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Full Length Research Paper

Effect of soil amendment with yeasts as bio-fertilizers on the growth and productivity of sugar beet

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The use of yeast as a bio-fertilizer in agriculture has received considerable attention because of their bioactivity and safety for human and the environment. This study evaluated the effect of soil amendment with three newly isolated yeast strains on the productivity and the external and internal structure of sugar beet to prove their application as bio-fertilizer. We conducted a two-year pot experiment to investigate the effects of *Kluyveromyces walti, Pachytrichospora transvaalensis* and *Sacharromycopsis cataegensis* on the growth and productivity of sugar beet. Soil was inoculated with three doses of each strain (0.0, 50.0 and 100.0 ml pot^{-1} with concentration of ~10^8 cfu ml^{-1}). Results showed that application of the yeasts significantly (*P < 0.05*) increased the photosynthetic pigments, soluble sugars, sucrose, and total soluble proteins of sugar beet. *K. walti* showed the best results among the three yeasts. It increased the sucrose content by about 43% of the control. Anatomy of the leaf and the root showed an increase in thickness of the blade, midvein, dimensions of the vascular bundles, and number and diameter of xylem vessels as the result of application of yeasts. Gas chromatography–mass spectrometry (GC-MS) analysis of the culture filtrates of the yeasts detected some beneficial secondary metabolites that could enhance the plant vigor and the physical and chemical properties of the soil. We assume that application of *K. walti*, *P. transvaalensis* and *S. cataegensis* as bio-fertilizers is a good alternative of the chemicals in the sustainable and organic farming and safe for human and environment.

Key words: Bio-fertilizer, sugar beet, yeast, anatomy, secondary metabolites.

INTRODUCTION

Organic farming strategy is growing rapidly all over the world to conserve human health and the environment, which became under risk because of the unbalance use of pesticides and chemical fertilizers. The dangerous effect is because the repeated use of chemical fertilizers destroys soil biota (Boraste et al., 2009). Organic farmingfarming is 'zero impact' on the environment (www.seedbuzz.com).

Bio-fertilizers are formulations of beneficial microorganisms, which upon application can increase the availability of nutrients by their biological activity and help to improve the soil health. Microbes involved in the formulation of bio-fertilizers not only mobilize N and P but is the process of producing crops and foods naturally. This method avoids the use of synthetic chemical fertilizers and genetically modified organisms to influence the growth of crops.

The main idea behind organic also secrete various plant growth and health promoting substances (Pandya and Saraf, 2010). Bio-fertilizers are low cost, effective and renewable source of plant nutrients to supplement chemical fertilizers (Boraste et al., 2009). In addition to their role in enhancing the growth of the plants, bio-fertilizers can act as biocontrol agents in the rhizosphere at the same time. This synergistic effect, when present,

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increases the role of application of bio-fertilizers in the sustainable agriculture.

Many attempts were made to prepare a bio-fertilizer from wastes using effective microorganism including bacteria and yeasts. Yeasts synthesize antimicrobial and other useful substances required for plant growth from amino acids and sugars secreted by bacteria, organic matter and plant roots (Boraste et al., 2009). *Saccharomyces cerevisiae* is considered as a new promising plant growth promoting yeast for different crops. Recently, it became a positive alternative to chemical fertilizers safely used for human, animal and environment (Omran, 2000). A growing number of studies indicate that plant root growth may be directly or indirectly enhanced by yeasts in the rhizosphere (Nassar et al., 2005; El-Tarabily and Sivasithamparam, 2006; Cloete et al., 2009). A wide diversity of soil yeasts have been researched for their potential as bio-fertilizers (Gomaa and Mohamed, 2007; Eman et al., 2008). Representatives of *Candida*, *Geotrichum*, *Rhodotorula*, *Saccharomyces*, and *Williopsis* are able to nitrify ammonium to nitrate via nitrite *in vitro* (Al-Falih, 2006). Whereas the *ascomycetous* genera *Williopsis* and *Saccharomyces* were able to oxidize elemental sulfur *in vitro* to produce phosphate, tetrathionate, and sulfate (Al-Falih and Wainwright, 1995).

On the other hand, sugar beet (*Beta vulgaris* L.) is the second important sugar crop after sugar cane; produce about 30% of total world production. Few preliminary studies revealed the suitability of growing sugar beet in Saudi Arabia. Nowadays, a great attention has been focused on the possibility of using natural and safe agents for promoting growth of sugar beet. Little information is available about the effect of application of yeasts as bio-fertilizers on the productivity and growth enhancement of sugar beet. Yeasts are a poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds. Searching new yeasts as bio-fertilizers and studying their productivity of bioactive chemical compounds expand our knowledge about their approached mechanisms to enhance the plant growth and soil characteristics.

We assume that a good understanding of the role of soil yeasts in the rhizosphere holds a key to future sustainable agricultural practices. Therefore, the aim of this study was to investigate the impact of soil amendment with *Kluyveromyces walti*, *Sccaromycopsis cataegensis* and *Pachytichospora transvaalensis* as bio-fertilizers on the growth parameters and productivity of sugar beet.

MATERIALS AND METHODS

Plant materials and yeasts

Seeds of sugar beet (*Beta vulgaris L.*) cv Hind were obtained from the agricultural commercial market, Egypt and used in this study. We obtained the yeast strains; *Pachytichospora transvaalensis* UFOSY-1240, *Kluyveromyces walti* UFOSY-1175 and *Sccaromycopsis cataegensis* UFOSY-1365 from Professor J.L.F. Kock, University of the Free State, South Africa for research purpose.

