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Production of Green Barley Sprouts on Rice Straw in the Presence of Microbial Inoculants and Elevated CO_2 as an Unconventional Dual Bio-Food and Fodders

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THE AIM of this investigation is to evaluate the nutritional quality and productivity of green barley sprouts and spent sprouts (roots and basal medium) cultivated on rice straw rather than using a conventional hydroponic system. To optimize the resulting products, barley was cultivated under a range of CO_2 concentrations and in the presence of up to three different microbial inoculants. Barley sprouts both treated with 800ppm CO_2 and grown on rice straw in the presence of a consortium of *Azotobacter chroococcum*, *Bacillus megaterium* and *Pseudomonas fluorescens* showed significantly greatest shoot length (16.4 and 15.33cm), shoot fresh weight (1315.03 and 1217.95g/m^2) and dry weight (185.11 and 171.45g/m^2) than sprouts subjected to the other treatments when harvested at the first and second cuts, respectively, beside the highest significant protein (21.79%) and lipid (2.14%) levels than sprouts subjected to the other treatments when harvested at the first cut. Spent sprouts subjected to the same treatment showed significantly highest protein (8.95%), and lipid (1.98%) levels, significantly lowest crude fiber (21.93%) and a lowest C/N ratio (31.8). The highest significant spent sprout yield (3302.1 g/m^2) was obtained when sprouts were grown in the absence of inoculants and under ambient air conditions. This study demonstrates the possibility of increasing the productivity and nutritive value of barley sprouts by cultivating sprouts on rice straw *via* different microbial inoculants in combination with elevated CO_2. The sprouts produced using this unconventional cultivation method was termed dual bio-barley fodder.

Keywords: CO_2 concentrations, Green barley sprout, Microbial inoculants, Rice straw, Spent sprout.

Introduction

The animal production sector is considered one of the most important agricultural subsectors. However, the shortage of fodder is considered one of the most fundamental and restrictive factors affecting livestock production, especially in developing countries (McGilloway, 2005). Sprouting is a simple, well-known technique that is used to germinate seeds of various forage crop species such as oats, barley, corn, and wheat to produce valuable green fodder (Muela et al., 2005). Sprouting is characterized by its high resulting yields, sustainable year-round production, efficient use of water (Fazaeli et al., 2012), and reduced growth time compared with the production of field-grown fodder (Guney et al., 2016). Moreover, sprouting uses 99% less land than that required for conventional production methods (Al-Karaki & Al-Momani, 2011), so it is valuable for countries that have little available land for cultivation or unsuitable land. Furthermore, the seed enzymes are activated by sprouting, as they change the starch, protein,

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and lipids within the grain to sugars, amino acids, and essential fatty acids, respectively, which are simpler forms for consumption (Fayed, 2011; Fazaeli et al., 2012). This explains why sprouts are much easier for animals to digest than dry seeds or other conventional fodder (Fazaeli et al., 2012). One of the main disadvantages of sprouting is the decrease in dry matter and the increase in moisture content compared to those produced by traditional methods (Dung et al., 2010; Peer & Leeson, 1985), which may negatively affect the consumption responses of animals (Saidi & Abo Omar, 2015). In this respect, Akbag et al. (2014) suggested that adding hay to sprouts would decrease the moisture content and increase the dry matter content.

Barley (*Hordeum vulgare* L.) is an annual cereal crop species cultivated in many dry areas of the world and serves both as the main animal fodder and as a base malt for beer and other beverages (Akbag et al., 2014; Guney et al., 2016; Helal, 2015).

The production of large amounts of agricultural byproducts such as rice straw is considered one of the key causes of various types of environmental harm as well as unsustainability in some agricultural systems as a result of poor management of rice straw (Zayed, 2018). Despite some rice straw is used as animal fodder, large amounts are disposed of by burning, causing air pollution (Helal, 2015; Zayed, 2018). However, using rice straw as fodder for ruminants has many disadvantages as a result of its poor nutritive value, low protein content, high fiber content, and low palatability (Polyorach & Wanapat, 2015); despite these drawbacks, few attempts have been made to ameliorate its nutritive value (Fayed, 2011).

Microbial inoculants have many capabilities that enable them to perform different tasks, such as biological nitrogen fixation, the production of various plant growth regulators that improve plant performance, and the degradation and solubilization of different compounds (Valentine et al., 2011; Zayed, 2012). Microbial inoculants were used as an alternative source to chemical fertilizers to improve plant nutrition and productivity as well as the nutrition quality of rice straw.

One of the major factors that is predicted to occur as a result of global climate change is increasing the annual rate of CO_2, which could have a major influence on plant growth and yield (Helal, 2015).

The aim of this study was to produce a new fodder product comprising green sprouts and rice straw. We investigated this aim by determining the growth performance and nutrient content of green barley sprouts in response to different microbial inoculants and in the presence of different CO_2 concentrations; beside the use rice straw as a basal medium for seed germination and sprout development to produce green sprouts and 'spent sprouts' (i.e., roots and rice straw) as animal fodder with good nutritional qualities. This new forage product (i.e., sprouts and spent sprouts) was termed a "dual bio-sprout".

Materials and Methods

This investigation was conducted in semi-automated climate chambers, in October 2016 and repeated in October of 2017 at the Central Laboratory for Agricultural Climate, Agriculture Research Center, Ministry of Agriculture and Land Reclamation, Egypt. The temperature was adjusted at ~29 ± 1℃

Microbial inoculants

Three different bacterial strains, namely, *Azotobacter chroococcum* (EMCCN 1004), *Bacillus megaterium* (EMCCN 1055), and *Pseudomonas fluorescens* (EMCCN 1067), were used in this study. They were kindly donated by the Microbial Inoculants Center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Although all the selected strains have common characteristics especially the production of phytohormones, each was selected for use in this investigation on the basis of one particular function: *Bacillus megaterium* as a cellulose decomposer, *Azotobacter chroococcum* as a nitrogen fixer, and *Pseudomonas fluorescens* as a plant growth-promoting rhizobacterium (PGPR).

Az. chroococcum was maintained on modified Ashby's medium (Abd El Malek & Ishac, 1968) for 5 days at 30°C, and *B. megaterium* was maintained on nutrient broth medium (Jacobs & Gerstein, 1960) for 24h at 30 °C. *Ps. fluorescens* was maintained on King's B medium (Schaad, 1980) for 5 days at 30°C.

