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Response of yield and its components in Barely (*Hordeum vulgar* L.) to Inoculation with Microorganisms and phosphorus Chemical fertilizer under newly reclaimed sandy soil

Elham A., Badr, M.H.M. Afifi ,Gehan Sh.H.Bakhoum and Gehan A., Amin

Field crops Department, National Research Center, Dokki, Giza, Egypt.

Abstract: Two field experiments were carried out during 2013/2014 and 2014/2015 winter seasons at the farm of research and production station, National Research Centre, Al Emam Malek Village, Nubria District, Al Behaira Governorate, Egypt, to study the effect Bio-fertilizer and different levels of phosphorus fertilizer on yield and yield components of barley (Giza 130 cultivar).The experimental treatments were arranged in split plot factorial based on a complete randomized block design with three replications . Results indicated that phosphorus fertilizer (100kg/fed) was significant effect on yield and its components also application of dual inoculation with *Azotobacter +Pseudomonas* give the highest significant effect on grain yield, harvest index, biological yield, plant height and protein content as compared to other treatments. The traits such as Grain yield, harvest index, biological and plant height were affected by interaction effects of both chemical and bio-fertilizers. The highest grain yield was thus due to the use of phosphorus fertilizer along with *Azotobacter+Pseudomonas* inoculation[Using a ratio of 75:100) recommended phosphorus fertilizer had no effect on yield and related traits. Thus, we can accept grain yield of barley by using 75 kg/fed phosphorus fertilizer and inoculation with *Azotobacter +Pseudomonas*] In general, the result of this investigation showed that the use of 75 chemical fertilizer along with dual inoculation (*Azotobacter +Pseudomonas*).
Keywords: Barley, *Azotobacter*, *Pseudomonas ,* Bio-fertilizer, phosphorus fertilizer.

Introduction

Barley is widely grown in the rain fed areas of the arid and semi arid Mediterranean regions where water is the main limiting factor affecting agricultural production . Barley is a widely distributed crop that can grow in a wide range of environmental conditions, even in semi-arid areas with 200–350 mm of annual rainfall,[1] It is the fourth most important crop in the world. Grain is used as feed, food, and malting purposes, while straw provides an important source of roughage for animals particularly in the dry areas , it is adapted to unfavorable conditions and environments

Phosphorus is one the most essential elements for plant growth after nitrogen. However, the availability of this nutrient for plants is limited by different chemical reactions especially in arid and semi-arid soils .Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, and transporting of the genetic traits. [3.]reported that one of the advantages of feeding the plants with phosphorus is to create deeper and more abundant roots. Phosphorus causes early ripening in pants, decreasing grain moisture, improving crop quality and is the most sensitive nutrient to soil pH [4.]Environmental problems caused by irregular applications of chemical fertilizers, inappropriate energy production methods and excessive consumption costs all have had harmful effects on

biological cycles, and destroyed farming stability systems; these factors altogether encourage the use of bio-fertilizers. Now-a-days attention to bio-fertilizer has been increased due to the advancement in countries research development, prices of chemical fertilizers and attention to sustainable agricultural systems[5.] The Plant Growth Promoting Rhizobacteria (PGPR)are group of bacteria that actively colonize plant roots and increase plant growth and yield. The mechanisms by which PGPRs promote plant growth are not fully understood, but are thought to be due to: (a) the ability to produce phytohormons (b) a symbiotic N2 fixation (c) against phytopathogenic microorganisms by producing siderophores the synthesis of antibiotics, enzymes and/or fungicidal compounds, and also (d) solubility of mineral phosphates and other nutrients[6.]. There are some evidences in support of bio-fertilizers including that plant growth and yield increase may be stimulated by plant growth promoting bacteria due to their ability of N2- fixing, phosphate solubility and production of plant growth hormones[7.]. Bio-fertilizer with 50% of chemical fertilizers (N and P) led to an increase in plant growth, plant height, branch numbers fresh and dry weight of safflower in comparison with chemical fertilizers application alone. Also the utilization of *Azetobacter* bio-fertilizer, biophosphate fertilizer, organic fertilizers,with half rate of chemical fertilizers increased the grain yield of safflower[8.]. Cereals Yield responses to inoculation may also depend on plant genotype, , bacterial strains and soil type as well as environmental conditions[9.]. The Excessive use of P causes environmental and economic problems.

