Pivotal Role of Bio and Mineral Fertilizer Combinations on Morphological, Anatomical and Yield Characters of Sugar Beet Plant (Beta vulgaris L.)

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ABSTRACT

Two field experiments were conducted at Sakha Research Station, Kafr El-Sheikh Governorate, Egypt during 2012/2013 and 2013/2014 seasons, to study the effect of mineral and bio-fertilizers as well as their combinations on morphology, anatomy, yield and quality of sugar beet. Bio-fertilizers were phosphorine, cerealine and yeast; however, mineral fertilizers were 50, 75 and 100% from recommended dose of nitrogen, phosphorus and potassium as well. The results revealed that the combined treatments of phosphorine, cerealine and yeast with 75% from recommended dose of NPK and phosphorine, cerealine and yeast with 50% from recommended dose of NPK showed the highest values of most studied characters; root length and diameter, shoot and root fresh weights, TSS, sucrose and purity percentages as well as root and sugar yield/fed in both studied growing seasons as compared with using each treatment alone as well as control treatment (100% NPK). However, the highest values of chlorophyll a and b, sucrose% as well as purity% were resulted from treatment of phosphorine, cerealine and yeast with adding 50% NPK in the two growing seasons. Interestingly that anatomical characters of root and leaf such as root diameter, number of vessels/bundle in root as well as leaf lamina and midrib thickness were increased with application of phosphorine, cerealine and yeast in addition with bio-fertilizers combined with 50% NPK as mineral fertilizers from recommended dose led to maximize sugar beet productivity and reduce the cost of mineral fertilization which being harmful for human health and cause environmental pollution.

Key words: Sugar beet, Beta vulgaris L., Bio-fertilizers, Mineral fertilizers, Morphology, Anatomy, Yield, Quality.

Introduction

Sugar beet (Beta vulgaris L.) belongs to Chenopodiaceae family. It is a biennial plant and one of the most important sugar crop in the world (Watson and Dallwitz 1992), which considered the second important sugar crop in Egypt and all over the world after sugar cane. About forty percent of world sugar production is obtained from sugar beet. The importance of sugar beet crop to agriculture is not confined only to sugar production, but also it is adapted to saline, sodic and calcareous soils.

Fertilizers play an important role in increasing sugar beet production. The main macronutrients present in inorganic fertilizers are nitrogen, phosphorus, and potassium which influence vegetative and reproductive phase of plant growth (Patil, 2010). Nitrogen fertilizer levels caused significant differences in all yield and quality characters of sugar beet (Kandil et al., 2002; Shewate et al., 2008 and Attia et al., 2011). Hafez and Abdelaal (2015) reported that increasing nitrogen level up to 150 kg N/ha significantly increased dry matter production, chlorophyll a, chlorophyll b and leaf area in maize plant. Phosphorus is one of the most important elements in the plant because of its role in the photosynthesis, transformation of sugars, transfer of genetic information and nutrient movement within the plant (Marschner, 1995). Potassium plays an important role in enzyme activation, charge balance and osmoregulation in plants (Cakmak, 2005). In sugar beet, K plays a significant role in biosynthesis and transfer of sucrose to storage roots (Winzer et al., 1996). It is assumed that P and K fertilizing increases both, yield and sugar beet quality. In addition, Monreala et al. (2007) stated that the highest values of quality parameters were obtained from the lowest level of nitrogen (30 kg N/ha). Mahmoud (2007) concluded that the highest top yield was obtained with application nitrogen at 90 kg/ha. Root and sugar yield significantly increased with potassium application at the rate of 114 kg ha⁻¹ (Abd El-Motagally and Attia, 2009). Abdelaal et al. (2015) reported that application of potassium at rate of 48 kg K₂O/ha and foliar spraying with some microelements significantly increased root diameter and root fresh weight, root and sugar yields/ha as well as sucrose%.

