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# Facilitating the Absorption of Nitrogen and Phosphorous by Using Some Specialized Microbes and its Reflection on the Yield and Quality of the Pepper Crop

*[1]El-Sayed El-B. Ibrahiem and [2]Heba. S. Shehata*

[1]Vegetable Research Departments, Horticulture Research Institute,
Agricultural Research Center, Giza, Egypt
[2]Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt

**Abstract:** Bio- agriculture is necessary for the future supported by good agricultural practices. The optimal direction in modern agriculture is to reduce high-cost chemical fertilizers that cause soil and water pollution and the demand is increasing all over the world for vegetables produced with bio-fertilizer, so the direction was to produce vegetables fertilized with bio-fertilizer. Two field experiments were carried out during the two successive seasons of 2021 and 2022 in clay soil at Kaha Vegetable Research Farm, Qalubia Governorate, Egypt to study the effect of two local bacterial strains, *i.e*., *pseudomonas monteilii* MB-4 and *Streptomyces rochei* MA-6 on sweet pepper plants (*Capsicum annum* L.) cv. Fares, $F_1$ hybrid grown under open field condition to reduce the use of nitrogen and phosphate fertilizers in addition to the quantity and quality improvements of the fruits. The results showed that using all applied bacterial strains treatment recorded significant increases for all growth parameters of pepper plants *i.e*. plant height, the total number of branches per plant, average stem diameter, both fresh and dry weight/plant, each of fresh weight, length and diameter of fruit as well as early and total pepper yield as compared with the recommended chemical fertilization rate NPK100% (T1) and/or the two deficiency levels of both N and P in both seasons. The highest total pepper yield was achieved by a mixture of bacterial strains at a mineral fertilization level of N 75% +P 33.3% +K100% (T9) followed by the T7 at a mineral fertilization level of N 75 % +P33.3 % +K100% compared to the rest of the treatment and T1 without applied bacterial strains in both seasons. The treatment (T9) led to an increment of the total yield by 37.6% in the average of the two seasons and a decrease in the total costs of production. It can be recommended that applied a mixture of bacterial strains or used *Streptomyces rochei* alone plus a chemical fertilization level of N 75 % +P33.3 % +K100% in both treatments to enhance pepper production.

**Key words:** *Pseudomonas monteilii* · *Streptomyces rochei* · Growth and yield of sweet pepper

## INTRODUCTION

Pepper (*Capsicum annuum* L.) is an important public vegetable crop in Egypt and also a national diet in many tropical and sub-tropical countries. It is an important source of several antioxidants due to its fruits containing vitamin C, phenolic compounds, flavonoids and many different carotenoids [1]. Egypt is one of the major countries of pepper production and ranked second after Nigeria in Africa and the first Arab according to FAO statistics [2]. The cultivation of microorganisms in the laboratory requires a nutrient environment that serves as a source of nutrients for growth.

Available Commercial media, *i.e*., Nutrient Agar and Mac Conkey Agar used for the growth of microorganisms are very expensive. Therefore, plant-based culture media that are natural environment (low-cost media rich in nutrients) has been introduced for the culturing of rhizobacteria as a sole growth milieu [3, 4].

Both fresh and dried leaves of *Mentha piperita* with various spices have been traditionally used as low-cost media rich in nutrients like carbohydrates, proteins, lipids, fibers and mineral elements, *i.e*., potassium, sodium, calcium, magnesium and phosphorus [5]. Plant growth-promoting rhizobacteria (PGPR) have the ability to colonize plant roots and increase plant growth by a wide

**Corresponding Author:** El-Sayed El-B. Ibrahiem, Vegetable Research Departments, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

range of mechanisms including the production of phytohormones, *i.e*., indole acetic acid (IAA), cytokinin and gibberellins. Also, PGPR can affect positively plant growth through several mechanisms like phosphate solubilization, siderophore production, biological nitrogen fixation, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), phytohormone production, exhibiting antimicrobial activity [6].

Nitrogen plays an important role in chlorophyll synthesis, photosynthesis, carbon dioxide assimilation and cell division in addition to improving vegetative growth and increasing yield [7]. Phosphorus is the second most abundant metallic element after nitrogen and both organic and inorganic forms are found in the soil. It has crucial roles in metabolic processes (quality of agricultural products, photosynthesis, energy transfer and resistance to plant diseases and pathogens). Phosphate solubilization in the rhizosphere is one of the most common modes of action implicated in PGPR that increases nutrient availability and has positive effects on plant growth [8]. The use of chemical fertilizers, particularly nitrogenous and phosphorous, has led to substantial pollution of soil, air and water. Excessive use of these chemicals exerts deleterious effects on soil microorganism, affects the fertility status of soil, pollutes the environment and also decline the productivity of crop [9, 10]. Kafi *et al*. [11] evaluated the effect of *P. putida* strain P3-57 with 100 % or 70 % of the recommended amount of NPK fertilizers on cucumber fruit yield and quality compared to Barvar NPK (commercial bio-fertilizer) under commercial greenhouse conditions.

The most important genera of rhizobacteria include *Pseudomonas, Streptomyces* and others studies by Adesemoye *et al.* [12] and Dinesh *et al*. [13] with rhizobacteria and inorganic fertilizers have reported an increase in plant growth due to increase nutrient uptake promoted by these microorganisms and/or the influence of inorganic fertilizers in increasing populations of rhizobacteria in the soil mainly producing indole acetic acid (IAA) and phosphorus solubilizing [14, 15]. Naseri and Mirzaei [16] reported that application of the rhizobacterial genera *Azotobacter* and *Azospirillum* reduced 50% of inorganic nitrogen and significantly increased the growth of sunflower plants. The objective of this study is to reduce the use of nitrogen and phosphate fertilizers as well as increase the yield and improve the quality characteristics by using *pseudomonas monteilii* and *Streptomyces rochei* on sweet pepper plants in the field.

## MATERIALS AND METHODS

Two field experiments were carried out during the two successive summer seasons of 2021 and 2022, at Kaha Vegetable Research Farm, Qalubia Governorate, Egypt. The present investigation was to study the effect of two bacterial strains, *i.e.*, *Pseudomonas monteilii* MB-4 and *Streptomyces rochei* Ma-6 under different levels of mineral fertilization on vegetative growth, yield and fruit characters of sweet pepper (*Capsicum annum* L.) cv. Fares, $F_1$ grown under open the field conditions. Seeds of sweet pepper were sown on 12$^{th}$ and 4$^{nd}$ of January in 2021 and 2022 seasons, respectively in seedling trays (209 cells) filled with a mixture of peat-moss and vermiculite (1:1 v/v) under unheated greenhouse conditions. The seedlings were transplanted after 50 days in both seasons in a randomized complete block design with three replicates. Physical and chemical analysis at the experimental site, which is characterized as clay soil (Table 1), was done before sowing.