B. megaterium 70ml/tray (10^8 cfu/ml) was added 10 days before cultivation of the grains (as it is used as a cellulose decomposer). Inoculated straw was sprayed every two days throughout the experiment period to maintain its WHC at 60%. By contrast, *Az. chroococcum* and *Ps. fluorescens* 70ml/tray (10^8–10^9 cfu/ml) for each of them were added after seedling emergence, and then again directly after the first cut (as they used as biofertilizers).

Physiological activities of the selected strains

Nitrogenase activity was assessed by performing an acetylene-reduction assay by adopting the technique described by Hardy et al. (1973).

Auxin (indole acetic acid; IAA) content was quantified using the colorimetric technique described by Glickmann & Dessaux (1995).

Cytokinins were quantified according to the technique reported by Fletcher & McCullagh (1971).

The gibberellic acid content was quantified according to the method described by Tien et al. (1979).

Cellulase activity was measured by the dinitrosalicylic acid method (DNS) as described by Miller (1959). One cellulase unit is known as the amount of enzyme that reducing sugar at the rate of 1µmol $ml^{-1}min^{-1}$ under assay condition.

Rice straw

Rice straw was collected from the Unit of Experimental and Agricultural Research, Faculty of Agriculture, Ain Shams University, Egypt. Chopped rice straw was soaked in tap water overnight and then sterilized at 121°C for 1h before use as a basal medium, according to the methods of Mohammadi & Abdallah (2007). The level of moister was maintained to 60% water holding capacity.

Grain

The barley (*Hordeum vulgare*) cultivar Giza 2000 was obtained from the Agricultural Research Center, Egypt. Healthy grains of uniform size were soaked overnight before used in the sprouting experiment. Rice straw medium and 75% seeding density (120g. dry grains) were used to produces barley green grass.

Experimental design and treatments

The experiment was of completely randomized design, with two factors (CO_2 concentration and microbial inoculant) and five replicates for each treatment. We used three concentrations of CO_2: ambient (approximately the average of CO_2 during the working months ranging from 350 to 400ppm according to: Central Laboratory for Agricultural Climate Egypt), 600ppm, and 800ppm. We used either a single microbial inoculant (*Az. chroococcum* or *B. megaterium*, or *Ps. fluorescens*), two of these inoculants, all three inoculants together, or control (without inoculation).

The experiment was conducted in 40× 24× 11cm trays (contain draining holes) filled with 250 g of sterilized rice straw (8-10cm in depth) as a basal medium for seed germination. Each tray was seeded with 120-grams of dry barley grains (1g contains approximately 24 seeds).

Two cuts of barley sprouts were harvested. The first cut was harvested 14 days after seeding by cutting the plants at the base of the sprout at a height of 1 cm above the top of the straw. After the first cut, sprouts were regularly watered every two days until new growth was observed (approximately 10 days after the first cut), after which the plants were cut and harvested (second cut). The basal medium, which comprised the straw and plant roots, is referred to as the "spent sprouts".

CO_2 concentrations

We used three semi-automated growth chambers that designed to evaluate the effects of the three carbon dioxide concentration treatments (ambient air, 600 and 800ppm CO_2).

Parameters measured

All the parameters were measured in the uniformly cut sprouts. For the measurement of growth parameters, at both the first and second cuts, ten green barley sprouts were collected randomly from each tray, then shoot height (cm), shoot fresh and dry weight (g/m^2) were measured. Based on the fresh and dry weight of these 10 barley sprouts and the seeding density in each tray, the fresh and dry weights of sprouts were estimated per square meter of tray.

Chemical parameters were assessed in green barley sprouts at the first cuts. Total protein, lipid,

and crude fiber contents of samples were assessed in accordance with the methods of Cunniff (1996). Total carbohydrates were determined by subtraction as described by Eneche (1999). The energy value was calculated using the Atwater factor method [(9× lipid) + (4× carbohydrate) + (4× protein)] as described by Eneche (1999). Iron, magnesium, and zinc were analyzed *via* a Perkin-Elmer 3300 atomic absorption spectrophotometer. Calcium was analyzed using a Perkin-Elmer Optima 2000 DV ICP in accordance with the method described by (Cunniff, 1996). Total phosphorus was determined calorimetrically in milligrams per 100 g of dry weight using the hydroquinone and sodium sulfite method described by Cunniff (1996). Total potassium was determined in milligrams per 100 g of dry weight using flame photometry according to the methods of Dewis & Freitas (1970).

Total chlorophyll was measured before the first and second cuts using a Minolta SPAD-501 chlorophyll meter. Three leaves were selected of each plant to measure the chlorophyll content (SPAD value), and five replicates were performed of each treatment.

Spent sprouts

At the end of the experiments (after the second cut), the remaining rice straw, which comprised barley roots, seed residue, and microbial inoculants was analyzed to determine its suitability for its uses as animal fodder. Specifically, spent yield (g/m^2), total proteins, lipids, and crude fiber percentage were estimated according to the methods of Cunniff (1996).

Statistical analysis

Data were statistically analyzed using the CoStat software package (version 6.303; CoHort Software, USA). Two-way ANOVA was performed in conjunction with a completely randomized design with replication (five replicates). Means were compared using Duncan's multiple range test (Waller & Duncan, 1969). For all statistical analyses, a p-value of less than or equal to 0.05 was considered statistically significant.

Results

Metabolic activities of the selected microbial strains

We found that all the microbial strains were able to produce phytohormones (cytokinins, IAA, and gibberellins) with different capabilities (Table 1). *Ps. fluorescens* showed the highest phytohormone production capability, of 2.8, 6.2 and 2.6µg ml^{-1}, for cytokinins, IAA, and gibberellins, respectively. Metabolic activity analyses confirmed that *A. chroococcum* was able to fix atmospheric N_2 at 134.1µmol C_2H_4 ml^{-1} hr^{-1}. and that *B. megaterium* showed a high capability for cellulase production of 3522.1µmol min^{-1}. These data recommend using *B. megaterium* as a cellulase decomposer by adding it to the rice straw 10 days before cultivation of the grains and using *Az. chroococcum* and *Ps. fluorescens* after seedling emergence as biofertilizers.

Production of green barley sprouts

Barley seeds revealed uniform germination rate in all the experiments. The results shown in Tables 2-5 reveal the effects of the tested factors on the growth performance of barley sprouts which could be categorized as follows: first, the single effect (one factor) which are different concentrations of carbon dioxide, or different microbial inoculants. Second, the combined effect of all the possible interactions between microbial inoculants and carbon dioxide.

TABLE 1. Metabolic activities of selected microbial strains.

