Middle East Journal of Agriculture Research
ISSN 2077-4605
Volume : 04 | Issue : 04 | Oct.-Dec. | 2015
Pages: 717-734

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

Khaled A. A. Abdelaal

Agricultural Botany Department, Faculty of Agriculture, Kafrelsheikh University, Egypt

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 + 50% NPK.
It can be concluded that application with phosphorine, cerealine and yeast as 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/fed 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/fed. 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/fed and foliar spraying with some microelements significantly increased root diameter and root fresh weight, root and sugar yields/fed 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/fed. Bio-fertilizer treatments produced the highest

Corresponding Author: Khaled A. A. Abdelaal, Agricultural Botany Department, Faculty of Agriculture, Kafrelsheikh University, Egypt.
E-mail: khaled_elhaies@yahoo.com

values of yield quality parameters, excluding TSS% (in the first and third seasons) and purity% (in the second season) as well as all yield characters in both seasons. Abou-Atteia 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 cerialine 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 Biozar 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 (phsphorine, 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 1^{st} and 4^{th} 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 m^2 (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 P_2O_5 and 50 kg K_2O per fed) as urea (46 % N), calcium superphosphate (15.5 % P_2O_5) and potassium sulphate (48 % K_2O). 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 converse 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 rats 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.

No.	Treatments
1	100% NPK (150, 100 and 50 kg/fed urea, P_2O5 and K_2O; respectively)
2	75% NPK
3	75% NPK + phosphorine 0.6 kg/fed.
4	75% NPK + cerealine 0.6 kg/fed.
5	75% NPK + Yeast
6	75% NPK + phosphorine 0.6 kg/fed + cerealine 0.6 kg/fed + Yeast
7	50% NPK
8	50% NPK + phosphorine 0.6 kg/fed.
9	50% NPK + cerealine 0.6 kg/fed.
10	50% NPK + Yeast
11	50% NPK + phosphorine 0.6 kg/fed+ cerealine 0.6 kg/fed + Yeast

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.

Soil analysis	2012/2013 season	2013/2014 season
Mechanical analysis		
Sand %	65.45	66.80
Silt%	21.65	20.90
Clay %	12.90	12.30
Textural class	Sandy	Sandy
Chemical analysis		
$CaCo_3$ (%)	1.521	1.521
Organic matter (%)	1.81	1.94
Avialable N (ppm)	25.45	25.65
Avialable P (ppm)	4.72	4.84
Avialable K (ppm)	280.18	280.23
PH	8.75	8.81

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 (cm^2/ 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).

Sucrose percentage was determined Polarimetrically on lead acetate extract of fresh macerated roots according to the method of Carruthers and Oldfield (1960).

8- 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).
9- 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.
10- 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 spectrophotometerically 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).

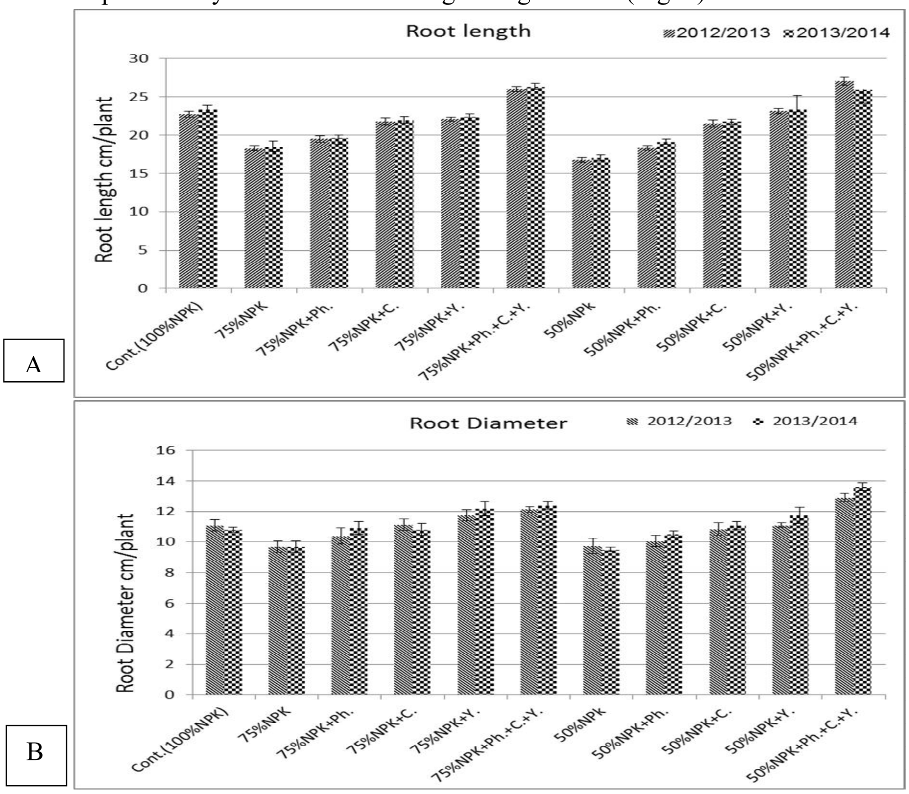

Fig. 1: Effect of bio and mineral fertilizers and their combinations on length and diameter of sugar beet roots during 2012/2013 and 2013/2014 seasons.

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 chllorophyll a and b is in consistence with that obtained by Stino *et al*. (2009) and Abdo *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).