Each plot area was 7.45 m² (the plot consisted of three rows, each row was 0.7 m wide and 3.55 m long) with a spacing of 40 cm between plants. All the treatments were fertilized with the corresponding rates of NPK, calcium superphosphate (15.5% $P_2O_5$) was added once before planting as a source of phosphorus. Both ammonium sulphate (20.5% N, as a source of nitrogen) and potassium sulphate (48% $K_2O$, as a source of potassium) were applied in four constant doses. Common cultural practices concerning sweet pepper production, such as surface irrigation, weed control and pest management, were conducted whenever necessary according to the recommendation of the Egyptian Ministry of Agriculture and Land Reclamation.

The following methods of soil physical and chemical examination were used:

- Particle size distribution of the soil samples was conducted according to pipette method described by Piper [17].
- Soil pH, soil calcium carbonate, electrical conductivity, organic matter content were all done as described by Page *et al*. [18].
- Cation exchange capacity was determined as the method of Chapman and Pratt [19].
- Available N, P and K were determined using the methods outlined by Jackson [20].
- Available Fe, Mn, Cu and Zn were extracted according to Lindsay and Norvell [21] and determined using atomic absorption spectrophotometry.

Table 1: Physical and chemical properties of the experimental soil during both seasons of 2021 and 2022.

| | Soil characters | | | | | | | | | | | | | | | | | Elements (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mechanical analysis (%) | | | | | Chemical analysis | | | | Soluble cations (M/L) | | | | Soluble anions (M/L) | | | | Macro | | | Micro | | | |
| Seasons | Coarse sand % | Fine sand % | Silt % | Clay % | Texture class | EC $dSm^{-1}$ | PH | CaCO3 % | Organic matter% | $Ca^{+2}$ | $Mg^{+2}$ | $Na^{+}$ | $K^{+}$ | $CO3^{-2}$ | $HCO^{-3}$ | $Cl^{-2}$ | $SO4^{-2}$ | N | P | K | Fe | Cu | Zn | Mn |
| 2021 | 39.9 | 9.5 | 28 | 53 | Clay | 2.63 | 7.7 | 3.3 | 1.7 | 6.9 | 3.85 | 15.6 | 0.19 | - | 2.3 | 9.5 | 13.2 | 85 | 6.5 | 57.6 | 3.3 | 6.5 | 2.21 | 2.22 |
| 2022 | 40.1 | 10.0 | 27 | 51.5 | Clay | 2.61 | 7.9 | 3.2 | 1.9 | 6.7 | 3.78 | 15.8 | 0.17 | - | 2.4 | 10.0 | 13.1 | 84 | 6.4 | 56.9 | 3.4 | 6.7 | 2.23 | 2.21 |

Table 2: Chemical and bio-fertilizer treatments used in this study.

| | |
|---|---|
| T1 | Full NPK % ( recommended mineral fertilization $N_{150\ kg} + P_2O_{5(60\ kg)} + K_2O_{(120\ kg)}$ / fed.) |
| T2 | 50 % N + 33.3 % $P_2O_5$ +100% $K_2O$ = ($N_{75\ kg}$+$P_{20\ kg}$+$K_{120\ kg}$) |
| T3 | 75 % N + 33.3 % $P_2O_5$ +100% $K_2O$ = ($N_{112\ 5kg}$+ $P_{20\ kg}$+$K_{120\ kg}$) |
| T4 | *Pseudomonas monteilii* MB-4 + T2 |
| T5 | *Pseudomonas monteilii* MB-4 + T3 |
| T6 | *Streptomyces rochei* Ma-6 + T2 |
| T7 | *Streptomyces rochei* Ma-6 + T3 |
| T8 | *Pseudomonas monteilii* MB-4 + *Streptomyces rochei* Ma-6 + T2 |
| T9 | *Pseudomonas monteilii* MB-4 + *Streptomyces rochei* Ma-6 + T3 |

Table 3: Some characteristics of microorganisms (Bacterial strains) used in the present study.

| Species | Strains | IAA* | GA** | T-Carb. ⫞ | Am-P† | Ph-Sol.‡ |
|---|---|---|---|---|---|---|
| *Pseudomonas monteilii* | MB-4 | 36.51 | 5.15 | 56 | +++ | 729.62 |
| *Streptomyces rochei* | Ma-6 | 10.06 | 27.86 | 89 | +++ | 867.85 |

*IAA: Indole acitic acid (µg/ml), **GA: Gibrellines (µg/ml), ⫞T-Carb.: Total carbohydrates (µg/ml),
†Am-P: Ammonia production and ‡Ph-Sol.: Phosphate solubilization (µg/ml).

The experiment was contained nine treatments as the following in Table 2.

**Bacterial Strains:** Both local *pseudomonas monteilii* MB-4 and *Streptomyces rochei* Ma-6 bacterial strains were kindly provided by Microbiology Department, Soils, Water and Environment, Research Institute (ARC), Giza, Egypt where both strains were isolated, purified and identified as mentioned by Ibrahim *et al*. [22]. The biofertilization with bacterial strains developed on plant based culture media temperature 30°C for 24-48 hours.

**Preparation of Plant-based Culture Media:** The vegetative parts (leaves and stems) of *Mentha viridis* and the succulent plants *Aloe vera* were washed, sliced and then blended with equal aliquots of distilled water (w/v) for 5 min in the blender. The resulting slurry homogenate was coarsely filtered through cheesecloth to obtain plant juice; almost 73- 82% of the plant's fresh weight was recovered as juice. The pH for juice was in the range of 5.8-6.5. The obtained plant juice was further diluted with distilled water (v/v) at 1:10, 1:20, 1:40, 1:80 and 1:100. Exclusively, such diluted juice was used to prepare the plant-based agar culture media (2% agar, w/v). The media was adjusted to pH 7.0 and autoclaved at 1.5 atm., 121°C for 20 mins. The cultures were then diluted 10 times with distilled water resulting $10^8$ colony-forming units (cfu) $mL^{-1}$. After the successful growth of microorganisms, pick up the individual colonies and purify them.

**Application Methods:** Three weeks after seedling transplanting, bacterial suspensions were applied at a rate of 20 L/fed. and repeated twice each 30 days interval. Controls (Non-inoculated) received fresh medium diluted 10 times. For mixed inoculation, mixtures of PGPR strains were prepared by combining equal proportions of each strain before applying them to the plant.

***In-vitro* Characterization of Bacterial Endophytes as Plant Growth-promoting in Table 3:**

- Total gibberellins (GA) was determined according to Udagwa and Kinoshita [23].
- The indole acetic acid (IAA) was determined according to Glickman and Dessaux. [24].
- The phosphate solubilization was analyzed quantitatively using the method of Mehta and Nautiyal [25].
- Total carbohydrate content was determined according to Dubois *et al*. [26].
- Bacterial strains were assessed for their potential for production of ammonia was determined according to Cappucino and Sherman [27].

**Data Recorded:**

**Growth Parameters:** A random sample of five pepper plants were taken from each plot after 70 days of transplanting to estimate the plant height (PH, cm), total number of branches per plant (NB), average stem diameter (SD, cm) and both fresh (FW) and dry (DW) weight of both leaves and stems (g/plant) as well as number of days from transplanting to 50% flowering per plot (DF).

**Fruit Yield and its Characteristics:** A sample of five fruits at marketable stage were taken randomly of sweet pepper fruits from the fourth picking to measure the average each of fruit length (cm), fruit diameter (cm) and fruit weight (g) in addition to early yield (ton/fed) was expressed as weight of the first two pickings and total yield (ton/fed) was expressed as weight of fruits for all harvestings.

