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Impact of Integration between Phosphate-Solubilizing Microorganisms and Yeast Extract on Wheat (*Triticum aestivum* L.) Productivity

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Combination between plant growth promoting rhizobacteria; *Pseudomonas fluorescens* and mycorrhizal fungi AM (*Glomus mosseae*) were applied to the rock-phosphate amended soil in two field experiments during 2013/2014 and 2014/2015 winter seasons. Interaction effects between those biofertilizers and foliar spraying with *Saccharomyces cerevisiae* extract on some microbial activities, growth characteristics as well as yield component of wheat were studied. Significant positive effects were obtained of phosphatase and available phosphorous content in rhizosphere of wheat after inoculation with *Ps. fluorescens*. Also, dual inoculation of *Ps. fluorescens* with AM in presence of rock phosphate gave maximum values of phosphatase, mycorrhizal infection percentage and available P in wheat rhizosphere in both seasons especially when foliar spraying with yeast extract was applied. Meanwhile, application of both dual inoculants either alone or with yeast extract spraying led to considerable improvement in growth characteristics, photosynthetic pigments as well as biochemical composition of wheat plants when compared with untreated ones. Also, dual inoculation with *Ps. fluorescens* and AM increased gibberellins, auxins and cytockinins content either in the presence or absence of yeast spraying. Similar positive trend was observed in yield and yield component with good quality of chemical composition of wheat grains especially when dual inoculation combined with yeast foliar spraying were applied. The obtained results confirm the positive influence of co-inoculation with phosphate-solubilizing microorganisms in presence of rock phosphate as an active tool to improve wheat yield, also, support the role of *Saccharomyces cerevisiae* extract for enhancing biofertilization performance.

Key words: Biofertilizers, mycorrhizae, *Ps. fluorescens*, yeast, rock phosphate, wheat, chlorophyll, endogenous hormones.

Wheat enjoys a privileged position amongst food grain crops in the world in general and particularly in Egypt where it serves as a staple food for the majority of the population. Hence, under the prevailing circumstances, restoration and maintenance of soil fertility is a basic and critical problem, particularly in the newly reclaimed soil. This can be accomplished by adding organic material, biological active substances and plant growth-promoting microorganisms, in addition to other field practices[7]**.** biofertilizers contain a variety of beneficial microorganisms and enzymes which accelerate and improve plant growth and protect plants from pests and diseases. Completely fermented organic matters, resulted in biofertilizers

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improve the physical properties of soils; enrich air aeration, water and nutrients retention capacity. Biofertilizers provide the cultivated plants with the macro as well as micronutrients, required for healthy growth therefore, improve yield and quality of agricultural crops, and reduce the overall cost of chemical fertilizers as well pesticides application, based on yield[25]. Clearly, there is an urgent need for sustainable agricultural practices on a global level. To overcome the ecological problems resulting from the loss of plant nutrients and to increase crop yield, microorganisms that allow more efficient nutrients use or increase nutrients availability can provide sustainable solutions for present and future agricultural practices[24]. An alternative approach for using of phosphate-solubilizers as a microbial inoculants is the use mixed cultures or co-inoculation with other microorganisms. On the other hand, it has been postulated that some phosphate solubilizing bacteria behave as mycorrhizal helper bacteria. Similarly, bacteria and their growth or activities are affected by fungi and their exudates in rhizosphere[22]. A promising trend for increasing the efficiency of biofertilizers was studied by using different mixture of nitrogen fixing bacteria, phosphate solubilizers and potassium solubilizer[30] found that application of triple inoculants not only increased nutritional assimilation of plant, but also improved soil properties. This was observed that half the amount of biofertilizers application had similar effects when compared with organic fertilizer or chemical fertilizer treatments. In Egypt, however, there is a dire need to make availabilities of co-inoculation with biofertilizers and transfer the technology to farmers. Many studies have indicated that yeast is one of the richest sources of high quality protein, especially the essential amino acids, the essential minerals and trace elements such as Ca, Co, Fe. Also, yeast extract is the best source of the B-complex vitamins, amino acids furthermore, bio-constituents especially, cytokinins[20]. Also, yeast extracts improved all vegetative growth parameters, flowering, total yield and quality of plants.

