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RESPONSE OF BARLEY GROWTH, YIELD AND MICROBIAL POPULATIONS IN THE RHIZOSPHERE TO SOIL APPLICATION OF YEAST AND FOLIAR SPRAY OF CITRIC AND ASCORBIC ACIDS.

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ABSTRACT

The effect of soil application of yeast (*Saccharomycec cerevicea*) each separately or in combination with foliar spray of either citric or ascorbic acid on some growth characters, yield, and yield components as well as on the microbial populations in the rhizosphere of barley plants was investigated in a field experiment.

Among all treatments, plants fertilized with recommended doses of N fertilizer scored the highest grain yield, straw yield, spike length, weight of grains per spike, 1000-grains weight and N-content in straw and grains.

Heavy inoculation of soil with yeast separately, or in combination with foliar spray of citric acid and\or ascorbic acid gave higher values in plant height, grain yield, number of grains per spike, 1000-grains weight compared to those fertilized with half dose of N-fertilizer only.

When yeast was applied as a soil inoculant combined with foliar spray of ascorbic acid, their effect on plant height, straw and grain yields, N content in straw and grains was higher than those inoculated with yeast along with foliar spray of citric acid.

The total numbers of bacteria, fungi and actinomycetes were increased by soil application of yeast separately or in presence of foliar spray of citric acid and\or ascorbic acid as compared to the plants fertilized with either recommended or half dose of N fertilizer especially at tillering stage. When yeast was combined with foliar spray of ascorbic acid, their combination gave the highest number of bacteria compared to the other tested treatments. As usual the lowest number of bacteria, fungi and actinomycetes was recorded in plants fertilized with ½ dose of the recommended N fertilizer.

Generally these results suggest that the heavy use of yeast as a source of nutrients and plant growth hormones along with foliar sprays of either citric or ascorbic acid might be considered as an alternative substitute of half dose of recommended N-fertilizer applied for barley crop.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is considered as one of the most suitable crops, which can be grown over a wide range of environmental conditions. In Egypt, barley cultivated area is mostly under rainfed conditions and newly reclaimed lands. Barley is used mainly for consumption as feed for animals and humans. Various agronomic factors affect the yield and quality of barley grains, the application of nitrogen fertilizer is considered the most important factor (Tofinga, 1990), increasing the rates of nitrogen fertilizer may increase the yield of grains but reduce the quality of grains (Withers and Dyer, 1990 and Conry, 1995).

Glycolysis and tricarboxylic acid cycles are the main sources of respiratory, plant growth, and all nutritional processes which reflect on plant growth and nutrient uptake, are dependent on organic acids level in plant tissues (Givan, 1979).

Ascorbic acid is a common antioxidant component in the apoplast and it affects plant growth and many plysiological processes. L-Ascorbic acid serves as a co-factor for many enzymes (Arrigoni and De Tullio, 2002; De Tullio *et al.*, 1999) and it contributes to the detoxification of reactive oxygen species (ROS) (Smirnoff and Wheeler, 2000; Conklin, 2001; Conklin and Barth, 2004). This antioxidant activity of AA is associated with resistance to oxidative stress and longevity both in animals and plants. Furthermore, the endogenous level of AA has recently been suggested to be important in the regulation of developmental senescence and plant defense against pathogens (Pastori *et al.*, 2003; Barth *et al.*, 2004; Pavet *et al.*, 2005).

Citric acid is also one of the organic acids presented in tricarboxylic acid cycle synthesized either from acetyl-COA, glycine and @-ketoglutrate or malic acid conversion to citric acid (Miernyk and Trelease, 1981).

Citric acid is also considered a physiological relevant factor leads to the activity of some plant enzymes (Reibstein *et al.*, 1986). It changes to ketoglumatic acid required for protein synthesis (Miflin and Lea, 1980).

Yeast (*Saccharomyces cervicia*) plays an important role in soil biofertility because of their capability for producing hormones, amino acids, cytokinines and vitamins (Monib *et al.*, 1982). Many investigators found that the application of yeast as a biologically active microorganism increased many crop growth and yield (Besada *et al.*, 1991; Al-Kahal, *et al.* 2002; and AL-Kahal, *et al.*, 2008).

Application of ascorbic acid and citric acid as foliar spray have been stated that these organic acids have a positive effect on plant growth and yield of many crops as mentioned by (Rabie and Negm 1992; Mousa *et al.*, 1994; Dalia, 1996).

Therefore, the aim of the present study is to investigate if the of application of yeast as a soil inoculant separately or in combination with foliar spray of ascorbic acid and citric acid could reduce the amount of nitrogen fertilizer needed for barley crop under field conditions and to study the effect of these treatments on barley growth, yield and yield components.

