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# GREEN PEA SPROUT RESPONSE TO MICROBIAL INOCULATION AND INCREASING ATMOSPHERIC $CO_2$ CONCENTRATION

[185]

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## ABSTRACT

Baby pea (*pisum sativum* L.) shoots is a new ready to eat baby leaf vegetable sprouts in Egypt. The overall quality change of baby pea shoots is greatly affected by surrounding environmental conditions especially increased elevation of carbon dioxide concentration in the air. This work focus on the impacts of predicted climate changes conditions on the quality of baby pea shoots by using two carbon dioxide concentrations (600 and 800 ppm) compared with ambient air (control) in interaction with three microbial inoculants and their combinations, in semi-automated growth chambers using tray sprouting method. The obtained results showed the largest yield of pea sprouts per unit area in 800 ppm $CO_2$ concentration with increasing about 20% more than ambient air (control) followed by 600 ppm with increasing about 9.4% than ambient air. Also, it revealed that using $CO_2$ at 800 ppm increased pea sprout crude protein content 37.8%, lipid 46.9% and energy 19.5% per unit area when compared to ambient air. While pea sprout treated by 800 ppm $CO_2$ and inoculated by combination of *Az. chroococcum* + *B. megaterium* + *Ps. fluorescens* recorded the highest significant shoot length in the second cut and highest significant chlorophyll in first and second being 13.25 cm, 57.3 and 58.9 µg Chl./cm (SPAD) and the highest significant protein, lipids, and ash content being 48.65, 4.95, 10.69% as well as the highest significant mineral values of P, Ca, Mg, Fe and Zn being 0.545, 3.535, 0.620% and 61.3 ppm respectively. Current study suggests that high $CO_2$ concentration in the presence of *Az. chroococcum* + *B. megaterium* + *Ps. fluorescens* improve the yield and the quality of baby pea shoots.

## INTRODUCTION

Today, research seems to be confirming that seed sprouts are the function food of the future, as was the food of the past. Therefore, the attention of experts dealing with the healthy nutrition turned more and more towards, the determination of the biological value of the nutritional sprouts **(Penas et al 2008; Abdallah 2008 and Mańton et al 2010)** The consumption of green leafy vegetables is recommended due to their high content of vitamins, minerals, and antioxidant phytochemicals, as well as low content of fat and carbohydrate **(Rico et al 2007).** The producers of fresh-cut sprouts seek for adding new varieties of leafy vegetables that are ready-to-eat to attract more consumers **(Martinez-sánchez et al 2012).**

Pea shoots were recently presented as a ready-to-eat baby–leaf vegetable and is recognized as a popular vegetable in some parts of Asia and Africa which also is gaining popularity in the United States and Europe **(Miles and Sonde 2003 and Santos et al 2014).**

Baby pea shoots is considered as a healthy, beneficial and highly nutritive new leaf vegetable sprout **(Ibrahim Mona 2015 and Ahmed et al 2018).**

Changes in earth's climate have been projected by the end of the 19$^{th}$ century because some atmospheric "greenhouse" gases are increasing at which Carbone dioxide ($CO_2$) one of them, **(IPCC 2001).** The naturally $CO_2$ concentration in ambient

air is 400 parts per million (ppm). However, doubling ambient $CO_2$ level (i.e 700 to 800ppm) which is predicted to occurs due to climate changes could make a significant and visible difference in plant growth and yield because $CO_2$ is utilized by plants for higher rates of photosynthesis during daytime **(Ludwig and Asseng 2006; Süß et al 2015 and Poudel and Dunn 2017).**

However, $C_3$ Photosynthetic pathways plants as peas are more responsive to higher $CO_2$ concentration than plants having a $C_4$ pathway. An increase in ambient $CO_2$ to 800 ppm can increase the yield of $C_3$ plants up to 40% to 100% **(Poudel and Dunn2017).**

The main consideration for biological management of plant growth is to utilize microbial inoculants that play a dynamic role in sustaining agriculture by improving their growth performance in a safer way **(Mcdaniel et al 2014).** Plant response to microbial inoculants could be associated with more than one mechanism at which microorganisms were suggested to have more than one function in stimulating plant growth that results in more than one consequence **(Cakmakci et al 2007)**, therefore, they have great capabilities to increase plant growth and yield under different conditions**.** These increments could be attributed to different mechanisms such as increasing nutrients uptake through solubilization and degradation of complicated compounds, nitrogen fixation which has special effect on the physiological processes of plants **(Valentine et al 2010; Zayed Mona 2012)** and stimulating plant growth either by production of plant growth promoting substances such as indole-3-acetic acid, cytokinins and gibberellins which able to encourage progressive effects on the plant growth and development or modulating endogenous plant hormone levels **(Gray 2004; Van Loon 2007; Ortíz-Castro et al 2008; and Ahemad and Khan 2011),** in addition to improving plant immunity against diseases by producing different antibiotics (**Atta et al 2012**).