This study aimed to evaluate application of plant growth promoting microorganisms with either endomycorrhizal fungi or P-solubilizing bacteria in presence of rock phosphate and their interaction effects with foliar application with yeast extract on growth, yield and yield components of wheat.

MATERIALS AND METHODS

Two field experiments were conducted on newly soil cultivated with wheat (*Triticum aestivum* L. c.v. Sakha 93) at El-Bostan region, El-Behera Governorate, Egypt during winter seasons of 2013/2014 and 2014/2015. Interaction effects between *Saccharomyces cerevisiae* extract and endomycorrhizal fungi (*Glomus mosseae*) individually or in combined with *Pseudomonas fluorescens* on growth and yield of wheat were studied. Some physical and chemical properties of the experimental soil were estimated according to[9,18] respectively as following (A):

Mycorrhizal inoculation

Arbuscular mycorrhizal fungus (*Glomus mosseae*) was obtained as spores from biofertilization unit, Fac. Agric. Ain Shams Univ, Egypt. Micorrhizal inoculum consisted of root, hyphae, spores and growth media from a pot culture of onion plants which was previously infected with *Glomus mosseae* and grown for 4 months in pot culture. The standard inoculum (400 kg/fed.) contained about 270 spores/g. Spores of the fungus were measured by a wet-sieving and decanting technique[14].

Bacterial inoculum

Pseudomonas fluorescens (pure local strains that exhibited high inorganic phosphate solubilization) were isolated and identified by[23] Botany Dept., microbiology branch, Fac. Agric. Benha Univ., Egypt. The selected bacterial strains were propagated in nutrient broth medium and incubated on a rotary shaker (180 rpm) at 30ÚC for 5 days. The obtained suspension (about 10^8 c.f.u./ml) were mixed with sterilized peat moss at the rate of 2:1 (V/W) under aseptic conditions. Arabic Gum (16%) was applied to the grains as an adhesive agent before mixing with peat inoculants. Each inoculum was used at a rate of 400 g/fed and thoroughly mixed with the grains, the coated seeds were left to air-drying in shade, then the seed became ready for sowing[6]

***Saccharomyces cerevisiae* extract**

Local isolate of *S. cerevisiae* was obtained from biofertilization unit, Fac. Agric. Ain Shams Univ., Egypt. *Saccharomyces* extract was

prepared using a technique described by[4]. A technique allowed yeast cells (*Saccharomyces cerevisiae*) to be grown and multiplied efficiently on YEMEG medium was used for 5 days on a shaker at 30˚C to produce beneficial bioconstituents, hence allowed such constituents to release out of yeast cells[27]. Two cycles of freezing and thawing for disruption of yeast cells and releasing their content were done. Chemical analysis of *Saccharomyces* extract stock solution was as following (B)

Experimental design

Grains of wheat (Sakha 93) were successfully washed with water and air-dried. Then, grains were soaked in cell suspension of *Pseudomonas fluorescens*. In uninoculated treatments, grains were treated with uninoculated media. In addition, over-head soil technique was carried out using freshly prepared suspensions which spread on soil surface adjacent to the seedlings at a rate of 1L of the inoculum (containing 10^8 cfu/ml) per each plot. This application was added three times during the growth period (20, 40 and 60 days after sowing. The grains were sown on the 15th and 17th of November in the two growing seasons, respectively. Foliar spray with *Saccharomyces cerevisiae* extract was applied in three times by 20 days intervals (20, 40 and 60 days after sowing) using 200 ml/L of yeast extract per each plot. The untreated plants were sprayed with tap water. The experiments were arranged in randomized complete block design with three replicates. The plot area was 10.5 m^2 (3x 3.5m). All plots received nitrogen fertilizers at the rate of 200 kg/fed urea (46 % N) in two equal doses (before the first and second irrigation). Potassium sulphate (48 % K_2O) was added before cultivation in both seasons at the rate of 100 kg/ fed. Calcium super phosphate (15.5% P_2O_5) was added to the treatments without rock phosphate at the rate of 150 kg/fed. While, rock phosphate was added with the same dose. Mycorrhiza was added just before sowing. The other required culture practices for growing wheat were followed as recommended.