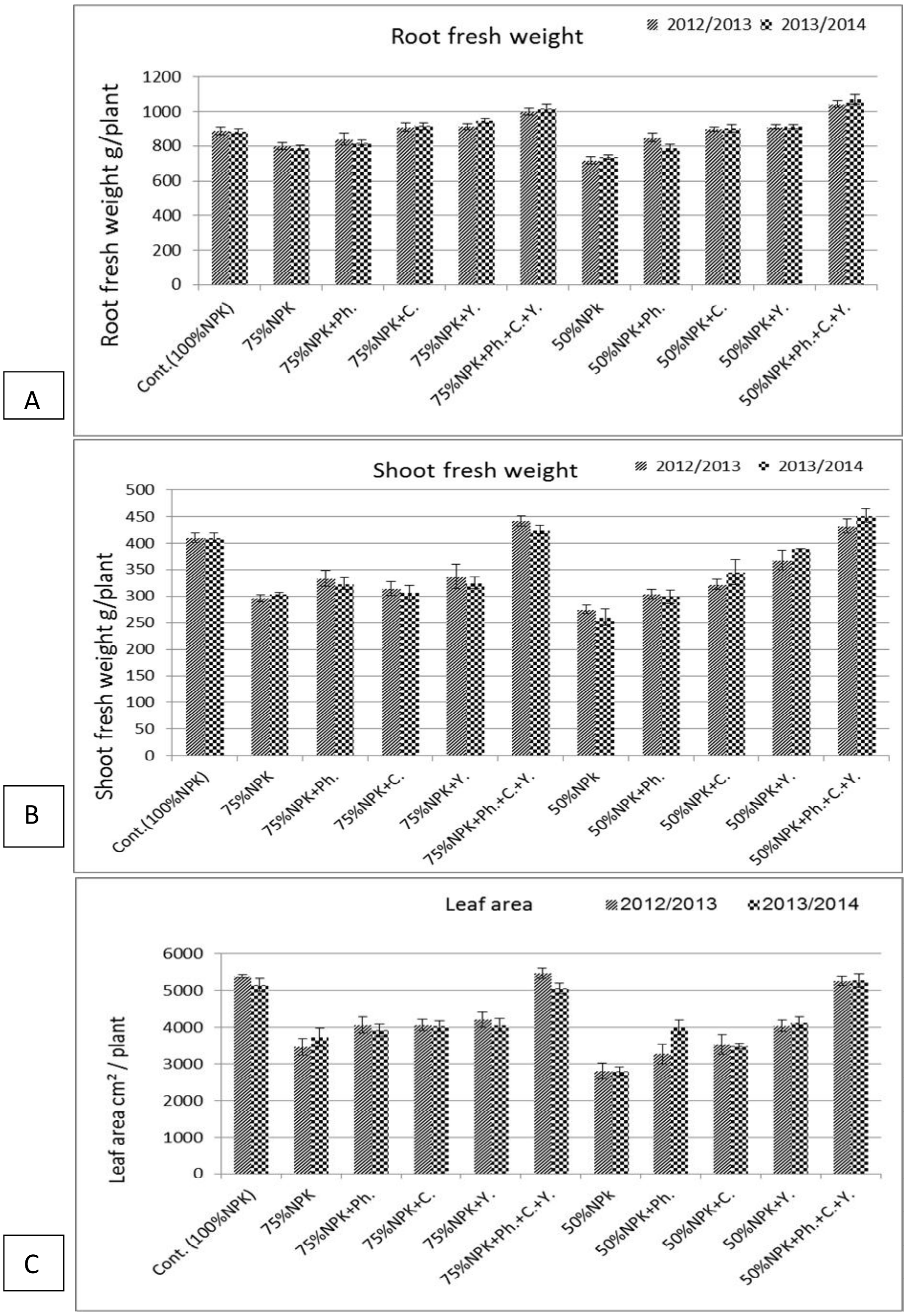

Fig. 2: Effect of bio and mineral fertilizers and their combinations on fresh weight of sugar beet root and shoot and leaf area during 2012/2013 and 2013/2014 seasons.

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

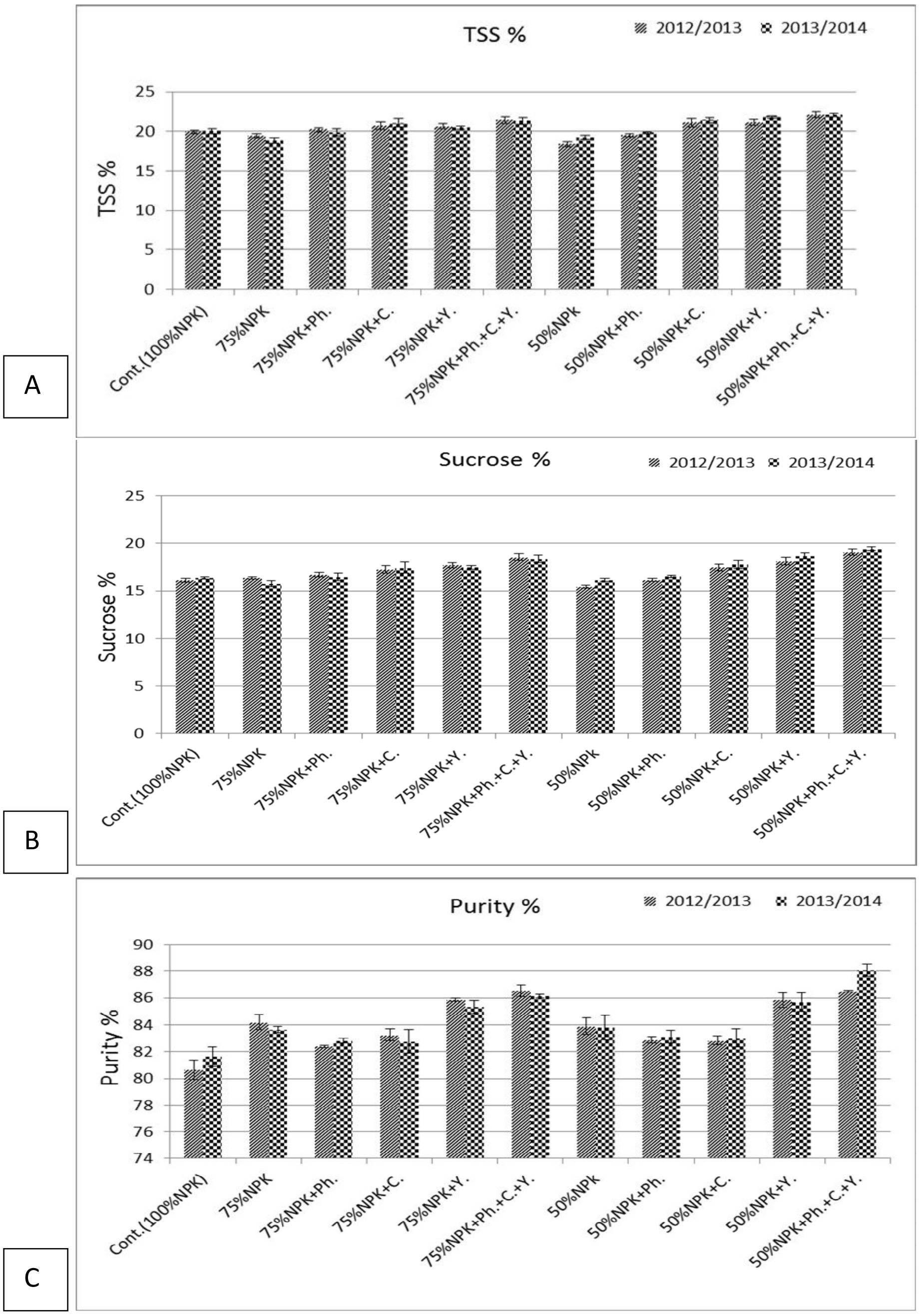

Fig. 3: Effect of bio and mineral fertilizers and their combinations on TSS, sucrose and purity% of sugar beet roots during 2012/2013 and 2013/2014 seasons.

