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Improvement of soybean growth and productivity by inoculation with two yeast species in new reclaimed sandy soil amended with humic acid

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The use of biofertilizers and organic matter can eventually reduce the need for inorganic synthetic fertilizers which are potentially more detrimental to the environment. The objective of this work was to study the impact of soil inoculation with *Rhodotorula mucilaginosa* MB151 and *Saccharomyces cerevisiae* 66 in a soil inoculated with *Bradyrhizobium japonicum* 110 and amended with different concentrations of humic acid (HA) or fertilized with full dose of N (nitrogen), P (phosphorus) and K (potassium) as full NPK control on soybean growth and productivity. Field inoculation experiments were carried out during two successive seasons in a sandyloamy soil. The total microbial count, the physiological and the yield parameters of soybean were determined. The two yeast strains produce indole acetic acid and gibberellins. All the growth parameters of soybean were significantly enhanced due to application of yeasts, especially *S. cerevisiae*. The treatment T11 (*S. cerevisiae* + 3% HA) gave the significantly highest increase in N% and consequently the crude protein percent (6.37, 6.43; 39.81, 40.19) of soybean seeds at both seasons respectively. The soybean seeds oil percent increased as the HA% increased in the different treatments during the first season in comparison with control T1 (full NPK). The treatments T12 (*S. cerevisiae* + 4% humic acid) and T13 (S. cerevisiae + 5% humic acid) gave increase in seeds oil % equal 1.2 times the control T1. T11 (*S. cerevisiae* + 3% HA) gave significant increase in seed yield and straw yield (3.816 and 3.838; 5.377 and 5.380 Mg.ha^{1}) during the two seasons, respectively. It could be concluded that application of yeasts in soil amendment with HA, through the numerous direct or indirect mechanisms of action, allow significant enhancement in soybean growth and productivity.

Key words: Organic matter, *Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae, Bradyrhizobium japonicum*, soybean.

INTRODUCTION

Excessive application of chemical fertilizers has led to health and environmental hazards. Therefore, sustainable

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ecological agriculture requires agricultural practices that are healthy to the environment and maintain the long-term balance of the soil ecosystem. In this context, use of microbial inoculants (biofertilizers) in agriculture represents an environmentally safely alternative to further applications of mineral fertilizers (Khan et al., 2007). The documented benefits of plant inoculation with beneficial microorganisms include reduced pathogen infection, improved fertilizer use efficiency, improved resistance such as drought, mineral deficiency and salinity (Kim et al., 2011; Amprayn et al., 2012). In addition, they produce phytohormones, siderophore and vitamin B12 that act as plant growth regulator (Pan et al., 2002).

Most of the research has focused on the use of particular bacterial species, commonly referred to as plant-growth promoting rhizobacteria (PGPR) (Vessey, 2003), or mycorrhizal fungi (Johansson et al., 2004); the role of other microbial species, including yeasts, has received less attention (Nassar et al., 2005).

Yeasts are unicellular fungi that proliferate primarily through asexual means and grow rapidly on simple carbohydrates (Botha, 2011). Because of their nutritional preference, yeast populations are generally an order of magnitude higher in the rhizosphere as opposed to the bulk soil (Botha, 2011). A diverse range of yeasts exhibit plant growth promoting characteristics, including pathogen inhibition (El-Tarabily and Sivasithamparam, 2006); phytohormone production and phosphate solubilization (Amprayn et al., 2012); nitrogen and sulphur oxidation (Al-Falih and Wainwright, 1995); siderophore production (Sansone et al., 2005), stimulation of mycorrhizal-root colonization (Alonso et al., 2008) and production of vitamin B12. Yeasts in the root zone may influence plant growth indirectly by encou-raging the growth of other plant growth promoting rhizo-microorganisms, through vitamin B12 production (Medina et al., 2004).

The application of composted organic matter to soil produces beneficial effects on the chemical, biochemical and physical quality of soil; increased soil microbial population and activity and its plant nutrition capacity (Arancon et al., 2004; Spaccini and Piccolo, 2009). Hence, a particular advantage of compost amendment to soil is the increase in colloidal humified organic matter that affects the quantitative and qualitative long term status of soil organic matter (Adani et al., 2007; Spaccini and Piccolo, 2009).

Moreover soil organic matter (SOM) is a basic component of the agroecosystem and acts as an essential link among the various chemical, physical and biological soil properties. It helps to prevent erosion and desertification and is a driving variable in environmental changes since it acts both as a source and as reservoir for carbon (Campitelli et al., 2006)

Soybean (*Glycine max* (L.) Merr.) is the most important oil seed crop in world with a seed protein content of 40-42% for human consumption and oil content of 20-22%. It is used as fodder for animal and is important in improved crop rotation systems (Carsky et al., 1997). When in symbiotic association with *Bradyrhizobium japonicum*, soybean plants can fix up to 200 kg N ha^{-1} yr^{-1} (Javaid and Mahmood, 2010).