Microbial activities

Microbial activities of the plants rhizosphere after 45 days from sowing were conducted. Mycorrhizal infection was microscopically estimated on a sample of fresh root as described by[15] after clearing and staining[28]. The samples were analyzed for phosphatase activity by the method given by[11]. Rhizosphere samples were analyzed for available phosphorus according to[2].

Sampling and collecting data

Nine plants of wheat from each treatment were randomly taken at 80 days after sowing to measure different morphological characteristics (plant height (cm), number of tillers/ plant, leaves dry weight (g/plant) and total leaf area (cm^2/plant).

Photosynthetic pigments

Chlorophyll a, b and carotenoids were colorimetrically determined in fresh leaves of wheat plants at 80 days after sowing during the two seasons according to the methods described by[29] and calculated as mg/g fresh weight.

Chemical composition

Samples from wheat leaves at 80 days after sowing and grains at harvest were taken to determine total nitrogen, phosphorus and potassium[1]. Also NPK uptake was calculated after determination of NPK according to[10]. Total carbohydrate was determined according to[12]. Crude protein was calculated according to the following equation: Crude protein= Total nitrogen x 5.75[1].

Endogenous phytohormones

Endogenous phytohormones were quantitatively determined in wheat shoots at 80 days after sowing in the second season using High-Performance Liquid Chromatography (HPLC) according to[19] for auxin (IAA), gibberellic acid (GA_3) and abscisic acid (ABA) while, cytokinins were determined according to[21].

Yield characteristics

At harvest, three plants were randomly taken /plot from each treatment for estimation of number of spikes/plant, grain yield (g)/plant, straw yield (g)/plant and weight of 1000 grains (g).

Statistical analysis

Data obtained in this study were statistically analyzed by using the least significant differences test (L.S.D) according to[26].

RESULTS AND DISCUSSION

Growth parameters

As shown in Table (1), the growth parameters of wheat plants as plant height, number of tillers/plant, dry weight of leaf/ plant and total leaf area/plant were significantly increased by

individual application of *Saccharomyces cerevisiae* extract and biofertilizers. Inoculation with *Ps. fluorescens* in the presence of AM significantly increased number of tillers and total leaf area/plant during the two seasons. In this regard,[23] reported that *Ps. fluorescens* possess a great variety of properties that are interest in the development of biofertilizers including production of growth promoting plant hormones (especially auxins, gibberellins and cytokinins) as well as N_2-fixation. Maximum stimulatory effect of the biofertilizers was obtained when they associated with *Saccharomyces cerevisiae* extract application in the two seasons. These results are in agreement with[31] who reported that yeast extract application positively affected the plant growth parameters. Moreover, yeast extract is a natural source of many growth substances (thiamine, riboflavin, niacin, pyridoxine and vitamins B_1. B_2, B_{12}), cytokinins and many of the nutrient elements as well as organic compounds i.e., protein, carbohydrates, nucleic acids and lipids[17] On the other hand, when the treatments that received rock phosphate treated with *Ps. fluorescens* and AM gave the highiest values of growth parameters[8] reported that certain microorganisms are capable of solubilizing rock phosphate and are collectively termed phosphate-solubilizing microorganisms (PSM). Rhizobacteria, from the genera *Pseudomonas* and *Bacillus* are among the most powerful phosphate solubilizing bacteria.