Bio-fertilizers led to retard nitrification for sufficiently longer time and increase the soil fertility (Jat et al., 2002). On the other hand, Badawi et al. (2004) found that bio-fertilization treatments caused a significant effect on TSS%, sucrose%, purity%, root, top and sugar yields/ha. Bio-fertilizer treatments produced the highest

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values of yield quality parameters, excluding TSS% (in the first and second seasons) and purity% (in the second season) as well as all yield characters in both seasons. Abou-Attea and Abdelaal (2007) indicated that bio-fertilizers gave the highest weight root and root diameter per sugar beet plant. Bio-fertilizers are low cost, effective and renewable source of plant nutrients to supplement chemical fertilizers (Boraste et al., 2009). Bio-fertilizers are useful substitutes to inorganic fertilizers which improve soil quality and it is a natural product carrying living microorganisms derived from the root or cultivated soil (Attarde et al., 2012 and Ramakrishnan, and Selvakumar, 2012). Inoculation seeds of sugar beet with bio-fertilizer + 60 kg N/ha produced the highest root weight per plant and per hectare compared with fertilizing plants with 100 kg N/ha alone (Favilli et al., 1993). Cakmakci et al. (2001) confirmed that cerealine caused an increase TSS%, sucrose%, purity% and sugar yield/fed. Shewate et al. (2008), Alaa et al. (2009) and Attia et al. (2011) found that bacterial inoculation of sugar beet seeds caused insignificant increases in root quality and growth parameters but it significantly increased root and sugar yields/fed. Kandil et al. (2004) and Amin et al. (2013) reported that inoculation seeds of sugar beet with bio-fertilizers significantly increased root, top and sugar yields/fed. Aly et al. (2009) recorded that inoculation with Azotobacter chroococcum and Bacillus megatherium saved about 25 kg N/fed of mineral fertilizer, which decreased the costs and the environmental pollution, in addition to the increase of sugar yield and recoverable sugar/fed. Furthermore, inoculation with Azospirillum increased sucrose content in sugar beet roots. The productivity and quality of sugar beet plants increased with fertilizing with 100 kg N/fed inoculated with mixture of bio-fertilizer (rizobacterin + phosphorine) and sprayed with micronutrients twice (Amin et al., 2013). Hashemi et al. (2014) reported that using Biozor had a positive impact on the sugar yield in sugar beet plant. Hussein et al. (2015) found that adding bio-fertilizer to dill plants significantly increased plant height, number of branches, root length, fresh and dry weights. Application of bio-fertilizers with chelated Nano fertilizers gave the highest chlorophyll a and b concentrations in sorghum plant (Mir et al., 2015). Abdelaal and Tawfik (2015) found significant increase in root length, root diameter and root fresh weight as well as root yield/fed in treated sugar beet plants with combination of bio-fertilizers and 105 kg mineral nitrogen.

Yeast is considering a source of bioactive and chemically novel compounds such as phytohormones, vitamins, enzymes, amino acids and minerals (Barnett et al., 1990 and Agamy et al., 2013). Application of active yeast extract on bean plants significantly increased plant height, number of branches/plant, number of leaves/plant, total leaf area/plant and dry weight of shoot/plant more than the control plants in the two seasons (Nassar et al., 2011). Ibrahim and Ibrahim (2014) indicated that combination of yeast with compost tea gave the highest uptake rate for P and enhance sugar beet growth.

El-Nagdy et al. (2010) reported that application of bio-fertilizer and 50% of the recommended dose from mineral fertilizers led to significant increase in diameter of the main stem, thickness of epidermis, cortex, secondary xylem, secondary phloem and pith diameter of flax plants. Anatomical studies of sugar beet root showed that bio-fertilizers increased the thickness of growth rings of sugar beet roots and average diameter of xylem vessels (Agamy et al., 2013).

Therefore the aim of this research was to study the effect of mineral fertilizers combined with bio-fertilizers on growth, anatomical structure, yield and its components of sugar beet plants to improve sugar beet production and saving the environment against pollution as well as decrease the side effect on human health.