Metabolic activities / Microorganisms	Nitrogenase activity (µmol C_2H_4 ml^{-1} h^{-1})	Cellulase activity (µmol min^{-1})	Phytohormone concentration		
			Cyto. (µg ml^{-1})	IAA (µg ml^{-1})	Gb. (µg ml^{-1})
Azotobacter chroococcum	134.1	-	2.4	1.3	1.8
Bacillus megaterium	-	3522.1	1.2	1.3	2.2
Pseudomonas fluorescens	-	-	2.8	6.2	2.6

- (-) means: No activity was detected.
- IAA: Indole acetic acid, Cyto.: Cytokinins, Gb.: Gibberellic acid.

TABLE 2. Effects of CO_2 concentration, microbial inoculants and their interactions on the growth parameters of green barley sprouts and their chlorophyll content (combined data of two experiments).

CO_2	Microbial inoculants	Shoot length (cm)		Shoot fresh weight (g/m^2)		Shoot dry weight (g/m^2)		Chlorophyll (µg Chl./cm)	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Ambient air	Control	13.78^{s}	12.72^{r}	318.33^{o}	299.62^{r}	44.81^{o}	40.94^{p}	28.6^{q}	29.9^{r}
	Az. chroococcum	14.36^{o}	13.30^{n}	411.72^{m}	385.34^{p}	57.96^{m}	52.82^{n}	34.0^{m}	34.6^{m}
	B. megaterium	14.24^{q}	13.17^{p}	349.03^{o}	322.60^{r}	49.13^{o}	45.41^{o}	32.0^{o}	33.4^{o}
	Ps. fluorescens	14.30^{p}	13.24^{o}	368.99^{n}	352.71^{q}	51.94^{n}	49.65n^{o}	31.1^{p}	32.9^{p}
	Az. chroococcum + B. megaterium	14.70^{k}	13.64^{k}	697.84^{h}	664.64^{j}	98.23^{h}	93.56^{h}	35.5^{j}	36.4^{k}
	Az. chroococcum + Ps. fluorescens	14.81^{i}	13.75^{i}	813.75^{f}	781.74^{g}	114.55^{f}	110.04^{e}	38.0^{g}	39.4^{h}
	B. megaterium + Ps. fluorescens	14.62^{l}	13.56^{l}	670.10^{h}	608.82^{k}	94.33^{h}	84.97^{i}	34.4^{l}	35.5^{l}
	Az. chroococcum + B. megaterium +Ps. fluorescens	14.92^{h}	13.86^{h}	972.78^{c}	946.94^{c}	136.94^{c}	128.03^{c}	39.8^{e}	41.4^{e}
	Means	**14.47C**	**13.41C**	**575.32C**	**545.30C**	**80.99C**	**75.68C**	**34.2C**	**35.4C**
CO_2 (600ppm)	Control	13.89^{r}	12.83^{q}	445.86^{l}	422.80^{o}	62.76^{l}	56.87^{m}	30.8^{p}	32.5^{q}
	Az. chroococcum	14.48^{m}	13.40^{m}	702.07^{gh}	682.59^{ij}	98.83^{h}	95.82^{gh}	34.9^{k}	36.5^{k}
	B. megaterium	14.34^{o}	13.26^{o}	492.55^{jk}	473.52^{mn}	69.33^{jk}	66.66^{kl}	32.2^{o}	33.9^{n}
	Ps. fluorescens	14.41^{n}	13.33^{n}	574.79^{i}	553.47^{l}	80.91^{i}	77.9 ^{j}	33.0^{n}	34.4^{m}
	Az. chroococcum + B. megaterium	14.83^{i}	13.74^{i}	793.37^{f}	770.21^{g}	111.68^{f}	104.49^{f}	38.1^{g}	39.8^{g}
	Az. chroococcum + Ps. fluorescens	14.95^{h}	13.86^{h}	868.75^{e}	842.26^{f}	122.29^{e}	118.56^{d}	40.7^{d}	41.8^{d}
	B. megaterium + Ps. fluorescens	14.76^{j}	13.69^{j}	734.95^{g}	702.73^{hi}	103.46^{g}	98.48^{g}	37.2^{hi}	38.5^{j}
	Az. chroococcum + B. megaterium +Ps. fluorescens	15.14^{g}	14.16^{g}	1102.36^{b}	1062.19^{b}	155.18^{b}	148.34^{b}	41.7^{c}	43.2^{c}
	Means	**14.60B**	**13.53B**	**714.34B**	**688.72B**	**100.56B**	**95.89B**	**36.1B**	**37.6B**
CO_2 (800ppm)	Control	14.41^{n}	13.32^{n}	468.36^{kl}	448.15^{no}	65.93^{kl}	63.08^{l}	32.1^{o}	33.9^{n}
	Az. chroococcum	15.65^{e}	14.57^{e}	734.95^{g}	711.45^{h}	103.46^{g}	100.15^{fg}	37.0^{i}	38.9^{ij}
	B. megaterium	15.56^{f}	14.46^{f}	514.04^{j}	493.07^{m}	72.36^{j}	69.41^{k}	32.9^{n}	34.4^{m}
	Ps. fluorescens	15.57^{f}	14.48^{f}	590.35^{i}	594.44^{k}	83.10^{i}	83.68^{i}	33.8^{m}	35.4^{l}
	Az. chroococcum + B. megaterium	15.97^{c}	14.87^{c}	912.36^{d}	884.72^{e}	128.43^{d}	124.54^{e}	39.1^{f}	40.8^{f}
	Az. chroococcum + Ps. fluorescens	16.19^{b}	15.12^{b}	943.16^{cd}	912.05^{d}	132.77^{c}	128.39^{c}	42.4^{b}	43.9^{b}
	B. megaterium + Ps. fluorescens	15.89^{d}	14.80^{d}	863.89^{e}	832.78^{f}	121.61^{e}	117.23^{d}	37.4^{h}	38.9^{i}
	Az. chroococcum + B. megaterium + Ps. fluorescens	16.40^{a}	15.33^{a}	1315.03^{a}	1217.95^{a}	185.11^{a}	171.45^{a}	43.5^{a}	45.0^{a}
	Means	**15.71A**	**14.62A**	**792.77A**	**761.83A**	**111.60A**	**107.24A**	**37.3A**	**38.9A**
Arithmetic means	Control	14.03^{H}	12.96^{H}	410.85^{H}	390.19^{H}	57.83^{H}	53.63^{H}	30.5^{H}	32.1^{H}
	Az. chroococcum	14.83^{E}	13.75^{E}	616.25^{E}	593.12^{E}	86.75^{E}	82.93^{E}	35.3^{E}	36.6^{E}
	B. megaterium	14.71^{G}	13.63^{G}	451.87^{G}	429.73^{G}	63.61^{G}	60.49^{G}	32.4^{G}	33.9^{G}
	Ps. fluorescens	14.76^{F}	13.68^{F}	511.38^{F}	500.21^{F}	71.99^{F}	70.41^{F}	32.6^{F}	34.2^{F}
	Az. chroococcum + B. megaterium	15.17^{C}	14.09^{C}	801.19^{C}	773.19^{C}	112.78^{C}	107.53^{C}	37.5^{C}	39.0^{C}
	Az. chroococcum + Ps. fluorescens	15.32^{B}	14.24^{B}	875.22^{B}	845.35^{B}	123.20^{B}	119.00^{B}	40.4^{B}	41.7^{B}
	B. megaterium + Ps. fluorescens	15.09^{D}	14.02^{D}	756.32^{D}	714.78^{D}	106.46^{D}	100.23^{D}	36.4^{D}	37.6^{D}
	Az. chroococcum + B. megaterium + Ps. fluorescens	15.49^{A}	14.45^{A}	1130.0^{A}	1075.69^{A}	159.08^{A}	149.27^{A}	41.7^{A}	43.2^{A}
LSD	CO_2 concentrations	0.0126	0.0129	11.4964	9.6569	1.6184	1.5693	0.1432	0.1319
	Microbial inoculants	0.0206	0.0211	18.7736	15.7697	2.6428	2.5626	0.2338	0.2154
	CO_2×Microbial inoculants	0.0357	0.0366	32.5168	27.3139	4.5775	4.4386	0.4050	0.3730