**Fruit Chemical Content:**

- Ascorbic acid (vitamin C) content in sweet pepper fruits tissues as mg/100g fresh weight according to the method described by AOAC [28]
- Elemental Analysis, *i.e.,* nitrogen, phosphorous and potassium content (%) in sweet pepper fruits tissues. Nitrogen was determined with the modified "Micro Kjeldahl" apparatus as described by Plummer [29]. Phosphorus was determined spectrophotometrically by using stannous chloride method according to AOAC [28]. Potassium was measured with flame photometer according to the method described by Jackson [30].
- Total chlorophyll content in sweet pepper fruits tissues (mg/g fresh weight) was determined according to the method of Lichtenthaler and Wellburn [31].

**Chemical Characters of Sweet Pepper Leaves:**

- Total leaf chlorophyll was measured at the flowering stage, using Minolta chlorophyll meter SPAD- 501 as SPAD units.
- Total nitrogen, phosphorus and potassium were determined in dry plant according to the methods described by Plummer[29]; Jackson [20] and Piper[17], respectively.

**UPGMA Clustering Dendrogram:** The cluster analysis was performed the NTSYS-pc program version 2.1 according to Rohlf [32] to construct at dendrogram tree by UPGMA (unweighted pair group method with arithmetic averages) proposed by Sneath and sokal [33].

**Economic Study:** To perform an economic analysis, the total cost of production and total income were calculated to estimate the total outlay, the cost of all agricultural inputs and practices was used at prevailing market rates. The total income was calculated by multiplying the total fruit production by the yield unit price in the local market. Net returns were calculated by subtracting the total cost from the total income. The benefit-cost ratio was calculated according to Boardman *et al*. [34] by dividing the gross return on total variable cost (Egyptian pound /fed).

**Statistical Analysis:** Data were subjected to the statistical analysis of variance according to Snedecor and Cochran [35] using Statistix 8 program and means were compared using L.S.D at 5%.

## RESULTS AND DISCUSSION

**Vegetative Growth Parameters:** Data obtained on days to 50% flowering of evaluated all applied bacterial strains treatment on pepper plants are presented in Table 4 and Fig. 1. Number of days to 50% flowering of bacterial strains treatments ranged from 60.8 & 61.6 to 64.9 & 65.1 days as compared to the control levels which ranged from 56.0 & 56.7 to 65.0 & 66.5 days in 1$^{st}$ and 2$^{nd}$ seasons, respectively. Among the bacterial strains, it was found that the lowest number of days to 50% flowering was recorded by T5 in average of both seasons followed by T9 and T4 with non-significant differences between them.

Changes in vegetative growth traits as affected by different fertilizer (bio-fertilizer and/or NPK) treatments on the pepper plants grown under open field condition are presented in Table 4 and Fig. 1.

Table 4 show the vegetative growth traits of sweet pepper plants treated with *Pseudomonas monteilii* (MB-4) and/or *Streptomyces rochei* (MA-6) for both nitrogen deficiency (50% & 75% N and 33.3% $P_2O_5$) as well as recommended application (100% NPK) control and the rest of control levels T2 and T3 treatments.

With respect to nitrogen deficiency; the results clearly indicated that all nitrogen levels had a significant effect on all vegetative growth characteristics. However, the 100% nitrogen rate (recommended dose) treatment had higher values than other N-deficiency treatments in all studied characters with non- significant differences between T1 (100%N) and T3 (75%N) in plant fresh weight and stem diameter in both seasons as well as plant dry weight in 2$^{nd}$ season.

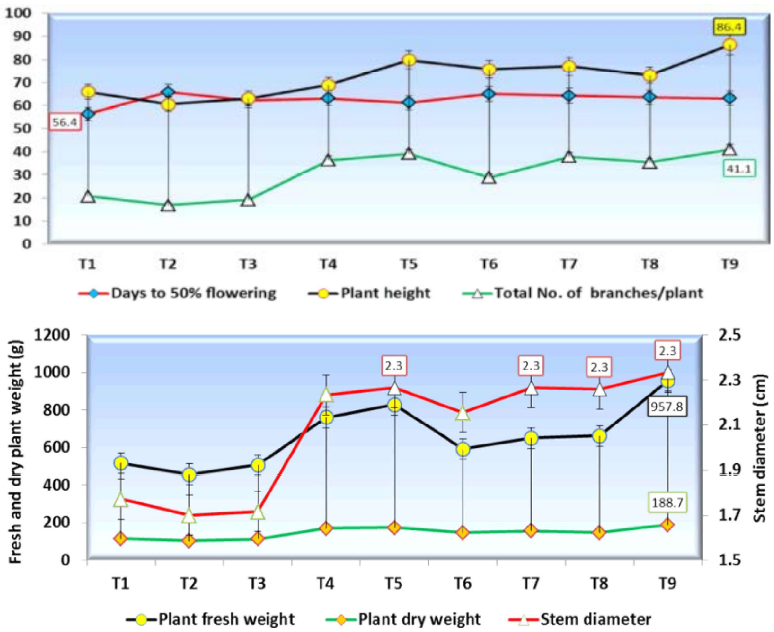


Fig. 1: Flowering date and vegetative growth traits (average of both seasons) as affected by synthetic and bio-fertilizer treatments on pepper plants at both 2021 and 2022 seasons

Table 4: Changes values in vegetative growth traits as affected by synthetic and bio-fertilizer treatments on pepper plants at both 2021 and 2022 seasons

| Traits | Season | Treatments T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | L.S.D 5% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Flowering | | | | | |
| DF | 2021 | 56 | 65 | 62 | 63.3 | 60.8 | 64.9 | 63.9 | 63.5 | 62.6 | 1.2 |
| | 2022 | 56.7 | 66.5 | 62.6 | 62.7 | 61.6 | 65.1 | 64.6 | 63.6 | 63.5 | 2.09 |
| | | | | | | Vegetative growth traits | | | | | |
| PH (cm) | 2021 | 66.9 | 61 | 63.1 | 67.2 | 79.1 | 76.2 | 78 | 73.8 | 86.9 | 2.5 |
| | 2022 | 65 | 60.2 | 63 | 70.6 | 80.6 | 75.4 | 76 | 72.6 | 85.8 | 1.7 |
| NB | 2021 | 20.4 | 16.6 | 19 | 36.2 | 39.4 | 28.3 | 37.4 | 35.3 | 40.8 | 1.2 |
| | 2022 | 21 | 17.1 | 19.5 | 36.5 | 39 | 29.2 | 38.5 | 35.6 | 41.4 | 1.4 |
| FW (g) | 2021 | 515.3 | 445.4 | 509.3 | 763.7 | 846 | 589.9 | 652.4 | 658.2 | 960.2 | 32.1 |
| | 2022 | 518.4 | 468.3 | 504.3 | 756.8 | 813.4 | 593 | 646.9 | 666.8 | 955.3 | 30.2 |
| DW (g) | 2021 | 116.3 | 102.4 | 113.6 | 169.3 | 174.6 | 147.8 | 157 | 145.4 | 187.9 | 1.5 |
| | 2022 | 114.7 | 105 | 113.2 | 167.5 | 173.1 | 142.8 | 153.8 | 146.6 | 189.4 | 2.2 |
| SD (cm) | 2021 | 1.77 | 1.67 | 1.73 | 2.24 | 2.26 | 2.16 | 2.26 | 2.25 | 2.34 | 0.1 |
| | 2022 | 1.77 | 1.73 | 1.7 | 2.23 | 2.27 | 2.15 | 2.27 | 2.27 | 2.33 | 0.09 |