Materials and Methods

Two field experiments were carried out at Sakha Research Station, Kafer El-Sheikh Governorate, Egypt during 2012/2013 and 2013/2014 seasons, to study the effect of chemical fertilizers (NPK) and bio-fertilizers (phosphorine, cerealine and yeast) on growth, anatomical structure and yield of sugar beet (Beta vulgaris L. var. Pleno).

Sugar beet seeds
Sugar beet seeds were sown at 1st and 4th of November in the first and second season (2012 and 2013); respectively. Four seeds were sown in hills and plants were thinned to one plant per hill at four true leaves stage. The experiments were laid out in a complete randomized block design with three replicates, each experimental basic unit included 5 ridges, each 60 cm apart and 3.5 m length, resulted an area of 10.5 m² (1/400 fed). The preceding summer crop was maize (Zea mays L.) in both seasons. Plants were kept free from weeds, which were manually controlled by hand hoeing at three times. The common agricultural practices for growing sugar beet according to the recommendations of Ministry of Agriculture were followed, except the factors under study.

NPK Fertilizers
The treatments of NPK were 100%, 75% and 50% from recommended doses of NPK (150 kg urea, 100 kg P₂O₅ and 50 kg K₂O per fed) as urea (46 % N), calcium superphosphate (15.5 % P₂O₅) and potassium sulphate (48 % K₂O). The whole amount of calcium superphosphate was added before sowing during soil preparation, but the amount of urea divided into two equal quantities, the first application was done after thinning and the
second one was carried out after 30 days from the first one. The dose of potassium sulphate was added with the first dose of urea.

Bio-fertilizers inoculation

Phosphorine and cerealine as commercial products were obtained from Bio-fertilizer Unit, Agricultural Research Center (ARC), Giza, Egypt. Phosphorine 0.6 kg/fed is a commercial bio-fertilizer contains a highly active dissolving bacteria, which reverse the insoluble tricalcium phosphate to the soluble mono-calcium phosphate and supplying the plants with its needs during growth stages. Cerealine 0.6 kg/fed is a free-living nitrogen-fixing bacteria, represents the best characterized genus of plant growth-promoting rhizobacteria. Sugar beet seeds var. Pleno were inoculated with phosphorine and cerealine at rates of 0.6 kg/fed for both. Before the inoculation, Arabic gum was used as an adhesive agent of bio-fertilizers to sugar beet seeds and then directly sown.

Yeasts are microorganisms which considered as bio-fertilizer. It was prepared by inoculating one L of nutrient broth with 10 gm of active commercial dry yeast and incubated for 48h, after that the one liter inoculums added to 10 L nutrient broth for yeast treatment. Yeast was applied at the rate of 4 liter/ plot after one month from planting and repeated with the same dose after two weeks from the first one. The experiment included the following treatments with three replicates in Table (1).

Table 1: Chemical and bio-fertilizers treatments.

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatments</th>
<th>2012/2013 season</th>
<th>2013/2014 season</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100% NPK (150, 100 and 50 kg/fed urea, P₂O₅ and K₂O, respectively)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>75% NPK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>75% NPK + phosphorine 0.6 kg/fed.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>75% NPK + cerealine 0.6 kg/fed.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>75% NPK + Yeast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>75% NPK + phosphorine 0.6 kg/fed + cerealine 0.6 kg/fed + Yeast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>50% NPK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>50% NPK + phosphorine 0.6 kg/fed.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>50% NPK + cerealine 0.6 kg/fed.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>50% NPK + Yeast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>50% NPK + phosphorine 0.6 kg/fed + cerealine 0.6 kg/fed + Yeast</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Soil samples were taken for conducting some physical and chemical analysis according to A.O.A.C. (2005) and all data were shown in Table (2).

Table 2: Physical and chemical soil characteristics at the experimental sites during both growing seasons.