Yeast culture preparation

Yeast cultures were prepared by growing the yeast strains in yeast extract malt extract broth (YMB) (yeast extract 3 gL^{-1}, malt extract 3 gL^{-1}, peptone 5 gL^{-1} and glucose, 10 gL^{-1}) at 25 ± 1°C with shaking (150 rpm) for 48 to 72 h. The yeast cells were pelletized by centrifugation (5000 r.p.m) for 10 min and resuspended in sterilized tap water to the desired concentration (~10^8 cfu ml^{-1}).

Experimental design

The pot experiment was conducted during the two-growing seasons on 15th September, 2010 and 2011. Each season extended for 5 months. Five seeds of sugar beet were sown in plastic pots (30-cm in diameter). Each pot was filled with 12 kg loamy soil (pH = 7.66, EC = 1.42 dS m^{-1}, $CaCO_3$, 5.72% and organic matter, 1.52%). Different doses of the three yeasts (0.0, 50.0 and 100.0 ml pot^{-1} with concentration of ~10^8 cfu ml^{-1}) and the final volume was completed to two liters per pot using tap water. The selection of the doses was based on preliminary studies (data not shown). This application was regularly repeated three-week intervals during the season. Each treatment was set in five replicates. After complete germination, plants in each pot were thinned to one plant. Pots were arranged in a complete randomized design in the greenhouse at temperature range 20 to 30°C, 12 h dark and 12 h light. Irrigation was done twice a week (Using two liters of tap water per pot in all treatments including control) and each pot was irrigated every two weeks with 50 ml pot^{-1} of Hoagland's nutrient solution.

Morphological measurements

Growth parameters including plant height, number of leaves per plant, root length and diameter, fresh and dry weight of the shoot as well as fresh and dry weight of roots were estimated in the treated plants after 150 days from sowing cultivation.

Photosynthetic pigments

Photosynthetic pigments (chlorophyll a, b and carotenoids) were determined in fresh leaf samples of 120-days old plants. Leaf samples (0.5 g) were homogenized in acetone (90% v/v), filtered and made up to a final volume of 50 ml. Pigment concentrations were calculated from the absorbance of extract at 663, 645 and 470 nm using the formulae of Lichtenthaler (1987) as given below:

Chlorophyll a (mg/g FW) = (11.75 × A663 - 2.35 × A645) × 50/500

Chlorophyll b (mg/g FW) = (18.61 × A645 - 3.96 × A663) × 50/500

Carotenoids (mg/g FW) = ((1000 × A470) – (2.27× Chl a)-(8.14×Ch b)/227) × 50)/500

Total soluble sugars

Total soluble sugars (TSS) were extracted by overnight submersion of fresh leaves in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 600 rpm. The supernatant was evaporated to complete dryness, and then dissolved in a known

volume of distilled water to be ready for determination of soluble sugars (Homme et al., 1992). TSS was analyzed by reacting of 0.1ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml of 72% H_2SO_4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using Spekol Spectrocololourimeter VEB Carl Zeiss (Yemm and Willis, 1994). Sucrose was estimated in fresh roots of sugar beet root by using Saccharometer according to the method described by A.O.A.C. (1995).

Total soluble proteins

Total soluble proteins content of the fresh leaves and roots was determined according to the method described by Bradford (1976) with bovine serum albumin as a standard. An amount of 2 g of samples was ground in a mortar with 5 ml of phosphate buffer (pH 7.6) and was then transformed to the centrifuge tubes. The homogenate was centrifuged at 8000 rpm for 20 min. The supernatant of different samples was put in separate tubes. The volume of all of the samples in tubes was then made equal by adding a phosphate buffer solution and the extraction were stored in the refrigerator at 4°C for further analysis. After extraction, 30 µl of different samples were taken out in separate tubes and were mixed with 70 µl of distilled water. In all of these separate sample tubes, 2.9 ml of the Coosmassic Brilliant Blue solution was then added and mixed thoroughly. The total volume was 3 ml in each tube. All tubes were incubated for 5 min at room temperature and then, the absorbance was recorded at 600 nm against the Blank. A standard curve of absorbance (600 nm) versus concentration (µg) of total soluble proteins was calculated.

Anatomical study

Samples of 150-days old from the middle of the fifth leaf from apex and root from 2 cm from base of the main root were taken. Samples were killed and fixed in F.A.A. solution (50 ml 95% ethyl alcohol + 10 ml formalin + 5 ml glacial acetic acid + 35 ml distilled water) for 48 h. Thereafter, samples were washed in 50% ethyl alcohol, dehydrated and cleared in tertiary butyl alcohol series, embedded in paraffin wax of 54 to 56°C mp. Cross sections with 20 µ thick were cut with a rotary microtome, adhered by Haupt's adhesive and stained with the crystal violet-erythrosin combination (Sass, 1961), cleared in carbolxylene and mounted in Canada balsam. Measurements were done, using a micrometer eyepiece and an average of 10 readings were calculated.

Gas chromatography–mass spectrometry (GC–MS) analysis

The three yeasts were grown on YMB at 25 ± 1°C with shaking (150 rpm) for 72 h. The yeast suspension was centrifuged at 10000 rpm for 15 min under cooling and the supernatant was filtered through cellulose membrane filter (0.45 µm), and then was extracted by chloroform solution. An aliquot of one µl extract (chloroform extract) of cell free extract was injected into the GC–MS (6890 N/5975B).