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

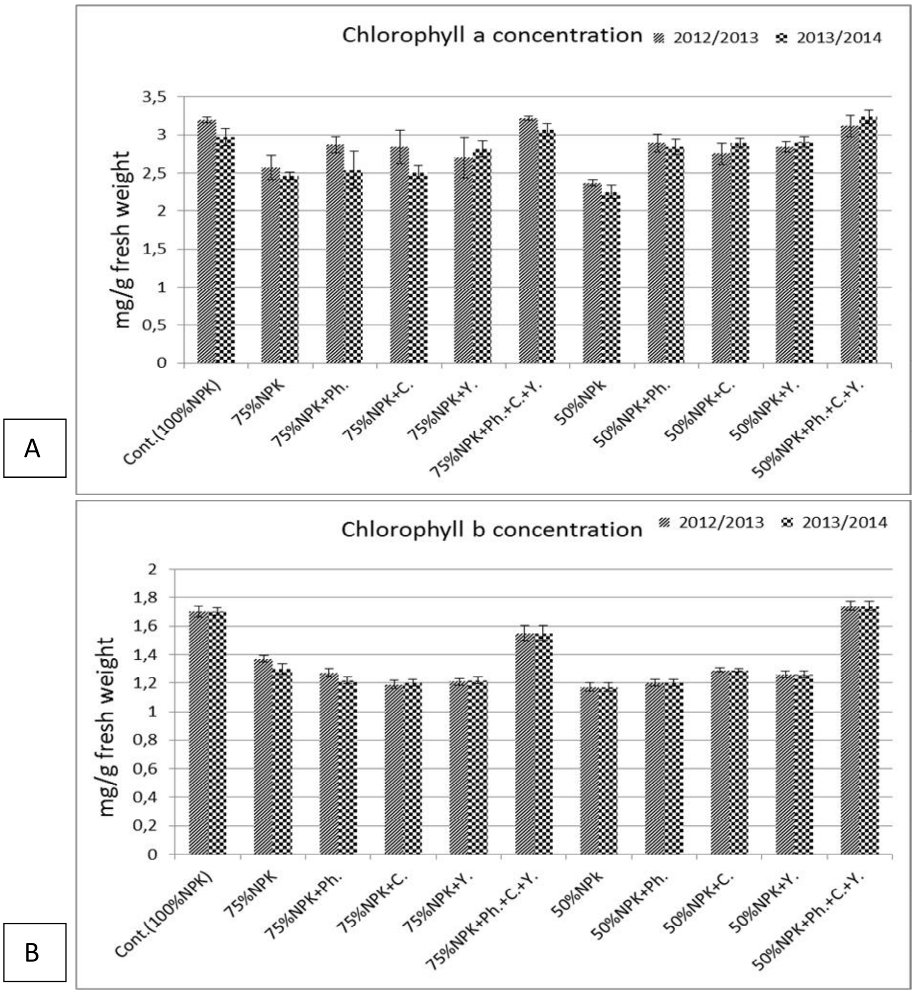

Fig. 4: Effect of mineral and bio-fertilizers and their combinations on chlorophyll a and b concentrations of sugar beet leaves during 2012/2013 and 2013/2014 seasons.
Cont. = control (100% NPK), Ph. = phosphorine, C. = cerealine, Y. = yeast.

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 Rengel 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).

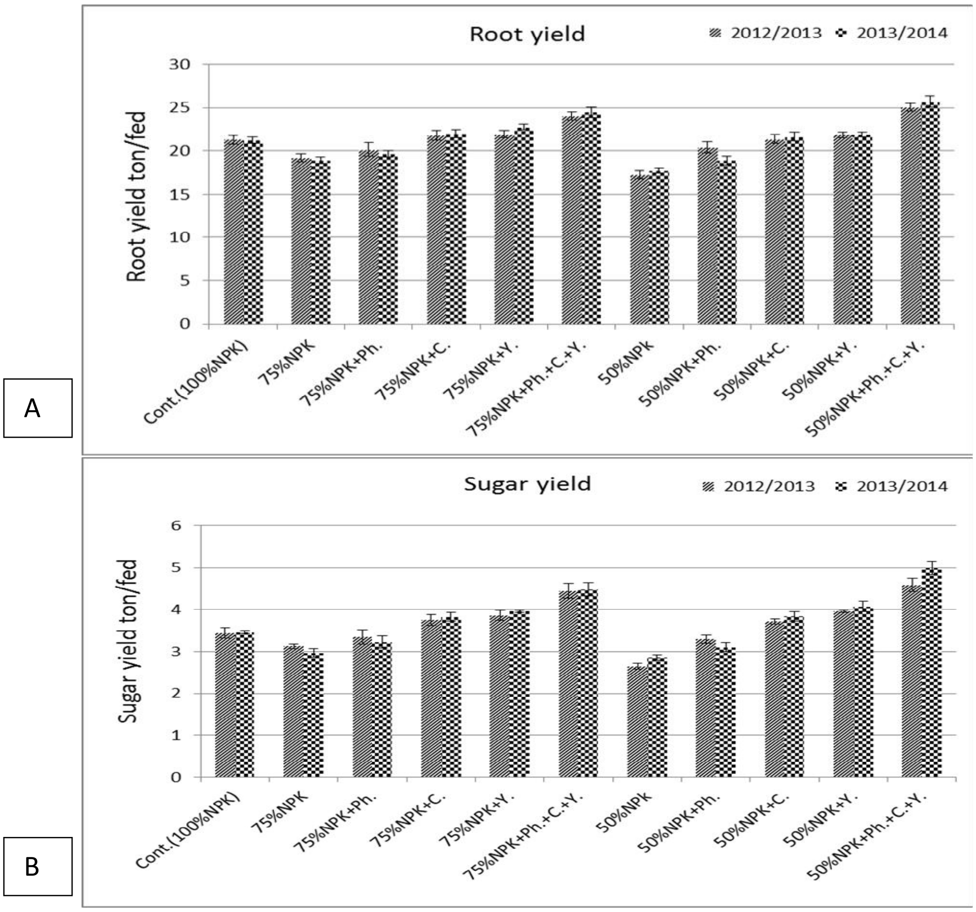

Fig. 5: Effect of mineral and bio-fertilizers and their combinations on Root and sugar yields of sugar beet plant during 2012/2013 and 2013/2014 seasons.
Cont. = control (100% NPK), Ph. = phosphorine, C. = cerealine, Y. = yeast.

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.

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

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).