<table>
<thead>
<tr>
<th>Soil analysis</th>
<th>2012/2013 season</th>
<th>2013/2014 season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand %</td>
<td>65.45</td>
<td>66.80</td>
</tr>
<tr>
<td>Silt %</td>
<td>21.65</td>
<td>20.90</td>
</tr>
<tr>
<td>Clay %</td>
<td>12.90</td>
<td>12.30</td>
</tr>
<tr>
<td>Textural class</td>
<td>Sandy</td>
<td>Sandy</td>
</tr>
<tr>
<td>Chemical analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCO₃ (%)</td>
<td>1.521</td>
<td>1.521</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td></td>
<td>1.94</td>
</tr>
<tr>
<td>Available N (ppm)</td>
<td></td>
<td>25.45</td>
</tr>
<tr>
<td>Available P (ppm)</td>
<td></td>
<td>4.72</td>
</tr>
<tr>
<td>Available K (ppm)</td>
<td></td>
<td>280.18</td>
</tr>
<tr>
<td>PH</td>
<td>8.75</td>
<td>8.81</td>
</tr>
</tbody>
</table>

At maturity (age of 210 days), the three middle rows of each plot were harvested, five plants selected randomly of each plot. The plants were separated into shoot and root and the following characters were determined:

Morphological, physiological characters and yield
1- Root length (cm). 2- Root diameter (cm). 3- Root fresh weight (g/plant).
4- Shoot fresh weight (g/ plant). 5- Leaf area (cm²/ plant).
6- Total soluble solids (TSS %) in roots was measured in juice of fresh roots by using Hand Refractometer.
7- Sucrose percentage was determined according to Le Docte (1927).
8- Sucrose percentage was determined Polarimetrically on lead acetate extract of fresh macerated roots according to the method of Carruthers and Oldfield (1960).
9- Purity percentage: which was estimated according to the following equation: Purity% = Sucrose% / TSS% x 100. It was determined as a ratio between sucrose % and TSS % of roots (Carruthers and Oldfield, 1960).
10- Root yield (ton/fed):- Plants of sugar beet from each plot were harvested topped to determine root yield as ton/fed on fresh weight basis.
11- Sugar yield (ton/fed) was calculated using the following equation:
Sugar yield (ton/fed) = Root yield (ton/fed) x sucrose /100.

**Determination of chlorophyll a and b concentrations**

Chlorophyll (chl.) concentration as mg/g fresh weight of one gram fresh leaves was extracted with 5 ml N,N-dimethyl-formamid for overnight at 5°C then estimated chlorophyll a and chlorophyll b spectrophotometrically at 663 and 647 nm as described by Moran and Porath (1982).

**Anatomical studies**

For anatomical studies, specimens from selected samples were taken during the second season 2013/2014 from the leaf and root at the age of 50 days from sowing. Samples were killed and fixed for at least 48 hours in formalin acetic acid alcohol (F.A.A.) solution [5 ml glacial acetic acid, 10 ml formalin and 85 ml ethyl alcohol 70%]. Samples were washed in 50% ethyl alcohol and dehydrated in a normal butyl alcohol series. The specimens were impeded in paraffin wax (56-58°C). Transverse sections (12-15 microns) thick were done with rotary microtome model 820. Paraffin sections were fixed on the slides with albumin, stained with safranin and light green and mounting in Canada balsam (Nassar and El-Sahar 1998). Slides were examined microscopically and photomicrographed.

**Statistical analysis**

The experiments were conducted in a complete randomized design with three replicates for each treatment. Data represent the mean ± SD. Student’s t-test was used to determine whether significant difference (P<0.05) existed between mean values according to O’Mahony (1986).

**Results and Discussion**

**Root length and diameter**

As a result of combined bio-fertilizers (phosphorine, cerealine and yeast) with 50% and 75% from recommended dose (NPK), one can see significant increase of root length and root diameter as compared with all treatments particularly 100% NPK in both growing seasons (Fig. 1).