These research focus on the impact of predicted climate change conditions (increased $CO_2$ concentration) and different microbial inoculants on the growth and yield of baby pea shoot (green sprouts). The main objectives are to evaluate the effect of increased $CO_2$ concentration on photosynthetic pigment (SPAD reading), chemical composition (proximate analysis), energy and mineral contents of pea shoot in the presence of different microbial inoculants.

## MATERIALS AND METHODS

This study was carried out during winter seasons of 2015 and 2016 at Central Laboratory for Agricultural Climate (CLAC), Agriculture Research Center (ARC), Ministry of Agriculture and Land Reclamation, in Semi-automated control environmental chambers. The experiment was designed to study the effect of different microbial inoculants and two $CO_2$ concentrations on pea sprout characters, yield and chemical composition in the two cutting.

### Microbial inoculants

Three different bacterial strains have various potential activities, namely *Azotobacter chroococcum, Bacillus megaterium* and *Pseudomonas fluorescens*, were used in this study. They were kindly provided by Microbial Inoculants Center, Fac. Agric., Ain Shams Univ., Cairo, Egypt. Each strain was maintained in its appropriate medium. *Azotobacter chroococcum* was maintained on Modified Ashby's medium **(Abd El-Malek and Ishac 1968)** for 7 days/30 °C, *Bacillus megaterium* was maintained on nutrient broth medium **(Jacobs and Gerstein1960)** for 24 h/30 °C while *Pseudomonas fluorescens* was maintained on King's B Medium **(Schaad 1980)** for 5 days/30 °C.

Seeds of pea (*Pisum sativum*), *Entesar* cultivar were obtained from Horticulture Research Institute (HRI), Agriculture Research Center (ARC). Clean seeds with uniform size were used.

Rice straw was collected from unit of Experimental and Agricultural Research, Faculty of Agriculture, Ain Shams University. Chopped rice straw was soaked overnight then sterilized at 121 °C/1 h. to be used as a bed media according to **Mohammadi and Abdallah (2007)**.

The experiment was carried out in (40 × 24 × 11cm) trays. 250 g of sterilized rice straw was added to each tray. Seeding density (dry seeds/$m^2$) was used to produce pea sprout according to **(Anwar Dina 2015).** Each treatment was repeated three times.

Three semi-automated growth chambers were previously designed for three carbon dioxide concentration treatments (ambient air, 600 and 800 ppm $CO_2$) using carbon dioxide pumping as reported by **Yossife et al (2017).**

### Experimental design and treatments

The experiment was designed in two factorials in complete randomized design with three replicates for each treatment. Factor A was $CO_2$ concentrations which were three carbon dioxide concentrations (ambient air, 600, and 800 ppm $CO_2$). Factor B was the addition of microbial inoculants which were *Azotobacter chroococcum, Bacillus megaterium* and *Pseudomonas fluorescens* and different combinations between all of them.

The experiment was subjected to three groups of treatments. Each group were subjected to one of $CO_2$ concentrations and eight sub-treatments from different combinations of microbial inoculants (Control, *Azotobacter chroococcum*, *Bacillus megaterium*, *Pseudomonas fluorescens*, (*Az. chroococcum* + *B. megaterium*), (*Az. chroococcum* + *Ps. fluorescens*), (*B. megaterium* + *Ps. fluorescens*), (*Az. chroococcum* + *B. megaterium* + *Ps. fluorescens*).

### Addition of microbial inoculants

*Bacillus megaterium* $10^8$ cfu/ml was added 10 days before the cultivation of seeds, while *Azotobacter chroococcum* and *Ps. fluorescens* $10^8 – 10^9$ cfu/ml were added during the experiment; the $1^{st}$ addition was after pea sprouts emergence and the $2^{nd}$ addition was after the $1^{st}$ cut.

Green sprouts (14 days old for $1^{st}$ cut and 12 days old for $2^{nd}$ cut) were harvested and dried in an oven at 60C° for 72hr. for measuring sprout characteristics and chemical analysis (proximate and minerals determinations).

### Growth parameters

The following parameters were measured in both first and second cut: Shoot length (cm), shoot fresh and dry weight ($g/m^2$), chlorophyll content (µg Chl. /cm) using SPAD.

### Chemical and biochemical parameters

Mineral contents (P%, K%, Ca% and Mg%, Fe ppm and Zn ppm), proximate analysis (moister, protein, lipids, carbohydrates, fiber and ash) were measured according to **AOAC (2012).** The energy value was calculated using the Atwater factor method [(9 x fat) + (4 x carbohydrate) + (4 x protein)] as described by **(Nwabueze 2007).**

The data were statistically analyzed using the CoStat package program **(Version 6.303; CoHort Software, USA)** by ANOVA analysis of variance using completely randomized design two ways with replication, and compare the means by Duncan's Multiple Range Test **(Waller and Duncan 1969).** All statistical determinations were made at $p \leq 0.05$.

