92.5cd	2.18b
50%N	23.1g	18.73e	24.02g	89.5e	2.23a
Cyano.	29.8e	19.58d	30.99e	94.0c	2.11c
Bact.	30.2de	20.41abc	31.41e	92d	2.09c
SMCE	30.7cd	20.45ab	31.93cd	93.0cd	2.09c
Cyano.+ bact.	31.2b	20.10c	32.45b	96.5ab	2.09c
Cyano.+ SMCE	31.1bc	20.14bc	32.34bc	96.0b	2.06d
Bact.+SMCE	30.8bc	20.42ab	32.03bc	98.0a	2.04d
Cyano.+bact.+SMCE	32.6a	20.47a	33.90a	98.0a	2.04d
Second season					
100%N	33.1c	20.13d	34.42c	96ab	2.08b
50%N	28.9d	19.33e	30.06d	93c	2.17a
Cyano.	33.5bc	20.23d	34.84bc	95bc	2.05bc
Bact.	34.9a	21.17c	36.30a	95bc	2.02c
SMCE	34.9a	21.17bc	36.30a	94bc	2.02c
Cyano.+ bact.	34.3abc	20.44d	35.67abc	98a	2.03bc
Cyano.+ SMCE	34.9a	21.53ab	36.30a	96ab	2.03bc
Bact.+SMCE	34.7ab	21.47abc	36.09ab	98a	2.01c
Cyano.+bact.+SMCE	35.1a	21.73a	36.50a	98a	2.02c

Cyano.= Cyanobacteria, Bac.= Bacteria, SMCE= spent mushroom-extract
Values within the same column followed by the same letters are not significantly different at 5% level (Duncan's multiple range test)
All biological treatments were combined with 50% of the recommended dose of mineral nitrogen fertilizer.

In first season all application recorded significant decrease while in second season the application which reach significant level were: Bact., SMCE, Bact+SMCE and the three combinations. Piratheepa *et al*. (2015) found that root colonizing bacteria have the ability to increase the seed germination rate, growth and yield of some crops such as *Vigna* species, pumpkin, chili and brinjal. PGPR make differences in plant growth by different mechanisms which can be mediated by direct or indirect methods and its activity on seed germination varied among the seed varieties and inoculum concentration.

Chemical contents:

Table (7) indicated that the effects of the applied amendments on chlorophyll contents of plant leaves showed no significant decrease compared with control (100% nitrogen). Enhancement of total chlorophyll content as a result of PGPR was recorded in cucumber by Elsharkawy *et al*. (2014) and Pii *et al*. (2015).

On the other hand, it is evident from the same table that the nitrogen and phosphorus content of plant leaves recorded significant increase in both seasons, but there was a decrease or non-significant increase with cyanobacteria in first season in nitrogen and in both seasons in phosphorus. Also spent mushroom compost extract showed no significant increase in phosphorous in second season. In case of potassium, the only application which recorded significant increase was the combined of the three amendments while other applications showed no significant either increase or decrease compared with the control. This result was markedly in alliance with concomitant enhancement of the vegetative growth (table3) and seed yield (table 6). In this connection, Hegazi *et al*. (2010) demonstrated that seed inoculation and/or soil drench with cyanobacteria under different nitrogen levels, i.e., 50 or 75 of recommend mineral nitrogen showed positive significant effects in NPK seed content of bean. Also, effectiveness of spent mushroom compost (SMC) as cucumber greenhouse soil application was recorded by Polat *et al*. (2009). SMC had positive influence of cucumber fruit nutrition e.g. N, P, K, Ca, Mg, Fe, Zn, Mn and Cu.

It is clear from the results presented in Table (7) that nitrate content of squash fruit recorded significant decrease, as compared with the control values, with the different applications. Fruit squash consumption with low nitrate level is beneficially for human health as it reduces the hazard effects cause by using excess mineral nitrogen fertilizer. Decrease nitrate content was also found in cabbage, Chinese cabbage and pepper by Liu *et al*. (2011) as a result of blue green algae application. The nitrite contents were decreased by 10.34%, 9.41% and 30.79%, respectively.

Table 7: Effect of various amendments on chlorophyll (SPAD) and NPK% of leaf and fruit nitrate content (mg/ kg fresh weight) of squash in winter season 2012/ 2013 and 2013/ 2014.