![Graph A](image1.png)

**Fig. 1:** Effect of bio and mineral fertilizers and their combinations on length and diameter of sugar beet roots during 2012/2013 and 2013/2014 seasons.

*Cont. = control (100% NPK), Ph. = phosphorine, C. = cerealine, Y. = yeast.*

Interestingly enough that bio-fertilizer treatments 50% NPK+ Yeast and 75% NPK+ Yeast did not show much different with the control treatment while other treatments (50% NPK, 50% NPK+ phosphorine, 50% NPK+...
cerealine, 75% NPK+ phosphorine and 75% NPK+ cerealine) decreased root length significantly (Fig. 1A). However, root diameter significantly decreased as a result of treatments (50% NPK, 50% NPK+ phosphorine and 75% NPK), while application of other treatments caused a significant increase in root diameter (Fig. 1B). The increasing of root length and diameter as a result of application bio-fertilizers may be due to its role in nitrogen fixation via free living bacteria which reduce the soil pH especially in the rhizosphere which led to increase the availability of most essential macro and micro-nutrients as well as excretion some growth regulators such as IAA and GA3 which play important role in formation a large root system and therefore increasing nutrient uptake as well as stimulating vegetative growth, hence increasing root length and diameter. Our results were supported by similar results obtained by Badawi et al. (2004) and Amin et al. (2013).

Root fresh weight, shoot fresh weight and leaf area
Presented data in Figure (2) showed that bio-fertilizers (phosphorine, cerealine and yeast) combined with 50% and 75% mineral fertilizers (NPK) significantly increased root fresh weight, shoot fresh weight and leaf area as compared with all treatments particularly 100% NPK (control treatment) in the two growing seasons. Interestingly that most of bio-fertilizers treatments significantly increased root and shoot fresh weight as compared with the control treatment, while mineral fertilizer treatments (50% and 75% NPK) decreased significantly root fresh weight (Fig. 2A). However, shoot fresh weight significantly decreased as a result of 50% NPK, 50% NPK+ phosphorine, 50% NPK+ cerealine, 75% NPK+ phosphorine, 75% NPK+ cerealine treatments as compared with control one (Fig. 2B). Accordingly, leaf area was decreased as a result of all treatments except two treatments 50% NPK+ (phosphorine, cerealine + Yeast) and 75% NPK+ (phosphorine, cerealine + Yeast) as compared with control one (Fig. 2C). It seems from these interesting results that combinations of bio-fertilizers (phosphorine, cerealine and Yeast) with mineral fertilizers (50% and 75% NPK) were effective on all mentioned characters compared with control treatment. These results may be due to the role of bio-fertilizers in improving growth by increasing the uptake and availability of most nutrients, consequently enhancement root and shoot fresh weight as well as leaf area. Similar results were reported by Maareg and Badr (2001), Badr (2004), Nakayan et al. (2009) and Amin et al. (2013).

Total soluble solids (TSS), sucrose and purity percentages
Data presented in Figure (3) cleared that application of bio-fertilizers was associated with significant effect on TSS, sucrose and purity percentages in the two growing seasons. Application of 50% NPK + cerealine, 50% NPK + Yeast, 50% NPK + (phosphorine, cerealine+ Yeast), 75% NPK+ cerealine and 75% NPK+ Yeast as well as 75% NPK + (phosphorine, cerealine + Yeast) significantly increased TSS% and sucrose% in both seasons compared with control (Fig. 3A and 3B). On the other hand, application of bio-fertilizers in combined with mineral fertilizers led to significant increase in purity% with all treatments compared with control one (Fig. 3C). Generally, it was observed that bio-fertilizers led to gradual tendency to improve all quality determinations as compared with control treatment in both seasons. This increase in TSS, sucrose and purity percentages with mixture of bio-fertilizers and mineral fertilizers may be due to the role of bio-fertilizers in improving growth by increasing the uptake and availability of most nutrients, consequently enhancement sucrose content in roots. These results are similar to that obtained by Cakmakci et al. (2001) and Amin et al. (2013).

