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Research Article
Effect of Inoculated *Azotobacter chroococcum* and Soil Yeasts on Growth, N-uptake and Yield of Wheat (*Triticum aestivum*) under Different Levels of Nitrogen Fertilization

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Abstract
Background: Mixed inoculants are used for many crops grown under field condition and many studies have shown that mixed inoculants containing mixture of bacterial species promote greater beneficial effects than single strain inocula. **Materials and Methods:** A pot experiment was conducted to study the effect of inoculation with *A. chroococcum* or plus yeast strains (*Saccharomyces cervisiae*, *Candida sake*, *Saccharomyces exiguous*, *Pichia membranifaciens* and *Cryptococcus laurentii*) on grain germination, growth of wheat (*Triticum aestivum* L.) cv., Giza-64 and to choose the best yeast strain for inoculation under field condition. **Results:** The results showed that the mixed inoculation of any of the yeast strains with *A. chroococcum* except yeast strain *Cryptococcus laurentii* resulted in significant ($p \leq 0.05$) increases in shoot fresh and dry weights, root fresh and dry weights. The most stimulative treatment on all plant growth parameters was that inoculated with *A. chroococcum*+*Candida sake*, scoring the following increases in germination, shoot fresh and dry weight and root fresh and dry weights making 134.09, 210.9, 30.23, 20.71 and 71.60%, respectively, compared with the single inoculation treatment with *A. chroococcum* alone. The response of wheat to co-inoculation with *A. chroococcum* and the selected yeast strain (*Candida sake*) was tested in season 2015/2016 under field condition in presence of different N levels (40, 60 and 80 kg N feddan^{-1}). The dual inoculation treatments of *A. chroococcum*+yeast strain *C. sake* under any of the N levels produced significant ($p \leq 0.05$) increases in fresh and dry weights of shoots and roots, N-uptake and grain yield of wheat compared to the single inoculation treatment with *A. chroococcum* alone. **Conclusion:** Dual inoculation with *A. chroococcum*+yeast strain *C. sake* along with 60 kg feddan^{-1} is recommended for wheat fertilization in Egypt since it gave highest grain yield and was equal to that obtained with dual inoculation+80 kg feddan^{-1}, thus saving 20 kg N-fertilizer feddan^{-1}. The magnified promotion induced by the mixture of the yeast strain *C. sake* and *A. chroococcum* may indicate a synergetic interaction between them.

Keywords: Biofertilizers, *Azotobacter chroococcum*, soil yeasts, *Candida sake*, inoculation, N-fertilization, germination, *Triticum aestivum*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There has been an increased interest in biological nitrogen fixation in the context of sustainable agriculture as a result of cost of mineral fertilizers and their possible harms to the environment. Non-symbiotic nitrogen fixing bacteria that live in the rhizosphere[1] and/or endophytically[2] often increase yields of cereals and other crops. Many bacterial species were identified to have nitrogen fixing properties including *Azospirillum* sp., *Azotobacter* sp., *Bacillus* sp., *Beijerinckia* sp., *Clostridium* sp., *Enterobacter* sp., *Pseudomonas* sp., etc.[1,3]. Many researchers have shown the positive effect of inoculation of wheat with *Azotobacter chroococcum* or yeast[4-7].

Yeasts were found in the different soils and rhizosphere of various plants[8,9]. Although the numbers of yeasts are low in comparison with other microorganisms, many investigators claimed that this group of organisms seems to play an important role in the soil fertility and they are capable for producing certain growth promoting substances as hormones, amino acid, vitamins, protein, organic acid and soluble and volatile exudates[10-14].

Usually, mixed inoculants are used for many crops grown under field condition and many studies have shown that mixed inoculants containing mixture of bacterial species promote greater beneficial effects than single strain inocula[15,16]. This was attributed in part to intensive population densities in mixed inocula and to the greater ability of the strains or species to cope with continually fluctuating conditions in the rhizosphere of inoculated plants[17]. Also, strains in mixed inocula can have synergetic effect on the survival and persistence of other community members that are less competitor but desirable strains[16]. The present investigation was carried out to study the effect of single and mixed inoculum of *Azotobacter chroococcum* and soil yeast strains (*Saccharomyces cervisiae*, *Candida sake*, *Saccharomyces exiguous*, *Pichia membranifaciens* and *Cryptococcus laurentii*) on growth and yield of wheat (*Triticum aestivum* L.).