The HP-5MS column was 30 m in length, 0.25 mm i.d., and 0.25 mm in thickness. The carrier gas was helium with average velocity 36 cm sec^{-1}, and flow 1 ml min^{-1}. The operating condition of GC oven temperature was maintained as follows: initial temperature 40°C for 9 min, 150°C for 8 min, at 15°C min^{-1} up to final temperature 310°C with isotherm for 3 min at 25°C min^{1}. The injector and detector temperatures were set at 250 and 280°C, respectively, according to the standard method 8270 EPA (Cakir et al., 2004). Identification of the components of the prepared extract was assigned by comparison of their retention indices, relative to a series of n-alkane indices on the capillary column and GC–MS spectra from the Wiley 6.0 MS data.

Statistical analysis

Treatments were arranged in a completely randomized design. Analysis of variance was performed using the SPSS software package. Analysis of variance (ANOVA) was performed on the data to determine the least significant difference (LSD) among treatment at $P < 0.05$ and Duncan's multiple range tests were applied for comparing the means (Duncan, 1955).

RESULTS

Growth parameters and yield

Results showed that addition of any of the three yeasts (*P. transvaalensis*, *K. walti* and *S. cataegensis*) as bio-fertilizer to the soil cultivated with sugar beet significantly increased the yield and enhanced the growth of the plants (Table 1). The two doses (50 and 100 ml pot^{-1}) increased the plant height, number of leaves, root length, root diameter, fresh and dry weight of shoots and roots significantly as compared to the control in almost cases. However, the highest dose (100 ml pot^{-1}) showed better results than the lowest one. *K. walti* involved in the highest increase in all parameters significantly compared to the untreated and treated plants.

Our results revealed that application of the three yeasts induced the formation of photosynthetic pigments (chlorophyll a and b). However, *K. walti* (100 ml $plant^{-1}$) involved in the highest increase in the pigments' contents (0.86 and 0.22 mg g^{-1} fresh leaves, respectively). The other two yeasts increased the pigments' content significantly, compared to the untreated control. Carotenoids content was either did not change because of the application of the yeast (*K. walti*, 100 ml pot^{-1}), or decreased significantly in the rest of the treatments (Table 2). Consequently, the content of total sugars in leaves and total soluble proteins in both leaves and roots increased significantly because of the application of the yeasts except in one case (*P. transvaalensis*, 50 ml pot^{1}). The data indicate that the three yeasts induced sucrose formation in the beet roots significantly as compared with the control. The highest dose (100 ml pot^{-1}) was the best inducer among the all cases. However, *K. walti* (100 ml/plant) caused the highest increase in the sucrose content. It increased the sucrose content by 42.45% of the yield of the control.

Anatomical studies

Root

Data in Table 3 and Figure 1 show that treatment of *P. transvaalensis, K. walti* and *S. cataegensis* increased the

Table 1. Effect of *Kluyveromyces walti, Pachytichospora transvaalensis* and *Saccharomycopsis cartaegensis* on growth parameters and yield of sugar beet plants.

Treatment	Plant height (cm)	Number of leaves plant^{-1}	Root length (cm)	Root diameter (cm)	FW of shoots plant^{-1} (g)	DW of shoots plant^{-1} (g)	FW of roots plant^{-1} (g)	DW of roots plant^{-1} (g)
Control	27.33^{d}	20.33^{c}	12.50^{c}	6.20^{d}	55.57^{f}	6.61^{d}	58.47^{d}	10.50^{b}
K. walti (50 ml pot^{-1})	34.00^{b}	23.00^{b}	14.00^{bc}	6.90^{bcd}	76.67^{d}	8.62^{c}	62.10^{d}	11.15^{b}
K. walti (100 ml pot^{-1})	36.00^{a}	26.67^{a}	16.83^{a}	8.23^{a}	96.19^{a}	11.37^{a}	87.26^{a}	15.78^{a}
P. transvaalensis (50 ml pot^{-1})	27.67^{d}	24.33^{ab}	13.67^{bc}	6.70^{cd}	71.11^{e}	9.09^{c}	62.41^{d}	11.52^{b}
P. transvaalensis (100 ml pot^{-1})	31.00^{c}	25.33^{a}	13.83^{bc}	7.17^{bc}	84.91^{b}	9.40^{bc}	78.66^{b}	14.95^{a}
S. cartaegensis (50 ml pot^{-1})	30.33^{c}	20.67^{c}	13.67^{bc}	7.73^{ab}	82.03^{bc}	10.19^{b}	74.72^{b}	14.85^{a}
S. cartaegensis (100 ml pot^{-1})	32.00^{c}	24.00^{ab}	16.00^{a}	8.23^{a}	78.34^{cd}	10.18^{b}	78.16^{b}	15.74^{a}
LSD ($P < 0.05$)	1.89	1.43	1.41	0.82	5.06	0.94	6.96	2.38

Values in the same column followed by the same letter(s) are not significantly different at LSD, $P < 0.05$.

Table 2. Effect of *K. waltii, P. transvaalensis* and *S. crataegensis* on photosynthetic pigment content, total soluble proteins content and total soluble sugars content of sugar beet plants.