Since, most of the research has focused on the use of PGPR and the role of other microbial species such as yeasts has received less attention, it is supposed that a good understanding of the role of soil yeasts in the rhizosphere hold a key to future sustainable agricultural practices.

Therefore, the objective of this work was to study the impact of soil inoculation with *Rhodotorula mucilaginosa* and *Saccharomyces cerevisiae* in a newly reclaimed soil inoculated with *B. japonicum* 110 and amended with different concentration of humic acid (HA) as organic matter on the growth parameters and productivity of soybean plants.

MATERIALS AND METHODS

Microbial strains and culture conditions

Yeast strains of *S. cerevisiae* 66 and *R. mucilaginous* MB151 were kindly provided by Microbiology Department, Soils, Water and Environment Research Institute (ARC), Giza, Egypt. The strains were grown on glucose peptone and yeast extract agar (GPY) medium (Difco, 1985). Whereas, *B. japonicum* 110 was kindly provided by the Biofertilizers Production Unit, Soil, Water and Environment Research Institute, Agricultural Research Center (ARC), Giza, Egypt. *B. japonicum* 110 was grown on yeast extract mannitol agar (YEM) medium (Vincent, 1970).

Plant growth-promoting characteristics of the two yeast strains

The ability of the two tested yeast strains to produce plant growth promoting hormones such as IAA was studied according to Glickmann and Dessoux (1995) while total gibberellins was studied according to the method described by Udagwa and Kinoshita (1961).

S. cerevisiae and *R. mucilaginosa* inocula preparation

The two yeasts *S. cerevisiae* and *R. mucilaginosa* were inoculated in 250 ml Erlenmeyer flasks containing 50 ml of liquid glucose peptone and yeast extract (GPY) medium. Then, they were incubated at 30°C for 48 h on a rotary shaker at 150 rpm.

Humic acid (HA) preparation

Mature compost with physical and chemical composition shown in Table 1 was used for extraction of humic acid substances. The extraction and the purification of humic acid (HA) were determined according to the methods described by Sanchez-Monedero et al. (2002) and Kononova (1966), respectively.

Elemental analysis [carbon (C), hydrogen (H), nitrogen (N), sulphur (S) and oxygen (O_2)] of the purified HA was performed by microanalyser (Table 2) as described by Goh and Stevenson (1971). The total acidity and carboxyl groups of HA were determined according to the method described by Dragunova (1958) and Schnitzer and Gupta (1965), respectively. However, phenolic groups were determined as described by Kononova (1966).

Table 1. Physical and chemical analysis of the used compost.

Macronutrient (%)			Organic carbon (%)	Organic mater (%)	C/N Ratio	EC (dS/m)	pH	Parasite
N	P	K						
1.35	0.52	0.55	25	43.1	18.5/1	3.21	7.6	Not detected

N: Nitrogen, P: phosphorous, K: potassium, C/N: carbon: nitrogen ratio EC: electrical conductivity.

Table 2. Characteristic of humic acid (HA) extracted from compost.

C%	N%	H%	S%	O_2%	Total acidity (mmole/100 g)	Carboxyl groups (mmole/100 g)	Phenolic groups (mmole/100 g)
50.0	4.1	5.0	1.0	39.9	425	195	230

C: carbon, N: nitrogen, H: hydrogen, S: sulfpher, O_2: oxygen.

Field trials

Two field experiments were carried out at Ismailia Research and Experimental Station, Ismailia Governorate, Egypt ((30° 35` 28.35`` N 32° 15` 6.56`` E), during the 2011 and 2012 summer seasons on a sandy loamy soil. This soil had the following physical and chemical characteristics: sand 70%; clay 29.3%, pH 7.73; electrical conductivity (EC)1.15 dSm^{-1}; organic carbon 0.143%; total N 624 ppm; available P 8.6 ppm; available K 348 ppm and $CaCO_3$ 1.5%. The experiments were conducted in a complete randomized plot design; where the plot size was 3 m in length x 3.5 m in width in 3 replicates. Each plot consisted of 6 lines with 3 m in length and 30 cm in width. Organic fertilizer, humic acid was randomly assigned to main plots with soil irrigation as 48 L ha^1 of humic acid with different concentration 1, 2, 3, 4 and 5%. In the sub-plot design, the two yeast species (*S. cerevisiae* and *R.mucilaginosa*) were distributed as biofertilizer. Their liquid cultures (10^8CFU) were added with soil irrigation at a rate of 24 L ha^1 in three equal doses after 15, 30 and 45 days of sowing. The soybean seeds [*Glycine max* (L.) Merrill] cv. Crawford was kindly provided by the Field Crops Research Institute, ARC, Giza, Egypt. The seeds were sterilized as described by Vincent, (1970) and then coated with *B. japonicum* 110 suspension (~10^8 cells.ml^1) using Arabic gum (40%) as an adhesive agent for 2 h before planting. The treated seeds were sown in hills (three seeds /hill, then after seed germination, the seedlings thinned to two seedlings/hill) on one side of the line at a distance of 20 cm apart.