That is, the over fertilization of P leads to pollution due to soil erosion and runoff water containing large amounts of soluble P. In addition, the use of P fertilizers has become a costly affair and there is a need for alternative sources[10.]

Materials and Methods

Two field experiments were conducted at research and production station, National Research Centre Al Emam Malek Village Nubaria District Al Behaira Governorate

Egypt 2013/2014and 2014/2015 winter seasons investigated the effect of bio and different levels of phosphorus fertilizer on yield and its components of barley (*Hordeum vulgar* L.) .The experiment was performed using factorial arrangement in phosphorus randomized complete block designed with three replications. Experimental factors included the chemical fertilizer ($P_2O_{5)}$ at the rate of (0, 50,75and 100kg/fed) and bio-fertilizer (non-inoculation, inoculating with *Azotobacter indicum,Pseudomonas Putida* and *Azotobacter+ Pseudomonas*

The inoculants bacteria, *Pseudomonas potida,* have a good ability to facilitate phosphorus uptake and Auxine hormone secretion which effectively enhances the plant growth at 5×108 cell g 1 population.. To inoculate the seed by biological fertilizers, the seed were first covered by Arabic Gum solution were applied according to experimental treatments. All the seeds were sown soon after inoculation in experimental plots of (3 x 2.5 m) it includes 15 rows, 20 cm apart and each of 2.5 m in length sowing. Dates experiments were on November 25th in both seasons and harvested on May. Commercial product was obtained farm the Agricultural Research center Ministry of Agriculture. Calcium super phosphate 15.5% P2O5 at(0,50,75 and 100) kg/fad as well as 48 kg K2O/fad as potassium were applied at sowing time and 100 kg N/fad in form of ammonium sulphate (20.5% N)

At harvest the data recorded are plant height (cm), number of spikes/m^2,numberof grain /spike ,1000-grain weight (g), biological yield (kg/fad), harvest index (grain yield/biological yield × 100),grain yield(kg/fed)and Crude protein content it was determined as the total nitrogen following the micro kjieldhl method according to[11.] and them total N content was multiplied by 5.7 to obtain the protein content in grain

Statistical analysis

The data were analyzed using [12.]and MSTATC software [13.]

Table 1: Physical and chemical soil characteristics.

Sand %	Silt %	Clay %	Soil texture	pH	O.M %	CaCO3 %	E.C (ds/m-)	Available nutrients (ppm)		
								N	P	K
91.2	3.7	5.1	sandy	7.3	0.3	1.4	0.3	8.1	3.2	20

The experimental soil was analyzed according to the method described by [14.]

Results and discussion

Plant height

Data presented in Table (2).Showed the use of chemical fertilizer caused the increase in plant height. Mean comparisons indicated that the lowest plant height was obtained by non-utilizing of phosphorus phosphorus fertilizer. But there was no significant difference among using 50, 75 and 100kg/fed recommended fertilizer. Bio-fertilizer also increased the height of barley plant. Dual inoculation with *Azotobacter*+ *Pseudomonas* and non-inoculation had the highest and the lowest plant heights respectively. The plant height related changes study in response to bio-fertilizer during growth season showed that height of barley plant increased due to inoculation in general plant height influenced by availability of water and nutrients through increasing nodes numbers and mid nodes length. [15] suggested that inoculation seeds with bio-fertilizer such as *Azespirillum*, *Azotobacter* and *Pseudomonas* caused improving plant growth trait due to inoculation nutrients uptake by plant microbial inoculation also led to improving the soil attributes such as organic matter content and increasing N content. [16] reported an 8.5% increase in plant height of corn by applying *Azotobacter* and *Pseudomonas* to the growing seed. the highest plant(cm)by using 75kg/fed phosphorus fertilizer and dual inculcation with *Azotobacter*+ *Pseudomonas* and lowest plant height (cm)belonged to non- -utilizing chemical fertilizer and non inoculation in Table(4)