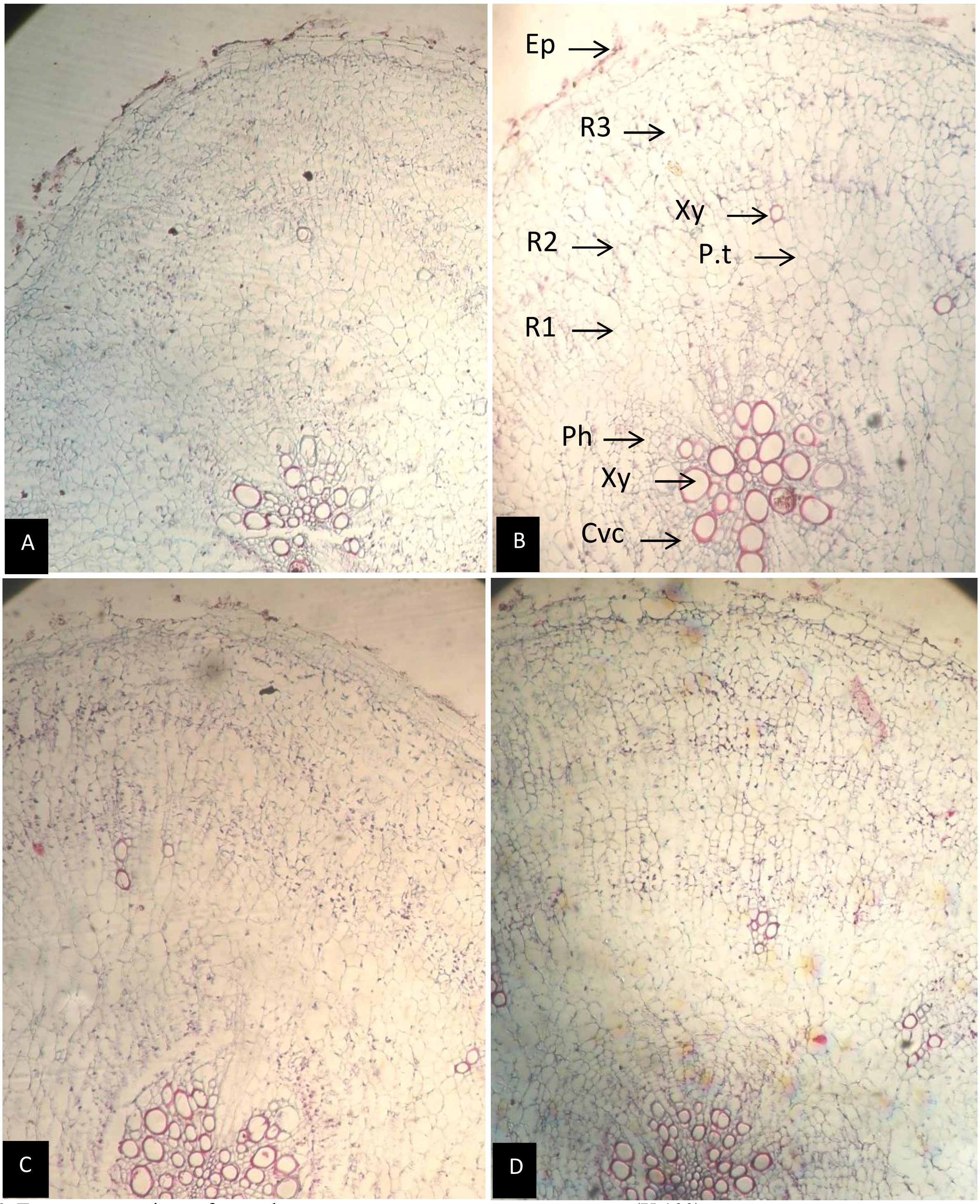

Fig. 6: Transverse sections of sugar beet roots. (X 100)
A-100% NPK (control).
B-75% NPK.
C-75% NPK+ phosphorine.
D-75% NPK + cerialine.
Details:
Ep: Epidermis, R1, R2, R3: growth rings, P.t: Parenchyma tissue, Xy: Xylem tissue, Ph: Phloem tissue, Cvc: Concentric vascular cylinder.

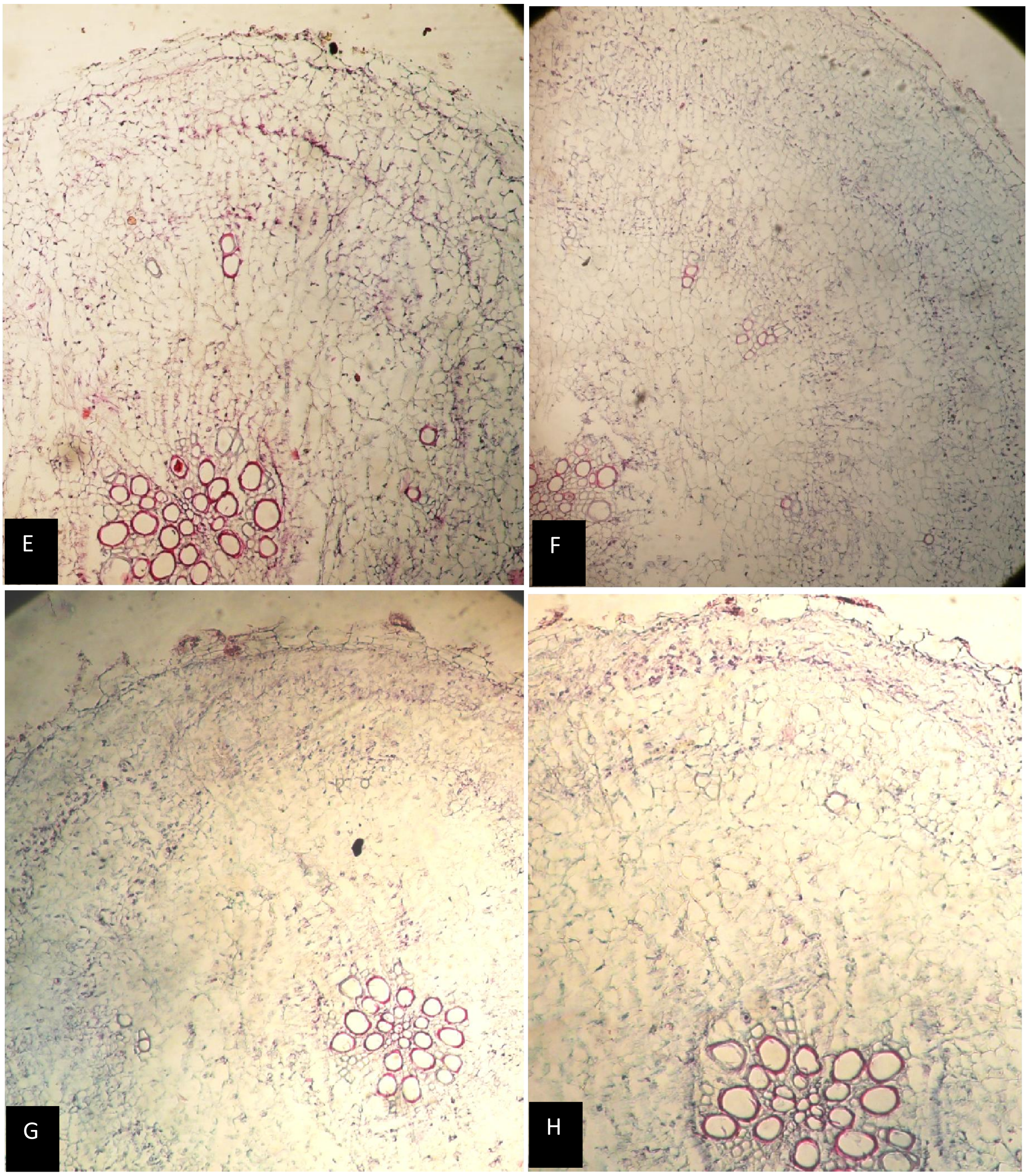

Fig. 7: Transverse sections of sugar beet roots. (X 100)