## RESULTS AND DISCUSSION

### Effect of $CO_2$ concentrations, microbial inoculants, and their interactions on green pea shoots length, weight, and chlorophyll

Green pea shoots are simply pea sprout cuts at early stages of growth before stem branches initiation. Data presented in **Table (1)** show that generally, first cut recorded increase in shoot length, shoot fresh weight and shoot dry weight when compared to the second cut in all treatments. While the second cut recorded increase in the chlorophyll reading (µg Chl/cm tissue) when compared to the first cut in all treatments. Also, increasing the concentration of $CO_2$ recorded significant increase in all parameters measured at which 800 ppm $CO_2$ recorded the highest significant values when compared to 600 ppm and ambient air $CO_2$ in all of shoot length, shoot fresh weight, shoot dry weight and chlorophyll in the first and second cut being 13.19, 12.15 cm, 1020, 928.5 $g/m^2$, 154.1, 140.2$g/m_2$ and 49.8, 51.6 µg Chl. /cm (SPAD), respectively

Concerning microbial inoculants, data show that combined interaction between *Azotobacter chroococcum*, *Bacillus megaterium,* and *Pseudomonas fluorescens* recorded the highest significant increase in shoot length, shoot fresh weight, shoot dry weight and chlorophyll in the first and second cut being 14.03, 12.92 cm, 1500.9, 1438.2 $g/m^2$, 226.5, 217.0 $g/m^2$, 51.4 and 53.0 µg Chl./cm (SPAD) in respective order.

Regarding the interaction between $CO_2$ concentrations and microbial inoculants, no significant difference was recorded in shoot length in the first cut, shoot fresh weight in the first and second cut and shoot dry weight in the first and second cut. While, pea sprout treated by 800 ppm $CO_2$ and inoculated by *Az. chroococcum* + *B. megaterium* + *Ps. fluorescens* recorded the highest significant shoot length in the second cut and highest significant chlorophyll in the first and second cut being 13.25 cm and 57.3 and 58.9 µg Chl. /cm (SPAD), respectively.

**Table 1.** Effect of $CO_2$ concentrations, microbial inoculants, and their interactions on green pea sprouts shoot characters and Chlorophyll (Combined data of two experiments)