Mycorrhizal colonization and soil enzymes

Results of mycorrhizal colonization percent shown in Table (2) exhibited a gradual increase with inoculation by AM fungi, while it showed no significant increase with individual application of *Ps. fluorescens* comparing to the control treatment. Mycorrhizal root infection, phosphatase activity and available phosphorous were significantly increased by application of of *Ps. fluorescens* in combination with AM fungi. The results were in agreement with those obtained by[16] who reported that organic compounds significantly increased colonization of mycorrhiza. It was also noticed from Table (2) that individual application of yeast extract or biofertilization with AM or *Ps. fluorescens* significantly increased phosphatase activity in wheat rhizosphere as compared to the control treatment. The combined inoculation with *Ps. fluorescens* and AM increased enzymes activity more than the individual inoculation. Also, the highest values of enzymes activity were recorded in rhizosphere of the plants that treated with yeast extract in the presence of biofertilizer especially the dual inoculation. This may be due to the mechanisms of *Ps. fluorescens* and AM on soil

Chemical properties of the experimental soil (A)

Particle size distribution %				Soil chemical properties			
Sand	Silt	Clay	Texture class	pH	$CaCo_3$ %	OM %	EC (ds/m^{-1})
65.25	10.21	24.54	Sandy clay loam	8.16	14.27	0.97	1.43

Soluble cations and anions m mol/L							Available nutrients (ppm)						
Ca^{++}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	N	P	K	Fe	Mn	Zn	Cu
7.92	4.25	9.19	0.63	0.00	2.96	11.81	25.8	3.1	13.7	2.1	0.62	0.39	0.36

Chemical analysis of *Saccharomyces* extract stock solution (B)

Amino acidmg D 100g dry weight				Vitamins and Carbohydratesmg D 100g dry weight			
Arginine	1.99	Threonine	2.09	Vit.B1	2.23	Biotin	0.09
Histidine	2.63	Tryptophan	0.45	Vit.B2	1.33	Nicotinic acid	39.88
Isoleucine	2.31	Valine	2.19	Vit.B6	1.25	Pantothenic acid	19.56
Leucine	3.09	Glutamic acid	2.00	Vit B12	0.15	P amino benzoic acid	9.23
Lysine	2.95	Serine	1.59	Thiamin	2.71	Folic acid	4.36
Methionine	0.72	Aspartic acid	1.33	Riboflavin	4.96	Pyridoxine	2.90
Phenyl alanine	2.01	Cysteine	0.23	Inositol	0.26	Total carbohydrates	23.2

Table 1. Growth characteristics of wheat as affected by phosphate solubilizing microbes and foliar application with yeast during two seasons.

Treatments	Plant height cm/ plant		No. of tillers /plant(g)		D.W. of leaf / plantcm^2		Total leaf area / plant		Plant height cm/ plant		No. of tillers /plant(g)/		D.W. of leaf plant cm^2		Total leaf area / plant	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
-Control	84.7	85.4	5.3	5.1	5.43	5.16	1189.5	1143.2	87.5	88.4	6.4	6.6	7.28	7.28	1364.7	1313.9
-Rock phosphate (R)	81.4	79.3	4.6	4.5	4.68	4.36	1121.4	1064.3	83.7	85.2	5.7	5.6	6.31	6.31	1287.4	1269.5
-*Ps. Fluorescens*	86.2	87.5	5.5	5.4	5.87	5.68	1236.9	1213.4	89.7	89.8	6.9	7	8.25	8.25	1461.9	1546.8
-AM	90.5	88.9	5.6	5.8	6.12	6.52	1322.4	1338.6	91.6	92.7	7.3	7.4	9.15	9.15	1621.4	1749.1
-*Ps. fluorescens* + AM	90.8	91.6	5.7	5.9	6.28	6.74	1302.2	1359.4	94.3	93.8	7.8	8	10.34	10.34	1832.3	1905.6
-AM + R	91.3	92.1	5.8	6	6.43	6.88	1320.8	1399.3	95.2	96.3	8.1	8.3	10.76	10.76	1928.5	1954.8
-*Ps. fluorescens* + R	92.7	93.8	6.1	6.3	7.18	7.56	1406.9	1463.4	97.6	98.4	8.5	8.7	11.64	11.64	1876.2	1907.5
-*Ps. fluorescens* +AM+R	94.8	96.2	6.2	6.4	7.65	7.74	1542.5	1486.8	99.1	99.7	9.9	9.8	13.11	13.11	2218.4	2114.7
LSD at 5%	2.23	2.41	0.41	0.34	0.51	0.56	107.6	104.3	2.15	2.13	0.45	0.52	0.54	0.54	110.5	112.4