DF(days to 50% flowering ), PH(plant height), NB(total No. of branches/plant), FW(plant fresh weight), DW(plant dry weight ), SD(stem diameter)

Exposure of untreated plants (treated with distilled water) to low mineral applications of T2 (50%N & 33.3% P) caused obvious inhibition of stem diameter, plant height, plant dry weight, plant fresh weight and total No. of branches/plant by 3.95, 8.11, 10.22, 11.61 and 18.6%, while exposure to low mineral applications of T3 (75%N & 33.3% P) caused inhibition of plant dry weight, plant fresh weight, stem diameter, plant height and total No. of branches/plant by 1.82, 1.94, 3.11, 4.40 and 7.00% in descending order as an average of both seasons (Fig. 1). In this regard, Marschner [36]; El-Tohamy *et al*. [37] and Abusetta [38] found that the growth of plants was negatively affected by the low chemical fertilization treatments, especially at 50% of N. Oppositely, the increase in plant growth by relatively high levels of nitrogen may be attributed to the beneficial effects of N on stimulating the meristematic activity, for producing more tissues and organs and cell enlargement, since N plays major roles in the synthesis of structural proteins and other several macro-molecules, in addition to its vital contribution in several biochemical processes in the plant related to growth.

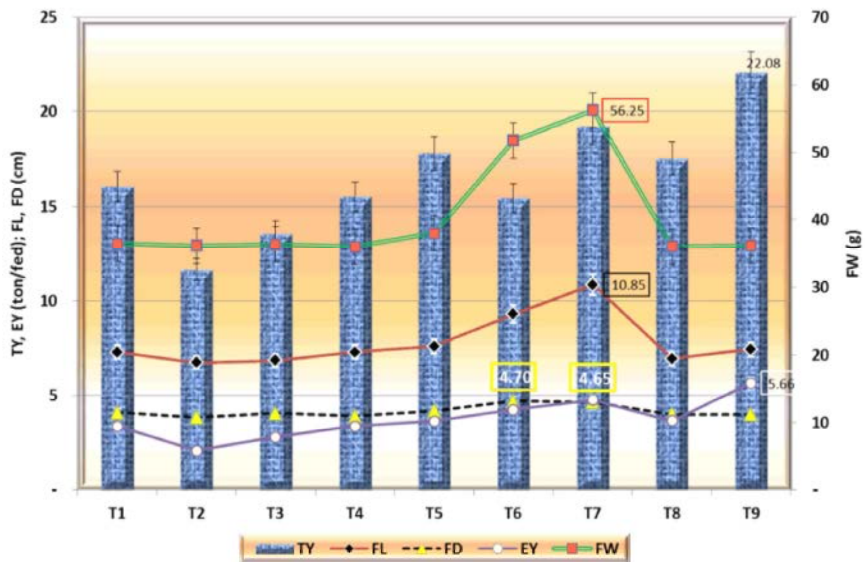


Fig. 2: Yield and fruit traits (average of both seasons) as affected by synthetic and bio-fertilizer treatments on pepper plants at both 2021 and 2022 seasons

Upon treatment of stressed plants with bacteria strains treatments, many significant increases in all vegetative growth traits were observed in both seasons compared to all control treatments (T1 of recommended NPK, in addition to both T2 and T3 stress treatments. T9 (mix+75%N+33.3%P+100%K) has significant maximum effects by 98.6, 85.3, 63.3, 31.9 and 30.9% (average of both seasons) for total No. of branches/plant, plant fresh weight, plant dry weight, stem diameter and plant height in descending order over the untreated plants of 100% NPK; in addition to 113.5, 89.0, 66.4, 37.0 and 36.2% as well as 143.9, 109.6, 81.9, 42.5 and 37.4% for total No. of branches/plant, plant fresh weight, plant dry weight, plant height and stem diameter in descending order over the untreated plants of 50 and 75% NPK, respectively. The beneficial effect of bacterial inoculation is a result of many components that work synergistically at different concentrations. These results are in agreement with those mentioned by Bacilio *et al*. [39] ; Chiquito-Contreras *et al*. [40]; He *et al*. [41] and Hernandez-Montiel *et al*. [42]. It is clearly noticed that all bio-fertilizer treatments applied along with 50% N gave statistically increase results as compared to the untreated plants of 100% recommended dose of N fertilizer, indicating the efficient role of the studied local bacterial strains for substitution of nitrogen N up to 50% [43].

The decline in the amount of nitrogen fertilization, without much reducing the trait leads to a lower cost of using nitrogen fertilizer and thus increases profit and also reduces pollution resulting from the use of nitrogen fertilizer [44]. Many investigators reported that applied *Pseudomonas sp* from tomato and sweet pepper plant rhizosphere, which have ability to produce antifungal metabolites, phosphate solubilization, HCN (Hydrocyanic Acid) and IAA (indole-3- acetic acid ) as Hamada *et al*. [45] and Omar *et al*. [8]. Moreover, using bacteria in fertilizer may be play a profound role in improving soil fertility and plant growth development via N2 fixation and releasing certain nutritive elements such Fe, Zn and Mn and some phytohormones such as gibberellins, auxins and cytokinins- substances which may encourage up taking and sufficient nutrients, subsequently enhance plant growth. [46]

**Yield and Its Attributed Traits:** Table 5 and Fig. 2 shows the yield and its attributed traits of sweet pepper plants treated with *Pseudomonas monteilii* (MB-4) and/or *Streptomyces rochei* (MA-6) for both nitrogen deficiency (50% & 75% N and 33.3% $P_2O_5$) as well as recommended application (100% NPK) treatments. As for nitrogen deficiency; the results clearly indicated that all nitrogen levels had a significant effect on all yield characteristics except fruit weight and fruit diameter in both seasons as well as fruit length in 1st season. However, the 100% nitrogen rate (recommended dose) treatment had higher values than other nitrogen rate deficiency treatments in all studied characters with non - significant differences between T3 (75%N) and T1 (100%N) in early yield (2nd season) as well as betweenT2 (50%N) and T3 (75%N) in total yield (1st season).