| $CO_2$ | Microbial inoculants | Pea shoot characters | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Shoot length (cm) | | Shoot fresh weight g/m2 | | Shoot dry weight g/m2 | | Chlorophyll SPAD (µg Chl/cm) | |
| | | 1st cut | 2nd cut | 1st cut | 1st cut | 2nd cut | 2nd cut | 1st cut | 2nd cut |
| Ambient air | Control | 12.19a | 10.93 o | 616.4 a | 577.6 a | 89.2 a | 87.1 a | 31.7 n | 35.7 q |
| | *Az. chroococcum* | 12.52a | 11.27lm | 737.4 a | 692.0 a | 111.2 a | 104.3 a | 37.1m | 40.6 n |
| | *B. megaterium* | 12.35a | 11.16 n | 652.5 a | 652.6 a | 99.8 a | 98.8 a | 35.5m | 36.7 p |
| | *Ps. fluorescens* | 12.36a | 11.20 n | 699.4 a | 651.1 a | 105.5 a | 98.2 a | 39.8 l | 40.3 n |
| | *Az. chroococcum +B. megaterium* | 13.58a | 12.31 h | 925.5 a | 863.1 a | 139.5 a | 130.1 a | 41.8 k | 44.4 k |
| | *Az. chroococcum +Ps. fluorescens* | 13.61a | 12.37 g | 969.9 a | 935.3 a | 146.2 a | 141.0 a | 44.7 ij | 46.1 i |
| | *B. megaterium + Ps. fluorescens* | 13.56a | 12.29 h | 808.1 a | 765.5 a | 121.8 a | 115.4 a | 41.5 k | 42.7lm |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 13.91a | 12.64 e | 1416.8 a | 1379.3 a | 213.7 a | 208.0 a | 47.0gh | 47.2 h |
| **Mean** | | **13.01C** | **11.77C** | **853.2 C** | **814.6 C** | **128.4 C** | **122.9B** | **39.9 C** | **41.7 C** |
| $CO_2$ (600 ppm) | Control | 12.27a | 11.16 n | 700.2 a | 636.4 a | 105.7 a | 95.9 a | 35.7m | 38.5 o |
| | *Az. chroococcum* | 12.60a | 11.42 k | 817.7 a | 756.6 a | 123.3 a | 114.0 a | 41.9 k | 43.0lm |
| | *B. megaterium* | 12.43a | 11.26 m | 718.0 a | 674.5 a | 108.3 a | 101.8 a | 36.2m | 40.0 n |
| | *Ps. fluorescens* | 12.46a | 11.30lm | 777.8 a | 711.9 a | 117.4 a | 107.3 a | 40.5kl | 43.3 l |
| | *Az. chroococcum +B. megaterium* | 13.67a | 12.51 f | 1003.2 a | 923.2 a | 151.2 a | 139.1 a | 45.7hi | 47.4 h |
| | *Az. chroococcum +Ps. fluorescens* | 13.70a | 12.53 f | 1043.4 a | 989.5 e | 157.3 a | 149.2 a | 47.4fg | 51.7 f |
| | *B. megaterium + Ps. fluorescens* | 13.66a | 12.49 f | 892.0 a | 828.0 a | 134.5 a | 124.8 a | 41.3kl | 44.4 k |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 14.02a | 12.87 b | 1500.9 a | 1440.5a | 226.5 a | 217.3 a | 49.9de | 52.9 e |
| **Mean** | | **13.10B** | **11.94B** | **931.7 B** | **870.1 B** | **140.5 B** | **131.2 B** | **42.3 B** | **45.1 B** |
| $CO_2$ ( 800 ppm) | Control | 12.36a | 11.31 l | 784.4 a | 695.4 a | 118.5 a | 105.0 a | 41.7 k | 42.5 m |
| | *Az. chroococcum* | 12.68a | 11.60 i | 905.5 a | 815.6 a | 136.7 a | 123.1 a | 48.8ef | 51.3 f |
| | *B. megaterium* | 12.52a | 11.46 jk | 820.6 a | 733.6 a | 124.0 a | 110.9 a | 44.1 j | 45.2 j |
| | *Ps. fluorescens* | 12.55a | 11.50 j | 867.4 a | 770.9 a | 131.1 a | 116.4 a | 47.1gh | 49.0 g |
| | *Az. chroococcum +B. megaterium* | 13.75a | 12.70cd | 1093.5a | 982.3 a | 165.0 a | 148.2 a | 53.4 c | 55.4 c |
| | *Az. chroococcum +Ps. fluorescens* | 13.78a | 12.73 c | 1137.9 a | 1048.5 a | 171.8 a | 158.3 b | 55.4 b | 56.7 b |
| | *B. megaterium + Ps. fluorescens* | 13.74a | 12.67de | 972.6 a | 887.0 a | 146.8 a | 133.9 a | 50.4 d | 53.6 d |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 14.17a | 13.25 a | 1584.9 a | 1494.8 a | 239.2 a | 225.6 a | 57.3 a | 58.9 a |
| **Mean** | | **13.19A** | **12.15A** | **1020.9A** | **928.5 A** | **154.1 A** | **140.2 A** | **49.8 A** | **51.6 A** |
| Average | Control | 12.28F | 11.13 G | 700.3 H | 636.5 G | 104.5 E | 96.0 E | 36.4 G | 38.9 H |
| | *Az. chroococcum* | 12.60D | 11.43 D | 820.2 E | 754.7 E | 123.7CD | 113.8CD | 42.6 E | 45.0 E |
| | *B. megaterium* | 12.43E | 11.29 F | 730.4 G | 686.9 F | 110.7DE | 103.8DE | 38.6 F | 40.7 G |
| | *Ps. fluorescens* | 12.46E | 11.33 E | 781.6 F | 711.3 F | 118.0DE | 107.3DE | 42.5 E | 44.2 F |
| | *Az. chroococcum +B. megaterium* | 13.67BC | 12.51 C | 1007.4C | 922.9 C | 151.9 B | 139.2 B | 46.9 C | 49.0 C |
| | *Az. chroococcum +Ps. fluorescens* | 13.70 B | 12.55 B | 1050.4B | 991.1 B | 158.4 B | 149.5 B | 49.2 B | 51.5 B |
| | *B. megaterium + Ps. fluorescens* | 13.65 C | 12.48 C | 890.9 D | 826.8 D | 134.4 C | 124.7 C | 44.4 D | 46.9 D |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 14.03 A | 12.92 A | 1500.9A | 1438.2A | 226.5 A | 217.0 A | 51.4 A | 53.0 A |
| L.S.D | $CO_2$ concentration | 0.0179 | 0.0170 | 15.7543 | 15.2488 | 9.1258 | 8.4132 | 0.5177 | 0.2552 |
| | Bio-fertilizers | 0.0294 | 0.0277 | 25.7268 | 24.9012 | 14.9024 | 13.7388 | 0.8453 | 0.4168 |
| | $CO_2$×biofertilizer | NS | 0.0481 | NS | NS | NS | NS | 1.4641 | 0.7218 |

Means in each column in each group followed by the same letter are not significantly different at the 5% level.
NS= not significant

**Proximate analysis and energy of pea sprout cuts as affected by microbial inoculants, $CO_2$ concentrations, and their interactions**

The results of the proximate analysis and energy of pea sprout shoot cut (1st cut) are summarized in **Table (2).** Pea shoot cut showed marked increase in moisture, protein, lipids and ash composition by increasing $CO_2$ concentration at which 800ppm $CO_2$ recorded the highest significant results when compared to ambient air (control) being 5.40, 42.5, 4.53 and 10.39% in respective order. While carbohydrate, crude fiber, and energy significantly decreased by increasing $CO_2$ concentration at which the highest significant decrease was recorded within 800 ppm $CO_2$ being 28.80, 8.34% and 326 kcal/g plant, respectively.

Concerning microbial inoculants, **Table (2)** show that sprout inoculated by combined interaction between *Az. chroococcum*+ *B. megaterium* + *Ps. fluorescens* recorded the highest significant increase in moisture, protein, lipids, and ash, as well as the highest significant decrease in carbohydrate and crude fiber, being 5.60, 44.1, 4.55 and 10.28%, 27.21, 8.26% respectively. While the highest significant decrease in energy was recorded with sprout inoculated by *Az. chroococcum* only being 325.85 kcal /g.