- Means in each column followed by the same letter are not significantly different at the 5% level.
- Letters used to distinguish the significance between the means of co_2 levels are not subscribed to avoid interference with microbial inoculants ' arithmetic means letters

TABLE 3. Effects of CO_2 concentration, microbial inoculants, and their interactions on the proximate analysis and energy of green barley sprouts.

CO_2	Microbial inoculants	Protein %	Lipids %	Carbo-hydrates %	Crude fiber %	Ash %	Energy (kcal/g)
Ambient air	Control	10.90^{p}	1.31^{o}	50.57^{a}	20.15^{a}	11.57^{q}	257.64^{a}
	Az. chroococcum	12.04^{lm}	1.38^{m}	48.11^{e}	21.17^{a}	11.72^{o}	253.02^{d}
	B. megaterium	11.10^{op}	1.34^{n}	50.03^{b}	20.32^{a}	11.67^{p}	256.63^{b}
	Ps. fluorescens	11.88^{m}	1.36^{mn}	48.36^{e}	21.15^{a}	11.71^{o}	253.17^{d}
	Az. chroococcum + B. megaterium	14.52^{i}	1.43^{l}	43.94^{l}	22.67^{a}	11.81^{m}	246.73^{h}
	Az. chroococcum + Ps. fluorescens	15.21^{h}	1.46^{k}	43.26^{m}	22.59^{a}	11.83^{lm}	247.01^{h}
	B. megaterium + Ps. fluorescens	13.21^{k}	1.41^{l}	46.16^{i}	21.83^{a}	11.77^{n}	250.17^{f}
	Az. chroococcum + B. megaterium + Ps. fluorescens	15.75^{g}	1.50^{j}	42.24^{n}	22.95^{a}	11.86^{d}	245.44^{i}
	Mean	**13.08C**	**1.40C**	**46.58A**	**21.60C**	**11.74C**	**251.23A**
CO_2 (600ppm)	Control	11.15^{o}	1.43^{l}	49.63^{c}	20.21^{a}	11.85^{kl}	255.98^{b}
	Az. chroococcum	12.23^{l}	1.53^{hi}	47.22^{g}	21.23^{a}	11.95^{i}	251.61^{e}
	B. megaterium	11.40^{n}	1.47^{k}	49.07^{d}	20.42^{a}	11.91^{j}	255.05^{c}
	Ps. fluorescens	12.00^{lm}	1.48^{jk}	47.64^{f}	21.21^{a}	11.93^{ij}	251.92^{e}
	Az. chroococcum + B. megaterium	16.50^{f}	1.58^{g}	41.19^{o}	22.73^{a}	12.13^{fg}	244.99^{i}
	Az. chroococcum + Ps. fluorescens	17.83^{e}	1.62^{f}	39.50^{p}	22.95^{a}	12.18^{e}	243.91^{j}
	B. megaterium + Ps. fluorescens	13.83^{j}	1.55^{hi}	44.80^{k}	21.86^{a}	12.11^{g}	248.48^{g}
	Az. Chroococcum +B. megaterium +Ps. fluorescens	19.54^{c}	1.72^{e}	37.32^{r}	22.99^{a}	12.30^{k}	242.89^{k}
	Mean	**14.31B**	**1.55B**	**44.55B**	**21.70B**	**12.04B**	**249.36B**
CO_2 (800ppm)	Control	13.29^{k}	1.53^{i}	46.90^{h}	20.29^{a}	12.07^{h}	254.54^{c}
	Az. chroococcum	17.63^{e}	1.63^{f}	41.01^{o}	21.33^{a}	12.29^{d}	249.17^{g}
	B. megaterium	14.44^{i}	1.56^{gh}	45.44^{j}	20.49^{a}	12.12^{fg}	253.52^{d}
	Ps. fluorescens	15.77^{g}	1.58^{g}	43.25^{m}	21.29^{a}	12.14^{ef}	250.29^{f}
	Az. chroococcum + B. megaterium	18.58^{d}	1.88^{c}	38.02^{q}	22.81^{a}	12.44^{c}	243.35^{jk}
	Az. chroococcum + Ps. fluorescens	20.15^{b}	1.96^{b}	35.89^{s}	23.13^{a}	12.51^{b}	241.80^{l}
	B. megaterium + Ps. fluorescens	16.42^{f}	1.86^{d}	41.10^{o}	21.94^{a}	12.42^{c}	246.79^{h}
	Az. chroococcum + B. megaterium +Ps. fluorescens	21.79^{a}	2.14^{a}	33.66^{t}	23.16^{a}	12.69^{a}	241.05^{l}
	Mean	**17.26A**	**1.77A**	**40.66C**	**21.81A**	**12.34A**	**247.56C**
Arithmetic means	Control	11.78^{H}	1.42^{H}	49.03^{A}	20.22^{G}	11.83^{H}	256.05^{A}
	Az. chroococcum	13.97^{E}	1.51^{E}	45.45^{D}	21.24^{E}	11.98^{E}	251.27^{D}
	B. megaterium	12.31^{G}	1.46^{G}	48.18^{B}	20.41^{F}	11.90^{G}	255.07^{B}
	Ps. fluorescens	13.22^{F}	1.47^{F}	46.42^{C}	21.21^{E}	11.93^{F}	251.79^{C}
	Az. chroococcum +B. megaterium	16.53^{C}	1.63^{C}	41.05^{F}	22.73^{C}	12.13^{C}	245.02^{F}
	Az. chroococcum +Ps. fluorescens	17.73^{B}	1.68^{B}	39.55^{G}	22.89^{B}	12.17^{B}	244.2^{G}
	B. megaterium + Ps. fluorescens	14.49^{D}	1.61^{D}	44.02^{E}	21.8^{D}	12.10^{D}	248.48^{E}
	Az. chroococcum + B. megaterium +Ps. fluorescens	19.03^{A}	1.78^{A}	37.74^{H}	23.03^{A}	12.29^{A}	243.13^{H}
LSD	CO_2 concentrations	0.0786	0.0082	0.1103	0.0675	0.0119	0.2758
	Microbial inoculants	0.1284	0.0134	0.1801	0.1102	0.0195	0.4504
	CO_2 × Microbial inoculants	0.2223	0.0231	0.3120	NS	0.0337	0.7801