Twelve treatments were included in the experiment and were arranged in a complete randomized plot design. The following treatments were used: Full NPK as control (T1); *R. mucilaginosa* without HA (T2); R. *mucilaginosa* + 1% HA (T3); *R. mucilaginosa*. + 2% HA (T4); *R. mucilaginosa* + 3% HA (T5); *R. mucilaginosa*. + 4% HA (T6); *R. mucilaginosa* + 5% HA (T7); *S. cerevisiae* without HA (T8);*S. cerevisiae* + 1% HA (T9); *S. cerevisiae* + 2% HA (T10); *S. cerevisiae* + 3% HA (T11); *S. cerevisiae* + 4% HA (T12); *S. cerevisiae*+ 5% HA (T13).

At soil preparation all plots received the recommended dose of phosphorus (15.5% P_2O_5) 360 kg ha^1 as calcium super phosphate and potassium (48% K_2O) 120 kg ha^1 as potassium sulphate, once after the first irrigation. Nitrogen (33.5% N) of 107.5 kg ha^1 as ammonium sulphate (36 nitrogen unit ha^1) was added during planting to activate nodulation. The plants were grown for 120 days, under field conditions. Water was supplied regularly as needed using sprinkler irrigation system.

Assays

Nodulation was estimated at 45 and 75 days after planting by counting the number of nodules (Nod no) in plant roots chosen randomly from each plots. Nodules were dried (60°C for three days) and the nodules dry weight (Nod DW) was measured. Nitrogenase activity was determined in an indirect way by acetylene reduction assay (ARA) according to Somasegaran and Hobben (1994). ARA was determined by GC using Hewlett Packard chromatography model HP (6890 GC) fitted with dual flam detector and 150 × 0.4 cm diameter stainless steel column fitted with propack - N × R 100-120 mesh. Nod No, Nod DW and ARA are the average of five plants from each treatment from each plot at 45 and 75 days.

Total nitrogen (N), phosphorous (P) and potassium (K) percentages (%) were determined in shoot dry matter and seeds of soybean at 45, 75 days and harvest according to Jackson (1958). The crude protein and oil percentage in seeds were also determined (AOCS, 1982). At harvest, shoot dry weight (Sh.DW) and pods number (Pods no.) were measured. The seeds and straw yield (Mg.ha^1) were also determined. All the tested parameters were determined during the two seasons.

Estimation of total microbial count in rhizosphere of soybean plants

The population dynamics of total microbial counts, including yeast were determined in the rhizosphere of soybean plants at 45 and 75 days by the plate count method according to Reinhold et al. (1985).

Statistical analysis

The data were analyzed statistically by applying Duncan's multiple range at P value 0.05 (Duncan, 1955), using a software Package "Costat", a product of Cohort software INC., Berkley, California.

RESULTS

Plant growth-promoting characteristics of the two yeast strains

Potentialities of the two yeast strains (*S. cerevisiae* and *R. mucilaginosa*) to produce phytohormones IAA and gibberellins were tested. Figure 1 shows that the two yeast strains have the ability to produce IAA and gibberellins. It was obvious that *S. cerevisiae* strain produced higher gibberellins (461 µg.ml^1) than *R.*

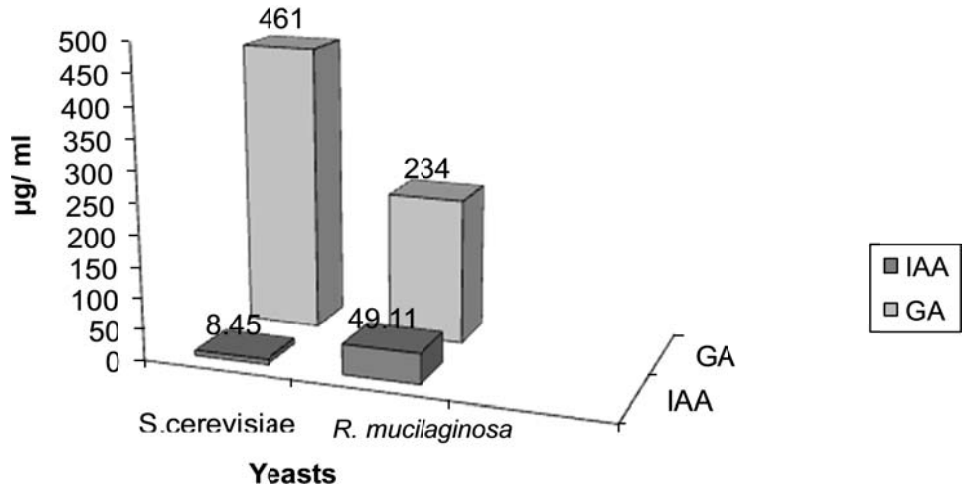

Figure 1. Growth hormones produced by the two yeast strains. IAA: indole acetic acid, GA: gibberellic acid.