MATERIALS AND METHODS

Microbial strains used: Five yeast strains (*Saccharomyces cervisiae, Candida sake, Saccharomyces exiguous, Pichia membranifaciens* and *Cryptococcus laurentii*) were previously isolated from soil, which showed the ability of yeast strains for solubilizing inorganic phosphate, secrete IAA and production of chelating substrates[12,18,19]. The strains were maintained onmalt-yeast-glucose-peptone agar (YM) slants at 4°C in a refrigerator. The used *Azotobacter chroococcum* strain (non-symbiotic nitrogen fixer) was obtained from Desert Research Center, Egypt, which is usually used in large-scale production of biofertilizer called "Azotobactrien". The strain was maintained on nutrient agar slants at 4°C in a refrigerator.

Pot experiment: Effect of inoculation with *Azotobacter chroococcum* alone or plus one of the yeast strains (*Saccharomyces cervisiae*, *Candida sake*, *Saccharomyces exiguous*, *Pichia membranifaciens* and *Cryptococcus laurentii*) on germination and growth of wheat (*Triticum aestivum* L.) cv., Giza-164 was tested in washed sandy soil. The experiment included the following treatments: (1) Grains inoculated with *Azotobacter chroococcum* alone and (2) Grains inoculated with *Azotobacter chroococcum* plus one of each of the five yeast strains. Fifteen wheat grains were planted per each plastic pot (15 cm in diameter) containing 2-3 kg of washed sandy soil. Three replicate pots were assigned to each inoculation treatment as well as the uninoculated control. The pots were irrigated every 3-4 days by tap water. The tested yeast strains and *Azotobacter chroococcum* were, respectively grown each in 100 mL^{-1} aliquot of malt-yeast-glucose-peptone (YM) broth medium and Burk's broth medium in 250 mL^{-1} Erlenmeyer flasks. The flasks were incubated at 25°C for 5 days in case yeast strains and at 28°C for 7 days in case *Azotobacter chroococcum*. The cultures contained about 10^9 viable cells of *Azotobacter* mL^{-1} and 10^7 viable cells of yeast mL^{-1}. Single or mixed inoculation was made by soaking the grains in the broth cultures for one hour before planting. After 25 days the plants were uprooted, washed and used for determination of shoot and root length, shoot and root dry weights. The most stimulative yeast strain on germination and growth of wheat was used in a field experiment.

Field experiment: During the season of 2015-16 a field experiment was conducted at the Experimental Farm of Faculty of Agriculture, Assiut University to test response of wheat to co-inoculation with *Azotobacter chroococcum* and the most stimulative yeast strain (*Candida sake*) in presence of different levels of N (40, 60 and 80 kg N $feddan^{-1}$). The physical and chemical properties of soil are presented in Table 1. The experimental split plot design with 6 replicates was employed. The main plots were devoted to the different levels of N-fertilization; 40, 60 and 80 kg N $feddan^{-1}$. The subplots were assigned for the treatment of *Azotobacter chroococcum* alone or plus yeast strain (*Candida sake*). The area of the experimental unit (subplot) was 1/400 (10.5 m^2) feddan. Grains of each separate plot, uninoculated or inoculated were broadcasted at the rate of 160 g plot^{-1} (60 kg feddan^{-1}), then slightly covered by surface scratching the seedbed before irrigation.

Preparation of inoculant and grains treatment: Sterilized peat moss was used as a carrier for inoculant preparations. The pulverized dry peat moss, was neutralized to pH 7 with $CaCO_3$ and $Ca(OH)_2$ and distributed in batches of 50 g each in polyethylene bags and autoclaved for 30 min at 121°C on 3 successive days. Aliquots of 25 mL^{-1} of *Azotobacter chroococcum* or yeast broth culture were used per 50 g of the sterilized carrier material. In case of mixed inoculation with *Azotobacter chroococcum* and yeast, the single peat inocula were mixed in equal weights just before grains inoculation.