- Means in each column followed by the same letter are not significantly different at the 5% level.
- Letters used to distinguish the significance between the means of co_2 levels are not subscribed to avoid interference with microbial inoculants ' arithmetic means letters.

TABLE 4. Effects of CO_2 concentration, microbial inoculants, and their interactions on the mineral content of green barley sprouts.

CO_2	Microbial inoculants	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Zn (ppm)
Ambient air	Control	0.26^{m}	1.82^{j}	0.84^{r}	0.21^{n}	147.08^{n}	21.67^{o}
	Az. chroococcum	0.32^{k}	1.97^{hij}	0.94^{mn}	0.27^{lm}	154.92^{k}	29.17^{l}
	B. megaterium	0.30^{l}	1.86^{j}	0.84^{r}	0.23^{n}	150.00^{m}	23.67^{n}
	Ps. fluorescens	0.37^{ij}	1.94^{ij}	0.90^{p}	0.25^{m}	151.25^{lm}	26.00^{m}
	Az. chroococcum + B. megaterium	0.40^{gh}	2.23^{efg}	0.95^{lm}	0.28^{kl}	159.42^{ij}	32.92^{i}
	Az. chroococcum + Ps. fluorescens	0.42^{ef}	2.53^{cd}	0.97^{kl}	0.31^{j}	160.92^{hi}	35.83^{h}
	B. megaterium + Ps. fluorescens	0.41^{ef}	2.33^{def}	0.93^{no}	0.29^{kl}	152.67^{kl}	31.08^{jk}
	Az. chroococcum + B. megaterium +Ps. fluorescens	0.46^{d}	2.65^{bc}	0.99^{k}	0.34^{fgh}	162.42^{h}	40.17^{de}
	Mean	**0.37C**	**2.17C**	**0.92C**	**0.27C**	**154.83C**	**30.06C**
CO_2 (600ppm)	Control	0.32^{kl}	1.96^{hij}	0.81^{c}	0.28^{kl}	153.33^{kl}	26.67^{m}
	Az. chroococcum	0.36^{j}	2.17^{efgh}	0.97^{kl}	0.32^{ij}	165.00^{g}	33.92^{i}
	B. megaterium	0.33^{k}	2.09^{ghi}	0.87^{q}	0.33^{hi}	157.33^{j}	29.92^{kl}
	Ps. fluorescens	0.38^{hi}	2.13^{fghi}	0.92^{o}	0.29^{k}	161.00^{hi}	32.42^{ij}
	Az. Chroococcum + B. megaterium	0.42^{ef}	2.94^{a}	1.14^{i}	0.38^{e}	172.33^{e}	38.50^{ef}
	Az. chroococcum + Ps. fluorescens	0.47^{d}	2.91^{a}	1.24^{ef}	0.40^{d}	177.25^{d}	41.67^{cd}
	B. megaterium + Ps. fluorescens	0.45^{d}	2.56^{c}	1.11^{j}	0.36^{f}	169.00^{f}	36.33^{gh}
	Az. chroococcum + B. megaterium +Ps. fluorescens	0.49^{c}	2.96^{a}	1.28^{s}	0.43^{c}	183.58^{c}	45.00^{b}
	Mean	**0.40B**	**2.47B**	**1.04B**	**0.35B**	**167.35B**	**35.55B**
CO_2 (800ppm)	Control	0.41^{fg}	2.25^{efg}	1.18^{h}	0.31^{j}	157.33^{j}	30.67^{jkl}
	Az. chroococcum	0.46^{d}	2.35^{de}	1.25^{de}	0.38^{e}	173.00^{e}	38.00^{fg}
	B. megaterium	0.43^{e}	2.28^{efg}	1.22^{g}	0.33^{ghi}	162.00^{h}	33.00^{i}
	Ps. fluorescens	0.46^{d}	2.32^{def}	1.23^{fg}	0.35^{fg}	166.33^{g}	35.67^{h}
	Az. chroococcum + B. megaterium	0.50^{bc}	2.83^{ab}	1.28^{c}	0.42^{c}	182.00^{c}	43.00^{c}
	Az. chroococcum + Ps. fluorescens	0.55^{a}	2.96^{a}	1.31^{b}	0.45^{b}	188.00^{b}	46.00^{b}
	B. megaterium + Ps. fluorescens	0.52^{b}	2.80^{ab}	1.26^{d}	0.40^{d}	176.67^{d}	40.33^{d}
	Az. chroococcum + B. megaterium +Ps. fluorescens	0.56^{a}	2.99^{a}	1.34^{a}	0.48^{a}	195.00^{a}	53.00^{a}
	Mean	**0.49A**	**2.60A**	**1.26A**	**0.39A**	**175.04A**	**39.96A**
Arithmetic means	Control	0.33^{H}	2.01^{D}	0.94^{H}	0.27^{G}	152.58^{H}	26.33^{H}
	Az. chroococcum	0.38^{F}	2.16^{C}	1.05^{E}	0.32^{E}	164.31^{E}	33.69^{E}
	B. megaterium	0.35^{G}	2.08^{CD}	0.98^{G}	0.30^{F}	156.44^{G}	28.86^{G}
	Ps. fluorescens	0.40^{E}	2.13^{C}	1.01^{F}	0.30^{F}	159.53^{F}	31.36^{F}
	Az. chroococcum + B. megaterium	0.44^{D}	2.67^{B}	1.12^{C}	0.36^{C}	171.25^{C}	38.14^{C}
	Az. chroococcum + Ps. fluorescens	0.48^{B}	2.80^{A}	1.17^{B}	0.39^{B}	175.39^{B}	41.17^{B}
	B. megaterium + Ps. fluorescens	0.46^{C}	2.57^{B}	1.10^{D}	0.35^{D}	166.11^{D}	35.92^{D}
	Az. chroococcum + B. megaterium +Ps. fluorescens	0.50^{A}	2.87^{A}	1.20^{A}	0.42^{A}	180.33^{A}	46.06^{A}
LSD	CO_2 concentrations	0.0061	0.0679	0.0057	0.0060	0.7754	0.6123
	Microbial inoculants	0.0099	0.1110	0.0093	0.0098	1.2662	0.9999
	CO_2 × inoculants	0.0172	0.1922	0.0162	0.0170	2.1932	1.7318