Table 3. Effect of inoculation with the two yeast strains on total microbial count (CFU ×10^6), yeast count (CFU ×10^4) in soybean rhizospheric soil amended with humic acid.

Treatment	Season 2011				Season 2012			
	T. count (CFU×10^6) /g rhizosphere soil		T. yeast (CFU×10^4) /g rhizosphere soil		T. count (CFU × 10^6) /g rhizosphere soil		T. yeast (CFU×10^4) /g rhizospher soil	
	45 days	75 days	45 days	75 days	45 days	75 days	45 days	75 days
Control full NPK (T1)	15	32	3	7	22	51	5	10
Rhodotorulamucilaginosa (T2)	33	37	18	25	32	40	16	29
R. + Humic acid (1%) (T3)	40	38	23	31	31	51	25	37
R. + Humic acid (2%) (T4)	44	47	29	36	49	66	33	41
R. + Humic acid (3%) (T5)	61	88	42	58	95	99	47	55
R. + Humic acid (4%)(T6)	55	78	40	49	73	84	46	54
R. + Humic acid (5%) (T7)	49	54	35	43	52	68	38	47
Saccharomyces cerevisiae (T8)	40	46	22	30	49	66	24	33
S. + Humic acid (1%) (T9)	48	51	28	37	67	83	31	40
S. + Humic acid (2%) (T10)	59	65	33	45	79	98	40	48
S. + Humic acid (3%) (T11)	88	101	50	62	106	130	55	66
S. + Humic acid (4%) (T12)	79	90	44	56	96	124	49	59
S. + Humic acid (5%) (13)	66	82	39	51	88	102	45	53

T. count: total microbial count, T. yeast: total yeast count, CFU: colony forming unit, g: gram, NPK: nitrogen, phosphorous and potassium, *R.*: *Rhodotorula, S.*: *Saccharomyces*.

mucilaginosa (234 µg.ml^1) whereas *R. mucilaginosa* produced more IAA (49.11 µg.ml^1) than *S.* cerevisiae (8.45 µg.ml^1).

Total microbial count in the rhizosphere of soybean plants in soil inoculated with the tested yeast strains and amended with humic acid

All the treatments showed increase in the dynamics of total microbial populations (CFU × 10^6.g^1 rhizosphere) and total yeast count (CFU × 10^4.g^1 rhizosphere) in comparison with the treatment T1 (full NPK) in soybean rhizospheric roots during the two seasons at 45 and 75 days (Table 3). However, in treatment T5 and T11 the increases of total microbial populations and total yeast count were higher than in all the other treatments in soybean rhizospheric roots, during the two seasons at 45 and 75 days. The total microbial and yeast count were increased by the inoculation with *S. cerevisiae* more than

Table 4. Nodules number, nodules dry weight (g) and acetylenes reduction assay (ARA) (µmole C2H4.g1 dry nodule) in soybean roots inoculated with the two yeast strains and amended with humic acid.

Treatment	Season 2011						Season 2012					
	Nod. no. plant^{1}		Nod DW (g.plant^{1})		ARA (µmol.g^{1}nodules)		Nod. no.plant^{1}		Nod DW (g.plant^{1})		ARA (µmol.g^{1}nodules)	
	45 d	75 d	45 d	75 d	45 d	75 d	45 d	75 d	45 d	75 d	45 d	75 d
Control full NPK (T1)	10.00	25.0	0.15	0.31	0.05	2.31	13.00	35.0	0.22	0.75	0.11	3.61
Rhodotorulamucilaginosa (T2)	20.60*	61.8*	0.36*	0.42*	0.31	6.55*	19.80*	63.0*	0.40*	0.51	0.41	22.53*
R. + Humic acid (1%) (T3)	22.90*	68.7*	0.48*	0.49*	0.61	10.10*	22.90*	68.0*	0.39*	0.60	0.65	24.51*
R. + Humic acid (2%) (T4)	24.00*	89.7*	0.40*	0.60*	1.31*	13.55*	22.80*	90.1*	0.40*	0.70	1.51*	28.00*
R. + Humic acid (3%) (T5)	33.3*	122.0*	0.57*	0.70*	2.31*	25.10*	32.40*	181.0*	0.56*	0.82	3.55*	42.20*
R. + Humic acid (4%)(T6)	29.9*	99.0*	0.53*	0.61*	2.22*	22.00*	28.90*	101.3*	0.49*	0.71	3.12*	33.35*
R. + Humic acid (5%) (T7)	28.40*	85.2*	0.43*	0.52*	1.33*	13.76*	26.13*	87.0*	0.41*	0.54	1.71*	27.52*
Saccharomyces cerevisiae (T8)	24.80*	74.4*	0.39*	0.66*	0.41	5.70*	24.13*	83.0*	0.43*	0.51	1.09	24.50*
S. + Humic acid (1%) (T9)	26.60*	78.0*	0.44*	0.70*	0.53	11.50*	27.30*	88.3*	0.49*	0.55	1.10	26.57*
S. + Humic acid (2%) (T10)	32.03*	96.9*	0.50*	0.74*	3.20*	28.30*	28.2*	110.0*	0.53*	0.81	4.11*	32.53*
S. + Humic acid (3%) (T11)	29.10*	135.0*	0.56*	0.76*	5.11*	44.18*	36.00*	155.0*	0.58*	0.87	6.15*	49.65*
S. + Humic acid (4%) (T12)	37.50*	112.5*	0.67*	0.85*	4.61*	41.30*	40.60*	117.1*	0.68*	0.83	4.80*	47.51*
S. + Humic acid (5%) (13)	30.70*	92.1*	0.55*	0.73*	3.78*	35.90*	30.13*	103.3*	0.56*	0.70	4.20*	46.4*
LSD at 0.05	1.77	1.85	0.09	0.06	1.02	1.21	3.04	3.59	0.11	0.14	1.04	1.68