E. 75% NPK + Yeast.
F. 75% NPK + phosphorine + cerialine + Yeast.
G.50% NPK.
H.50% NPK+ phosphorine.

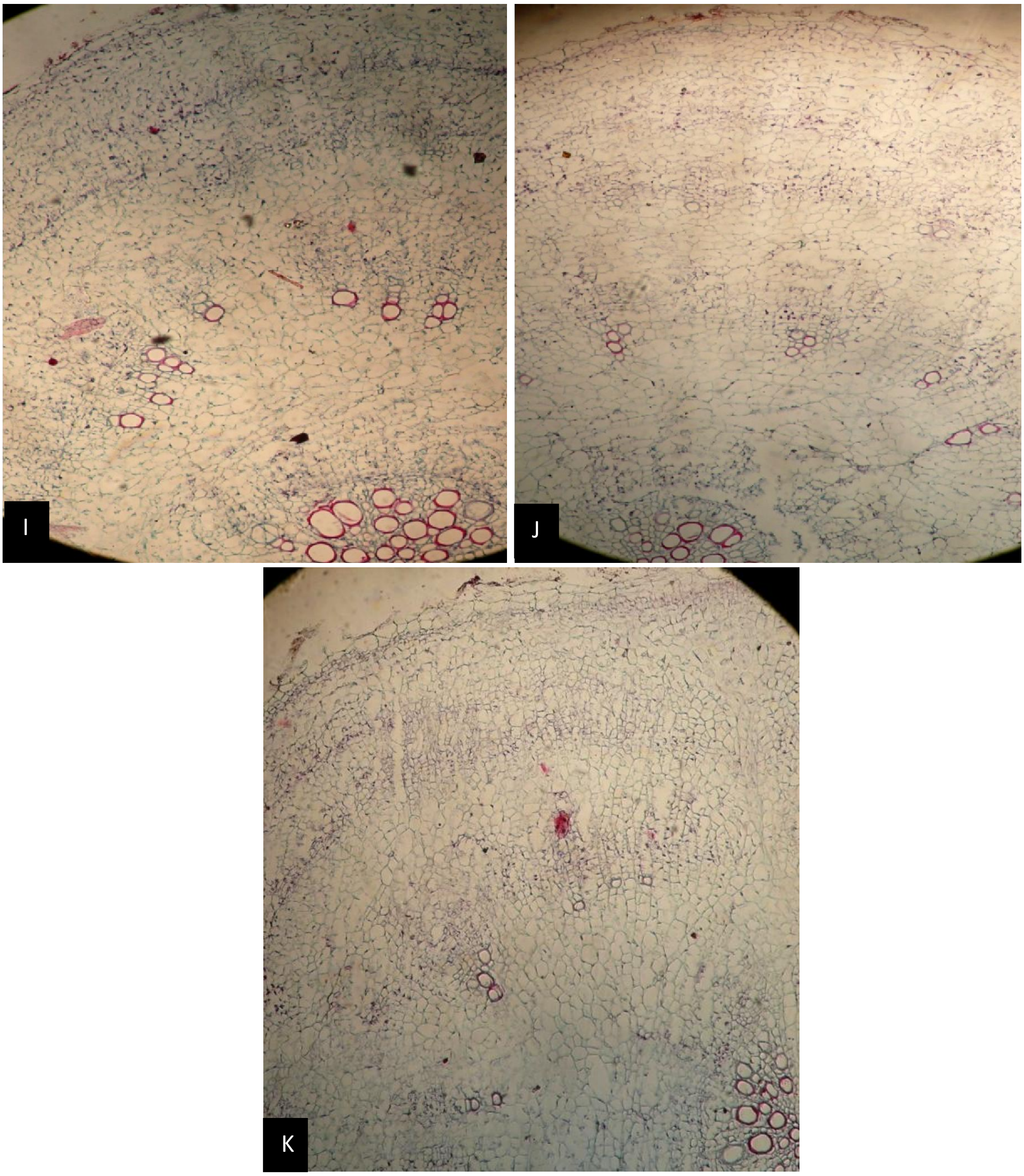

Fig. 8: Transverse sections of sugar beet roots. (X 100)

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

Treatments	Thickness of lamina (µ)	Thickness of midvein (µ)	No. of xylem rows/midvein bundle	No. of vessels/midvein bundle
100% NPK	298.13	432.83	7	22
75% NPK	269.61	565.73	9	21
75% NPK + phosphorine.	324.55	572.52	8	25
75% NPK + cerialine	362.69	583.57	9	27
75% NPK + Yeast	390.06	607.62	10	27
75% NPK + phosphorine + cerialine + Yeast	392.17	614.59	9	30
50% NPK	251.46	514.54	11	21
50% NPK + phosphorine.	372.91	578.51	9	27
50% NPK + cerialine	388.24	581.98	8	27
50% NPK + Yeast	389	589.08	9	28
50% NPK + phosphorine + cerialine + Yeast	397.77	593.56	9	29

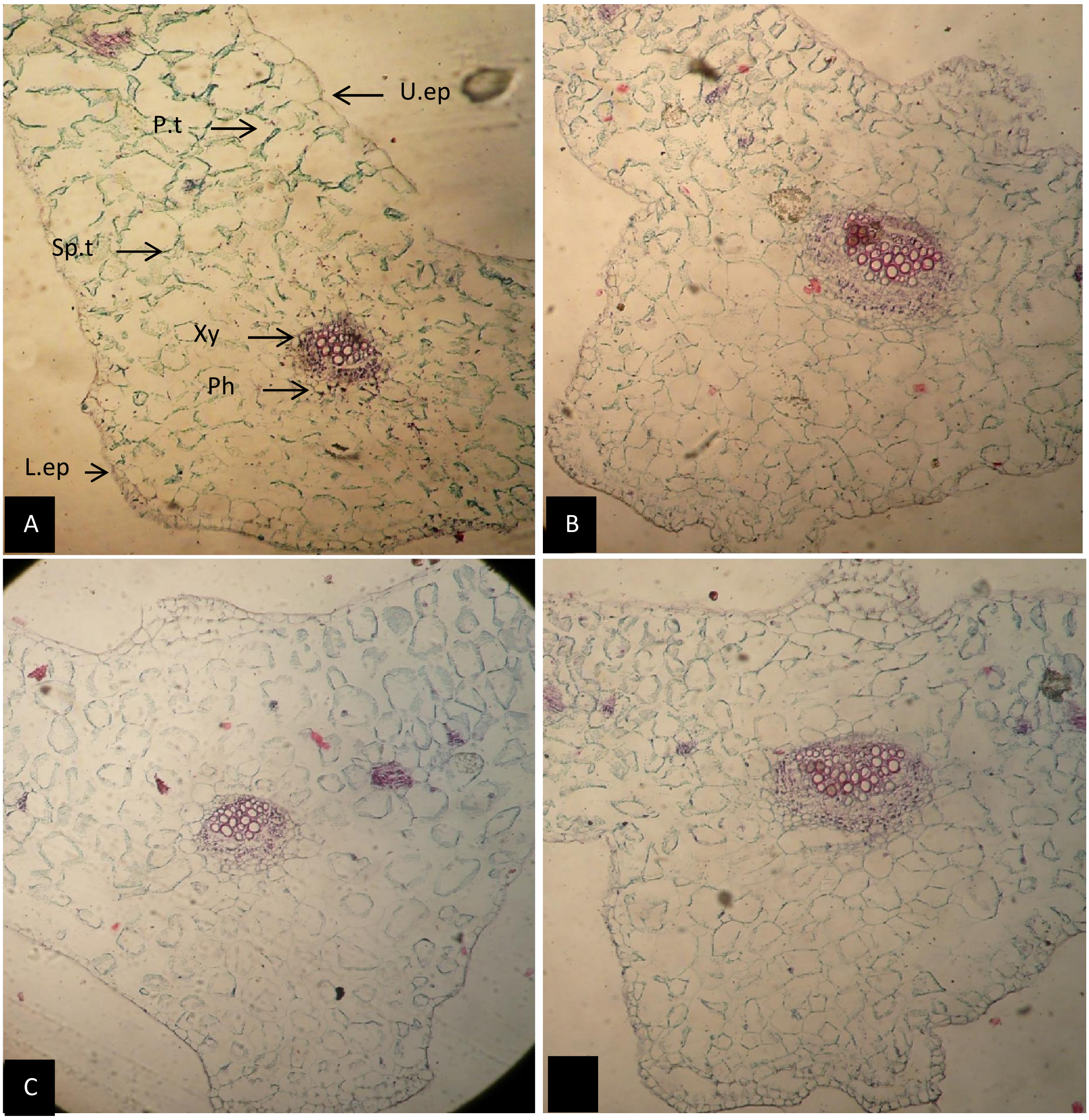

Fig. 9: Transverse sections of sugar beet leaves. (X 100)

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

Details:

U.ep: Upper epidermis, P.t: Palisade tissue, Sp.t: Spongy tissue, Xy: Xylem tissue, Ph: Phloem tissue, L.ep: Lower epidermis.