Number of spikes/m^2

Table(2) showed the highest spikes/m^2 were obtained by using 100kg/fed recommended phosphorus fertilizer and the least number of spikes/m^2 obtained in non-utilizing phosphorus fertilizer. The use of 75and100kg/fed phosphorus fertilizer had no effect on spike**s/m**^{2}. Bio-fertilizer also had significant effect on number of spikes/.m^2. The least number of spikes.m-2 observed in non inoculating treatments with bacteria which was the cause of increasing numbers in spikes.m^2in reverse order. effect of bio-fertilizers on spike was positive in this experiment. In other words, using N (urea) and P (Triple superphosphate) fertilizers at appropriate stages provide better nutrient uptake and plant photosynthesis through improving bio-fertilizers. In a study on the effect of N fertilizer, [17] reported the increase in spike numbers per plant of barley through application of N fertilizer. They related it to the fact that N might have increased cytokinin content of xylem sap through which spike number per plant increased. These studies are in agreement with the results of present study.

Number of grains/spike

Table(2) showed applying phosphorus fertilizer alone caused increased number of grains/spike. The lowest and highest number of grains/spike was belonged to non-utilizing chemical fertilizer and using (100 kg/fed)recommended chemical fertilizer, respectively. But there was no significant effect between 75 and 100kg/fed recommended phosphorus fertilizer. Bio-fertilizer improved the wheat growth and growth parameters. The performance of the plants was better in inoculated treatments in comparison with the control. The results showed that the number of barley grains/spike were significantly increased by N2-fixing and P-solubilizing PGPR strains application as compared with the control.

Table(2)The effect of chemical and bio fertilizer in barely (combined analysis of two seasons)

Treatment	Plant height(cm)	Number of Spike /m^2	Number of Grains /Spike	1000grian W(g)
Phosphorus fertilizer (P_2O_5)				
0 kg/fed	76.8b	460.9b	30.8b	34.2b
50 kg/fed	81.5a	479.2ab	32.5ab	39.7a
75 kg/fed	83.1a	493.2a	33.6a	41.3a
100 kg/fed	84.4a	519.8a	34.2a	41.5a
Biofertilizer				
Non-inoculation	70.8c	461.2b	27.3c	35.4b
Azotobacter	76.7b	486.3ab	29.9b	38.9a
Pseudomons	78.9ab	488.2ab	32.9ab	40.2a
Azot+Pseu	82.9a	498.6a	35.4a	42.1a

The highest and lowest number of grains/spike was obtained by applying dual inoculation with *Azotobacter* and *Pseudomonas* and non-inoculation treatment, respectively Table(2). This indicated the ability of bio-fertilizers applications with different levels of chemical fertilizers, IAA + cytokine produced by *Azotobacter* caused increasing of preserved material through growing the side rhizomes and increasing the leaf and root weights; the cause to improve vegetative growth and raise number of grains/spike[18.]. The obtained results are in agreement with[17.] in barely plant.