Table 2. Phosphatase, mycorrhizal infection percentage and soil-P content as affected by phosphate solubilizing microbes and foliar application with yeast during two seasons

Treatments	Without yeast extract						With yeast extract					
	Phosphatase activity as µg P/g dry soil		Mycorrhizal infection %		P ppm		Phosphatase activity as µg		Mycorrhizal infection % P/g dry soil		P ppm	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
-Control	34.2	30.5	14.2	11	5.22	4.48	37.4	39.6	19.4	18.6	4.9	4.88
-Rock phosphate (R)	40.8	36.2	12.2	16.5	6.53	6.71	47.9	48.4	22.5	24	6.8	6.82
-*Ps. fluorescens*	50.4	50.8	35.4	38.9	7.45	7.26	56.2	58.6	32.2	34.3	8.56	8.24
-AM	51.6	51.2	60.8	61.2	7.8	7.7	61	60.7	73.1	75.4	8.86	8.76
-*Ps. fluorescens* + AM	52.4	51.9	65.2	66.3	8.12	7.84	71.4	71.6	78.5	76.8	9.23	8.94
-AM + R	50.7	50.9	61.3	62.8	8.14	7.68	72.8	72.5	85.3	82.8	8.24	7.75
-*Ps. fluorescens* + R	51.3	51	30.2	31.9	8.72	8.63	78.8	87.2	35.5	36.9	8.76	8.33
-*Ps. fluorescens* +AM+R	55.7	58.4	70.5	71.3	8.87	8.95	84.3	95.6	93.5	94.1	9.89	9.70
LSD at 5%	3.6	3.9	6.1	6.3	0.7	1.1	3.7	3.8	6.5	6.7	0.9	1.11

Table 3. Photosynthetic pigments as affected by phosphate solubilizing microbes and foliar application with yeast during two seasons

Treatments	Without yeast extract								With yeast extract							
	Chlorophyll a mg/g F.W		Chlorophyll b mg/g F.W		Carotenoids mg/g F.W		Total pigments mg/g F.W		Chlorophyll a mg/g F.W		Chlorophyll b mg/g F.W		Carotenoids mg/g F.W		Total pigments mg/g F.W	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
-Control	0.49	0.5	0.31	0.32	0.38	0.39	1.18	1.21	0.63	0.66	0.42	0.46	0.5	0.52	1.55	1.64
-Rock phosphate (R)	0.46	0.44	0.29	0.27	0.32	0.3	1.07	1.01	0.52	0.55	0.36	0.39	0.41	0.44	1.29	1.38
-*Ps. fluorescens*	0.52	0.55	0.33	0.35	0.41	0.42	1.26	1.32	0.68	0.71	0.51	0.53	0.56	0.58	1.75	1.82
-AM	0.56	0.58	0.36	0.38	0.47	0.48	1.39	1.44	0.72	0.75	0.57	0.59	0.62	0.66	1.91	2
-*Ps. fluorescens* + AM	0.55	0.6	0.34	0.37	0.49	0.46	1.38	1.43	0.77	0.8	0.62	0.61	0.69	0.7	2.08	2.11
-AM + R	0.62	0.64	0.47	0.48	0.53	0.54	1.62	1.66	0.82	0.83	0.63	0.65	0.73	0.75	2.18	2.23
-*Ps. fluorescens* + R	0.66	0.68	0.51	0.54	0.57	0.58	1.74	1.8	0.86	0.87	0.67	0.68	0.72	0.73	2.25	2.28
-*Ps. fluorescens* +AM+R	0.73	0.78	0.56	0.59	0.63	0.65	1.92	2.02	0.94	0.96	0.71	0.73	0.78	0.79	2.43	2.48
LSD at 5%	0.2	0.22	0.17	0.19	0.18	0.16	0.28	0.26	0.26	0.24	0.19	0.21	0.22	0.23	0.29	0.27