Nod DW: nodule dry weigh, ARA: acetylene reduction assay, g: gram, d: day, NPK: nitrogen, phosphorous and potassium, R.: *Rhodotorula*, S: *Saccharomyces*, LSD at 0.05: least significant difference at P value 0.05. *: Significant result.

the inoculation with *R. mucilaginosa.* Moreover, the enhancement in the total microbial populations and yeast count increased in the second season.

Root-nodulation related characters of soybean plants

Nod no., Nod Dw. per plant and ARA were significantly higher in almost all the treatments in which plants were inoculated with the *B. japonicum* combined with each of the two yeast species *R. mucilaginosa* or *S. cerevisiae* and humic acid as compared to the treatment T1 (full NPK) (Table 4). Furthermore, the data in Table 4 showed that the amendment of soil with different concentrations of HA improved soybean nodulation as well as the related characters. During the two seasons, the treatments T5, T6, T11 and T12 gave the significantly highest values of Nod no, Nod DW and ARA. The nitrogenase activity values increased significantly with the treatment T5 inoculated with *R. mucilaginosa* + 3% HA + *B. japonicum* (25.10; 42.2 µmol ethylene h^{-1} .g^{1} Nod DW) at 75 days during the two seasons, respectively. However, the increase in nitrogenase activity values in treatment T11 inoculated with *S. cerevisiae* + 3% HA + *B. japonicum* during the two seasons was higher than that in the treatment T5 at 45 and 75 days, respectively. In the second season, the results come in the same trend as the first one (Table 4), even at 45 or 75 days of growth. The treatments T5 and T11 proved that they are still the superior ones that gave the significant highest values of nitrogenase activity.

Shoot inorganic mineral contents of soybean dry shoots

N, P and K percentages in shoot dry matter were increased in inoculated plants with *R. mucilaginosa* + *B. japonicum* + HA and *S. cerevisiae* + *B. japonicum* + HA in both growth

Table 5. Effect of inoculation with the two yeast strains and amendment with humic acid on N, P and K percentage in soybean shoots during the two successive seasons.

Treatment	Season 2011						Season 2012					
	N (%)		P (%)		K (%)		N (%)		P (%)		K (%)	
	45 d	75 d	45 d	75 d	45 d	75 d	45 d	75 d	45 d	75 d	45 d	75 d
Control full NPK (T1)	1.33	2.31	0.37	1.73	1.82	0.77	1.51	2.20	0.36	1.70	1.90	1.00
Rhodotorulamucilaginosa(T2)	0.90	1.45	0.29	0.49	1.30	0.75	0.95	1.65	0.30	0.60	1.51	0.82
R. + Humic acid (1%) (T3)	0.96	1.65	0.31	0.53	1.51	0.83	1.00	1.76	0.33	0.63	1.56	0.85
R. + Humic acid (2%) (T4)	1.00	1.81	0.34	0.85	1.79	0.80	1.21	1.93	0.34	0.94	1.83	0.87
R. + Humic acid (3%) (T5)	1.40	2.51*	0.40	1.77	1.93*	1.00*	1.52	2.61*	0.42*	1.91*	1.59	1.05
R. + Humic acid (4%)(T6)	1.45	2.31	0.36	1.52	1.91	0.92*	1.33	2.33*	0.35	1.72	2.00*	0.92
R. + Humic acid (5%) (T7)	1.30	1.95	0.34	1.31	1.81	0.88	1.37	2.00	0.35	1.56	1.95	0.90
Saccharomyces cerevisiae (T8)	0.94	1.54	0.30	0.55	1.40	0.77	1.10	1.75	0.39	0.60	1.75	0.80
S. + Humic acid (1%) (T9)	0.98	1.60	0.33	0.61	1.53	0.80	1.21	1.82	0.40	0.66	1.87	0.83
S. + Humic acid (2%) (T10)	1.10	1.85	0.32	0.87	1.81	0.86	1.33	1.96	0.40	1.00	1.96	0.90
S. + Humic acid (3%) (T11)	1.40	2.56*	0.43*	1.82	2.00*	1.10*	1.62	2.67*	0.50*	2.00*	2.11*	1.21*
S. + Humic acid (4%) (T12)	1.37	2.41	0.36	1.56	1.94*	0.95*	1.48	2.56*	0.45*	1.93*	1.96	1.00
S. + Humic acid (5%) (13)	1.31	1.99	0.35	1.41	1.90	0.90*	1.43	2.44*	0.42*	1.72	1.99	0.95
LSD at 0.05	0.168	0.141	0.059	0.102	0.099	0.116	0.127	0.122	0.052	0.17	0.095	0.185