Treatment	Photosynthetic pigments (mg g^{-1} fresh leaves)			Total soluble metabolites (mg g^{-1} fresh matter)			
	Chl. a	Chl. b	Carotenoids	Proteins in leaves	Proteins in roots	Sugars in leaves	Sucrose in roots
Control	0.61^{e}	0.14^{c}	0.47^{a}	7.38^{b}	2.47^{d}	15.39^{d}	115.44^{g}
K. walti (50 ml pot^{-1})	0.66^{d}	0.17^{b}	0.37^{b}	7.31^{a}	3.08^{bc}	17.36^{b}	123.04^{e}
K. walti (100 ml pot^{-1})	0.86^{a}	0.22^{a}	0.48^{a}	8.65^{a}	3.44^{b}	21. 31^{a}	164.45^{a}
P. transvaalensis (50 ml pot^{-1})	0.69^{c}	0.21^{a}	0.40^{c}	7.52^{b}	2.90^{c}	17.16^{b}	117.65^{f}
P. transvaalensis (100 ml pot^{-1})	0.74^{b}	0.19^{b}	0.42^{b}	8.42^{a}	3.14^{bc}	20.85^{a}	141.13^{c}
S. cartaegensis (50 ml pot^{-1})	0.67^{d}	0.14^{c}	0.38^{d}	7.42^{b}	3.46^{b}	16.45^{c}	128.22^{d}
S. cartaegensis (100 ml pot^{-1})	0.69^{c}	0.18^{b}	0.38^{d}	7.80^{b}	4.09^{a}	20.77^{a}	150.45^{b}
LSD ($P < 0.05$)	0.01	0.02	0.01	0.50	0.33	0.55	1.23

Values in the same column followed by the same letter(s) are not significantly different at LSD, $P < 0.05$.

thickness of growth rings of sugar beet roots by increasing the average diameter of the cells. Similarly, average diameter of secondary xylem vessels was also increased as compared to control. The maximum growth of rings' thickness (833.33) and average diameter of the cells (29.17) was obtained by 100 ml pot^{-1} of *K. walti.*

Leaf

Table 4 and Figure 2 show that inoculation of the soil cultivated with sugar beet plants with *P. transvaalensis*, *K. walti* and *S. cataegensis* increased the thickness of the leaf blade and mid-vein by increasing length and width of the vascular bundles. The average diameter of the vessels and average number of vessels/bundle increased significantly as compared to the control. In consistence with the obtained results from morphological and physiological analysis, the maximum increase was obtained as the result of application of 100 ml pot^{-1} of *K. walti*. It increased

Table 3. Effect of *K. walti*, *P. transvaalensis* and S. *cataegensis* on anatomical structure of root of sugar beet plants.

Treatment	Average number of vessels bundle^{-1}	Growth rings		Secondary growth	
		Thickness (µ)	Diameter (µ)	Number of vessels row^{-1}	Diameter of vessel (µ)
Untreated plant	35.0	446.7	20.6	4	26.3
K. walti (50 ml pot^{-1})	52.7	583.3	22.9	4	27.5
K. walti (100 ml pot^{-1})	63.3	883.3	23.3	5	27.5
P. transvaalensis (50 ml pot^{-1})	47.33	550.0	20.2	4	27.5
P. transvaalensis (100 ml pot^{-1})	60.0	633.3	21.7	6	29.6
S. cataegens (50 ml pot^{-1})	36.0	583.3	22.9	4	29.5
S. cataegens (100 ml pot^{-1})	46.0	625.0	26.3	5	26.9

Table 4. Effect of *K. walti, P. transvaalensis* and S. *cataegensis* on anatomical structure of leaf blade of sugar beet plants.

Treatment	Midvein thickness (µ)	Blade thickness (µ)	Dimensions of vascular bundles (µ)		Average diameter of vascular bundles (µ)
			length	width	
Control	1875.0	260	350.0	340.0	33.3
K. walti (50 ml pot^{-1})	2400.0	220	243.3	223.3	38.8
K. walti (100 ml pot^{-1})	2875.0	280	583.3	500.0	40.0
P. transvaalensis (50 ml pot^{-1})	2012.5	210	533.3	456.7	34.2
P. transvaalensis (100 ml pot^{-1})	2312.5	230	550.0	550.0	35.8
S. cataegens (50 ml pot^{-1})	2312.5	250	386.7	313.3	32.5
S. cataegens (100 ml pot^{-1})	2337.5	260	500.0	423.3	37.1