MATERIALS AND METHODS

A field experiment was conducted in the season 2006-2007 at Bahteem farm, Kalubia Governorate, ARC, Giza, Egypt. The experimental soil texture was sandy clay loam soil. Some physical and chemical analyses of the soil are presented in Table (1).

The experiment included 5 treatments, plants fertilized with recommended dose of N fertilizer; plants fertilized with half dose of N fertilizer; plants amended with Yeast (*Saccharomyces cervicia*) as soil inoculant at a rate of 5 litter / feddan and the inoculation was repeated twice, plants amended with Yeast (*Saccharomyces cervicia*) combined with foliar spray ascorbic acid at a rate of 1 g/ L, plants amended with Yeast (*Saccharomyces cervicia*) combined with foliar spray of citric acid at a rate of 1 g/ L. Spraying of both ascorbic and citric acids was repeated twice during vegetation period. Barley Seeds cultivar Giza 123 are provided by Crop Res. Institute, ARC. Plots area was 4.2 m^2.

Table (1): Some physical and chemical properties of Soil

Properties	Value
Sand %	60
Silt %	15
Clay %	25
$CaCO_3$ %	2.10
Textural class	Sandy clay loam loam
pH 1:5	7.60
E.C. dSm^{-1} 1:5 (soil: water) extract)	1.09
O.C. %	0.38
O.M. %	0.67
Total N %	0.09
Soluble cations (meq l^{-1})	
Ca^{++}	6.11
Mg^{++}	3.31
Na+	2.78
K+	0.29
Soluble anions (meq l^{-1})	
$CO3^{-}$	—
HCO_3^-	2.29
$SO^{=}_4$	10.20

The treatments were assigned in a randomized complete design with four replicates. Super phosphate 15.5% P_2O_5 was applied basically at a rate of 100 kg/fed. Nitrogen fertilizer in the form of urea (46% N) was applied in two rates as recommended dose (5o kg fed^{-1}) and half recommended dose (25 kg fed^{-1}), which were divided into three additive doses each.The other cultural practices, i.e., irrigation and weed control were carried out as recommended by the Egyptian Ministry of Agriculture and Land Reclamation. Data were recorded at harvest for the following parameters and were taken as follows: -,

1- Vegetative growth characters in terms of Plant height and straw yield.
2- Yield ad yield components grain yield; spike length; N o. of grains/ spike, weight of grains/ spike, 1000-grain weight, N- content in straw and grain yields.
3- Total count of bacteria, fungi and actinomycetes in the rhizosphere of barley plants were determined using soil extract agar medium (Allen, 1959), Rose Bengal streptomycin agar (Martin, 1950) and Williams and Davis (1965), respectively.
4- Total nitrogen in straw and grains was determined by microkjeldahl procedure (Piper, 1950).
5- Statistical analysis of collected date was done according to Snedecore and Cochran (1980).

RESULTS AND DISCUSSION

Effect of foliar spray of ascorbic acid or citric acid and or soil inoculation with yeast on:

A- Barley growth, yield and its components.

Data in Table (2) revealed that there was an increase in plant height at harvest in all treatments compared to the plants fertilized with half dose of nitrogen fertilizer (control treatment). The increase was not significantly affected by application of yeast or inoculation of yeast along with foliar spray of citric acid. Application of ascorbic acid combined with soil inoculation with yeast gave significant increase in plant height compared to the plants fertilized with half doses of nitrogen fertilizer. Soil inoculation of yeast gave a slight increase in plant height compared to control, but the difference was not significant.

As indicated in Table (2) plants fertilized with recommended doses of N fertilizer scored the highest straw yield followed by those treated with ascorbic acid and yeast inoculation. Application of yeast alone or along with foliar spray of citric acid gave insignificant increase in straw yield compared with plants fertilized with half dose of N fertilizer. These findings suggested that foliar spray of ascorbic acid had a favorable effect on barley growth characters. The increase in the growth and development of plants in response to antioxidant treatment might be due to the enlargement of cell division and/ or cell enlargement and / or the influence on DNA replication (Smirnoff, 1996). Dalia (1996) found that shoot dry weight of wheat plants was significantly increased by foliar spray of citric acid or ascorbic acid. The increase in shoot dry weight of wheat plants may be due to the effect of the organic acids on many metabolic and physiological processes, mineral uptake and phyto-hormone balance.