Regarding the interaction between $CO_2$ concentrations and microbial inoculants, no significant differences were recorded between the treatments in moisture and crude fiber in the sprout. While pea sprout treated by 800 ppm $CO_2$ and inoculated by *Az. chroococcum*+ *B. megaterium* + *Ps. fluorescens* recorded the highest significant increase in protein, lipids, and ash as well as the highest significant decrease in carbohydrate being 48.65, 4.95, 10.69 and 21.77%, respectively. While the highest significant decrease in energy was recorded within sprout treated by 600ppm $CO_2$ and inoculated by *Az. chroococcum*+ *B. megaterium* + *Ps. fluorescens* being 324.31 kcal/g.

**Minerals content of pea sprout cuts as affected by $CO_2$ concentrations, microbial inoculants, and their interactions**

Data in **Table (3)** generally show that cutting pea sprout shoot 14 days after seed sowing (1st cut) recorded increase in all minerals values with medium $CO_2$ concentration (600 ppm) followed by ambient air concentration while the lowest minerals values of P, K, Ca, Mg, Fe and Zn were recorded with higher $CO_2$ concentration (800 ppm). These data indicated that increasing $CO_2$ concentration to 800 ppm affected the translation of minerals from pea seed's cotyledons and roots to pea sprout shoots which recorded the lower contents.

Regarding microbial inoculants, **Table (3)** shows that inoculating pea sprout by combination of *Az. chroococcum*+ *B. megaterium* + *Ps. fluorescens* recorded the highest significant increase in all minerals content in green pea sprout being 0.419, 2.391, 3.11, 0.508%, 85.3 and 51.1 ppm, respectively, followed by those inoculated by *Az. chroococcum*+ *Ps. fluorescens* being 0.360, 2.281, 2.993, 0.469 % and 83.0, 49.1 ppm.

The combined interaction between $CO_2$ concentration and microbial inoculants reveal that no significant difference in the K% and Fe ppm in pea sprout.

The highest significant mineral values of P, Ca, Mg, Fe and Zn were recorded with pea sprout treated by 600 ppm $CO_2$ and inoculated by combination of *Az. chroococcum* + *B. megaterium* + *Ps. fluorescens* being 0.545, 3.535, 0.620% and 61.3 ppm, respectively. Followed by those inoculated by *Az. chroococcum* + *Ps. fluorescens* in the same concentration being 0.460, 3.390, 0.588% and 57.3 ppm respectively. While the lowest P, Ca, Mg and Zn values were recorded inpea sprouts treated by 800 ppm $CO_2$ either un-inoculated by microbial inoculants (control) or inoculated by *B. megaterium*.

Carbone dioxide ($CO_2$) level increased in the atmosphere from 270 ppm two hundred years ago to 370 - 400 ppm today due to fossil fuel use and deforestation, and it is expected to double its concentrations in the coming centuries. Most researchers focus on the growth performance of plants as affected by elevated $CO_2$ due to their ability to acquire $CO_2$ through photosynthesis. Therefore, different researchers reported that elevating $CO_2$ directly improve photosynthetic processes in plants especially those with the $C_3$ photosynthetic pathway suggesting a wide range of physiological, biochemical and morphological responses **(Amthor 2001; Kimball et al 2002; Leakey et al 2009 and Yossife et al 2017).** In general, higher $CO_2$ concentrations increase plant production due to higher rates of photosynthesis and water utilization (**Ludwig and Asseng 2006)**. Results obtained in this investigation are in line with those reported by **Jitla et al (1997); Sage Rowan (2002**) and **Centritto et al (1999)** who mentioned that high $CO_2$ generally stimulate the

**Table 2.** Effect of $CO_2$ concentrations, microbial inoculants, and their interactions on proximate analysis (g/100g) and energy (kcal/g) of green pea sprouts shoot (Combined data of two experiments)