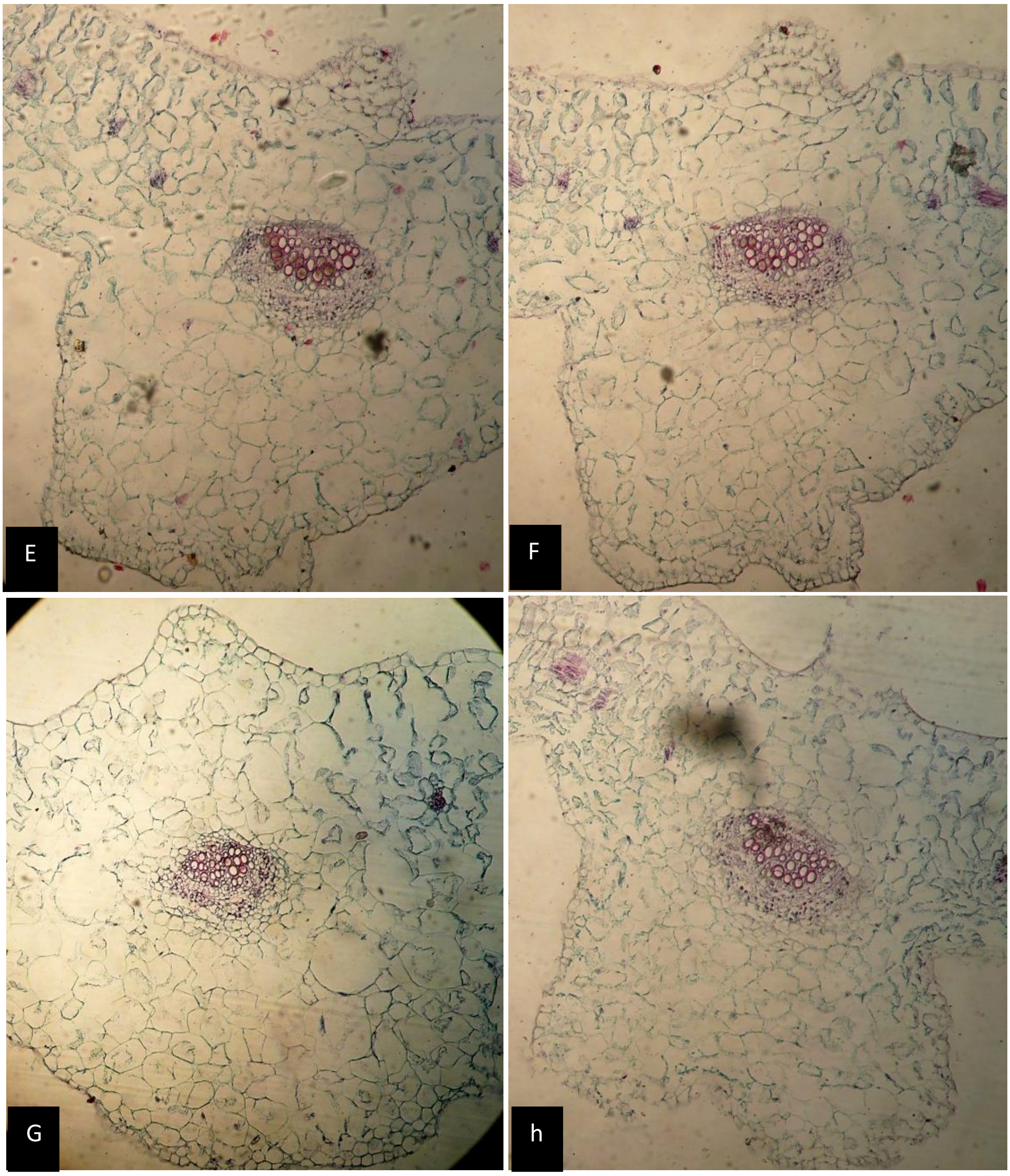

Fig. 10: Transverse sections of sugar beet leaves. (X 100)

E. 75% NPK + Yeast.
F. 75% NPK + phosphorine + cerialine + Yeast.
G.50% NPK.
H.50% NPK+ phosphorine.

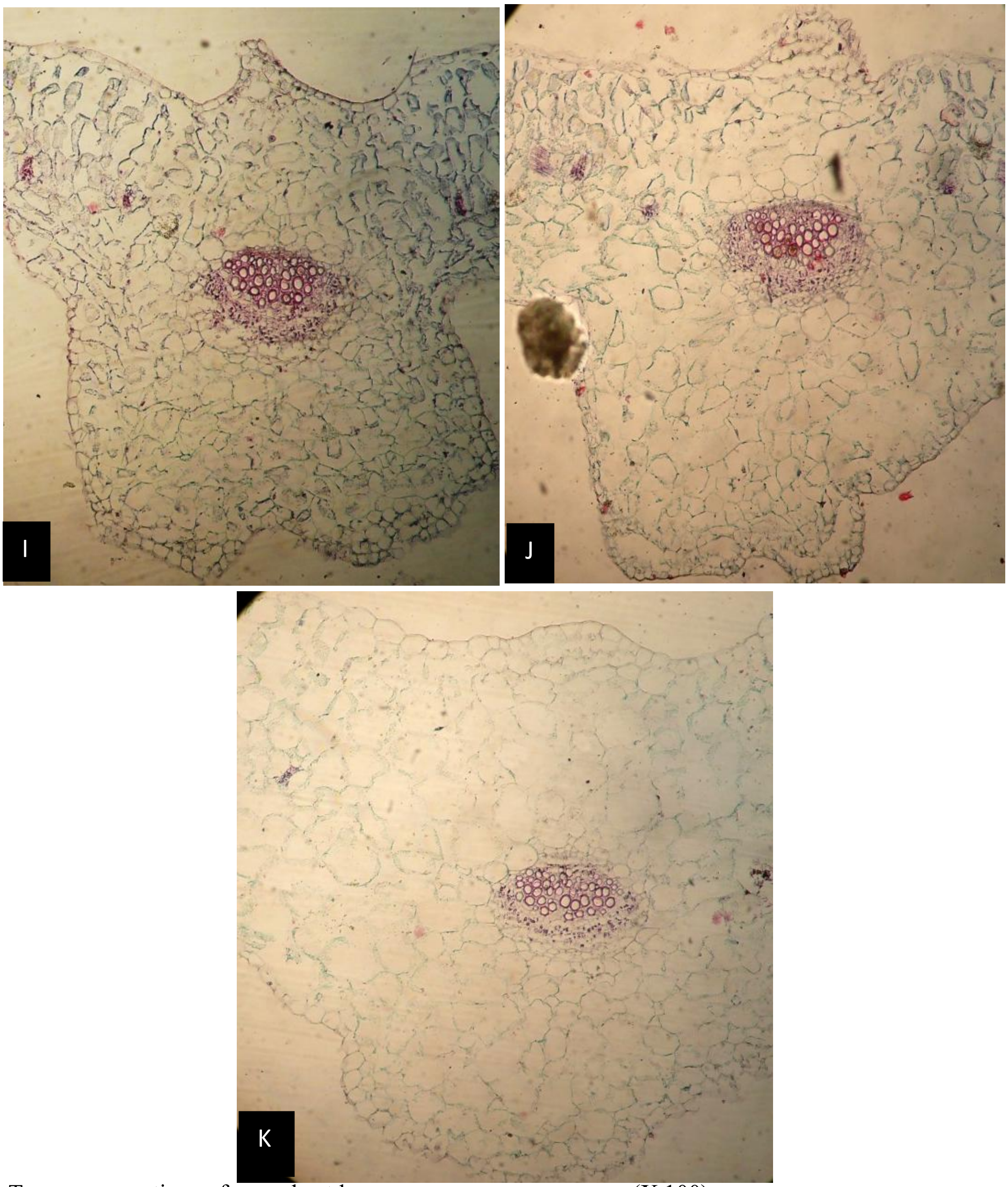

Fig. 11: Transverse sections of sugar beet leaves. (X 100)

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

Acknowledgement

This research was supported by Plant Pathology and Biotechnology Lab., (under accreditation of ISO/17025) and EPCRS Excellence Centre, Dept. of Agric. Botany, Fac. of Agric., Kafr-Elsheikh University, Kafr-Elsheikh, Egypt. The EPCRS Excellence Centre Project funded by Management Supporting Excellence (MSE), Development Projects Management Unit, Ministry of Higher Education, Egypt.