Chlorophyll a and b concentrations
Application of bio-fertilizers (phosphorine, cerealine + yeast) combined with mineral fertilizers (NPK) resulted in significant increase of chlorophyll concentrations with all treatments compared with 75% NPK and 50% NPK without bio-fertilizers in both seasons (Fig. 4). The highest concentrations of chlorophyll a were produced from fertilizing beet plants with 75% NPK+ (phosphorine, cerealine + Yeast), 50% NPK+ (phosphorine, cerealine + Yeast) and control (100 % NPK) in both seasons, respectively. However, the combination of 75% NPK with other bio-fertilizers and 50% NPK with other bio-fertilizers led to no significant effect on chlorophyll a concentration. However, the lowest concentrations of chlorophyll a were recorded with 75% NPK without bio-fertilizers and 50% NPK without bio-fertilizers in both seasons (Fig. 4A). Importantly that the highest concentration of chlorophyll b was recorded with application of 50% NPK+ (phosphorine, cerealine and Yeast), 75% NPK+ (phosphorine, cerealine and Yeast) and control (100% NPK), respectively (Fig. 4B). These results may be due to the promoting role of bio-fertilizers in enhancing cell division and elongation producing more leaf area and chlorophyll concentrations. The increase in chlorophyll formation could be attributed to the role of cytokinins delaying the aging of leaves by reducing the degradation of chlorophyll and enhancing the protein and RNA synthesis (Castelfranco and Beale 1983). The positive effect of bio-fertilizers on chlorophyll a and b is in consistence with that obtained by Stino et al. (2009) and Abdou et al. (2012). Chlorophyll concentration was elevated as a result of bio-fertilizer treatments, this could perhaps connected with the increased of the antioxidant enzyme activities such as catalase, peroxidase and polyphenol oxidase as found recently by Abdelaal et al. (2014) and Hafez et al. (2014).
Fig. 2: Effect of bio and mineral fertilizers and their combinations on fresh weight of sugar beet root and shoot and leaf area during 2012/2013 and 2013/2014 seasons.

Cont. = control (100% NPK), Ph. = phosphorine, C. = cerealine, Y. = yeast.
Fig. 3: Effect of bio and mineral fertilizers and their combinations on TSS, sucrose and purity% of sugar beet roots during 2012/2013 and 2013/2014 seasons.

Cont. = control (100% NPK), Ph. = phosphorine, C. = cerealine, Y. = yeast.
Fig. 4: Effect of mineral and bio-fertilizers and their combinations on chlorophyll a and b concentrations of sugar beet leaves during 2012/2013 and 2013/2014 seasons.

Cont. = control (100% NPK), Ph. = phosphorine, C. = cerealine, Y. = yeast.

Root and sugar yields

Combinations of bio-fertilizers and mineral fertilizers tended to a significant effect on root and sugar yields (Fig. 5). Application of 50% NPK + cerealine, 50% NPK + Yeast, 50% NPK + (phosphorine, cerealine + Yeast), 75% NPK+ cerealine, 75% NPK+ Yeast and 75% NPK + (phosphorine, cerealine + Yeast) significantly increased root and sugar yields in the two growing seasons compared with control (100% NPK). However, root and sugar yields significantly decreased as a result of treatment with 50% NPK in both seasons. The increase in root and sugar yields with application of bio-fertilizers and mineral fertilizers may be due to the role of nitrogen and potassium in building up metabolites and activation of enzymes that associate with accumulation of carbohydrates, which translated to developing roots as well as increasing division and elongation of cells, consequently increasing root size also the role of bio-fertilizers in making available nutrient elements and active substances such as auxins, gibberellins, cytokinins, amino acids and enzymes which play active roles in improving crop growth and gave the highest yield (Bahr and Gomaa, 2002; Hussain et al., 2002 and Mirabal Alonso et al., 2008). These results fairly agreed with those found by Rangel and Damon (2008); Abd El-Motagally and Attia (2009); Zengin et al. (2009); Alaa et al. (2009); Fibach-Paldi et al., (2012) and Hellal et al. (2013).
Fig. 5: Effect of mineral and bio-fertilizers and their combinations on Root and sugar yields of sugar beet plant during 2012/2013 and 2013/2014 seasons.