N: nitrogen, P: phosphorous, K: potassium, d: day, NPK: nitrogen, phosphorous and potassium, R: *Rhodotorula*, S: *Saccharomyces*, LSD at 0.05: least significant difference at P value 0.05, *: Significant result.

periods 45 or 75 days during the two seasons, especially the treatments T5 and T11 that received 3% HA (Table 5).

Data in Table 5 showed that the significantly highest value of N% in soybean shoot dry matter were in treatment T5 (2.51 and 2.61, during the first and second season, respectively) at 75 days. In addition, treatment T11 gave the significantly highest value of N% (2.56 and 2.67, at 75 days), during the first and second season, respectively.

P % increased significantly in treatment T5 during the second season at 45 and 75, but the increase was insignificant in the first season as compared to control T1 (full NPK). However, T11 showed significant P % increase in both seasons at 45 and 75 days as compared to the control T1. Furthermore, T11 gave significant K% in both seasons at 45 and 75 days compared to the control T1.

Comparing the data obtained in Table 6, it was found that treatments T5, T6, T11 and T12 significantly increased N% and consequently the crude protein of soybean seeds in both seasons. However, the highest of them (6.37, 6.43; 39.81, 40.19) were obtained in treatment T11 at both seasons, respectively. In addition, results in Table 6 shows that the seed oil % increased as the HA % increased in the different treatments during the first season in comparison with control T1 (full NPK). On the other hand, the seed oil % decreased in all treatments as compared to control T1 in the second season.

Table 7 shows the effect of the different treatments on the NPK content of soybean straw. The data proved that T5 and T11 gave significant increase in NPK % in soybean straw during the first season as compared to the treatment T1 (control full NPK). However, in the second season, T5 and T11 gave the significant increase in straw N% only. On the other hand, T11 showed the highest value of N% (1.55) and P% (0.53) in second season.

As shown in Table 8, the yield components of soybean plants inoculated with some yeast strains in soil amended with humic acid proved that, the treatment T11 in both seasons is considered the best treatment. It showed the significant highest plant Sh.DW (44.6 and 44.5 g plant^{1}) and the significant highest number of pods per plant (32.3 and 32.5). In addition T11 showed significant increase in seed yield (3.816 and 3.838 Mg.ha^{1}) as well as it gave significant increase in straw yield (5.377 and 5.380 Mg.ha^{1}) during the two seasons, respectively.

DISCUSSION

For a sustainable agriculture system, it is necessary to utilize renewable inputs which can maximize the ecological benefits and minimize the environmental hazards. The present study have assessed the influence of two yeast strains (*R. mucilaginosa* and *S. cerevisiae*) in a soil amended with *B. japonicum* 110 and different concentrations of humic acid (HA) on growth and productivity of soybean plants under two field experiments.

The increase of total microbial count and total yeast

Table 6. Crude protein, oil and total nitrogen (%) in seeds of soybean plants inoculated with some yeast strains in soil amended with humic acid.

Treatment	Season 2011			Season 2012		
	Protein (%)	Oil (%)	Nitrogen (%)	Protein (%)	Oil (%)	Nitrogen (%)
Control full NPK (T1)	34.37	20.00	5.50	35.00	30.80	5.60
Rhodotorulamucilaginosa(T2)	28.44	18.80	4.50	28.75	18.10	4.60
R. + Humic acid (1%) (T3)	30.00	19.60	4.80	31.69	19.20	5.07
R. + Humic acid (2%) (T4)	32.50	21.00*	5.20	34.56	21.30	5.53
R. + Humic acid (3%) (T5)	38.75*	20.80*	6.20*	38.31*	21.60	6.13*
R. + Humic acid (4%)(T6)	37.50*	21.70*	6.00*	37.69*	20.90	6.03*
R. + Humic acid (5%) (T7)	34.19	22.20*	5.47	35.19	21.90	5.63
Saccharomyces cerevisiae (T8)	28.56	18.63	4.57	29.19	18.70	4.67
S. + Humic acid (1%) (T9)	31.25	19.30	5.00	32.69	19.17	5.23
S. + Humic acid (2%) (T10)	33.31	22.03*	5.33	32.93	22.43	5.27
S. + Humic acid (3%) (T11)	39.81*	21.83*	6.37*	40.19*	22.53	6.43*
S. + Humic acid (4%) (T12)	37.69*	23.30*	6.03*	37.69*	23.40	6.03*
S. + Humic acid (5%) (13)	36.88*	23.43*	5.90*	36.44*	23.10	5.83
LSD at 0.05	0.332	0.51	0.18	1.21	1.08	0.24

NPK: nitrogen, phosphorous and potassium, R: *Rhodotorula*, S: *Saccharomyces*, LSD at 0.05: least significant difference at P value 0.05, *: Significant result.