References

Abdelaal, Kh. A. A. and Sahar F. Tawfik, 2015. Response of sugar beet plant (*Beta vulgaris* L.) to mineral nitrogen fertilization and bio-Fertilizers, Int. J. Curr. Microbiol. App. Sci., 4(9):677-688.

Abdelaal, Kh. A. A., Shimaa A. Badawy and Shahrzd M. Neana, 2015. Effect of foliar application of microelements and potassium levels on growth, physiological and quality characters of sugar beet (*Beta vulgaris* L.) under newly reclaimed soils, J. Plant Production, Mansoura Univ., Egypt, 6 (1): 1259-1280.

Abdelaal, Kh. A. A., Y. M. Hafez, M.M. Badr, W.A. Youseef and Samar M. Esmail,2014. Biochemical, histological and molecular changes in susceptible and resistant wheat cultivars inoculated with stripe rust fungus *Puccinia striiformis* f.sp. *tritici*, Egyptian Journal of Biological Pest Control, 24(1):421-429.

Abdel-motagally, M., F. Fathy and K. Attia, 2009. Response of sugar beet plants to nitrogen and potassium fertilization in sandy calcareous soil. J. Agric. & Bio., 11: 695-700.

Abdo, Fatma, A., Nassar, Dali, M. A., Gomaa, Elham F. and Nassar, Rania M. A., 2012. Minimizing the harmful effects of Cadmium on vegetative growth, leaf anatomy, yield and physiological characteristics of Soybean plant [*Glycin max* (L.) Merrill] by foliar spray with active yeast extract or with garlic cloves extract. Research Journal of Agriculture and Biological Science, 8(1): 24-35.

Abou-Atteia, F.A.M. and Kh. A. A. Abdelaal, 2007. Effect of bio and mineral fertilization on the main insect pests and some characters of sugar beet plants, J. Agric. Sci. Mansoura Univ., Egypt, 32(2): 1471-1485.

Agamy, R., M.Hashem, and S. Alamri, 2013. Effect of soil amendment with yeasts as bio-fertilizers on the growth and productivity of sugar beet. African Journal of Agricultural Research, Vol. 8(1): 46-56.

Alaa, I.B., N.M.M. Awad and Sahar M. Ibrahim, 2009. Productivity and quality of sugar beet as affected by rates of potassium and some micronutrients under two locations. Minufiya J.Agric.Res., 34(6):2131-2144.

Aly, M.H.A., Zeinab R.M. and A.M.H. Osman, 2009. Effect of seed inoculation and foliar application with *Azospirillum brasiliense* and/or *Bacillus megatherium* on productivity and quality of sugar beet. Egypt. J. Appl. Sci., 24 (2A): 56-70.

Amin, Gehan A., Badr, Elham A. and M.H.M. Afifi, 2013. Root yield and quality of sugar beet (*Beta vulgaris* L.) in response to biofertilizer and foliar application with micronutrients. World Applied Sciences Journal. 27(11): 1385-1389.

A.O.A.C., 1995. Association of Official Analytical Chemists. Official methods of analysis, 16th Edition, AOAC International, Washington, DC.

Attarde, S. B., S. D. Narkhede, R. P. Patil and S. T. Ingle, 2012. Effect of organic and inorganic fertilizers on the growth and nutrient content of *Abelmoschus esculentus* L. (okra crop). International Journal of Current Research, 4(10):137-140.

Attia, A.N., E.M. Said, S.E. Seadh, Samia S. El-Maghraby and M.E.M. Ibrahim,2011. Effect of sowing methods and weed control treatments on growth of sugar beet and weed characters under nitrogen fertilizer levels. J. Plant Production, Mansoura Univ., Egypt, 2(6): 773-785.

Badawi, M.A., A.A. Kandil, M.S. Sultan, A.N. Attia and S.E. Seadh, 2004. Effect of planting dates, biofertilization and NK combination levels on: II- Yield and quality of sugar beet. The 4th Scientific Conf. of Agric. Sci., 7-9 Dec. 2004, Fac. of Agric. Assiut Univ., pp: 747- 762

Bahr, Amany A. and A.M. Gomaa, 2002. The integrated system of bio-and organic fertilizers for improving growth and yield of triticale. Egypt. J. Appl. Sci., 17(10):512-523.

Barnett, J.A., R.W. Payne and D. Yarrow, 1990. Yeasts, characteristics and Identification. Cambridge University Press, London, 999pp.

Boraste, A., K.K. Vamsi, A. Jhadav, Y. Khairnar, N. Gupta, S. Trivedi, P. Patil, G. Gupta, M. Gupta, A.K. Mujapara and B. Joshi, 2009. Bio-fertilizers: A novel tool for agriculture. Int. J. Microbial. Res., 1(2):23-31.

Cakmak, I., 2005. The role of potassium in alleviating detrimental effects of abiotic stresses in plants. Journal of Plant Nutrition and Soil Science, 168: 521-530.

Cakmakci, R., F. Kantar and F. Sahin, 2001. Effect of N^2-fixing bacterial inoculation on yield of sugar beet and barley. J. of plant Nutrition and Soil Sci., 164(5): 527-531.

Carruthers, A. and J.F.T. Old Field, 1960. Methods for the assessment of beet quality. Int. Sugar J., 63: 72-74.

Castelfranco, P.A. and S.I. Beale, 1983. Chlorophyll biosynthesis recent advances and areas of current interest. Ann. Rev. Plant Physiol., 34:241-278.

Cloete, K.J., A.J. Valentine, M.A. Stander, L.M. Blomerus and A. Botha, 2009. Evidence of symbiosis between the soil yeast *Cryptococcus laurentii* and a sclerophyllous medicinal shrub, *Agathosma betulina* (Berg.) Pillans. Microb. Ecol., 57:624-632.

El-Nagdy, G. A., Dalia M. Nassar, Eman A. Elkady and Gelan S.A.El-Yamanee, 2010. Response of flax plant (*Linum usitatissimum* L.) to treatments with mineral and bio-fertilizers from nitrogen and phosphorus. Journal of American Science, 6(10): 207-216.

Favilli, F., R. Pastorelli, and A. Gori, 1993. Response of sugar beet to *Azospirillum* bacterization in field experiments. Agric. Mediterr. 123:281-285.

Fibach-Paldi S, S. Burdman and Y. Okon, 2012. Key physiological properties contributing to rhizosphere adaptation and plant growth promotion abilities of *Azospirillum brasilense*. FEMS Microbiol. Lett. 326: 99–108.

Hafez, E. M. and Kh. A.A. Abdelaal, 2015. Impact of Nitrogen fertilization levels on morphophysiological characters and yield quality of some maize hybrids (*Zea mays* L.) Egypt. J. Agron., 37(1):35-48.

Hafez, Y. M., Mourad, Y. Rasha, M. Mansour and Kh. A.A. Abdelaal, 2014. Impact of non-traditional compounds and fungicides on physiological and biochemical characters of barely infected with Blumeria graminis f. sp hordei under field conditions, Egyptian Journal of Biological Pest Control, 24(1):445-453.