The grains of each separate plot in polyethylene bag, were inoculated by adding 10 mL of 40% Arabic gum solution and after mixing, the peat inoculant was added and thoroughly mixed with the grains until uniformly surface coated. The inoculant was added to grains at a rate of 15 g/100 g grains. Peat inocula contained 10^8 viable cells of *Azotobacter chroococcum* g^{-1} and 10^6 viable cells of yeast g^{-1}.

Plant sampling, growth measurements and yield: Five-plant samples were taken from each subplot after 60 days from sowing. Immediately after sampling, the plants were transferred to the laboratory and roots were washed with tap water to remove loose soil. Fresh and dry weights of shoots and roots were determination. At harvest, the total grain yield of subplots were determined then calculated per hectare (hectare = 2.381 feddan). The nitrogen contents of dried shoots were determined by the semi-micro-Kjeldahl technique[20].

Table 1: Some physical and chemical characteristics of the composite soil sample used in the field experiment

Properties	Values
Clay	47.8
Silt	29.5
Sand	22.7
Texture grade	Clayey
Total $CaCO_3$ (%)	2.65
EC (dS cm^{-1}) (1:1)	1.22
pH (1:1 suspension)	7.47
Total nitrogen (%)	0.06
Organic matter (%)	1.30
Available P soil (mg g^{-1})	8.67

Statistical analysis: The data reported in this study are mean values of only five replicates. The differences among treatments were tested by the ANOVA and mean values of the treatments were compared by Duncan's multiple range test at $p\leq 0.05$. Statistical analysis of the data was performed by using the statistical computer program[21].

RESULTS AND DISCUSSION

Pot experiment: A pot experiment was carried out during the season of 2014/2015 to test effect of inoculation with *Azotobacter chroococcum* alone or mixed with one of the following five yeast strains (*Saccharomyces cervisiae*, *Candida sake*, *Saccharomyces exiguous*, *Pichia membranifaciens* and *Cryptococcus laurentii*) on germination and growth of wheat grown on washed sandy soil. The results presented in Table 2 show that in general, the mixed inoculation treatments with any of the yeast strains, increased the percentage of seed germination compared with the single inoculation treatment. Also, the results show that the mixed inoculation with *Azotobacter chroococcum* and any of the yeast strains, except yeast strain *Cryptococcus laurentii*, significantly ($p\leq 0.05$) increased the shoot fresh and dry weights, root fresh and dry weights. The most stimulative treatment was that inoculated with *Azotobacter chroococcum*+*Candida sake*, scoring the following increases in germination, shoot fresh and dry weights and root fresh and dry weights: 20.55, 18.49, 28.67, 33.80 and 35.05, respectively, compared with the single inoculation treatment with *Azotobacter chroococcum* alone. These stimulations in per cent grain germination of wheat and promotions of root and shoot weights are probably due to hormonal effects. Many investigators reported that the yeasts are capable of producing growth promoting substances, as auxins and hormones (IAA and GA3), amino acids and vitamins in their cultures[22,23]. The results obtained in the present

Table 2: Effect of inoculation* with *A. chroococcum* alone or plus yeast strains on germination and growth of wheat** on washed sandy soil

		Shoot weight (per plant)		Root weight(g plant^{-1})	
Inoculation treatments	Germination (%)	Fresh (g)	Dry (g)	Fres	Dry
A. chroococcum (Azoto.)	79.3^{d}	3.46^{d}	1.36^{d}	0.71^{e}	0.097^{c}
Azoto.+*S. cervisiae*	86.3^{bc}	3.87^{b}	1.66a^{b}	0.84^{bc}	0.115^{ab}
Azoto.+*C. sake*	95.6^{a}	4.10^{a}	1.75^{a}	0.95^{a}	0.131^{a}
Azoto. *S. exiguous*	88.5^{b}	4.20^{a}	1.67^{ab}	0.88^{b}	0.117^{ab}
Azoto.+*P. membranifaciens*	84.0^{c}	3.70^{c}	1.58^{bc}	0.75^{e}	0.105^{bc}
Azoto.+*C. laurentii*	81.0^{d}	3.54^{d}	1.40^{d}	0.78^{de}	0.102^{bc}

*Grains were soaked for 1 h in 5 days broth cultures, **Grown for 25 days, values in column followed by the same letter (s) are not significant by Duncan's multiple range test at 5% level investigation are in harmony with those obtained by Ahmed *et al.*[24] who indicated that supplemented of organic fertilizers with *Azotobacter*, yeast and their interaction produced significant increment in all growth characters of wheat plants compared to application of *Azotobacter* alone, although yeast did not fix nitrogen but produce growth promoting substances.