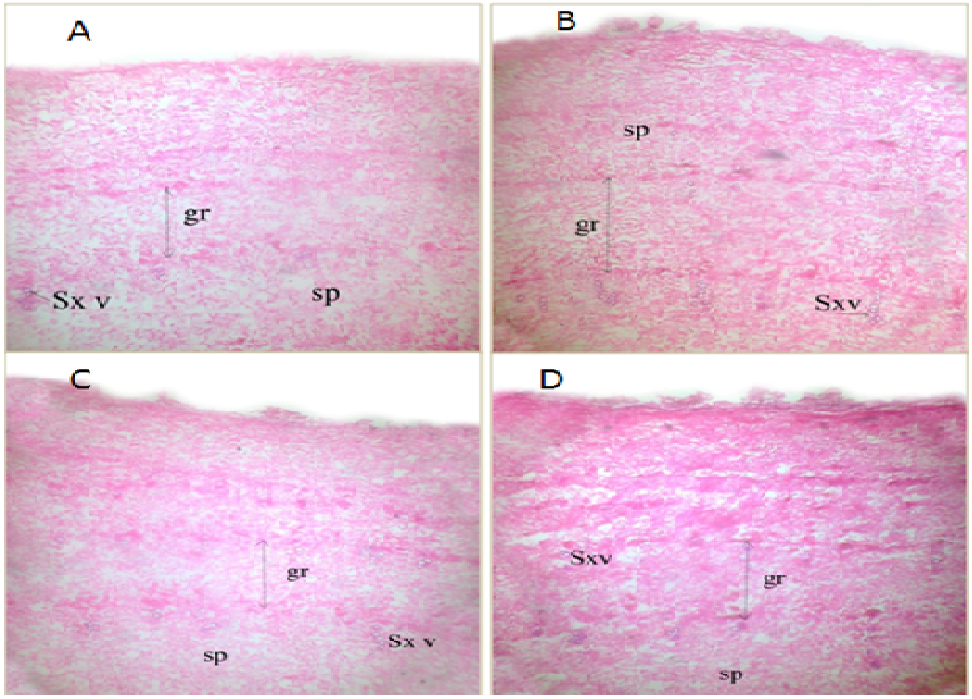

Figure 1. Transections of sugar beet root as affected by application of yeasts. A) untreated plant, B) *K.walti* (100 ml pot^{-1}), C) *P. transvaalensis* (100 ml pot^{-1}), D) *S. cataegensis* (100 ml pot^{-1}), gr; growth ring, Sx v; secondary xylem vessels and sp; storage parenchyma.

Table 5. Chemical composition chloroform extract of the culture filtrate of K. *walti*, *P. transvaalensis* and S. *cataegensis*.

Chemical compound	Abundance (%)	RT
K. walti		
Diisodecyl trimethyladipate	9.227	36.388
3-Methylundecane	9.117	36.114
1-(4'-Methoxy-6-methoxy-quinolin-2-yl)-3-methyl-pyrazol-5-ol	6.388	36.639
Dioctyl adipate	5.728	35.700
6-Ethyl-oct-3-ylheptatyloxalate	5.385	36.517
Didecyl sebacate	4.623	35.793
Decyl ether	4.038	36.197
Decane	3.407	36.882
Bis(6-ethyloct-3-yl)oxalate	3.261	35.536
2-(3-Methyl-2-butenyl)-4-nitrophenol	3.100	23.150
P. transvaalensis		
2-Hexyl-1-decanol	7. 345	21.332
2-(3-Methyl-2-butenyl)-4-nitrophenol	6 .872	23.158
Bacchotricuneatin c	5 .514	21. 441
2,4-Bis (1,1- dimethylethyl) phenol	5 .486	16.974
Methyl 14-methylpentadecanoate	3.983	23.485
2,2-Dimethoxy-1,2-diphenylethanone	3 .773	22.998
1,3 -Bis(1,1- dimethylethyl) benzene	3 .766	12.698
4-Methyl-2-undecene	3 .384	10.052
Dibutylcyanamide	3 .248	13.518
4-Isopropylphenyl methyl phathalate	3 .033	20.227
S. *cataegensis*		
3-methylundecane	27.392	35.746
Decane	7.96	36.699
Dihexyl hexanedioate	5.806	36.429
Diisodecyl trimethyladipate	5.461	35.347
1-(Tert-butoxycarbonyl)-2-methoxy-3-(5-phenyl-4-pentynyl)piperidine	4.316	37.007
Diisooctyl adipate	3.562	36.234
4-Methylundecane	2.746	36.633
4-Hydroxy-3-(3-methyl-2-butenyl)nitrobenzene	2.064	23.159
Capric ether	1.887	36.338
1-(6'-Methoxy-4'-methyl-2'-quinolyl)-3-methyl-1H-pyrazol-5-ol)	1.873	37.301

RT= Retention time.

the midvein thickness to 2875 µ and the average diameter of the vessels to 40 µ.

Analysis of the secondary metabolites

Table 5 and Figures 3 to 5 shows that the culture filtrate of the three yeasts contains many aliphatic and aromatic compounds. We considered only the top ten secondary metabolites from each yeast. Methyl undecane or its derivatives were common among the metabolites of the three yeasts.

Adipate compounds were detected in the filtrate of K. *walti* and *S. cataegensis* as dioctyl adipate and diisooctyl adipate, respectively. Didecyl sebacate was detected in the culture filtrate of K. *walti* in considerable concentration (5.385%). Sebacate is an organic compound which is the diester of sebacic acid and 2-ethylhexanol.

DISCUSSION

Our results showed that all growth parameters of sugar beet plants were significantly enhanced as the result of application of yeasts, especially *K. walti*. Increase in fresh and dry weight of the root is a good indicator for enhancement of the yield.

Increase in vegetative growth of plants because of the application of bio-fertilizers was reported in previous works