Table 5: Changes values in yield and its attributed traits as affected by synthetic and bio-fertilizer treatments on pepper plants at both 2021 and 2022 seasons

| | Treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Traits | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | L.S.D at 5% |
| | | | | | 1st season | | | | | |
| FW (g) | 36.0 | 35.9 | 35.8 | 35.5 | 37.4 | 51.4 | 55.9 | 35.6 | 35.6 | 1.03 |
| FL (cm) | 7.2 | 6.8 | 6.7 | 7.0 | 7.3 | 9.3 | 11.0 | 6.9 | 7.4 | 0.78 |
| FD (cm) | 4.2 | 3.9 | 4.1 | 4.1 | 4.4 | 4.8 | 4.8 | 4.0 | 4.0 | 0.69 |
| EY (t/fed) | 3.221 | 2.010 | 2.499 | 3.245 | 3.492 | 4.081 | 4.475 | 3.434 | 5.353 | 0.451 |
| TY (t/fed) | 15.135 | 11.340 | 12.641 | 14.812 | 17.431 | 14.842 | 18.543 | 16.66 | 21.227 | 2.301 |
| | | | | | 2nd season | | | | | |
| FW (g) | 36.9 | 36.4 | 36.9 | 36.5 | 38.7 | 52.1 | 56.6 | 36.5 | 36.7 | 0.95 |
| FL (cm) | 7.4 | 6.7 | 7.0 | 7.6 | 7.9 | 9.3 | 10.7 | 7.0 | 7.5 | 0.53 |
| FD (cm) | 4.0 | 3.8 | 4.0 | 3.7 | 4.0 | 4.6 | 4.5 | 4.0 | 4.0 | 0.25 |
| EY (t/fed) | 3.522 | 2.136 | 3.117 | 3.497 | 3.767 | 4.41 | 5.063 | 3.884 | 5.976 | 0.456 |
| TY (t/fed) | 16.942 | 11.961 | 14.462 | 16.205 | 18.151 | 16.023 | 19.93 | 18.383 | 22.933 | 2.185 |

FW (fruit weight), FL(fruit length),FD(fruit diameter),EY(early yield),TY(total yield)

Exposure of untreated plants to low mineral fertilizer applications T2 (50%N & 33.3% P) caused obvious inhibition of FW, FD, FL, TY and EY by 0.82, 6.10, 7.53, 27.36 and 38.51% respectively, while exposure to T3 (75%N & 33.3% P) caused inhibition by 0.27, 1.22, 6.16, 15.51 and 16.71 % respectively in descending order as an average of both seasons (Fig. 2).

In this regard, Marschner [36]; El-Tohamy *et al*. [37] and Abusetta [38] found that the yield was negatively affected by the low chemical fertilization treatments, especially at 50% of nitrogen. Oppositely, the increase in yield by relatively high levels of nitrogen may be attributed to the beneficial effects of nitrogen on stimulating the meristematic activity, for producing more tissues and organs and cell enlargement, since nitrogen plays major roles in the synthesis of structural proteins and other several macro-molecules, in addition to its vital contribution in several biochemical processes in the plant related to growth.

Upon treatment of stressed plants with bacteria strains treatments, many significant increases in all yield traits were observed in both seasons compared to all control treatments (T1 of recommended 100% NPK, in addition to both T2 and T3 stress treatments except for T4, T8 and T9 treatments which showed a slight increase in most fruit and yield traits, *i.e*., fruit weight and fruit diameter in both seasons as well as fruit length in 1st season.

Applied treatment T7 (*Streptomyces rochei* MA-6 plus 75%N+33.3%P+100%K) has significant maximum effects by 54.3 and 48.6% (average of both seasons) for FW and FL, respectively over the untreated plants of 100% NPK; in addition to 55.6 and 60.7% as well as 54.7 and 58.4% over the untreated plants of 50 and 75% NPK, respectively. It is clearly noticed that treatments containing *Pseudomonas* or *Streptomyces* applied along with 75% N (T5 and T7) as well as *Streptomyces* applied along with 50% N (T6) gave statistically increase results in fruit qualities as compared to the untreated plants of 100% recommended dose of N fertilizer, indicating the efficient role of the studied local bacterial strains for substitution of nitrogen N up to 50% Gholve *et al*. [43].

However, the highest total pepper yield was achieved by a mixture of bacterial strains at a mineral fertilization level of 75% N +33.3% P +100% K (T9) followed by T7 (*Streptomyces* applied along with 75% N) as compared to the rest treatments and untreated control (T1) in both seasons. The T9 treatment led to an increase in production the total yield by 37.7% in the average of the two seasons and a decrease in the total costs of producing when compared to the recommended rate of chemical fertilization 100% NPK (T1).

The beneficial effect of bacterial inoculation is a result of many components that work synergistically at different concentrations. These results are in agreement with those mentioned by Bacilio *et al*.[39], Chiquito-Contreras *et al*. [40], He *et al*. [41] and Hernandez-Montiel *et al*. [42] They reported that pepper plant inoculated with of mixture *Pseudomonas putida* rhizobacteria and Inorganic fertilization 75% increases productivity by up to 36% when compared with inorganic fertilizer at 100% (without treatment). The decline in the amount of nitrogen fertilization, without much reducing the trait leads to a lower cost of using nitrogen fertilizer and thus increases profit and also reduces pollution resulting from the use of nitrogen fertilizer [46].

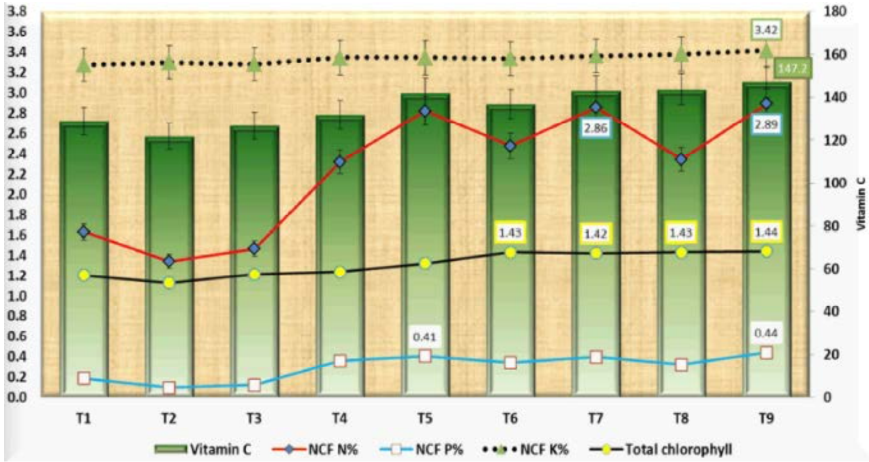


Fig. 3: Elemental analysis of fruits (average of both seasons) as affected by synthetic and bio-fertilizer treatments on pepper plants at both 2021 and 2022 seasons.