Cont. = control (100% NPK), Ph. = phosphorine, C. = cerealine, Y. = yeast.

Anatomical studies
Root anatomy
The transverse sections through the root of sugar beet plant are shown in Figures (6, 7 and 8) and their data are presented in Table (3).

Table 3: Effect of mineral and bio-fertilizers and their combinations on anatomical structure of sugar beet roots during 2014 season.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Diameter of root (μ)</th>
<th>No. of growth rings</th>
<th>Number of vessels/bundle</th>
<th>Average diameter of vessel (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% NPK</td>
<td>1029.74</td>
<td>2</td>
<td>22</td>
<td>21.7</td>
</tr>
<tr>
<td>75% NPK</td>
<td>974.66</td>
<td>3</td>
<td>21</td>
<td>21.0</td>
</tr>
<tr>
<td>75% NPK + phosphorine.</td>
<td>1164.82</td>
<td>2</td>
<td>25</td>
<td>23.6</td>
</tr>
<tr>
<td>75% NPK + cerealine</td>
<td>1209.76</td>
<td>3</td>
<td>26</td>
<td>23.2</td>
</tr>
<tr>
<td>75% NPK + Yeast</td>
<td>1063.36</td>
<td>3</td>
<td>26</td>
<td>24.3</td>
</tr>
<tr>
<td>75% NPK + phosphorine + cerealine + Yeast</td>
<td>1216.5</td>
<td>3</td>
<td>27</td>
<td>26.0</td>
</tr>
<tr>
<td>50% NPK</td>
<td>929.12</td>
<td>2</td>
<td>21</td>
<td>21.4</td>
</tr>
<tr>
<td>50% NPK + phosphorine.</td>
<td>1170.34</td>
<td>2</td>
<td>25</td>
<td>25.9</td>
</tr>
<tr>
<td>50% NPK + cerealine</td>
<td>1231.44</td>
<td>2</td>
<td>26</td>
<td>26.4</td>
</tr>
<tr>
<td>50% NPK + Yeast</td>
<td>1203.16</td>
<td>3</td>
<td>27</td>
<td>26.6</td>
</tr>
<tr>
<td>50% NPK + phosphorine + cerealine + Yeast</td>
<td>1408.74</td>
<td>3</td>
<td>28</td>
<td>27.8</td>
</tr>
</tbody>
</table>

Results showed that application of 75% NPK + bio-fertilizers increased root diameter, number of growth rings, and number of xylem vessels as well as average diameter of vessel of sugar beet roots compared with 75% NPK alone and 100% NPK (control) (Fig. 6A-D). In the same trend data in Figures (7 and 8) pointed out that the highest values of root diameter, number of growth rings and number of xylem vessels as well as average diameter of vessel were recorded with application of 75% NPK + (phosphorine, cerealine + Yeast) and 50% NPK + Yeast as well as 50% NPK + (phosphorine, cerealine + Yeast) (Figs 7F, 8J and 8K). This results may be
due to the important role of bio-fertilizers specially Yeast in enhancement root growth and increase cell division and elongation (Cloete et al., 2009). These results were supported by similar results which obtained by El-Nagdy et al. (2010) and Agamy et al. (2013).

**Fig. 6:** Transverse sections of sugar beet roots. (X 100)

A-100% NPK (control).
B-75% NPK.
C-75% NPK + phosphorine.
D-75% NPK + cerialine.