| $CO_2$ | Microbial inoculants | Proximate analysis % | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Moisture | Protein | Lipids | Carbohydrates | Fiber | Ash | Energy (Kcal. /g) |
| Ambient air | Control | 4.57 a | 32.54k | 3.35 k | 41.62 a | 8.77 a | 9.16 l | 326.75cd |
| | *Az. chroococcum* | 4.71 a | 36.73 j | 3.55 j | 36.97 b | 8.62 a | 9.41 k | 326.79cd |
| | *B. megaterium* | 4.63 a | 36.65 j | 3.49 jk | 37.32 b | 8.55 a | 9.36 k | 327.23bc |
| | *Ps. fluorescens* | 4.67 a | 36.66 j | 3.51 jk | 37.23 b | 8.53 a | 9.39 k | 327.18 bc |
| | *Az. chroococcum +B. megaterium* | 4.86 a | 38.25 i | 3.88 i | 35.12 c | 8.47 a | 9.42 k | 328.39 a |
| | *Az. chroococcum +Ps. fluorescens* | 4.92 a | 38.33 i | 3.92 i | 34.91 c | 8.44 a | 9.47 jk | 328.27 a |
| | *B. megaterium + Ps. fluorescens* | 4.77 a | 38.22 i | 3.77 i | 35.42 c | 8.43 a | 9.39 k | 328.49 a |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 5.22 a | 39.21h | 4.13 h | 33.50 e | 8.33 a | 9.61 i | 327.99 ab |
| **Mean** | | **4.80 B** | **37.0C** | **3.70 C** | **36.51 A** | **8.52 A** | **9.40 C** | **327.64 A** |
| $CO_2$ (600 ppm) | Control | 4.79 a | 39.44g | 3.45 jk | 34.06 d | 8.69 a | 9.56 ij | 325.09 hi |
| | *Az. chroococcum* | 5.22 a | 39.87 f | 4.21 gh | 31.98 f | 8.56a | 10.16 gh | 325.28fh |
| | *B. megaterium* | 5.19a | 39.77 f | 4.11 h | 32.32 f | 8.49a | 10.11 h | 325.38fh |
| | *Ps. fluorescens* | 5.23 a | 39.84 f | 4.17 gh | 32.15 f | 8.48a | 10.13 h | 325.49fh |
| | *Az. chroococcum +B. megaterium* | 5.49 a | 41.56d | 4.33 eg | 29.97 ij | 8.45 a | 10.19 gh | 325.13 hi |
| | *Az. chroococcum +Ps. fluorescens* | 5.65 a | 41.59d | 4.44 de | 29.62 j | 8.42 a | 10.27 eg | 324.86 hi |
| | *B. megaterium + Ps. fluorescens* | 5.44a | 41.51d | 4.25 fh | 30.23 hi | 8.40a | 10.17 gh | 325.21 gi |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 5.77 a | 44.45b | 4.56 cd | 26.36 l | 8.31 a | 10.55 b | 324.31 i |
| **Mean** | | **5.35 A** | **41.0B** | **4.19B** | **30.84 B** | **8.47A** | **10.14 B** | **325.09 C** |
| $CO_2$ ( 800 ppm) | Control | 4.83 a | 39.84 f | 4.16 gh | 32.34 f | 8.62 a | 10.22 fh | 326.12dg |
| | *Az. chroococcum* | 5.27 a | 40.77e | 4.43 df | 30.62 gh | 8.56a | 10.34cf | 325.47fh |
| | *B. megaterium* | 5.22 a | 40.63e | 4.27eh | 31.12 g | 8.47a | 10.28dg | 325.49fh |
| | *Ps. fluorescens* | 5.23 a | 40.67e | 4.32 eg | 31.03 g | 8.43 a | 10.32 cf | 325.67eh |
| | *Az. chroococcum +B. megaterium* | 5.58 a | 43.29c | 4.71 bc | 27.81 k | 8.20 a | 10.41 cd | 326.79 cd |
| | *Az. chroococcum +Ps. fluorescens* | 5.67 a | 43.33c | 4.75 b | 27.63 k | 8.17 a | 10.45 bc | 326.56 ce |
| | *B. megaterium + Ps. fluorescens* | 5.55 a | 43.20c | 4.63 bc | 28.08 k | 8.15 a | 10.39 ce | 326.79 cd |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 5.82 a | 48.65a | 4.95 a | 21.77 m | 8.13 a | 10.69 a | 326.22 df |
| **Mean** | | **5.40 A** | **42.5A** | **4.53A** | **28.80C** | **8.34B** | **10.39A** | **326.14B** |
| Average | Control | 4.73 E | 37.2D | 3.65 F | 36.01 A | 8.69 A | 9.65 E | 325.99 C |
| | *Az. chroococcum* | 5.07 D | 39.1C | 4.07 D | 33.19 C | 8.58 B | 9.97 CD | 325.85 C |
| | *B. megaterium* | 5.01 D | 39.0C | 3.96 E | 33.59 B | 8.50 B | 9.92 D | 326.03C |
| | *Ps. fluorescens* | 5.04 D | 39.0C | 4.00DE | 33.47BC | 8.48 BC | 9.95 CD | 326.11BC |
| | *Az. chroococcum +B. megaterium* | 5.31BC | 41.0B | 4.31BC | 30.97DE | 8.37CD | 10.01BC | 326.77A |
| | *Az. chroococcum +Ps. fluorescens* | 5.41 B | 41.0B | 4.37 B | 30.72 E | 8.34 D | 10.06 B | 326.56AB |
| | *B. megaterium + Ps. fluorescens* | 5.25 C | 40.9B | 4.22 C | 31.24 D | 8.33 D | 9.98CD | 326.83A |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 5.60 A | 44.1A | 4.55 A | 27.21 F | 8.26 D | 10.28 A | 326.17BC |
| L.S.D | $CO_2$ concentration | 0.0852 | 0.0799 | 0.0589 | 0.1910 | 0.0677 | 0.0406 | 0.2934 |
| | Bio-fertilizers | 0.1391 | 0.1305 | 0.0963 | 0.3119 | 0.1105 | 0.0663 | 0.4792 |
| | $CO_2$×biofertilizer | NS | 0.2260 | 0.1667 | 0.5402 | NS | 0.1148 | 0.8299 |