Table 4. Nitrogen, phosphorous, potassium uptake and total carbohydrates in wheat leaves as affected by phosphate solubilizing microbes and foliar application with yeast during two seasons

Treatments	Without yeast extract								With yeast extract							
	N-uptake		P-uptake		K-uptake		Total carbohydrates mg/g D.W		N-uptake		P-uptake		K-uptake		Total carbohydrates mg/g D.W	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
-Control	123.5	125.4	17.7	18.7	128.5	130.4	421.9	428.7	145.6	147.2	28.6	29.8	186.4	191.5	438.7	440.3
-Rock phosphate (R)	93.8	95.7	17.5	19.4	112.7	115.2	415.8	410.3	112.7	120.8	28.5	28.3	140.4	143.2	431.7	437.4
-*Ps. fluorescens*	127.4	132.2	18.9	21.5	134.7	137.6	422.4	431.8	154.8	156.5	30.3	31.4	198.3	197.6	450.9	458.2
-AM	142.3	146.5	24.4	25.2	120.4	122.5	438.3	442.7	160.4	163.6	32.7	33.5	167.5	175.3	455.3	453.8
-*Ps. fluorescens* + AM	148.8	150.2	26.8	27.5	126.9	130.7	447.5	450.4	168.5	270.7	34.8	34.2	176.6	178.4	458.8	456.4
-AM + R	152.6	155.7	28.3	28.9	145.7	148.5	452.8	457.3	171.2	175.4	38.5	38.6	218.3	221.2	469.7	464.2
-*Ps. fluorescens* + R	158.2	163.4	29.4	30.2	155.9	160.3	460.5	462.6	177.6	181.5	42.2	42.7	226.4	230.5	478.3	474.4
-*Ps. fluorescens* +AM+R	166.9	169	32.8	33.5	169.4	173.9	464.7	470.8	183.4	185.7	44.6	44.8	253.9	257.7	486.7	493.5
LSD at 5%	18.8	21.4	5.6	7.3	23.7	25.6	31.4	35.7	23.7	26.4	7.3	8.5	21.4	18.7	37.5	39.7

Table 5. Endogenous phytohormones in wheat shoots as affected by phosphate solubilizing microbes and foliar application with yeast during second season

Treatments	Without yeast extract				With yeast extract			
	Auxins µg/g fresh t weigh	Gibberellins µg/g fresh weight	Cytokinins µg/g fresh weight	Abscisic acid (ABA) µg/g fresh weight	Auxins µg/g fresh weight	Gibberellins µg/g fresh weight	Cytokinins µg/g fresh weight	Abscisic acid (ABA) µg/g fresh weight
-Control	41.18	55.35	33.7	1.65	52.14	61.75	43.57	1.63
-Rock phosphate (R)	37.29	52.26	29.34	2.85	40.67	54.64	32.75	2.15
-*Ps. Fluorescens*	45.7	56.48	38.96	1.62	57.45	63.95	46.29	1.37
-AM	48.63	58.15	40.42	1.57	63.24	65.36	52.86	1.35
-*Ps. fluorescens* + AM	51.34	60.32	44.56	1.55	70.66	66.82	59.39	1.28
-AM + R	56.78	64.83	46.43	1.48	74.15	67.37	62.17	1.17
-*Ps. fluorescens* + R	68.57	65.76	50.32	1.2	87.52	69.24	66.11	1.15
-*Ps. fluorescens* +AM+R	75.14	69.54	54.67	1.12	98.54	73.6	73.27	1.05