Hashemi, G., A. Farnia, M. Rahnamaeian and M. Shaban, 2014. Changes in Carbohydrates and Sugar Yield in Sugar Beet (*Beta vulgaris* L.) under different biofertilizers and irrigation closed time, Int. J. Adv. Biol. Biom. Res., 2 (8), 2350-2355.

Ibrahim, Heba A.K. and S. M. Ibrahim, 2014. Effect of some organic extracts on essential nutrients uptake of sugar beet under saline conditions. Desert Research Journal of Agriculture and Biological Sciences, 10(1): 53-64.

Hellal, F.A., S.A.A. El-Sayed and R.M. Zewainy, 2013. Effects of natural potassium source enriched with compost on nutrient uptake and yield of sugar beet grown in newly reclaimed soils. Middle East Journal of Agriculture Research, 2(3): 101-107.

Hussain, T., A. D. Anjum, and J. Tahir, 2002. Technology of beneficial microorganisms. Nat. Farm. Environ. 3:1-14.

Hussein, A.H.S., M.Z. Atef, S.M. Abou Dahab, E. N. Abou-Zeid, M. S. Ali and Nabila Y. Naguib, 2015. Dill (*Anethum graveolens* L.) Growth and yield responses to Gamma radiation and bio-Fertilization, International Journal of Life Science and Engineering, 1(2):68-74

Jat, M. L. and S. S. Pal, 2002. Relative efficiency of Pusa microemulsion-coated and prilled urea in rice (*Oryza sativa* L.) – wheat (*Triticum aestivum*) cropping system on Ustochrepts of Indo-Gangetic Plain. Indian J. Agric. Sci., 72(9): 548-550.

Kandil, A.A., M.A. Badawi, S.A. El-Moursy and U.M.A. Abdou, 2002. Effect of planting dates, nitrogen levels and biofertilization treatments on: II- Yield, yield components and quality of sugar beet (*Beta vulgaris*, L.). J. Agric. Sci. Mansoura Univ., Egypt, 27(11): 7257-7266.

Kandil, A.A., M.A. Badawi, S.A. El-Moursy and U.M.A. Abdou, 2004. Effect of planting dates, nitrogen levels and bio-fertilization treatments on 1: growth attributes of sugar beet (*Beta vulgaris*, L). Sci. J. King Faisal Univ., (Basic and applied sciences), 5(2-1425): 227-237.

Le-Docte, A., 1927. Commercial determination of sugar beet root using the Sachr Le-Docta process. International Sugar J., 29: 488-492. (C.F. Sugar beet Nutrition, April, 1927 Applied Sciences Publishers LTD, London, A.P. Draycott).

Maareg, M.E. and Sohir, T. Badr, 2001. Impact of three soil biofertilizers applied separately and in combinations with a nematicided on *Meloidogyne incognita* infecting sugar beet. Egyptian J. of Agronematology, 4(1-2): 1-9.

Mahmoud, E. K., 2009. Effects of different organic and inorganic fertilizers on Cucumber yield and some soil properties, World Journal of Agricultural Sciences, 5(4):408-414.

Mahmoud, I.I., 2007. Effect of nitrogen, potassium and manganese fertilization on growth and yield of sugar beet, J. Adv. Agric. Res. Fac. Agric. Saba Basha, Egypt, 12(4): 693-704.

Marschner, H., 1995. Mineral nutrition of higher plants, Academic Press, London, UK, pp. 889.

Milford, G.F.J., M.J. Armstrong, P.J. Jarvis, B.J. Houghton, D.M. Bellett-Travers, J. Jones and R.A. Leigh 2000. Effect of potassium fertilizer on the yield, quality and potassium uptake of sugar beet crops grown on soils of different potassium status. J. Agric. Sci., 135: 1-10.

Mirabal, Alonso L., D. Kleiner and E. Ortega, 2008. Spores of the mycorrhizal fungus *Glomus mosseae* host yeasts that solubilize phosphate and accumulate polyphosphates. Mycorrhiza, 18:197-204.

Mir, S., A. Sirousmehr and E. Shirmohammadi, 2015. Effect of nano and biological fertilizers on carbohydrate and chlorophyll content of forage sorghum. International Journal of Biosciences, 6(4):157-164.

Monreala, J.A., E.T. Jiméneza, E. Remesala, R. Morillo-Velardeb, S. García-Mauriñoa and C. Echevarríaa, 2007. Proline content of sugar beet storage roots: Response to water deficit and nitrogen fertilization at field conditions. Enviro. and Exper. Botany, 60(2): 257-267.

Moran, R. and D. Porath, 1982. Chlorophyll determination in intact tissues using N,N-Dimethyl formamide. Plant Physiol., 69: 1370-1381.

Nassar, M.A. and K.F. El-Sahhar, 1998. Botanical preparations and microscopy (Microtechnique). Academic Bookshop, Dokki, Giza, Egypt, 219 pp. (In Arabic).

Nakayan, P., F.T. Shen, M.H. Hung and C.C. Young, 2009. Effectiveness of *Pichia* sp. CC1 in decreasing chemical fertilization requirements of garden lettuce in pot experiments. As. J. Food. Ag-Ind. Special. S66- S68.

O'Mahony, M., 1986. Sensory evaluation of food: Statistical Methods and Procedures. CRC Press, pp. 487.

Patil N.M., 2010. Biofertilizer effect on growth, protein and carbohydrate content in *Stevia rebaudiana* var. *Bertoni,* Recent Research in Science and Technology, 2(10): 42-44.

Radford, S. P.J., 1967. Growth analysis formulae, their use and abuse. Crop Sci., 7: 171-175.

Ramakrishnan, K. and G. Selvakumar, 2012. Effect of biofertilizers on enhancement of growth and yield on Tomato (*Lycopersicum esculentum* Mill.). International Journal of Research in Botany, 2(4): 20-23.

Rania M.A. Nassar, A.M. Yasser and Dalia M.A. Nassar, 2011. Effect of foliar spray with active yeast extract on morphological, anatomical and yield charactreristics of kidney bean (*Phaseolus vulgaris* L.) Australian Journal of basic and Applied Science, 5(5): 1071-1079.

Rengel, Z and P. Damon, 2008. Crops and genotypes differ in efficiency of potassium uptake and use. Physiol. Plant., 133(4): 624-636.

Shewate, S.R., P.V. Ghodke and A.S. Patil, 2008. Effect of nitrogen levels and varieties on sugar beet growth, yield and quality. Cooperative Sugar, 39(11): 29-33.

Stino, R.G., A. T. Mohsen, M. A. Maksouds, M.M.M. Abd El- Migeed, A.M. Gomaa and A.Y. Ibrahim, 2009. Bioorganic fertilization and its impact on apricot young trees in newly reclaimed soil. American-Eurasion, J. Agric. Environ. Sci., 6(1):62-69.

Watson, L. and M. J. Dallwitz, 1992. The Families of Flowering Plants: Descriptions, illustrations, identification, and information retrieval. Version: 15th October1998. Http://biodiversity.uno.edu/delta/.

Winzer, T., G. Lohaus and H. Heldt, 1996. Influence of phloem transport, N-fertilization and ion accumulation on sucrose storage in the tap roots of fodder beet and sugar beet. Journal of Experimental Botany, 47: 863-870.

Zengin, M., G., Fatma, M.Y. Atilla and S. Gez Gin, 2009. Effect of potassium, magnesium and sulphur containing fertilizers on yield and quality of sugar beets (*Beta vulgaris* L.). Turk J. Agric., 33: 495-502.