- Means in each column followed by the same letter are not significantly different at the 5% level.
- Letters used to distinguish the significance between the means of co_2 levels are not subscribed to avoid interference with microbial inoculants ' arithmetic means letters.

TABLE 5. Effects of CO_2 concentration and microbial inoculants on spent yield, and the protein, lipid, fiber, and energy content of spent green barley sprouts.

CO_2	Microbial inoculants	Protein %	Lipids %	Crude fiber %	Energy (kcal/g)	Spent yield (g/m^2)	C/N ratio
Ambient air	Control	6.21^{t}	1.08^{o}	33.37^{a}	204.70^{o}	3302.1^{a}	51.6
	Az. chroococcum	6.60^{o}	1.20^{l}	27.53^{d}	227.92^{k}	3187.5^{b}	48.5
	B. megaterium	6.45^{q}	1.11^{n}	26.44^{e}	231.75^{j}	3197.9^{b}	49.0
	Ps. fluorescens	6.48^{p}	1.16^{m}	27.55^{d}	227.23^{l}	3142.4^{c}	48.5
	Az. chroococcum + B. megaterium	6.78^{l}	1.31^{k}	25.48^{i}	235.50^{g}	3090.3^{d}	46.3
	Az. chroococcum + Ps. fluorescens	6.80^{l}	1.35^{ij}	26.49^{e}	227.74^{k}	3045.1^{e}	46.0
	B. megaterium + Ps. fluorescens	6.66^{n}	1.29^{k}	25.12^{k}	236.92^{f}	2993.1^{f}	46.7
	Az. chroococcum + B. megaterium +Ps. fluorescens	6.97^{j}	1.43^{g}	23.28^{s}	240.44^{bc}	2958.3^{g}	44.6
	Mean	**6.62C**	**1.24C**	**26.91A**	**229.03B**	**3114.6A**	**47.6**
CO_2 (600ppm)	Control	6.28^{s}	1.14^{mn}	30.22^{b}	216.67^{n}	2875.0^{h}	49.4
	Az. chroococcum	6.88^{k}	1.54^{f}	26.45^{e}	232.03^{ij}	2822.9^{ij}	45.0
	B. megaterium	6.63^{no}	1.32^{jk}	24.51^{l}	238.99^{e}	2888.9^{h}	46.5
	Ps. fluorescens	6.71^{m}	1.37^{hi}	26.17^{f}	232.29^{i}	2836.8^{i}	45.6
	Az. chroococcum + B. megaterium	7.45^{f}	1.54f	24.31^{n}	239.40^{d}	2812.5^{ij}	40.9
	Az. chroococcum + Ps. fluorescens	7.67^{d}	1.87^{c}	25.61^{h}	232.03^{ij}	2802.1^{j}	39.7
	B. megaterium + Ps. fluorescens	6.87^{k}	1.54^{f}	24.11^{q}	240.72^{b}	2753.5^{k}	44.2
	Az. chroococcum + B. megaterium +Ps. fluorescens	7.78^{c}	1.71^{d}	22.41^{t}	243.20^{a}	2697.9^{l}	39.0
	Mean	**7.03B**	**1.50B**	**25.47B**	**234.42A**	**2811.2B**	**43.8**
CO_2 (800ppm)	Control	6.37^{r}	1.33^{jk}	29.25^{c}	218.95^{m}	2503.5^{m}	47.6
	Az. chroococcum	7.39^{g}	1.55^{f}	25.98^{g}	231.75^{j}	2416.7^{n}	40.9
	B. megaterium	6.88^{k}	1.37^{hi}	24.25^{o}	237.02^{f}	2375.0^{o}	43.9
	Ps. fluorescens	7.03^{i}	1.40^{gh}	25.40^{j}	231.91^{ij}	2350.7^{op}	42.6
	Az. chroococcum + B. megaterium	7.60^{e}	1.60^{e}	24.17^{p}	236.84^{f}	2333.3^{p}	39.3
	Az. chroococcum + Ps. fluorescens	7.89^{b}	1.91^{b}	24.41^{m}	234.97^{h}	2017.4^{r}	37.6
	B. megaterium + Ps. fluorescens	7.33^{h}	1.54^{f}	23.33^{r}	240.14^{c}	2125.0^{q}	39.6
	Az. chroococcum + B. megaterium +Ps. fluorescens	8.95^{a}	1.98^{a}	21.93^{u}	242.91^{a}	1965.3^{s}	31.8
	Mean	**7.43A**	**1.58A**	**24.84C**	**234.31A**	**2260.9C**	**40.4**
Arithmetic means	Control	6.29^{G}	1.18^{G}	30.95^{A}	213.44^{G}	2893.5^{A}	49.5
	Az. chroococcum	6.96^{D}	1.43^{D}	26.65^{B}	230.57^{F}	2809.0^{B}	44.8
	B. megaterium	6.65^{F}	1.27^{F}	25.07^{E}	235.92^{D}	2820.6^{B}	46.4
	Ps. fluorescens	6.74^{E}	1.31^{E}	26.37^{C}	230.48^{F}	2776.6^{C}	45.5
	Az. chroococcum + B. megaterium	7.28^{C}	1.48^{B}	24.65^{F}	237.24^{C}	2745.4^{D}	42.2
	Az. chroococcum + Ps. fluorescens	7.45^{B}	1.71^{A}	25.50^{D}	231.58^{E}	2621.5^{E}	41.1
	B. megaterium + Ps. fluorescens	6.95^{D}	1.46^{C}	24.19^{G}	239.26^{B}	2623.8^{E}	43.5
	Az. chroococcum + B. megaterium +Ps. fluorescens	7.90^{A}	1.70^{A}	22.54^{H}	242.18^{A}	2540.5^{F}	38.4
LSD	CO_2 concentration	0.0122	0.0121	0.07	0.1347	10.2231	
	Microbial inoculants	0.02	0.0197	0.1144	0.2199	16.6942	
	CO_2 × Microbial inoculants	0.0346	0.0341	0.1981	0.3809	28.9152	

- Means in each column followed by the same letter are not significantly different at the 5% level.
- Letters used to distinguish the significance between the means of co_2 levels are not subscribed to avoid interference with microbial inoculants ' arithmetic means letters.