Table 7. Effect of inoculation with the two yeast strains and amended with humic acid on N, P and K percentage in soybean straw.

Treatment	Season 2011			Season 2012		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
Control full NPK (T1)	1.20	0.04	0.07	1.34	0.44	0.09
Rhodotorulamucilaginosa (T2)	0.92	0.23*	0.07	0.91	0.23	0.08
R. + Humic acid (1%) (T3)	0.99	0.25*	0.08	0.94	0.26	0.08
R. + Humic acid (2%) (T4)	1.05	0.31*	0.08	1.05	0.32	0.08
R. + Humic acid (3%) (T5)	1.47*	0.44*	0.09*	1.49*	0.44	0.09
R. + Humic acid (4%)(T6)	1.32*	0.41*	0.09*	1.37	0.42	0.09
R. + Humic acid (5%) (T7)	1.18	0.38*	0.08	1.23	0.41	0.08
Saccharomyces cerevisiae (T8)	0.90	0.22*	0.05	0.92	0.21	0.08
S. + Humic acid (1%) (T9)	0.98	0.25*	0.07	0.99	0.27	0.08
S. + Humic acid (2%) (T10)	1.12	0.28*	0.07	1.13	0.33	0.08
S. + Humic acid (3%) (T11)	1.51*	0.49*	0.09*	1.55*	0.53*	0.09
S. + Humic acid (4%) (T12)	1.41*	0.43*	0.08	1.46*	0.45	0.09
S. + Humic acid (5%) (13)	1.28*	0.39*	0.08	1.28	0.44	0.09
LSD at 0.05	0.054	0.018	0.013	0.045	0.023	0.009

N: nitrogen, P: phosphorous, K: potassium, d: day, NPK: nitrogen, phosphorous and potassium, R: *Rhodotorula*, S: *Saccharomyces*, LSD at 0.05: Least Significant Difference at P value 0.05, *: Significant result.

count in the rhizosphere of soybean plants proved that inoculation with both yeast strains + *B. japonicum* + organic matter (humic acid) increased the microbial populations (Fierer et al., 2007; Botha, 2011). The increase of total microbial count and yeast populations in soil amended with organic matter was due to the act of simple organic carbon compounds found in humic acid associated with root exudates of soybean plants that are readily assimilated by yeasts and other microorganisms (Cloete et al., 2009; Botha, 2011).

Our study illustrated that the different treatments used led to enhancement of the plant growth, because yeasts are capable of directly enhancing the plant growth by the production of plant growth regulators (El-Tarabily and

Table 8. Yield components of soybean plants inoculated with the two yeast strains in soil amended with humic acid.

Treatment	Season 2011				Season 2012			
	Sh.DW g.plant^{1}	Pods no. Plant^{1}	Seeds yield (Mg.ha^{1})	Straw yield (Mg.ha^{1})	Sh.DW g.plant^{1}	Pods no. Plant^{1}	Seeds yield (Mg.ha^{1})	Straw yield (Mg.ha^{1})
Control full NPK (T1)	41.5	21.8	3.580	5.16	43.5	25.3	3.601	5.208
Rhodotorulamucilaginosa (T2)	30.7	19.7	2.281	3.508	32	19.4	2.164	3.544
R. + Humic acid (1%) (T3)	33.2	22.3	2.448	3.869	32.7	22.8	2.440	3.952
R. + Humic acid (2%) (T4)	35.7	23.7*	2.756	4.261	34.7	24.6	2.873	4.239
R. + Humic acid (3%) (T5)	41.2	29.9*	3.420	5.016	38.4	30.8*	2.703	4.947
R. + Humic acid (4%)(T6)	39.1	27.9*	3.265	4.748	36.6	28.6*	2.451	4.751
R. + Humic acid (5%) (T7)	37.3	25.8*	2.947	4.674	34.9	27.6*	3.163	4.599
Saccharomyces cerevisiae (T8)	33	19.7	2.252	3.698	35.8	20.3	2.230	3.815
S. + Humic acid (1%) (T9)	37.6	23.2*	2.778	3.751	36.9	23.9	2.67	4.280
S. + Humic acid (2%) (T10)	39.4	25.4*	3.016	4.465	38.7	25.9	3.018	4.526
S. + Humic acid (3%) (T11)	44.6*	32.3*	3.816*	5.377*	44.5	32.5*	3.838*	5.380*
S. + Humic acid (4%) (T12)	43*	30.3*	3.590	5.168	40.9	31.8*	3.654*	5.004
S. + Humic acid (5%) (13)	40.5	27.7*	3.375	4.834	39.3	28.7*	3.373	4.783
LSD at 0.05	0.943	0.853	0.031	0.048	1.295	1.26	0.035	0.005

Sh.DW: shoot dry weight, Pods no.: pods number, g: gram, Mg: mega gram, ha: hectare, NPK: nitrogen, phosphorous and potassium, R: *Rhodotorula*, S: *Saccharomyces*, LSD at 0.05: least significant difference at P value 0.05, *: Significant result.