Field experiment

Main effects of microbial inoculation and N levels: Data presented in Table 3 show the main effects of microbial inoculation with *Azotobacter chroococcum* alone or with yeast strain (*Candida sake*) in presence of different N levels (40, 60 and 80 kg N feddan^{-1}) and the different N levels. The results show that wheat inoculation with *A. chroococcum* or *A. chroococcum*+yeast strain *C. sake* caused significant ($p\leq 0.05$) increases in fresh and dry weights of shoots and roots and grain yield compared with the uninoculated control. Also, the dual inoculation treatments of *A. chroococcum*+yeast strain *C. sake* produced significant increases in N-uptake per plant. The dual inoculation with *A. chroococcum* +yeast strain *C. sake* resulted in 5.9 an increase in grain yield of compared with single inoculation treatment of *A. chroococcum*. As such, the magnified promotion induced by the mixture of the yeast strain *C. sake* and *A. chroococcum* may indicate a synergetic interaction between them. The increase in the root mass of plant induced by the inoculated yeast strain *C. sake* may also indicate direct hormonal effects. Many investigators claimed that yeasts are capable for producing certain growth promoting substances as hormones, amino acid, vitamins, protein, organic acid and soluble and volatile exudates[10-13]. Moreover, Kanti and Sudiana[10] found that 9 yeast strains out of the 23 isolates, belonged to genera of *Debaromyces*, *Pichia*, *Rhodotorula* and *Candida* that were isolated from soil, had the ability to dissolve $Ca_3(PO_4)_2$. Vassileva *et al.*[11], pointed to the importance of yeasts as well-known is the production of organic acids (especiallycitric) and their high survival rate under extreme soil conditions in transformation of rock phosphates and insoluble carbonate leading to increases in available phosphorus Fe and other micronutrients. They recorded the simultaneous solubilization of rock phosphate and calcium carbonate by free and agar encapsulated cells of yeast strain *Yarrowia lipolytica* as a result of citric acid production in repeated batch-shake-flask fermentation medium. They indicated that *Yarrowia lipolytica* and other acid producing yeasts could be successfully applied for rock other reports showed the ability of soil yeasts *Yarrowia lipolytica* and *Saccharomyces cervisiae* to solubilize inorganic phosphate compounds by production of organic acids, especially citric acid[11,12,23].

Data of the main effect of N levels (Table 3) show that increasing the N levels from 40 up to 80 kg feddan^{-1} had a significant ($p\leq 0.05$) influence on the fresh and dry weights of shoots and roots, N-uptake and grain yield. The optimum N level was 80 kg feddan^{-1}, which recorded the highest values in growth parameters and grain yield of wheat plant when compared with the other two N-fertilization levels (40 and 60 kg feddan^{-1}).

Interaction effect of microbial inoculation and N levels on wheat: The data presented in Table 4 show that the promoting effects at the different N levels, the highest on plant growth, N-uptake and grain yield were obtained from the dual inoculation with *A. chroococcum* plus yeast strain *C. sake*. But, the dual inoculation of wheat with *A. chroococcum*+yeast strain *C. sake* at the N level of 60 kg feddan^{-1} gave grain yield equal to that obtained at the N level of 80 kg feddan^{-1}. This mean that inoculation of wheat by *A. chroococcum*+yeast strain *C. sake* could save about 20 kg of N-fertilization feddan^{-1}. The benefits of the dual inoculation of wheat with *A. chroococcum*+yeast strain *C. sake* was also obvious at the N level of 40 kg feddan^{-1}, which gave higher uninoculated treatment at the N level 60 kg feddan^{-1}. These results indicate that wheat inoculation with *A. chroococcum*+yeast strains *C. sake* could save approximately 20 kg N-fertilizer feddan^{-1} at any N-fertilization