Shoot length, weight, and chlorophyll content of sprouts

Shoot length and shoot fresh weight and dry weight were greater at the first cut than at the second cut for all treatments (Table 2). However, the chlorophyll content at the second cut (µg Chl./cm tissue) was higher than at the first cut for all treatments. Furthermore, when barley sprouts were grown under increased CO_2 concentrations, all parameters were significantly increased ($P<0.05$) compared with those of barley sprouts grown under ambient air (control). Significantly highest values were obtained when barley sprouts were grown under the 800ppm CO_2 treatment than under the 600ppm CO_2 or ambient air treatments, with the following mean values obtained at 800 ppm from the first and second cuts, respectively: shoot length, 15.71 and 14.62cm; shoot fresh weight, 792.77 and 761.83g/m^2; shoot dry weight, 111.60 and 107.24g/m^2; and chlorophyll, 37.3 and 38.9µg Chl./cm.

Sprouts grown on rice straw treated with all three combined microbial inoculants showed significant increases ($P\leq0.05$) in all parameters measured compared with those grown on rice straw treated with a single microbial inoculant (Table 2). Sprouts grown on rice straw treated with a combined inoculum of *Az. chroococcum*, *B. megaterium*, and *Ps. fluorescens* showed the greatest increases, with the following mean values obtained at the first and second cuts, respectively, for all treatments with three inoculants: shoot length, 15.49 and 14.45 cm; shoot fresh weight, 1130 and 1075.69 g/m^2; shoot dry weight, 159.08 and 149.27 g/m^2; and chlorophyll, 41.7 and 43.2µg Chl./cm.

Sprouts grown under the highest CO_2 concentration (800ppm CO_2) and in the presence of all three microbial inoculants showed significantly greatest values than those obtained under other treatment conditions, with the following mean values obtained at the first and second cuts, respectively: shoot length, 16.4 and 15.33cm; shoot fresh weight, 1315.03 and 1217.95g/m^2; shoot dry weight, 185.11 and 171.45g/m^2; and chlorophyll, 43.5 and 45.0 Chl./cm.

Proximate analysis and energy content of sprouts

The protein, lipid, crude fiber, and ash% of 14-day-old (first cut) barley shoots were significantly the highest at 800ppm than at 600ppm CO_2 or ambient air treatments ($P\leq0.05$). The highest mean values were obtained for barley shoots subjected to 800 ppm CO_2 ($P\leq0.05$) (i.e., protein, 17.26%; lipid, 1.77%; crude fiber, 21.81%; and ash, 12.34%). The carbohydrate and energy content significantly decreased ($P\leq0.05$) in response to increased CO_2 concentration. The lowest mean carbohydrate (40.66%) and energy content levels (247.56kcal/g) were obtained for treatments subjected to the 800 ppm CO_2 treatment ($P\leq0.05$).

Sprouts grown on rice straw in the presence of *Az. chroococcum*, *B. megaterium*, and *Ps. fluorescens* as combination, showed significantly higher ($P\leq0.05$) protein, lipid, crude fiber, and ash% ($P\leq0.05$) and significantly lower ($P\leq0.05$) carbohydrate and energy content under all CO_2 concentrations than sprouts grown on rice straw in the presence of one or two inoculants or the control. The following mean values were obtained for the triple inoculant treatment: protein (19.03%), lipid (1.78%), crude fiber (23.03%), ash (12.29%) carbohydrate (37.74%), and energy content (243.13kcal/g).

Proximate analysis and analysis of the energy content of shoots of barley sprouts grown under the highest CO_2 concentration (800ppm CO_2) and in the presence of all three microbial inoculants revealed that significantly highest mean protein (21.79%), lipid (2.14%), crude fiber (23.16%), and ash (12.69%) values and significantly lowest mean carbohydrate (33.66%) and energy (241.05kcal/g) values were obtained for these sprouts than for sprouts subjected to other treatments.

Concentration of mineral in sprouts

In general, all mineral values of 14-day-old (first cut) barley shoots significantly increased ($P\leq0.05$) with increasing CO_2 concentration (Table 4). Barley sprouts grown under the highest CO_2 concentration (800ppm CO_2) showed the highest significant mean in P (0.49%), K (2.6%), Ca (1.26%), Mg (0.39%), Fe (175.04ppm), and Zn (39.96ppm) values.

Barley sprouts grown in the presence of all three microbial inoculants showed significantly highest mean in P (0.50%), K (2.87%), Ca (1.2%), Mg (0.42%), Fe (180.33 ppm), and Zn (46.06 ppm) levels than sprouts subjected to other inoculant treatments.

Barley sprouts grown under the highest CO_2

concentration (800ppm CO_2) and in the presence of all three microbial inoculants contained the highest significant mean of P (0.56%), K (2.99%), Ca (1.34%), Mg (0.48%), Fe (195.0 ppm), and Zn (53.0ppm) levels.

Proximate analysis%, energy content, spent yield and C/N ratio of spent barley sprouts

Spent barley sprouts that consisted of rice straw (the basal medium for sprout production), plant roots, and microbial inoculants were assessed to estimate its nutritional quality to be used as animal fodder.

The proximate protein, lipid, and energy content levels of spent barley sprouts significantly increased ($P \leq 0.05$) in response to increasing CO_2 concentration (Table 5). Spent sprouts grown under the highest CO_2 concentration (800ppm CO_2) showed significantly highest mean protein (7.43%), lipid (1.58%), and energy (234.45 kcal/g) content values and a significantly lowest mean crude fiber (24.84%) and C/N ratio (40.4) than spent sprouts produced under other treatment conditions. The highest spent yield (3114.6 g/m^2) was recorded for spent spouts produced in ambient air.

Among the microbial inoculant treatments, spent barley sprouts grown in the presence of all three microbial inoculants showed significantly highest mean protein (7.9%), lipid (1.7%), and energy (242.18kcal/g) levels and a significantly lowest crude fiber (22.54%) and C/N ratio (38.4) than spent sprouts subjected to other treatments (Table 5). The highest significant mean spent sprout yield was produced by sprouts that were grown in the absence of microbial inoculants (2893.5g/m^2).