Table 6: Changes values in elemental analysis of fruits as affected by synthetic and bio-fertilizer treatments on pepper plants at both 2021 and 2022 seasons

| Traits | Treatments T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | L.S.D at 5% |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1st season | | | | | |
| N% | 1.62 | 1.35 | 1.46 | 2.31 | 2.81 | 2.48 | 2.84 | 2.33 | 2.87 | 0.11 |
| P% | 0.18 | 0.09 | 0.11 | 0.36 | 0.41 | 0.33 | 0.4 | 0.31 | 0.43 | 0.03 |
| K% | 3.2 | 3.22 | 3.19 | 3.3 | 3.32 | 3.33 | 3.37 | 3.35 | 3.39 | 0.02 |
| V.C* | 129.47 | 120.83 | 125.67 | 132.27 | 142.5 | 136.3 | 144.67 | 143.37 | 147.9 | 0.95 |
| T-ch** | 1.19 | 1.13 | 1.2 | 1.22 | 1.32 | 1.42 | 1.44 | 1.42 | 1.43 | 0.03 |
| | | | | | 2nd season | | | | | |
| N% | 1.64 | 1.33 | 1.47 | 2.33 | 2.83 | 2.47 | 2.87 | 2.36 | 2.91 | 0.04 |
| P% | 0.19 | 0.1 | 0.13 | 0.36 | 0.4 | 0.35 | 0.39 | 0.33 | 0.45 | 0.03 |
| K% | 3.35 | 3.38 | 3.37 | 3.39 | 3.37 | 3.34 | 3.35 | 3.41 | 3.44 | 0.02 |
| V.C* | 128.27 | 122.84 | 127.6 | 131.57 | 141.3 | 137.17 | 141.43 | 144.2 | 146.4 | 1.86 |
| T-ch** | 1.21 | 1.12 | 1.22 | 1.25 | 1.32 | 1.44 | 1.4 | 1.44 | 1.45 | 0.04 |

*V.C (Vitamin C mg/100g FW), **T-ch. (total chlorophyll content mg/g fresh weight)

Many investigators reported that applied *Pseudomonas sp* from tomato and sweet pepper plant rhizosphere, which have ability to produce antifungal metabolites, phosphate solublization, HCN (Hydrocyanic Acid) and IAA (indole-3- acetic acid ) as Hamada *et al*. [45] and Omar *et al*. [8]. Moreover, using bacteria in fertilizer may be play a profound role in improving soil fertility and plant growth development via N2 fixation and releasing certain nutritive elements such Fe, Zn and Mn and some phytohormones such as gibberellins, auxins and cytokinins substances which may encourage up taking and sufficient nutrients, subsequently enhance plant growth [46]. Increased yield was linked to greater nutrient absorption by plants, which resulted in such good fruit development and yield. The use of bacterial strains on sweet pepper plants may have resulted in an excess of N and P in the soil solution and increased root development, thereby expanding root uptake area.

**Chemical Properties:** Data in Table 6 and Fig.3 revealed that all adding bacterial strains treatments significantly increased N, P and K% as well as Vitamin C and total chlorophyll content in the sweet pepper fruits when compared with the control levels (T1, T2 and T3) in both seasons. In the current study, the T9 treatment gave the highest significant value of N % (2.87 & 2.91%) and P% (0.43 & 0.45%) in the sweet pepper fruits followed by treatment T7 and T5 treatment in descending order in 1st and 2nd season, respectively. While the control (T1) recorded 1.62 and 1.64 N%, as well as 0.18 and 0.19 P% in 1st and 2nd seasons, respectively.

The obtained results in the current study revealed that the K% rate in the fruits is convergent because of the addition of the element potassium at a constant rate for all treatments under this study conditions but, T9 gave the highest value in both seasons. Regarding Vitamin-C concentration in the sweet pepper fruits, all treatments of

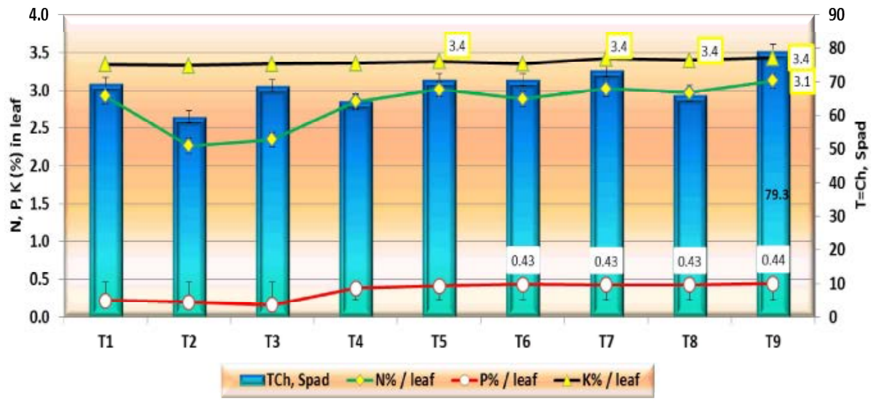


Fig. 4: Elemental analysis of leaves (average of both seasons) as affected by synthetic and bio-fertilizer treatments on pepper plants at both 2021 and 2022 seasons.

Table 7: Changes values in elemental analysis of leaves as affected by synthetic and bio-fertilizer treatments on pepper plants at both 2021 and 2022 seasons

| | Treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Traits | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | L.S.D at 5% |
| | | | | | 1st season | | | | | |
| N% | 2.91 | 2.25 | 2.39 | 2.84 | 3.05 | 2.85 | 2.98 | 2.93 | 3.11 | 0.032 |
| P% | 0.19 | 0.177 | 0.153 | 0.4 | 0.423 | 0.43 | 0.43 | 0.423 | 0.427 | 0.032 |
| K% | 3.3 | 3.29 | 3.32 | 3.34 | 3.35 | 3.35 | 3.38 | 3.36 | 3.4 | 0.07 |
| T-ch* (SPAD) | 68.5 | 56.9 | 68.4 | 63.5 | 70.4 | 70.3 | 73.3 | 66 | 78.4 | 2.2 |
| | | | | | 2nd Season | | | | | |
| N% | 2.93 | 2.28 | 2.3 | 2.86 | 2.97 | 2.92 | 3.06 | 3 | 3.14 | 0.044 |
| P% | 0.24 | 0.213 | 0.167 | 0.36 | 0.4 | 0.43 | 0.42 | 0.43 | 0.453 | 0.032 |
| K% | 3.38 | 3.36 | 3.37 | 3.38 | 3.42 | 3.36 | 3.46 | 3.44 | 3.46 | 0.04 |
| T-ch* (SPAD) | 70.5 | 62.1 | 69.2 | 65.1 | 71 | 71 | 73.5 | 66 | 80.2 | 3.7 |

*T-ch. (total chlorophyll content)

bacterial strains exhibited significant increases over the control levels and T9 (inoculated mixture of bacterial strains at a mineral fertilization level of (75% N +33.3% P + 100% K) has the highest values in both seasons which recorded 147.90 and 146.40 mg/100g fresh weight, while the control T1 recorded 129.47 and 128.27 mg/100g fresh weight in 2021 and 2022 seasons, respectively.

Concerning the effect of bacterial strains treatments on minerals constituents in pepper leaves, *i.e.*, N, P and K percentages as well as total chlorophyll content (SPAD unit) in the fresh tissues of pepper leaves, Table 7 and Fig. 4 showed significant increases compared to the control levels during the two seasons. T9 induced the highest significant increment in total chlorophyll content followed by T7 and T5 treatments in descending order in both seasons. Also, T9 produced the highest values of N (3.11 and 3.14%), P (0.427 and 0.453%) and K (3.40 and 3.46) concentrations, whereas T1 (full NPK, control) exhibited the significantly lowest values of N (2.91 and 2.93%), P (0.190 and 0.240 %) and K (3.30 and 3.38%) in the pepper leaves in 2021 and 2022 seasons, respectively.