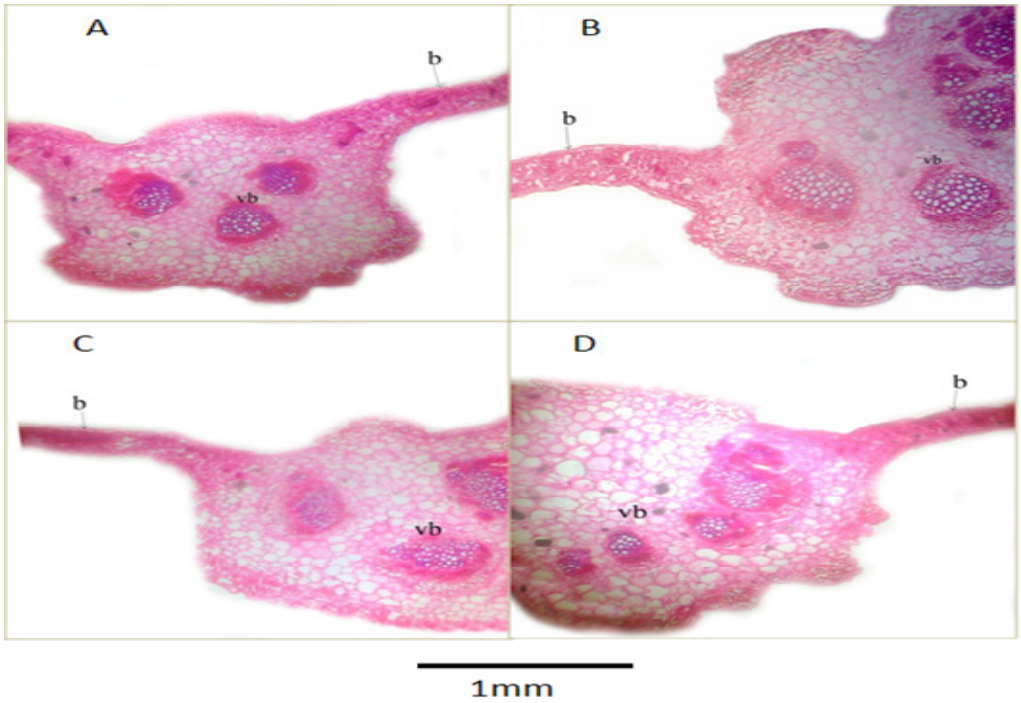

Figure 2. Transections of sugar beet leaf blade as affected by application of yeasts. (A) Untreated plant, (B) *K. walti* at 100 ml pot^{-1}, (C) *P. transvaalensis* 100 ml pot^{-1}, (D) *S. cataegensis* at 100 ml pot^{-1}; b, blade; vb, vascular bundles.

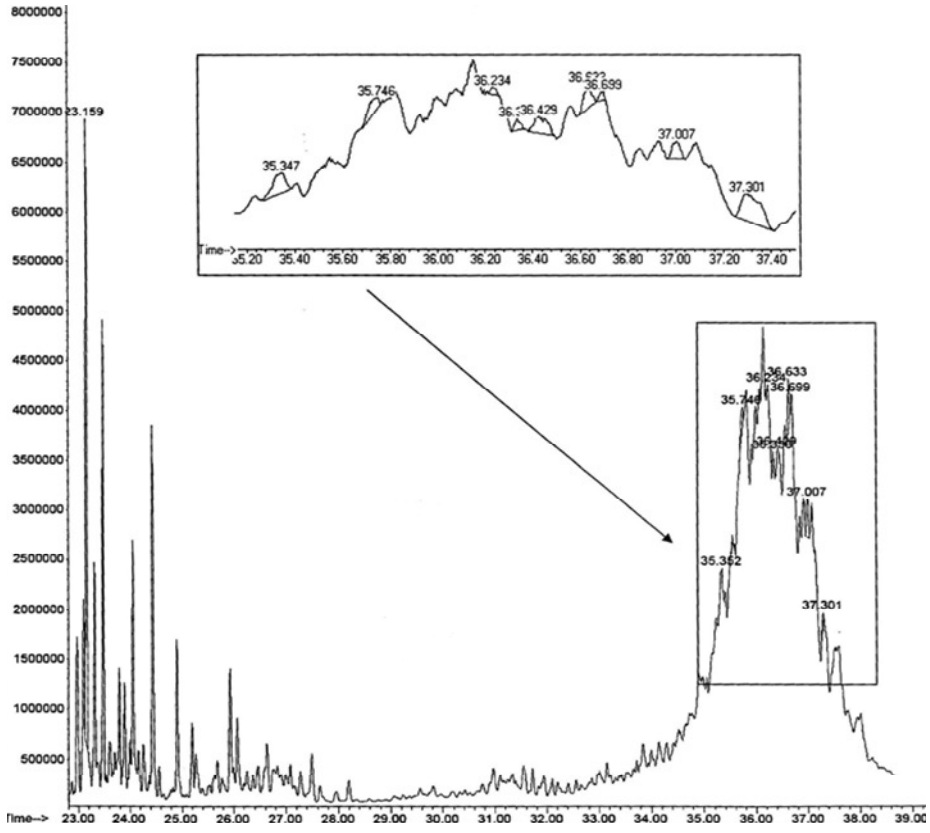

Figure 3. GC-MS chart of chloroform extract of the culture filtrate of *K. walti.*