Table 6. Yield components of wheat as affected by phosphate solubilizing microbes and foliar application with yeast during two seasons

Treatments	Without yeast extract								With yeast extract							
	No. of spikes / plant		Weight of 1000 grains		Grain yield g/plant		Straw yield g/plant		No. of spikes / plant		Weight of 1000 grains		Grain yield g/plant		Straw yield g/plant	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
-Control	6.49	6.27	49.64	48.24	7.15	7.32	10.17	10.12	6.91	6.9	51.2	51.24	10.55	10.62	10.3	10.35
-Rock phosphate (R)	6.18	6.13	44.23	44.18	6.54	6.48	10.28	10.32	6.48	6.53	55.43	56.58	11.84	11.98	10.78	10.62
-*Ps. fluorescens*	6.44	6.6	51.78	51.7	8.93	8.84	10.43	10.28	6.96	6.92	58.68	58.8	12.63	12.74	10.83	10.94
-AM	7.23	7.14	52.34	52.48	9.32	9.18	10.63	10.56	7.45	7.53	59.14	59.2	13.4	13.48	11.73	11.86
-*Ps. fluorescens* + AM	7.62	7.53	53.43	53.12	9.74	9.63	10.88	10.75	7.74	7.78	62.53	63.9	13.64	13.6	12.9	12.95
-AM + R	7.8	7.75	55.17	54.28	10.26	10.15	11.38	11.4	7.86	7.89	61.37	61.29	14.16	14.1	13.5	13.55
-*Ps. fluorescens* + R	7.92	7.87	56.26	55.69	10.93	10.85	11.52	11.47	8.13	8.22	61.76	62.59	14.9	14.75	13.62	13.77
-*Ps. fluorescens* +AM+R	8.76	8.63	57.75	58.47	11.63	11.55	11.63	11.66	9.28	9.34	64.54	64.97	15.8	15.85	13.83	13.96
LSD at 5%	0.34	0.36	1.86	1.83	0.48	0.42	0.67	0.62	0.37	0.39	1.88	1.86	0.5	0.52	0.69	0.64

properties. Addition of yeast extract may be of special importance in restoring optimal levels or organic matter for plant growth and for microbial activity, which associated with enzymes activity[31]. These results showed a good agreement with[3] who reported an increase in enzymes activity with application of yeast extract. Also, the microbial population and soil enzymes in the rhizosphere could be built up for the efficient utilization of nutrients.

Photosynthetic pigments

Data in Table (3) indicated that different photosynthetic pigments i.e., chlorophyll a, b and carotenoids in wheat leaves were positively responded to application of yeast extract, biofertilizers and their combinations during the two seasons. Moreover, the interaction between yeast extract and dual inoculation with *Ps. fluorescens* and AM gave the highest values of total pigments during the two seasons as compared with individual treatments and control plants. Generally, these results are to be considered as a good explanation to the obtained data regarding the favorable role of biofertilizers and yeast extract on growth parameters (Table 1) that enhanced photosynthetic efficiency and increased dry matter accumulation[13] found that application of *Ps. fluorescens* improved chlorophyll a, b and charotenoids content of wheat leaves.

Nutrients uptake and some bioconstituents in leaves

Table (4) clearly indicates that application of both yeast extract and biofertilizers significantly increased NPK uptake and total carbohydrates in wheat leaves at 80 days after sowing during the two seasons as compared with control treatment. Moreover, combination between yeast extract and dual inoculation with *Ps. fluorescens* and AM increased NPK uptake compared with control treatment especially when plants received rock phosphate instead of super phosphate. Furthermore, the addition of yeast extract associated with both biofertilizers increased nutrients uptake with an pronounced effect, and parallel trend for their increases in the soil (Table 2). This may be attributed to the enhancing effect of mycorrhiza on soil physical properties to release nutrients in the rhizosphere, which supply a power of available nutrients to plants. The obtained data were in agreement with[16]. In addition[8] reported that mycorrhizal fungi have also been shown to increase P uptake. Regarding total carbohydrate, the same positive trend was observed with application of yeast extract and biofertilizers. All treatments showed a significant increase and the maximum one obtained by the interaction between *Ps. fluorescens* and AM in the presence of yeast extract. In this respect, high content of total carbohydrates is a direct result for high rates of photosynthesis with great efficiency that was preceded with large photosynthetic area (Table 1) and high content of photosynthetic pigments (Table 3). The present results are in agreement with those of[13].