Means in each column followed by the same letter are not significantly different at the 5% level.
NS= not significant

**Table 3.** Effect of $CO_2$ concentrations, microbial inoculants, and their interactions on minerals content of pea sprout shoots (Combined data of two experiments)

| $CO_2$ | Microbial inoculants | Minerals content | | | | | |
|---|---|---|---|---|---|---|---|
| | | P% | K% | Ca% | Mg% | Fe(ppm) | Zn(ppm) |
| Ambient air | Control | 0.232 jk | 1.818 a | 2.780 i | 0.313 k | 67.8 a | 31.3 n |
| | *Az. chroococcum* | 0.288 h | 1.922 a | 2.885 fg | 0.398 h | 76.3 a | 41.7 gi |
| | *B. megaterium* | 0.265 hi | 1.818 a | 2.808 hi | 0.320 k | 71.2 a | 33.0 ln |
| | *Ps. fluorescens* | 0.328 fg | 1.860 a | 2.840 gh | 0.353 j | 72.0 a | 35.3 kl |
| | *Az. chroococcum +B. megaterium* | 0.315 g | 1.947 a | 2.933 ef | 0.415 gh | 82.2 a | 44.8 ef |
| | *Az. chroococcum +Ps. fluorescens* | 0.372 de | 1.985 a | 2.967 e | 0.442 f | 82.2 a | 47.0 de |
| | *B. megaterium + Ps. fluorescens* | 0.345 ef | 1.885 a | 2.867 g | 0.365 ij | 76.5 a | 40.7 hi |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 0.395 d | 2.067 a | 3.145 cd | 0.470 e | 84.0 a | 47.8 d |
| **Mean** | | **0.318 B** | **1.913 B** | **2.903 B** | **0.385 B** | **76.5B** | **40.2 B** |
| $CO_2$ (600 ppm) | Control | 0.320 fg | 2.795 a | 2.868 g | 0.423 fg | 83.3 a | 43.5 fg |
| | *Az. chroococcum* | 0.372 de | 2.900 a | 3.091 d | 0.523 d | 89.5 a | 55.0 bc |
| | *B. megaterium* | 0.342 fg | 2.818 a | 2.930 ef | 0.442 f | 85.7 a | 45.0 ef |
| | *Ps. fluorescens* | 0.430 c | 2.872 a | 2.965 e | 0.478 e | 87.5 a | 47.3 de |
| | *Az. chroococcum +B. megaterium* | 0.375 d | 2.922 a | 3.180 c | 0.560 c | 93.0 a | 53.8 c |
| | *Az. chroococcum +Ps. fluorescens* | 0.460 b | 2.953 a | 3.390 b | 0.588 b | 95.0 a | 57.3 b |
| | *B. megaterium + Ps. fluorescens* | 0.442bc | 2.910 a | 2.960 e | 0.515 d | 90.7 a | 48.3 d |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 0.545 a | 3.067 a | 3.535 a | 0.620 a | 97.2 a | 61.3 a |
| **Mean** | | **0.411 A** | **2.905 A** | **3.115 A** | **0.519 A** | **90.2A** | **51.5 A** |
| $CO_2$ ( 800 ppm) | Control | 0.158 l | 1.750 a | 2.470 m | 0.237 n | 59.5 a | 31.2 n |
| | *Az. chroococcum* | 0.208 k | 1.873 a | 2.556 l | 0.325 k | 67.8 a | 36.7 jk |
| | *B. megaterium* | 0.178 l | 1.792 a | 2.453 m | 0.253mn | 62.3 a | 32.0 mn |
| | *Ps. fluorescens* | 0.232 jk | 1.803 a | 2.463 m | 0.282 l | 64.8 a | 33.0 ln |
| | *Az. chroococcum +B. megaterium* | 0.208 k | 1.893 a | 2.573 kl | 0.362 ij | 71.7 a | 39.0 ij |
| | *Az. chroococcum +Ps. fluorescens* | 0.248 ij | 1.905 a | 2.623 jk | 0.377 i | 71.8 a | 43.0 fh |
| | *B. megaterium + Ps. fluorescens* | 0.262 hi | 1.845 a | 2.537 l | 0.268lm | 66.7 a | 34.5 km |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 0.318 fg | 2.038 a | 2.673 j | 0.433 fg | 74.8 a | 44.2 fg |
| **Mean** | | **0.227 C** | **1.863 C** | **2.544 C** | **0.317 C** | **67.4C** | **36.7 C** |
| Average | Control | 0.237 F | 2.121 F | 2.706 G | 0.324 G | 70.2 F | 35.3 F |
| | *Az. chroococcum* | 0.289 D | 2.232 C | 2.844 D | 0.416 D | 77.9 C | 44.4 C |
| | *B. megaterium* | 0.262 E | 2.143EF | 2.731FG | 0.338 F | 73.1 E | 36.7 F |
| | *Ps. fluorescens* | 0.330 C | 2.178DE | 2.756 F | 0.371 E | 74.8 D | 38.6 E |
| | *Az. chroococcum +B. megaterium* | 0.299 D | 2.254BC | 2.896 C | 0.446 C | 82.3 B | 45.9 C |
| | *Az. chroococcum +Ps. fluorescens* | 0.360 B | 2.281 B | 2.993 B | 0.469 B | 83.0 B | 49.1 B |
| | *B. megaterium + Ps. fluorescens* | 0.349 B | 2.213CD | 2.788 E | 0.383 E | 77.9 C | 41.2 D |
| | *Az. chroococcum +B. megaterium +Ps. fluorescens* | 0.419 A | 2.391 A | 3.118 A | 0.508 A | 85.3 A | 51.1 A |
| L.S.D | $CO_2$ concentration | 0.0090 | 0.0239 | 0.0190 | 0.0074 | 0.7709 | 0.9094 |
| | Bio-fertilizers | 0.0148 | 0.0390 | 0.0310 | 0.0120 | 1.2588 | 1.4850 |
| | $CO_2$×biofertilizer | 0.0256 | NS | 0.0537 | 0.0208 | NS | 2.5722 |