Sivasithamparam, 2006; Cloete et al., 2009). After growth for 75 days, soybean plants inoculated with *S. cerevisiae* + 3% HA + *B. japonicum* gave higher nodule number, nodule dry weight and nitrogenase activity. Moreover, many authors (Abd El-monem et al., 2008) studied a wide diversity of soil yeasts for their potential as bio-fertilizers. Organic fertilizers consisting of combinations of yeast strains as well as organic and inorganic components are already commercially available, which declares that some of the products are capable of re-establishing the sustainability of ecosystems, as well as enhancing the productivity of farmland for various crops (Pang et al., 2003; Botha, 2011). Our data proves that *S. cerevisiae* and *R. mucilaginosa* have the ability to produce IAA and gibberellins. Plant performance can also be increased as a result of the production of plant growth regulators compounds includes indole-3-acetic acid, indole-3-pyruvic acid, gibberellins and polyamines by yeasts (Botha, 2011).

Soil yeasts representing the genera *Candida*, *Saccharomyces, Geotrichum, Rhodotorula* and *Williopsis* have the potential to contribute to the nitrogen and sulphur cycles within soil (Al-Falih, 2006; Botha, 2011). In addition, these yeasts may be able to solubilize insoluble phosphates thus making these nutrients more readily available to plants (Botha, 2011).

Furthermore, contents of N, P and K were also higher in plants inoculated with both yeast types + *B. japonicum* in soil amended with humic acid as organic matter after growth for 45 and 75 days. The increasing N, P and K levels affected positively the plant growth, in addition to the increase of total yeast count in the soybean rhizosphere. This can be explained on the basis that yeasts are capable of indirectly enhancing the plant growth (El-Tarabily and Sivasithamparam, 2006; Cloete et al., 2009). Singh et al. (1991) found that inoculation of legumes with *S. cerevisiae* increases nodulation as well as *Arbuscular mycorrhiza* (AM) fungal colonization therefore a variety of yeasts are known to occur in the rhizosphere (Botha, 2011), and the interaction between *Mycorrhizal* fungi and soil yeasts is expected.

Alonso et al. (2008) found that yeast genera *Cryptococcus* and *Rhodotorula* were able to solubilize low soluble phosphorus sources and accumulate polyphosphates, affected root growth of rice seedlings and it was suggested that a tripartite interaction exists between the plants, AM fungi and microorganisms. Another research group concluded that both *Ascomycetous* and *Basidiomycetous* yeasts may exert a positive effect on *Glomus mosseae* colonization of cowpea as a result of vitamin B12 production, which stimulates AM development (Boby et al., 2008).

Application of humic acid + *B. japonicum* + yeasts resulted in the increase of soybean yield and other yield traits. This increases could be mainly attributed to the directly or indirectly enhancement in the rhizosphere by yeasts (El-Tarabily and Sivasithamparam, 2006; Cloete et al., 2009).

The results showed increase in seeds oil and protein contents, especially in the first season. The increase of

crude protein % mainly due to the increase of N percentage which indicate that both bio- organic matter can provide plants with essential nutrients elements required for oil and protein formation (Schmidt et al., 2000; Mekki and Ahmed, 2005). Furthermore, yeast is also a natural source of cytokinins that stimulates cell proliferation and differentiation, controlling shoot and root morphogenesis and chloroplast maturation which lead to vegetative growth stimulation (Ezz El-Din and Hendawy, 2010).

The reduction in N, P and K in soybean straw may be due to the increase of translocation rate of their element during flowering and seed formation stages. This is due to the fact that N, P and K are used for numerous plant growth processes (Miller, 2000).

In conclusion, plant growth promoting yeasts (PGPY) in addition to soil amendment with HA can be a true success story in sustainable agriculture. In fact, through their numerous direct or indirect mechanisms of action, PGPY and HA may allow significant reduction in the use of chemical fertilizers. These beneficial events producing plant growth promotion and increases in crops yield, can take place simultaneously or sequentially. There is important synergism observed on plant growth when the inoculants used contain a mixture of organisms. In order to have future beneficial inoculants for field grown crops, one approach should consider performing inoculation assays containing a mixture of soil organisms and amended soil with HA. This association could contain a mixture of PGPY stimulating plant growth at different growth stages, and showing one or more of the known PGPY mechanisms of action. It could also stimulate beneficial symbiotic organisms like AM fungi, rhizobia and *Mycorrhizae* helper bacteria (Son et al., 2001; Antoun and Prėvost, 2005).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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