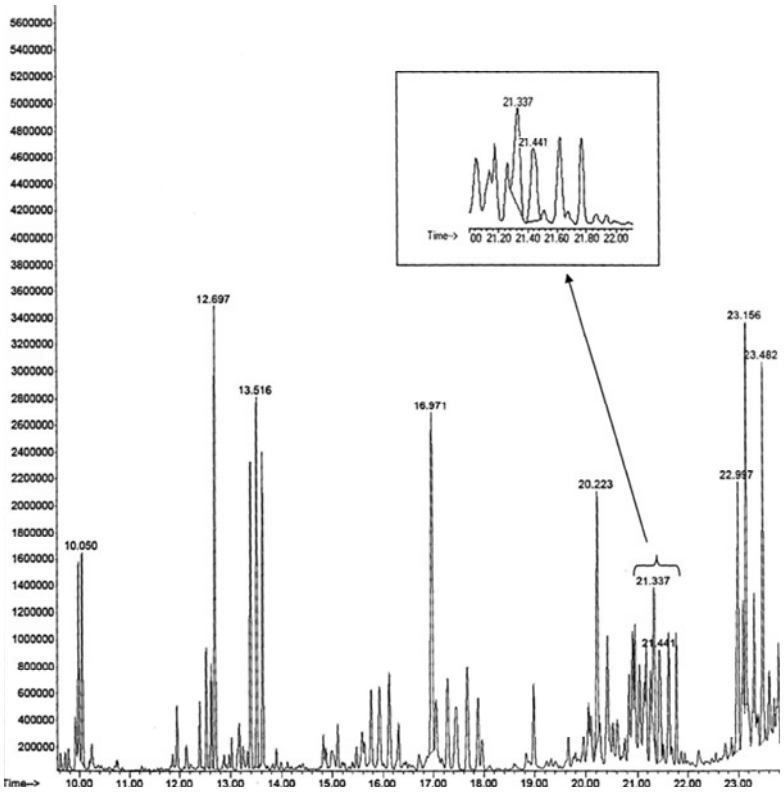

Figure 4. GC-MS chart of chloroform extract of the culture filtrate of *P. transvaalensis*.

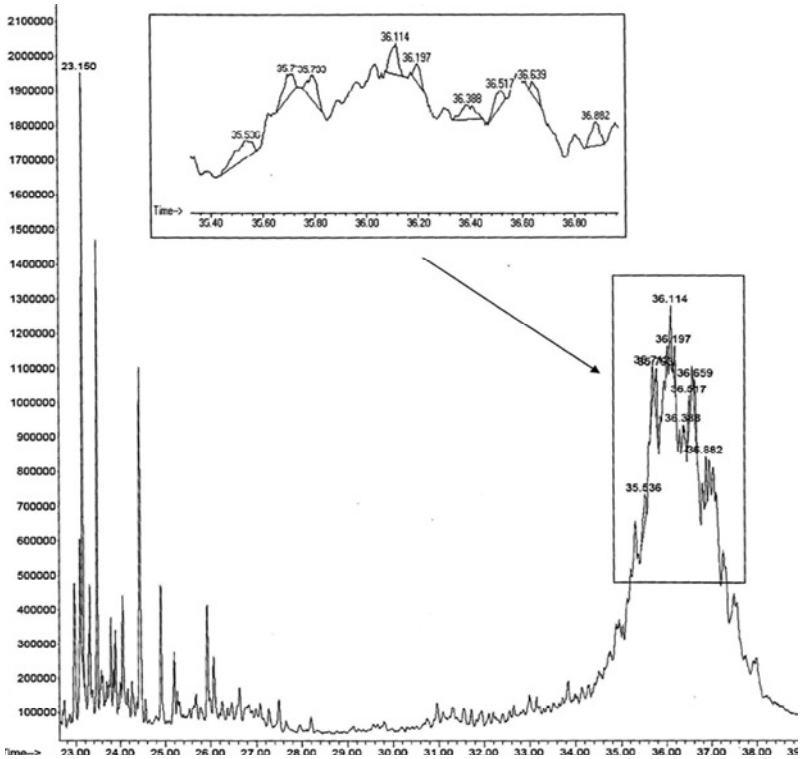

Figure 5. GC-MS chart of chloroform extract of the culture filtrate of *S. cataegensis*.

(Mahdi et al., 2010). Saber (1994) stated that this increment might be due to the availability of soil microorganisms to convert the unavailable forms of nutrient elements to available forms by generating of carbon dioxide from bio-fertilizers (Kurtzman and Fell, 2005). In agreement with our results, Wali Asal (2010) indicated that yeast has good efficiency on growth characters of wheat plants. Nakayan et al., (2009) reported that combination of yeast strain *Pichia* sp. CC1 and a half dose of chemical fertilizer (½CF) increased lettuce dry weight to 107%. The positive effect of yeast is supported by the findings of Mekki and Ahmed (2005). They stated that the increase in yield components because of yeast treatment is mainly attributed to the effect of yeast, which can play a very significant role in making available nutrient elements for plants. In addition, yeast content of macro and micronutrients, growth regulators and vitamins stimulate the plant to build up dry matters (Mirabal Alonso et al., 2008; Hesham and Mohamed, 2011).

The promoting effect of yeasts could be due to the biologically active substance produced by these bio-fertilizers such as auxins, gibberellins, cytokinins, amino acids and vitamins (Bahr and Gomaa, 2002). Afifi et al. (2003) obtained similar results where they found that inoculation of maize with *Rhodotorula* and *Azotobacter* in the presence of half the recommended doses of NPK induced growth parameters to match those of the recommended doses of NPK.

In this study, the application of the three yeasts induced the formation of photosynthetic pigments (chlorophyll a and b). However, *K. walti* (100 ml plant^{-1}) involved in the highest increase in the pigments' contents (0.86 and 0.22 mg g^{-1} fresh leaves, respectively). The difference among yeast strains efficiency to enhance the growth of plants was reported (Amprayn et al., 2012). For example, Nassar et al. (2005) mentioned that yeast isolates vary greatly in their efficiency for IAA production.

Data indicate that the three yeasts induced sucrose formation in the beet roots significantly as compared with the control. The positive effect of yeasts on chl. a and b is in consistence with the result obtained by Hayat (2007) and Stino et al. (2009), who stated that the increase in chl. a and b leads to a consequent increase in total carbohydrates, because the yeast application could enhance role in cell division, cell elongation producing more leaf area. Hussain et al. (2002) reported that *Saccharomyces* sp. is among the microorganisms, which improve crop growth and yield by increasing photosynthesis, producing bioactive substances, such as hormones and enzymes and controlling soil diseases.