Among the treatments, spent sprouts of barley grown under the highest CO_2 concentration (800ppm CO_2) in the presence of all three microbial inoculants showed significantly highest protein (8.95%), lipid (1.98%), and energy (242.91 kcal/g) content levels and significantly lowest crude fiber (21.93%) and C/N ratio (31.8) than spent sprouts subjected to other treatments. The highest significant spent barley yield (3302.1g/m^2) was produced by sprouts growing in the absence of microbial inoculants under ambient air conditions.

Discussion

During the past two hundred years, the concentration of carbon dioxide in the atmosphere has increased from 270ppm to 370–400ppm owing to the usage of fossil fuels and deforestation, and the concentration is predicted to double in the coming centuries. Most investigators are interested in the growth performance of plants as affected by elevated CO_2 due to their capability to fix CO_2 *via* photosynthesis. Many researchers have reported that elevating the CO_2 concentration directly improves photosynthetic processes in plants, particularly those with the C_3 photosynthetic pathway, and these researchers have implicated various physiological, biochemical, and morphological responses (Kimball et al., 2002). The results obtained in this investigation are in line with those reviewed by Gray & Brady (2016) who mentioned that elevated CO_2 generally stimulates the photosynthetic process, which leads to increased carbon uptake and assimilation, thereby increasing plant growth rates (Prior et al., 2011); however, this stimulation is contingent upon the availability of water and nitrogen (Gray & Brady, 2016). Furthermore, increasing atmospheric CO_2 causes photo-respiratory inhibition and decreases water use in plants, both of which stimulate plant growth and yield (Leakey et al., 2009; Myers et al., 2017). Also in this concern, Prior et al. (2011) reported that elevated CO_2 stimulates photosynthesis that leads to increasing carbon uptake and assimilation, thereby increasing plant growth.

The highest mineral levels of barley sprouts were detected in the results when the CO_2 concentration was 800ppm. This could suggest that elevated CO_2 concentrations improved the transport of minerals from barley seeds and the transport from the roots to the shoots.

Plant growth-promoting bacteria can excrete phytohormones such as auxins, cytokinins, and gibberellins, thereby improving the growth of plants (Bakonyi et al., 2013). The selected strains used in this study showed different metabolic activity levels, which give each of them a different advantage for use as a microbial inoculant. These results are consistent with those of Kumar & Singh (2001), Zayed (2018). In particular, we found that barley sprouts growing on rice straw in the presence of microbial inoculants showed enhanced growth compared with the control, and the consortium of *Az. chroococcum*, *B. megaterium*, and *Ps. fluorescens* had the greatest effect on plant growth performance, in terms of

shoot length and fresh and dry weight, which corresponds with the results reported by Zayed (2012). Furthermore, Cakmakci et al. (2007) reported that inoculating plants with N_2-fixing bacteria significantly increased the uptake of N, Fe, Mn, and Zn by barley seedlings compared with that of uninoculated plants (control) which corroborates and interprets the results obtained. The capability of bacteria to fix nitrogen is sometimes accompanied by other mechanisms, such as the production of phytohormones, antibiotics, the solubilization of different minerals and the degradation of different compounds, which have been suggested to be the mechanisms by which plant growth-promoting rhizobacteria (PGPRs) improve plant growth (Cakmakci et al., 2007).

The concentration of CO_2 in the soil is 10 to 15 times higher than that in the atmosphere; therefore, increasing the concentration of atmospheric CO_2 would not be expected to have a direct and/or high level of influence on soil microorganisms. However, elevated atmospheric CO_2 may affect the rice straw ecosystem indirectly by inducing plant responses that influence belowground processes (Drigo et al., 2008).

Plant-microbial interactions depend on root exudates (Hartmann et al., 2008) and plant metabolites (Rasche et al., 2009), which are affected by plant physiology that in turn is associated with CO_2 concentrations, which have an indirect influence on microorganisms present in the plant rhizosphere. Plants exposed to elevated CO_2 exhibit significant increases in photosynthesis, shoot growth, and root production (Milchunas et al., 2005). These results could lead to an assumption about increasing the flow of carbon from shoots to roots (Kimball et al., 2002), which stimulate belowground processes, especially root production and exudation (Sadowsky & Schortemeyer, 1997). This phenomenon in turn stimulates microorganisms present in rice straw, which causes an increase in microbial biomass (Cardon et al., 2001), activity (Rice et al., 1994), and in the rate of organic matter decomposition (Gill et al., 2002). High concentrations of CO_2 can also enhance the activities of microbial enzymes; Dhillion et al. (1995) reported that activities of dehydrogenase, cellulase, phosphatase, and xylanase were increased in the root region due to elevated CO_2. Furthermore, Dakora & Drake (2000) reported that elevated CO_2 stimulates the nitrogen fixation process as a consequence of increasing the activity of nitrogenase. These outcomes support the results obtained by this investigation, where the spent sprouts in the presence of a combination of microbial inoculants exhibited improvements in proteins, lipids, ash and energy as well as decreases in carbohydrates, crude fiber, and in the C/N ratio in response to increasing CO_2 concentrations.

Decreased crude fiber values and a decreased C/N ratio in spent sprouts inoculated with microbial inoculants have been reported previously by different research groups, from various perspectives. Among these, the most convincing interpretations are the capability of microbial inoculants to use rice straw as a source of carbon to produce energy for their growth (Akinfemi et al., 2010), and that the presence of *Azotobacter* sp. as a nitrogen fixer results in an increase in the nitrogen content of the treated straw, thereby supplying plants and other microbial inoculants present in the straw with nitrogen that increases their growth, activities and secretion of extracellular enzymes (Kadiri, 1999). All these factors improve microbial growth and activities that lead to the loss of organic matter *via* fermentation processes and subsequently improve plant growth and root exudates, which support improved protein and lipid percentages in spent sprouts as well as organic matter and dry matter degradation (Zayed, 2018). Although decreasing the organic matter and dry matter values was mentioned as the main drawback in the use of sprouts as a fodder and however it led to a decrease in the yield of spent sprouts, it led to decrease in the C/N ratio of the spent sprouts which cause improving in its nutritive qualities.

Conclusion

This investigation elucidated the possibility of using rice straw as a basal medium for the production of a new product termed dual bio-sprouts. Using different microbial inoculants in combination with increasing the CO_2 concentration to 800 ppm increased the sprout yield and enhanced the nutritional quality of the sprouts and spent sprouts.

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