Previous research on pepper plants showed similar results and the beneficial impacts of microbes could be attributed to their high amounts of photosynthetic activity [47]. Increased N, P and K% levels impacted plant growth significantly, as well as increased N% concentrations in leaves may optimisation chlorophyll contents and higher photosynthesis rates [48]. The study of detailed the release of phosphate by *Streptomyces Sp*. via the effects of released malic acid and gluconic acids [49]. Phosphorus is necessary for the formation of roots as well as the increase of growth and flowering [50]. According to Olanrewaju *et al.* [51] using plant growth-promoting bacteria is a critical activity for sustainable agriculture and a good substitute for environmentally risky chemical fertilisers and pesticides. These bacteria can increase nutrient availability, biosynthesize metal chelators and phosphorus solubilizers, produce phytohormones, control phytopathogens and alleviate abiotic stress in plants.

**UPGMA Clustering Dendrogram:** Construction of dendrogram based on 10 fruit traits*, i.e.*, FW, FL, FD, N%, P%, K%, V.C and total chlorophyll as well as both early

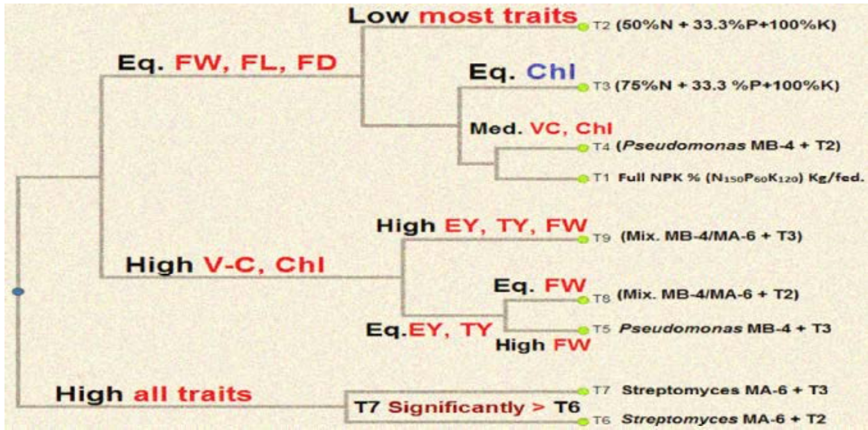


Fig. 5: Dendogram using average linkage (between groups), for nine fertilizer treatments based on ten fruit and yield traits.
FW (fruit weight), FL(fruit length), FD(fruit diameter),EY(early yield),TY(total yield), V.C (Vitamin C mg/100g FW), Chl (chlorophyll content mg/g fresh weight)

Table 8: Economic analysis and benefit cost ratio of different fertilization treatments of the experiment.

| Treatments | Total yield[1] | Gross return[2] | Total cost[3] | Net return[4] | IF[5] | Order |
|---|---|---|---|---|---|---|
| T1 | 16.039 | 80195 | 15050 | 65145 | 5.33 | 9th |
| T2 | 11.651 | 58255 | 10100 | 48155 | 5.77 | 7th |
| T3 | 13.552 | 67760 | 11975 | 55785 | 5.66 | 8th |
| T4 | 15.509 | 77545 | 10610 | 66935 | 7.31 | 4th |
| T5 | 17.791 | 88955 | 12485 | 76470 | 7.12 | 6th |
| T6 | 15.433 | 77165 | 10610 | 66555 | 7.27 | 5th |
| T7 | 19.237 | 96185 | 12485 | 83700 | 7.70 | 3rd |
| T8 | 17.522 | 87610 | 10670 | 76940 | 8.21 | 2nd |
| T9 | 22.08 | 110400 | 12545 | 97855 | 8.80 | 1st |

[1]Total yield (ton/fed) as average of two seasons, [2]Gross return as total yield (ton/fed) × 5000 L.E /ton
[3]Total cost: Bio-fertilizer = 510 L.E /fed, represent 60 L.E. strains + 450 L.E. agricultural laborer for its adding whereas Mineral fertilization = 15050 L.E., T1; 10100 L.E., T2 and 11975 L.E., T3
[4]Net return = Gross return-Total cost, [5]IF: The investment factor (The benefit-cost ratio) was calculated by dividing the gross income by total cost

and total yield/fed. of pepper plants treated with 9 fertilizer treatments including N and P deficiency levels as illustrated in Fig. 5.

In general, it shows three large classes. In a hard selection, across the approved cut-off point, six groups were formed, each of T9, T2 and T3 formed a single cluster (each) that T9 (Mix.(MB-4/MA-6) + T3) has the highest values for EY, TY, N, P, K, V.C, total chlorophyll and equal other traits whereas, both T2 (50%N + 33.3%P+100%K) and T3 (75%N + 33.3 %P+100%K) have the lowest values for most traits compared to full NPK treatment (T1). However, cluster I was composed of the two (T6 & T7) treatments which have high values in all traits (T7>T6). Cluster II contained both T5 and T8. It was characterized by high N, P, K, V.C and Chl of fruit as well as equal significantly equal to T1 (control) in other traits except for FW in which T5 (*pseudomonas monteilii* MB-4 + T3) has a high value. Cluster IV contained both T1 and T4 within class 3 which have significantly equal values for FW, FD and FL. Interestingly, the distribution of fertilizer treatments by the dendogram was consistent with the previous aggregation of these treatments and confirms the results and discussion.

**Economic Analysis:** Data in Table 8 show a comparison study among the treatments of fertilizer types as an economic assessment of pepper yield. However, the outputs were; gross return per feddan calculated as “yield × price of pepper”. The net return was calculated as “Gross return/fed. - Total cost/fed”. The investment factor (IF) was calculated as “Gross return/Total cost”. Results in Table 6 illustrate that application of T1 (full NPK) treatment had the highest cost (15050 L.E); whereas the T2 treatment (50%N deficiency) had the lowest cost (10100 L.E). As for gross return per feddan, T9 recorded the highest gross return, followed by T7 and T5, in descending order. Also, T9 treatment had the highest net return followed by T7, but the lowest net return was obtained with T2 treatment. However, the treatment of T9 had the highest investment factor (8.80).

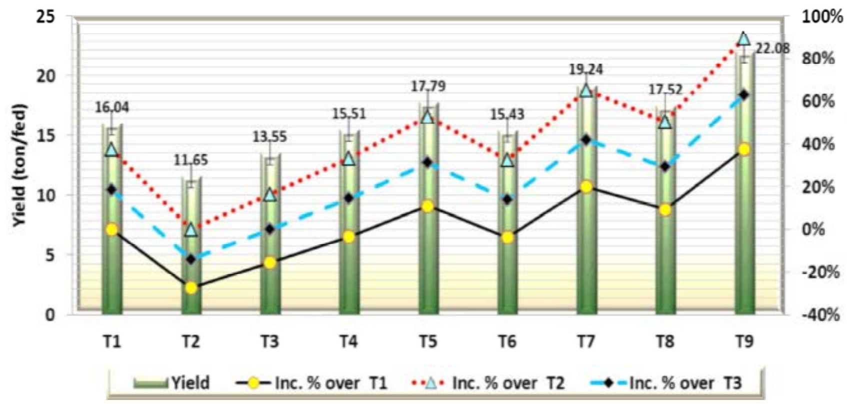


Fig. 6: Value and percentage (%) of yield (over corresponding control) as average of two seasons.