Details:
Ep: Epidermis, R1, R2, R3: growth rings, P.t: Parenchyma tissue, Xy: Xylem tissue, Ph: Phloem tissue, Cvc: Concentric vascular cylinder.
**Fig. 7:** Transverse sections of sugar beet roots. (X 100)

- E. 75% NPK + Yeast.
- F. 75% NPK + phosphorine + ceraline + Yeast.
- G. 50% NPK.
- H. 50% NPK + phosphorine.
Fig. 8: Transverse sections of sugar beet roots. (X 100)

I. 50% NPK + cerealine.
J. 50% NPK + Yeast.
K. 50% NPK + phosphorine + cerealine + Yeast.

Leaf anatomy
The obtained results in Table (4) and Figs. (9, 10 and 11) showed that thickness of the leaf lamina and midvein as well as number of xylem rows/midvein bundle and number of vessels/bundle increased by application of bio-fertilizers with mineral fertilizers. The best results were obtained with treatments 75% NPK + Yeast, 75% NPK + (phosphorine, cerealine + Yeast) and 50% NPK + Yeast as well as 50% NPK + (phosphorine, cerealine + Yeast) compared with another treatments (Figs. 10E, F and 11J, K). This results may be due to the role of bio-fertilizers in production of the phytohormones which activate cell division and elongation of leaves and consequently enhancement sugar beet growth (Mirabal Alonso et al., 2008 and Cloete et al., 2009). These results agreed with those obtained by Nassar et al. (2011) and Agamy et al. (2013).
Table 4: Effect of mineral and bio-fertilizers and their combinations on anatomical structure of sugar beet leaves during 2014 season.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Thickness of lamina (μ)</th>
<th>Thickness of midvein (μ)</th>
<th>No. of xylem rows/midvein bundle</th>
<th>No. of vessels/midvein bundle</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% NPK</td>
<td>298.13</td>
<td>432.83</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>75% NPK</td>
<td>269.61</td>
<td>565.73</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>75% NPK + phosphorine.</td>
<td>324.55</td>
<td>572.52</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>75% NPK + ceraline</td>
<td>362.69</td>
<td>583.57</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>75% NPK + Yeast</td>
<td>390.06</td>
<td>607.62</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>75% NPK + phosphorine + ceraline + Yeast</td>
<td>392.17</td>
<td>614.59</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>50% NPK</td>
<td>251.46</td>
<td>514.54</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>50% NPK + phosphorine.</td>
<td>372.91</td>
<td>578.51</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>50% NPK + ceraline</td>
<td>388.24</td>
<td>581.98</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td>50% NPK + Yeast</td>
<td>389.00</td>
<td>589.08</td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td>50% NPK + phosphorine + ceraline + Yeast</td>
<td>397.77</td>
<td>593.56</td>
<td>9</td>
<td>29</td>
</tr>
</tbody>
</table>

Fig. 9: Transverse sections of sugar beet leaves. (X 100)

A. 100% NPK (control), B. 75% NPK, C. 75% NPK + phosphorine, D. 75% NPK + ceraline.

Details:

Fig. 10: Transverse sections of sugar beet leaves. (X 100)

E. 75% NPK + Yeast.
F. 75% NPK + phosphorine + cerealine + Yeast.
G. 50% NPK.
H. 50% NPK + phosphorine.
Fig. 11: Transverse sections of sugar beet leaves. (X 100)

I. 50% NPK + cerealine.
J. 50% NPK + Yeast.
K. 50% NPK + phosphorine + cerealine + Yeast.

Conclusion

It can be concluded that application of bio-fertilizers combined with mineral fertilizers significantly increased the growth and yield characters of sugar beet plants. Generally, it could be recommended that fertilizing sugar beet with mixture of phosphorine, cerealine + Yeast and 50% NPK led to increase the productivity and quality of sugar beet plants. This is very important to save costs of chemical fertilizers and decrease its side effect on human health and environmental pollution as well.
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References