1000-grain weight

Using chemical fertilizer had significant effect on 1000-grain fertilizer (Table 2). According to the results the highest (41.5g) and lowest (34.2 g) of 1000-grain weights were obtained by using 100kg/fed recommended phosphorus fertilizer and non-utilizing chemical fertilizer, respectively. In this study, there was any significant difference between 50 and75kg/fed phosphorus fertilizer. The results of this study revealed that 1000-grain weight was affected by bio-fertilizer(*Azotobacter*, *Pseudomonas* and dual inoculation with *Azotobacter* and *Pseudomonas*).Thus, it seems that bio-fertilizers are the direct cause to improve this trait (Table 2) The positive effects of bio-fertilizers i.e. improving photosynthetic activities by increasing water and nutrients uptake and this resulted in more assimilation and improving of plant growth, as a result, 1000-grain weight showed an increase as compared with non inoculation treatment, such as leaves number, spike length and height that finally caused the increasing assimilates production. Probably using chemical fertilizer provided suitable nutritious condition to reproducing and acting of *Azotobacter* and *Pseudomonas* bacteria, because these bacteria need to P and N to growing and fixing P and N. Bio-fertilizer treatment provided more suitable condition to bio activities of soil as compared with chemical treatment and they resulted in increasing 1000-grain weight uptake of nutrients by root[19.] also reported positive affect of *Azotobacter* on 1000-grain weight in wheat. Also, increasing of 1000- grain weight reported by [9.] and [17.]in barley

Grain yield

As shown in Table(3) using phosphorus fertilizers cause increasing of grain yield. Mean comparisons showed that using 100kg/fed recommended phosphorus fertilizer and non-utilizing of chemical fertilizer had the highest (2003.7kg/fed) and lowest (1341.3kg/fed) grain yields, respectively. Also, showed significant differences for grain yield within the different levels of bio-fertilizer.

Table(3)The effect of chemical and bio fertilizer in barely (combined analysis of two seasons)

Treatment	Grain yield(kg/fed)	Harvest index %	Biological yield(kg/fed)	Protein content%
Phosphorus fertilizer (P_2O_5)				
0 kg/fed	1341.3d	36.4c	3682.8c	12.02b
50 kg/fed	1702.8c	39.5b	4300.6b	12.9a
75 kg/fed	1875.4b	42.1a	4457.2b	13.1a
100 kg/fed	2003.7a	41.8a	4791.4a	13.2a
Biofertilizer				
Non-inoculation	1510.8d	40.9c	3688.6b	11.8b
Azotobacter	1739.5c	42.4b	4099.2c	12.2a
Pseudomons	1852.1b	42.5ab	4358.5b	11.9ab
Azot+Pseu	1960.5a	43.2a	4542.6a	12.4a

The highest and the lowest grain yields were observed in dual inoculation with *Azotobacter* + *Pseudomonas* and non-inoculation treatment, respectively Table(3). This showed that using dual inoculation with *Azotobacter*+ *Pseudomonas* would have contribution in grain yield as compared to other inoculation treatments, followed by *Azotobacter* and then *Pseudomonas*. Thus, it seems that adding inoculation with *Azotobacter*+ *Pseudomonas* caused the increasing effect of bacterial inoculation of seeds on grain yield of barley. Therefore, most efficient bacteria were *inoculation with Azotobacter*+ *Pseudomonas*. This result can indicate synergistic relation to mixing mentioned bacteria with each other to increase grain yield of barley. Thus it can be concluded that positive relation between barley plant and these bacteria is forced and resulted in increasing grain yield. The highest grain yield produced by using 100kg/fed recommended phosphorus

fertilizer and dual inculcation with *Azotobacter*+ *Pseudomonas* and lowest grain yield belonged to non-utilizing chemical fertilizer and non inoculation in Table(4)

Harvest index

Harvest index indicates transforming percent photosynthetic matters from vegetative organs (source) to seeds (sink) [20.]phosphorus fertilizer significantly affected the harvest index of barleyTable(3). The Mean comparisons indicated that the lowest harvest index (36.4%) was obtained by nonutilizing of chemical fertilizer and using 75 & 100kg/fed recommended phosphorus fertilizer had the highest harvest index (42.1% and41.8%).Table(3). Results of this study revealed significant difference due to bio-fertilizer application upon harvest index .