Table 3: Main effects of inoculation with *Azotobacter chroococcum* or plus yeast strain *Candida sake* on growth, N-uptake and yield of wheat plants at the N-fertilization levels

Treatments	Shoot (g plant^{-1}) Fresh	Shoot (g plant^{-1}) Dry	Root (g plant^{-1}) Fresh	Root (g plant^{-1}) Dry	N-uptake (mg plant^{-1})	Grain yield (t ha^{-1})
Level of N fertilization:						
40 kg N feddan^{-1}	51.50^{b}	16.07^{c}	4.35^{a}	1.69^{c}	0.549^{b}	8.00^{c}
60 kg N feddan^{-1}	59.20^{b}	17.58^{b}	4.69^{b}	1.84^{b}	0.552^{b}	8.47^{b}
80 kg N feddan^{-1}	62.83^{a}	19.20^{a}	5.76^{a}	2.24^{a}	0.671^{a}	9.07^{a}
Inoculation treatment						
Uninoculated	50.06^{c}	15.54^{c}	4.00^{c}	1.29^{c}	0.489^{b}	7.92^{c}
Azoto.	64.68^{b}	19.35^{b}	5.80^{b}	2.31^{b}	0.672^{ab}	8.64^{b}
Azoto.+ *C. sake*	70.03^{a}	21.13^{a}	7.05^{a}	2.81^{a}	0.752^{a}	9.15^{a}

The values in column followed by the same letter (s) are not significant by Duncan's multiple range test at 5% level

Table 4: Interaction effects of inoculation with *Azotobacter chroococcum* or plus yeast strain *Candida sake* on growth, N-uptake and yield of wheat plants at the N-fertilization levels

Treatments	Shoot (g plant^{-1})		Root (g plant^{-1})			
N level × inoculation	Fresh	Dry	Fresh	Dry	N-uptake (mg plant^{-1})	Grain yield (t ha^{-1})
40						
Uninoculated	51.50^{e}	15.54^{f}	4.00^{e}	1.29^{e}	0.489^{e}	8.92^{e}
Azoto.	58.62^{d}	17.58^{e}	4.51^{de}	1.80^{d}	0.545^{e}	8.08^{e}
Azoto.+*C. sake*	64.23^{c}	19.20^{c}	5.61^{cd}	2.23^{c}	0.619^{cd}	9.27^{bc}
60						
Uninoculated	59.20^{d}	16.44^{ef}	4.69^{de}	1.84^{d}	0.552^{de}	8.47^{d}
Azoto.	63.84^{c}	19.19^{c}	5.31^{de}	2.12^{c}	0.661^{c}	9.35^{bc}
Azoto.+*C. sake*	71.00^{b}	21.30^{b}	6.96^{bc}	2.77^{b}	0.760^{b}	10.14^{a}
80						
Uninoculated	62.83^{c}	18.10^{cd}	5.76^{cd}	2.24^{c}	0.671^{c}	9.35^{bc}
Azoto.	70.97^{b}	21.29^{b}	7.57^{ab}	3.02^{b}	0.810^{ab}	10.03^{a}
Azoto.+*C. sake*	76.30^{a}	22.87^{a}	8.60^{a}	3.44^{a}	0.878^{a}	10.15^{a}

The values in column followed by the same letter (s) are not significant by Duncan's multiple range test at 5% level

level used. Afifi *et al.*[25] reported that inoculation of maize with *Rhodotorula* and *Azotobacter* in the presence of half the recommended doses of NPK induced results for growth parameters matched those of the recommended doses of NPK. Such enhancing effect of the dual application of *Azotobacter*+yeast may be attributed to the associative action of microorganisms especially when choose and applied in right way. The applications of biofertilizers in agriculture are suggested as a sustainable way of increasing crop yields and economize their production as well[26]. Ahmed *et al.*[24] reported that use of chicken manure in combination with *Azotobacter chroococcum* and yeast (*Candida trpoicalis*) can meet the nutrient requirement of sustainable wheat production under desert soil conditions. Moreover, mixed inoculums, generally had more favorable effect on the majority of studied parameters than single inoculants. These results indicated that mixed inoculants promote greater beneficial effects than single strain inocula and this depends on selecting the most compatible efficient strains. Results from all these and other experiments suggest that mixed inoculation of microbial strains should be the future trends of biofertilizers application[27,28].