With regards to the effect of 2 different doses of nitrogen fertilizer, it was noted that that plants fertilized with recommended doses of N fertilizer scored the highest grain yield, which recorded 14.11 ardab/fed, while plants fertilized with half dose of N fertilizer scored the lowest grain yield, which recorded 11.20 ardab/fed. Bulman and Smith (1993) reported that grain yield of barley was increased with increasing the applied nitrogen dose, which in turn increased in number of spikes. Regarding to the effect of foliar spray of either citric acid or ascorbic in combination with yeast inoculation on grain yield, there was an insignificant increase in grain yield due to the plants treated with yeast besides ascorbic acid compared to those fertilized with full dose of N-fertilizer. Concerning to the spike length, it was noticed that the highest spike length was recorded in plants fertilized with recommended doses of N fertilizer, followed by those treated with yeast combined with foliar spray of ascorbic acid. In this respect, Dalia (1996) found that 1000-grains weight of wheat was not affected by different concentrations of foliar spray for citric acid and\or ascorbic acid. Data in Table (2) revealed that there were significant increases in the number and weight of grains per spike of barley with increasing nitrogen fertilizer rate. Increases in the number and weight of grains per spike with nitrogen application have been reported by Bulman and

Smith (1993). The number and weight of grains per spike were not significantly affected by inoculation with yeast and or foliar spray of citric acid or ascorbic acid. Concerning 1000-grain weight, it was significantly affected by increasing rate of nitrogen fertilizer. The highest value was recorded in plants amended with full dose of nitrogen fertilizer. The lo west one was recorded in plants received half dose of nitrogen fertilizer.

A perusal of data on nitrogen percentage by grains plus straw exhibited that the highest value of N-content in barley shoots at harvest and in grains in the treatments that received full dose of nitrogen fertilizer. Nitrogen percentage in barley grains was significantly increased by foliar spray of ascorbic acid in presence of soil inoculation with yeast compared to the plants treated with yeast only. The positive response to ascorbic acid application could be explained on the basic that ascorbic acid contribute in the respiration system which reflects upon the nutrients uptake and transport, as mentioned by (Mona-Mahmoud, 1994).

Table (2): Effect of foliar spray with organic acids and soil application of yeast on barley growth and yield and its components under field conditions

Treatments	Plant height (cm)	Straw yield (ton /fed.)	Grain yield ardab/ fed	Spike length (cm)	No. of grains/ spike	Weight of grains/ Spike (g)	1000-grains weight (g)	N content (%)	
								Grains	Straw
½ dose of N	110.00	2.70	11.20	8.20	44	12.32	52.52	1.88	0.42
Full dose of N	115.00	3.40	14.11	8.90	51	14.22	57.72	2.61	O.47
yeast	112.50	2.65	11.34	8.30	47	13.32	55.80	1.94	0.37
Citric acid + yeast	113.75	2.50	10.37	8.00	52	14.02	55.87	1.83	0.36
Ascorbic acid + yeast	117.50	3.35	13.90	8.50	49	13.37	53.52	2.37	0.42
L.S.D. at 0.05	6.69	0.60	2.45	0.83	3.37	0.89	4.94	0.18	0.02

B- Microbial populations in the rhizosphere of barley plants:

The total numbers of bacteria, fungi and actinomycetes in the rhizosphere of barley plants in at tillering and harvest stages are illustrated in Tables (3), (4) and (5).

Table (3): Effect of foliar spray of organic acids and yeast on total bacteria count in rhizosphere soil of barley plants (cfu g^{-1} rhizosphere soil)

Treatments	Total count of bacteria at tillering stage (x 10^6)	Total count of bacteria at harvest (x 10^6)
½ dose of N	66.50	45.00
Full dose of N	73.25	46.50
Yeast	86.00	81.00
Citric acid + yeast	88.75	62.50
Ascorbic acid + yeast	102.00	71.00

Table (4): Effect of foliar spray of organic acids and yeast inoculation on total fungi count in rhizosphere soil of barley plants (*cfu g^{-1} rhizosphere soil)

Treatments	Total count of fungi at tillering stage (x 10^3)	Total count of fungi at harvest (x 10^3)
½ dose of N	44.50	14.25
Full dose of N	50.75	13.00
Yeast	80.00	22.75
Citric acid + yeast	52.25	25.00
Ascorbic acid + yeast	67.75	31.50

Table (5): Effect of foliar spray of organic acids and\or yeast inoculation on total actinomycetes count in rhizosphere soil of barley plants (*cfu g^{-1}rhizosphere soil)

Treatments	Total count of actinomycetes at tillering stage (x 10^4)	Total count of actinomycetes at harvest (x 10^4)
½ dose of N	49.25	35.50
Full dose of N	58.50	37.00
Yeast	66.00	44.25
Citric acid + yeast	60.75	34.50
Ascorbic acid + yeast	61.25	32.75