Table 7. Chemical composition of wheat grains as affected by phosphate solubilizing microbes and foliar application with yeast during two seasons

Treatments	Without yeast extract				With yeast extract			
	Total carbohydrates mg/g D.W		Crude protein mg/g D.W		Total carbohydrates mg/g D.W		Crude protein mg/g D.W	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
-Control	723.8	726.9	111	112.7	736.3	733.5	114.9	116.3
-Rock phosphate (R)	712.5	718.7	98.9	100.6	728.4	732.1	105.9	107.3
-*Ps. fluorescens*	735.4	737.5	116.2	117.3	747.6	743.8	118.9	121.6
-AM	738.7	740.2	119	119.6	750.4	752.5	122.1	122.8
-*Ps. fluorescens* + AM	742.8	746.4	120.2	121.3	758.4	760.3	123.4	123.9
-AM + R	753.2	755.3	122.5	121.9	768.5	767.6	124	124.6
-*Ps. fluorescens* + R	757.4	762.2	123.1	123.6	777.4	770.8	125.1	125.7
-*Ps. fluorescens* +AM+R	769.5	773.6	124.2	124.8	785.2	792.6	126.3	126.8
LSD at 5%	15.35	17.37	9.32	7.54	17.42	16.33	9.45	8.74

Endogenous phytohormones

According to the data in Table (5), *Ps. fluorescens* gave maximum values of auxins in wheat shoots compared with all treatments, but inoculation of these bacteria with yeast extract or yeast extract with AM led to a decrease in auxins content compared to control. Gibberellins and cytokinins were improved by inoculation with *Ps. fluorescens* or AM in presence of rock phosphate and reached the highest values when the biofertilizers were supported by yeast extract. Many investigators reported the role of plant growth promoting rhizobacteria such as *Ps. fluorescens* in the production of hormones such as gibberellins, auxins and cytokinins[23]. On the other hand, abscisic acid, as growth inhibitor, was decreased with using AM or yeast extract application while dual inoculation with AM and *Ps. fluorescens* in the presence of rock phosphate recorded maximum reduction of abscisic acid content in wheat shoots.

Yield and its components

Data in Tables (6 & 7) showed that, number of spikes, grain yield, weight of thousand grains and straw yield of wheat as well as chemical composition of wheat grains significantly increased in response to any of the tested biofertilizer in presence of rock phosphate compared to control. Also, yeast extract had positive effect on the same parameters. Moreover, yeast extract application triggered and increased the positive effects of *Ps. fluorescens* and AM inoculation when wheat plants were inoculated with both biofertilizers in the presence of rock phosphate. The stimulatory effect of yeast extract with dual inoculation on wheat yield would be expected since these applications promoted growth parameters (Table 1), microbial activities (Table 2), increased photosynthetic pigments (Table 3), increased nutrients uptake and total carbohydrates (Table 4) as well as endogenous phytohormones (Table 5) as previously resulted and discussed in this work. These findings are supported by[5,7,31]. They reported that the combined application of rhizobacteria and mycorrhiza increased plant yield. This study clearly indicated that yeast extract could have positive effect on plant growth and yield by acting as soil enhancer and as well as by improving its physical properties. Also, the combined application of yeast extract with the potent biofertilizers is a good tool for growth and yield promotion as well as improving soil health, particularly in newly soil. Moreover, the application of rock phosphate as a P fertilizer has the potential to become widespread once the bioavailability of P that improved crop yields and plant tissue P content. Microbial induced rock phosphate solubilization represent a solution for making P more plant-available.

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