CONCLUSION

Dual inoculation of wheat with *Azotobacter chroococcum* and one of the following yeast strains (*Saccharomyces cervisiae*, *Candida sake*, *Saccharomyces exiguous*, *Pichia membranifaciens* or *Cryptococcus laurentii*) resulted in significant increases in shoot fresh and dry weights, root fresh and dry weights, except the yeast strain *Cryptococcus laurentii*. At the field condition, the most stimulative treatment on all of measured plant growth parameters, N-uptake and grain yield was that inoculated with *Azotobacter chroococcum*+*Candida sake*. Dual inoculation of wheat with *A. chroococcum*+yeast strain *C. sake* and fertilization at the N level of 60 kg feddan^{-1} is recommended for wheat fertilizer in Egypt since it save the highest grain yield and was equal to that obtained with dual inoculation+80 kg N feddan^{-1}. The variation in amount of improvements induced by the different yeast strains, point to the importance of selecting the most compatible efficient strain for inoculation with the *Azotobacter chroococcum* species.

SIGNIFICANCE STATEMENTS

Biofertilizers are the formulation of living microorganisms, which are able to fix atmospheric nitrogen and convert insoluble phosphorus to available one for the use of plants. Bio-fertilization is very safe for human, animal and environment to get lower pollution and save fertilization cost. In addition, their application in soil improves soil biota and minimizes the sole use of chemical fertilizers. One of these biofertilizers is non-symbiotic nitrogen fixing bacteria (*Azotobacter chroococcum*) also yeasts were found in the different soils and rhizosphere of various plants. In the recent years mixed inoculants are used for increasing the effective microbial strains used as biofertilizers, whereas many studies have shown that mixed inoculants containing mixture of bacterial species promote greater beneficial effects than single strain inocula. So, this investigation study the effect of inoculation with *A. chroococcum* or plus yeast strains on growth and yield of wheat plants and selecting the most compatible efficient yeast strain for inoculation with the *Azotobacter chroococcum*.