Means in each column followed by the same letter are not significantly different at the 5% level.
NS= not significant

photosynthetic process that leads to significant high growth rate. As well, **Drake et al (1999) and Amthor (2000)** reported that increasing atmospheric $CO_2$ is causing respiratory inhibition and water balance of the plants that stimulates plant growth and yield. Also, **Stephen et al (2011**) reported that elevated $CO_2$ stimulates photosynthesis that leads to increase carbon (C) uptake and assimilation, thereby increasing plant growth.

The important of PGPB could be manifested on their ability to excrete phytohormones such as auxins and gibberellins, etc., thereby improving the growth and early development of plants **(Zayed Mona 2012; Ba'konyi et al 2013; Zayed Mona et al 2013 and Selim and Zayed Mona 2017)**. In this subject, (**Kloepper and Beauchamp 1992)** mentioned that inoculation of wheat by the *Azotobacter* sp. and *Bacillus* sp. increased its yield. Based on our results, microbial inoculant treatments improved the growth performance of pea when compared to control at which the combination between *Azotobacter chroococcum, Bacillus megaterium* and *Pseudomonas fluorescens* recorded the best effect on plant growth performance which could be displayed in shoot length, fresh and dry weight, which agree with the results mentioned by **Zayed Mona (2012) and and Selim and Zayed Mona (2017).** Also, **Cakmakci et al (2007)** reported that inoculation of plants with $N_2$ fixing bacteria significantly increased the uptake of N, Fe, Mn, and Zn by barley seedlings when compared to the uninoculated plants (control). Also, plant responses to $N_2$ fixing bacteria could be associated with other mechanisms, rather than direct $N_2$ fixation such as production of hormones which has been suggested as one of the mechanisms by which PGPR stimulate plant growth **(Cakmakci et al 2007)**.

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# استجابة نبت البسلة الخضراء للأضافات الميكروبيه وزيادة تركيز ثاني أكسيد الكربون في الغلاف الجوي

# [185]

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**الموجـــــــز**

يتاثر انتاج نبت البسلة الاخضر *Pisum sativum* L. بشكل كبير بالحالة البيئية المحيطة به خاصة مع زيادة تركيز ثاني أكسيد الكربون في الهواء الجوى. وفى هذا العمل تم التركيز على تأثيرات الظروف المناخية المتنبأ بها على التغيرات الداخلية لجودة نبت البسلة باستخدام تركيز ثاني أكسيد الكربون بمقدار 600 و800 جزء في المليون مقارنة بالهواء الجوى المحيط ، وكذلك تاثيراللقاحات الميكروبيه بإضافه ثلاثة انواع من الميكروبات والخلط بينهم وذلك فى غرف خاصة للإنبات نصف اتوماتيك.

وقد أظهرت النتائج أن أكبر إنتاجية من نبت البسلة في وحدة المساحة كانت في تركيز 800 جزء في المليون من ثاني أكسيد الكربون مع زيادة حوالي 20٪ أكثر عن الهواء الجوى المحيط تليها 600 جزء في المليون مع زيادة حوالي 9.4٪ من ثاني أكسيد الكربون $CO_2$ في الجو المحيط. وعند 800 جزء في المليون كانت هناك زيادة فى محتوى البروتين الخام الكلى من البسلة 37.8٪ والدهون 46.9٪ والطاقة بنسبة 19.5٪ لكل وحدة مقارنة بالهواء الجوى المحيط في حين انخفض محتوى الكربوهيدرات بنسبة 5.3٪ ، وقد أوضحت نتائج الدراسة أن نبت البسلة ينمو في أعلى تركيز من ثاني أكسيد الكربون مع الحفاظ على الجودة الداخلية المثلى.

**الكلمات الدالة:** البسلة، النبت، $CO_2$، اللقاحات الميكروبيه

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