* cfu = Colony formed unit^{-1}

Generally, the total numbers of bacteria, fungi and actinomycetes were increased by soil application of yeast separately or in presence of foliar spray of citric acid or ascorbic acid as compared to the plants fertilized with recommended or half dose of N fertilizer especially at tillering stage. Soil amended with yeast as an inoculant gave the highest number of fungi and actinomycetes in the rhizosphere of barley plants at tillering stage. When yeast was combined with foliar spray of ascorbic acid, their combination gave the highest number of bacteria compared to the other applied treatments. As usual the lowest number of bacteria, fungi and actinomycetes was recorded in plants fertilized with ½ dose of recommended N fertilizer. From the abovementioned results, it could be concluded that yeast plays an important role in stimulation the propagation of bacteria, fungi and actinomycetes in the rhizosphere of barley plants, This trend could be attributed to that *Saccharomyces cervisia* (yeast) produce hormones, such auxins, indole acetic acid besides, considered as source of nutrients and vitamins especially vitamin B_{12}, (Monib *et al.*, 1982). Therefore, the application of yeast as a biologically active microorganism stimulated the propagation of microbial communities in the rhizosphere of barley yield compared with plants fertilized with full dose or 1/2 N-fertilizer .Besada *et al.* (1991) found that inoculation of barley under field conditions with VA Mycorrhizae and mixed cultures of asymbiotic N_2-fixers and yeast gave the highest dry weight of plants. N-percentage in grains, straw and grain yields. On the other hand, application of yeast to soil may stimulate the propagation of other microorganisms especially N_2- fixing microorganisms, which may provide supply of soluble N in the rhizosphere of barley plants. Treating barley plants with ascorbic acid or citric acid in presence of yeast as a soil inoculant, stimulate also the propagation of bacteria, fungi and actinomycetes, this may be due to that foliar spray with these organic acids might increase the organic acids excreted from the roots into the soil and consequently increase the availability of nutrients, which it may be utilized by microorganisms.

CONCLUSION

Generally, the present results suggest that the heavy use of yeast as a source of nutrients and plant growth hormones along with foliar spray of either citric or ascorbic acid may be considered as an alternative substitute of half dose of recommended N-fertilizer applied for barley crop. However, this experiment needs more studies in different sites to enable the researchers to establish the phenomena of recommendation.

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تأثير إستخدام الرش الورقى بحمض الستريك وحمض الاسكوربيك والخميرة كلقاح للتربة على النمو والمحصول وعلى المجموعات الميكروبية فى منطقة الريزوسفير لمحصول الشعير

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اجريت تجربة حقلية لدراسة اثر استخدام الخميرة كلقاح للتربة منفردة او مع الرش الورقى سواء بحمض الستريك او بحمض الاسكوربيك على بعض صفات النمو ،وعلى محصول الحبوب ومكوناته بالاضافة الى دراسة تأثيره على المجموعات الميكروبية فى منطقة تربة الريزوسفير لمحصول الشعير. النباتات التى سمدت بالمعدل الموصى به من السماد النيتروجينى اعطت اعلى قيم من طول السنبلة ووزن الحبوب فى السنبلة ووزن ال ١٠٠٠ حبة و محصول الحبوب والقش ومحتوى النيتروجين فى القش والحبوب .

وقد ادى استخدام الخميرة سواء بمفردها او مع استخدام الرش الورقى بحمض الستريك او حمض الاسكوربيك الى زيادة فى طول النبات ومحصول الحبوب وعددالحبوب فى السنبلة ووزن الحبوب فى السنبلة ووزن ال ١٠٠٠ حبة.

أعطى الرش الورقى بحمض الاسكوربيك مع الخميرة كلقاح للتربة ، قيم اعلى فى طول النبات ومحصولى الحبوب والقش فى السنبلة ومحتوى النيتروجين فى القش والحبوب.

اما بالنسبة لتاثير استخدام الرش الورقى بحمض الستريك او بحمض الاسكوربيك مع الخميرة او استخدام الخميرة بمفردها على المجموعات الميكروبية ، فدلت النتائج على زيادة اعداد البكتيريا والفطريات والاكتينوميستات فى منطقة تربة الريزوسفير لمحصول الشعير مقارنة بالنباتات التى سمدت بالمعدل الموصى به او نصفه من السماد النيتروجينى وذلك فى مرحلة التفريع .ومن بين جميع المعاملات وجد ان تلقيح التربة بالخميرة مع استخدام الرش الورقى بحمض الاسكوربيك اعطى اعلى اعدا للبكتريا فى منطقة تربة الريزوسفير لمحصول الشعير. وجد ان النباتات التى سمدت بنصف المعدل الموصى به من السماد النيتروجينى اعطت اقل قيم بالنسبة لاعداد الميكروبات . وبصفة عامة فان الاستخدام المكثف للخميرة كمصدر للعناصر الغذائية ومنظمات النمو مع الرش الورقى بحمض الستريك او بحمض الاسكوربيك من الممكن اعتباره بديل عن استخدام نصف المعدل الموصى به من السماد النيتروجينى لمحصول الشعير .