Treatments	Chlorophyll contents (SPAD)	N %	P %	K %	Nitrate (mg/kg fresh weight)
First season					
100%	50.7a	4.58c	0.659d	3.167bc	1106a
50%	47.3ab	4.13d	0.573e	2.750e	1014b
Cyano.	47.8ab	4.58c	0.653d	3.180bc	977c
Bac.	47.6ab	4.86b	0.686c	3.148bcd	918d
SMCE	48.5ab	4.98ab	0.699c	3.168bc	804f
Cyano.+ bac.	44.9b	4.99ab	0.699c	3.098d	885e
Cyano.+ SMCE	51.2a	5.15a	0.759b	3.140cd	932d
Bac.+SMCE	49.7a	5.11a	0.738b	3.210b	825f
Cyano.+bac.+SMCE	49.9a	5.11a	0.820a	3.418a	984bc
Second season					
100%	51.03a	4.62d	0.651d	3.238b	1183a
50%	46.35b	4.20e	0.541e	2.928c	1076b
Cyano.	48.50ab	4.80c	0.660cd	3.310b	977c
Bac.	47.45ab	4.99b	0.676c	3.290b	970cd
SMCE	50.67a	4.82c	0.663cd	3.250b	819f
Cyano.+ bac.	45.35b	5.57a	0.726b	3.308b	885e
Cyano.+ SMCE	51.33a	5.57a	0.746b	3.340ab	830f
Bac.+SMCE	48.67ab	5.49a	0.775a	3.330ab	793f
Cyano.+bac.+SMCE	48.45ab	5.57a	0.779a	3.440a	929d

Cyano.= Cyanobacteria, Bac.= Bacteria, SMCE= spent mushroom-extract.
Values within the same column followed by the same letters are not significantly different at 5% level (Duncan's multiple range test).
All biological treatments were combined with 50% of the recommended dose of mineral nitrogen fertilizer.

Economic study:

Data in Table (8) showed the total income per plastic house under various amendments. It is evident from such data that using bact., cyano., SMCE, cyano+SMCE, cyano+bact., bact.+SMCE or cyano+bact.+SMCE, respectively gave higher total income/ plastic house (540 m^2) than 100 % N (control) or 50 %N. Generally, we can say that using this amendments gave the highest squash fruit yield with high quality and net income to growers, it addition to substitute for more expensive enviro-polluted fertilizers. This could become a significant factor in the alleviation of poverty in poor farming communities.

Table 8 : Effect of various amendments on net income/ plastic house (540 m^2) of squash plants during the average of two seasons of 2012/ 2013 and 2013/ 2014.

Treatments	Average total yield (ton/ 540 m^2)	Total income (L.E.) before adding the cost of the amendments	Amendments cost (L.E./540 m^2)	Total income (L.E.) after adding the amendments without cost of agriculture practices
100%N	2.325	13950	200	13750
50%N	1.913	11478	100	11378
Cyano.	2.512	15072	102	14970
Bact.	2.689	16134	103	16031
SMCE	2.508	15048	100	14948
Cyano.+ bact.	2.426	14556	105	14451
Cyano.+ SMCE	2.460	14760	102	14658
Bact.+SMCE	2.368	14208	103	14105
Cyano.+bact.+SMCE	2.361	14166	105	14061

Cyano.= Cyanobacteria, Bac.= Bacteria, SMCE= spent mushroom-extract.
All biological treatments were combined with 50% of the recommended dose of mineral nitrogen fertilizer.
**Price of sell of one ton of squash fruits during the season= 6000 L.E.*
**price of sell ammonium nitrate = 100 L.E. / 50 kg.*
**price of sell cyanobacteria= 2 L.E. / 540m^2.*
** price of sell bacteria= 3 L.E. / 540m^2.*
** price of sell SMCE= 0 L.E. / 540m^2.*

Conclusion:

Data revealed that spent mushroom compost extract (SMCE), PGPR bacteria and N_2-fixing cyanobacteria had a promise impact to compensate about half dose of mineral nitrogen fertilizer for squash cultivation under drip irrigation system in plastic house. Results of this study highlighted the dual effect of the combination of the three amendments on the vegetative growth and reduction of nitrate toxicity in edible part of plant as an indicator to use these amendments in organic agriculture. To meet the increasing demand for food security, biological biofertilizers will be needed as a sustainable eco-friendly low cost biofertilizer for vegetables crops.

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