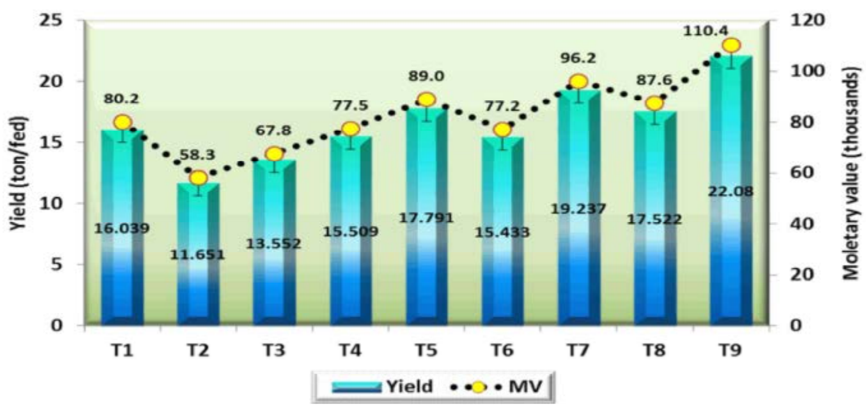


Fig. 7: Monetary value advantage (thousand pounds /fed) as affected by bio-fertilizer treatments over the average of both seasons.

Table 9: Value and percentage (%) of yield (over corresponding control) as average two seasons.

| Treatments | Ton/ fed. | Increment % over mineral fertilizer levels T1 | T2 | T3 | Range % of responses in fruit and yield traits T1 | T2 | T3 |
|---|---|---|---|---|---|---|---|
| T1 | 16.04 | 0.0% | 37.7% | 18.4% | 0.00 | 0.8%FW - 62.6%EY | 0.3%FW - 20.1%EY |
| T2 | 11.65 | -27.4% | 0.0% | -14.0% | (-38.5%EY) - (-0.82%FW) | 0.00 | (-26.2%EY) - (-0.6%FW) |
| T3 | 13.55 | -15.5% | 16.3% | 0.0% | (-16.7%EY) - (-0.27%FW) | 0.6%FW - 35.5%EY | 0.00 |
| T4 | 15.51 | -3.3% | 33.1% | 14.4% | (-4.9%FD) - 0%FL | (-0.4%FW) - 62.6%EY | (-3.7%FD) - 20.1%EY |
| T5 | 17.79 | 10.9% | 52.7% | 31.3% | 2.4%FD - 10.9%TY | 5.3%FW - 75.1%EY | 3.7%FD - 31.3%TY |
| T6 | 15.43 | -3.8% | 32.5% | 13.9% | (-3.8%TY) - 42%FW | 22.1%FD - 104.8%EY | 13.9%TY - 51.2%EY |
| T7 | 19.24 | 19.9% | 65.1% | 42.0% | 13.4%FD - 54.3%FW | 20.8%FD - 130.1%EY | 14.8%FD - 69.8%EY |
| T8 | 17.52 | 9.2% | 50.4% | 29.3% | (-4.8%FL) - 9.3%TY | (-0.3%FW) -76.5%EY | (-1.2%FD) - 30.3%EY |
| T9 | 22.08 | 37.7% | 89.5% | 62.9% | (-2.4%FD)-68%EY | 0%FW - 173.3%EY | (-1.2%FD) - 101.7%EY |

FW (fruit weight), FL (fruit length), FD (fruit diameter), EY (early yield), TY(total yield)

In general, the previous results illustrated that the fertilizing of pepper by combined mixed strains with T3 inorganic fertilizer was the best treatment for yield, quality and nutrient uptake than individual application as conventional chemical forms or other bio-fertilizers. Furthermore, fertilizer applications as T9 and T7 maximize the profitability of yield in both deficiency experiments (T2 and T3). In addition, the application of these previously mentioned treatments (T9 and T7) could be declined the load or request on chemical-fertilizer, as well as enhance the use efficiency of applied N-fertilizers and consequently could reduce the added rate of N. Therefore, the application of bio-fertilizer along with chemical forms as mixed fertilizer has become very important.

**A Brief Comparison of Results and Conclusion:** Field experiments were carried out to study the effect of the application 6 different bio-and/or inorganic fertilizer treatments on the growth and productivity of sweet pepper plants cultivated in clay soil under abiotic treatment*, i.e., Pseudomonas monteilii* (MB-4) and/or *Streptomyces rochei* (MA-6) for both nitrogen deficiency (50% & 75% N) and 33.3% $P_2O_5$ as well as recommended application (100% NPK) treatments. Accordingly, comparing the performance of the bio/inorganic fertilizer treatments on the basis of fruit yield (ton/fed.) under nitrogen deficiency (Common NPK fertilization T1 in Egypt as general control) and highest desirable response for yield (% over corresponding control) under various treatments as well as the effects of the fertilizer treatments on other traits was done. The best treatments, which are classified on the basis of these parameters, are shown in Table 9 and Fig. 6. All the six studied bio-treatments were classified as a good effect source in a desirable trend on vegetative growth, NPK of fruits, vitamin-C as well as both early and total yield/fed. Five out of these 6 treatments exhibited significant increases in fruit chlorophyll contents. Three out of these five treatments namely: T6, T7 and T9 exhibited a significant desirable positive increment for the early yield as well as the superiority (equal or significant increment) for the positive effect of fruit traits and most other traits. Two out of these three treatments (T7 and T9) recorded the highest desirable positive increment for total yield as well as both chlorophyll and N% of leaf contents over the general control (full NPK, T1), along with these three treatments, T5 exhibited significant increases in fruit weight and leaf N% content and equally increment in all other traits, indicating the possibility of combining both high yield and good quality characters under abiotic conditions. The treatments, which exhibited significant positive increment for yield, were also combined with significant/highly significant desirable negative or positive (due to the point of view) five or more important studied characters particularly vegetative growth, average fruit weight ….etc. However, treatment with high yield effects did not necessarily produce high other traits, especially qualitative traits and *vice versa*.

Also, obtained values shown in Table 8 and Fig. 7 indicated that the highest cash advantage (110.4 thousand L.E.) was achieved from T9 [mix bacterial strains (*Pseudomonas monteilii MB-4* plus *Streptomyces rochei* MA-6) interacted with T3 ($N_{112.5kg}$+ $P_{20kg}$+$K_{120kg}$)], followed by T7 and T5 (96.185 and 88.955 thousand L.E.), i.e., individual *Streptomyces rochei* MA-6 or *Pseudomonas monteilii* MB-4, respectively interacted with T3 ($N_{112.5\ kg}$+ $P_{20\ kg}$+$K_{120\ kg}$) which higher than all the monetary values resulting from fruit yield. Our results reveal that the abovementioned bio-fertilizer treatments might be of prime importance for traditional agricultural procedures for high yield and/or some of its important components.

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