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REFERENCES

1. Dobereiner, J., 1997. Biological nitrogen fixation in the tropics: Social and economic contributions. Soil Biol. Biochem., 29: 771-774.
2. Hecht-Buchholz, C., 1998. The apoplast-habitat of endophytic dinitrogen-fixing bacteria and their significance for the nitrogen nutrition of nonleguminous plants. J. Plant Nutr. Soil Sci., 161: 509-520.
3. Kennedy, I.R. and Y.T. Tchan, 1992. Biological nitrogen fixation in non-leguminous field crops: Recent advances. Plant Soil, 141: 93-118.
4. Tawfik, M.M. and A.M. Gomaa, 2005. Effect of organic and biofertilizers on the growth and yield of wheat plants. Egypt. J. Agric. Res., 2: 711-725.
5. Abbasdokht, H., 2008. The study of *Azotobacter chroococcum* inoculation on yield and post harvest quality of wheat. Proceedings of the International Meeting on Soil Fertility Land Management and Agroclimatology, October 29-November 1, 2008, Kusadasi, Turkey, pp: 885-889.
6. Badr, E.A., O.M. Ibrahim and M.F. El-Kramany, 2009. Interaction effect of biological and organic fertilizers on yield and yield components of two wheat cultivars. Egypt. J. Agron., 31: 17-27.
7. Bahrani, A., J. Pourreza and M.H. Joo, 2010. Response of winter wheat to co-inoculation with azotobacter and Arbescular Mycorrhizal Fungi (AMF) under different sources of nitrogen fertilizer. Am. Eurasian J. Agric. Environ. Sci., 8: 95-103.
8. Rosa, C.A., P.B. Morais, S.R. Santos, P.R. Peres-Neto, L.C. Mendonca-Hagler and A.N. Hagler, 1995. Yeast communities associated with different plant resources in sandy coastal plains of Southeastern Brazil. Mycol. Res., 99: 1047-1054.
9. Ganter, P.F., 2006. Yeast and Invertebrate Associations. In: Biodiversity and Ecophysiology of Yeasts, Rosa, C.A. and G. Peter (Eds.). Springer, New York, ISBN: 9783540309857, pp: 303-370.
10. Kanti, A. and I.M. Sudiana, 2002. Diversity and ecological perspective of soil yeast in Gunung Halimun national park. Berita Biologi, 6: 25-32.
11. Vassileva, M., R. Azcon, J.M. Barea and N. Vassilev, 2000. Rock phosphate solubilization by free and encapsulated cells of *Yarowia lipolytica*. Process Biochem., 35: 693-697.
12. Hesham, A.E. and M.M. Hashem, 2011. Molecular genetic identification of yeast strains isolated from Egyptian soils for solubilization of inorganic phosphates and growth promotion of corn plants. J. Microbiol. Biotechnol., 21: 55-61.
13. Sampedro, I., E. Aranda, J.M. Scervino, S. Fracchia, I. Garcia-Romera, J.A. Ocampo and S. Godeas, 2004. Improvement by soil yeasts of arbuscular mycorrhizal symbiosis of soybean (*Glycine max*) colonized by *Glomus mosseae*. Mycorrhiza, 14: 229-234.
14. Boby, V.U., A.N. Balakrishna and D.J. Bagyaraj, 2007. Effect of combined inoculation of an AM fungus with soil yeasts on growth and nutrition of cowpea in sterilized soil. World J. Agric. Sci., 3: 423-429.
15. De Freitas, J.R., 2000. Yield and N assimilation of winter wheat (*Triticum aestivum* L., var. Norstar) inoculated with rhizobacteria. Pedobiologia, 44: 97-104.
16. Goddard, V.J., M.J. Bailey, P. Darrah, A.K. Lilley and I.P. Thompson, 2001. Monitoring temporal and spatial variation in rhizosphere bacterial population diversity: A community approach for the improved selection of rhizosphere competent bacteria. Plant Soil, 232: 181-193.
17. Pierson, E.A. and D.M. Weller, 1994. Use of mixtures of fluorescent pseudomonads to suppress take-all and improve the growth of wheat. Phytopathology, 84: 940-947.
18. Abo-Elyousr, K.A.M. and H.M. Mohamed, 2009. Note biological control of fusarium wilt in tomato by plant growth-promoting yeasts and rhizobacteria. Plant Pathol. J., 25: 199-204.
19. Mohamed, H.M. and A.K. Metwally, 2014. Effect of combined inoculation of *Rhizobium* with soil yeasts on nodulation, growth and yield of common bean (*Phaseolus vulgaris* L.) under field condition. Am. J. Plant Nutr. Fertil. Technol., 4: 1-10.
20. Bremner, J.M. and G.S. Mulvany, 1982. Nitrogen Total. In: Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties, Page, A.L. (Ed.). American Society of Agronomy, Madison, WI., USA., pp: 595-622.
21. StatSoft., 1995. Statistica for Windows (Computer Program Manual). StatSoft Inc., Tulsa, OK.
22. Reed, G. and T.W. Nagodawithana, 1991. Yeast Technology. 2nd Edn., Van Nostrand Reinhold, New York.
23. Nassar, A.H., K.A. El-Tarabily and K. Sivasithamparam, 2005. Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea mays* L.) roots. Biol. Fertil. Soils, 42: 97-108.
24. Ahmed, M.A., A.G. Ahmed, M.H. Mohamed and M.M. Tawfik, 2011. Integrated effect of organic and biofertilizers on wheat productivity in new reclaimed sandy soil. Res. J. Agric. Biol. Sci., 7: 105-114.
25. Afifi, M.H., F.M. Manal and A.M. Gomaa, 2003. Efficiency of applying biofertilizers to maize crop under different levels of mineral fertilizers. Annal. Agric. Sci., 41: 1411-1420.
26. Sharma, A.K., 2003. Biofertilizers for Sustainable Agriculture. Agrobios, India, Pages: 407.
27. Subashini, H.D., S. Malarvannan and P. Kumaran, 2007. Effect of biofertilizers (N-fixers) on the yield of rice varieties at Puducherry, India. Asian J. Agric. Res., 1: 146-150.
28. Rajankar, P.N., D.H. Tambekar and S.R. Wate, 2007. Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of Purna river basin. Res. J. Agric. Biol. Sci., 3: 701-703.