The increase in the total soluble proteins content could be attributed to the growth hormones produced by yeast (Gaballah and Gomaa, 2004; Khalil and Ismael, 2010), direct stimulation of the synthesis of protein (Stino et al., 2009), providing plants with essential nutrient elements required for protein formation (Hayat, 2007). Previously. Castelfranco and Beale (1983) stated that the increase in photosynthetic pigment formation could be attributed to the role of yeast cytokinins delaying the aging of leaves by reducing the degradation of chlorophyll and enhancing the protein and RNA synthesis.

Anatomical studies of the root showed that *P. transvaalensis*, *K. walti* and *S. cataegensis* increased the thickness of growth rings of sugar beet roots and average diameter of secondary xylem vessels. A growing number of studies indicate that plant root growth may be directly or indirectly enhanced by yeasts in the rhizosphere (El-Tarabily and Sivasithamparam 2006; Cloete et al., 2009). Warring and Philips (1973) stated that yeast is rich in tryptophan which consider precursor of IAA (indole acetic acid) which stimulate cell division and elongation.

Yeasts are grown rapidly on simple carbohydrates, often through fermentative as well as respiratory pathways (Botha, 2011). As a consequence of their nutritional preference, yeast populations are generally an order of magnitude higher in the rhizosphere as opposed to the bulk soil (Cloete et al., 2009; Botha, 2011). A diverse range of yeasts exhibit plant growth promoting characteristics, including pathogen inhibition (El-Tarabily and Sivasithamparam, 2006; Sansone et al., 2005); phytohormone production (Nassar et al., 2005); phosphate solubilisation (Mirabal Alonso et al., 2008); N and S oxidation (Al-Falih and Wainwright, 1995); siderophore production (Sansone et al., 2005) and stimulation of mycorrhizal-root colonization (Mirabal Alonso et al., 2008). Also, the anatomy of leaf showed that yeasts increased the thickness of the leaf blade and midvein by increasing length and width of the vascular bundles. We assume that the increase in the thickness of the leaf blade and the midvein is expected and consequent effect of the overall enhancement of the plant nutrition, production of the phytohormones, cell division and elongation (El-Tarabily and Sivasithamparam, 2006; Nassar et al., 2005; Mirabal Alonso et al., 2008). To the best of our knowledge, this is the first report about the effect of yeast application on the anatomical structure of the sugar beet leaves.

GC-MS analysis approves the presence of some important chemical compound like Methylundecane, Adipate, didecyl sebacate and Bacchotricuneatin c. Methylundecane is an aliphatic natural product belonged to semiochemicals, which are defined as chemicals that mediate communication between individual organisms. Although some semiochemicals are released purposefully (sex pheromones, the scent of flowers), others are released as a consequence of normal metabolism, but nevertheless still convey information (Am et al., 1992). It was reported that adipic acid may be released into the environment in various waste streams from its production and use in the manufacture of synthetic fibers, plasticizers, resins plastics, and as a food acidulant (Mitchell et al., 1982). Sebacate is an organic compound which is the diester of sebacic acid

and 2-ethylhexanol. It is an oily colorless liquid and used as a plasticizer. We could assume that releasing such compounds in soil might improve the physical and chemical properties of soil that increase water holding capacity, prevent nutrient leaching and add more mineral nutrients to the soil, especially in the rhizosphere zone.

This assumption could be supported by finding of many authors; for example, Botha (2006), who reported that yeasts, such as cryptococci, may affect soil texture by producing extracellular substances that form connective bridges between soil particles or sand grains, thereby contributing to aggregate formation. Genera such as *Cryptococcus*, *Lipomyces*, and *Rhodotorula* are well known producers of extracellular polymeric substances (Vishniac, 1995; Cho et al., 2001).

These substances usually form a capsule enveloping the yeast cell (Kurtzman and Fell, 1998) and may contribute to biofilm formation (Joubert et al., 2003). The ability to resist desiccation and predation has been attributed to capsule formation (Steenbergen et al., 2003), Some soil yeasts are found to assimilate intermediates of lignin degradation, that is, ferulic acid, gallic acid, 4-hydroxybenzoic acid, protocatechuic acid and vanillic acid (Sampaio, 1999; Botha, 2011). The diterpene compound "Bacchotricuneatin c" and its relatives were isolated from plant species and show biological activities (Simirgiotis et al., 2000). Fatty acid was detected in the filtrate of *S. cataegensis* in the form of capric ether. We could state that production of such chemical compounds by the yeasts could serve as precursors or intermediates of beneficial compounds for the plants like growth hormones, fungicides, soil particles aggregators, or plants could assimilate them into valuable compounds. Hence, these compounds could directly or indirectly enhance the growth and the productivity of the sugar beet plants. Because of lacking of the literatures dealing with the production of such compounds by yeasts, we could conclude that yeasts are a poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds. Study the production of such products and their bioactivities in this field holds exciting promise.

Conclusion

Our results are promising in the field of bio-fertilizers. Application of yeasts increased the sugar content sugar beet by about 43%. It significantly enhanced the overall growth of the treated plants. The mechanisms which could be involved include the bioavialbility of macro and micronutrients, production of growth hormones, and reduction of the phytopathogens' growth. In addition, they could improve the physical and chemical properties of soil that increase water holding capacity, prevent nutrient leaching and add more mineral nutrients to the soil. We assume that studying the production of yeasts' secondary metaboilites and their bioactivities in the rhizospher holds exciting promise. We recommend further study dealing with the identification of the secondary metabolites of the yeasts and their bioactivities in the rhizosphere as well as their direct and indirect relationships with the plant growth and productivity.

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