Dual inoculation with *Azotobacter*+ *Pseudomonas* and non-inoculation had the highest and lowest harvest index, respectively. Harvest index of seeds inoculated with bacterial bio-fertilizer increased as compared with non inoculation treatment. Thus, it is indicated that growth improving bacteria increased harvest index by affecting on sharing dried weight of plant and allocated more dry matter to the grain. In Table(4) interaction effects of chemical fertilizer and bio-fertilizer also indicated that using 100 kg/fed recommended phosphorus fertilizer and non-utilizing fertilizer and non inoculation had the highest and lowest harvest index, respectively.

Biological yield

Biological yield was significantly affected by use of phosphorus fertilizer Table(3). This result indicated that the biological yield was increased by the application of phosphorus fertilizer Biological yield was highest and lowest using 100kg/fed recommended phosphorus fertilizer and non-utilizing chemical fertilizer. Results in Table(3) showed a significant difference among bio-fertilizers on biological yield. The use of bio-fertilizers resulted in improving this trait, but dual inoculation with *Azotobacter*+ *Pseudomonas* had the highest non inoculation the lowest the biological yield Table(3). Bio-fertilizer had a positive effect on biological yield. Also, interaction effect of chemical and bio-fertilizers showed that increasing chemical fertilizer and using biofertilizer caused increasing biological yield. Using 100kg/fed recommended chemical fertilizer and dual inoculation with *Azotobacter*+ *Pseudomonas* had the highest biological yield and non-utilizing chemical fertilizer and non-inoculation had lowest biological yield, respectively Table(4). However, the interaction effect shown, using 75 and 100kg/fed recommended phosphorus fertilizer and dual inoculation with *Azotobacter*+ *Pseudomonas* were not statistically different. Previous study revealed that corn inoculation with *Azespirilum* increased the biological yield[21.] In addition, [22.]reported that inoculating microrhizim with vetch caused the increase of biological yield. They indicated that the reason of this is due to improving and better uptaking of nutrients and they suggested that it is finally led to increasing accumulationof dried matter in vetch. [23.]concluded that application coexistent Mycorrhiza fungus caused the improving of physiological characteristics of soil and also increasing plant yield.

Table (4) Interaction effect of chemical and bio fertilizer in barely(combined analysis of two seasons)

Chemical fertilizer P_2O_5	Biofertilizer	Plant height (cm)	Grain yield (kg/fed)	Harvest index(%)	Biological yield (kg/fed)	Protein content(%)
0 kg/fed	Non-inoculation	68.4e	2666.6e	36.4h	7306.6g	10.9c
	Azotobacter	73.9de	2776e	36.9gh	7506.6g	11.5b
	Pseudomons	87.9ab	2736e	36.1gh	7576g	11.2bc
	Azot+Pseu	87.8ab	2866.6e	37.6fg	7613.3g	11.5b
50 kg/fed	Non-inoculation	78.06cd	2800e	37.2gh	7520g	11.7b
	Azotobacter	84.8abc	3520c	38.7f	9093.3de	12.7a
	Pseudomons	84.1abc	3776c	40.4e	9333.3cd	12.5a
	Azot+Pseu	88.03ab	4114.6b	41.8cde	9840bc	12.9a
75 kg/fed	Non-inoculation	78.6cd	322.1d	41.5de	7758.8fg	12.4a
	Azotobacter	85.3abc	3368c	38.3f	87773.3e	12.3a
	Pseudomons	88.06ab	4160b	44.4ab	9360cd	12.4a
	Azot+Pseu	91.6a	4533.3a	44.3ab	10238.6ab	12.9a
100 kg/fed	Non-inoculation	83.8bc	3531.3c	43.1bc	8202.6f	12.4a
	Azotobacter	83.3bc	4146.6b	44.7ab	9264de	12.5a
	Pseudomons	84.8abc	4496a	44.2ab	10213.3ab	12.4a
	Azot+Pseu	87.2ab	5604.2a	45.3a	10393.7a	12.8a

Protein content

Effect of chemical fertilizer use was significant on barley Protein content.. Grain protein content of barley was higher in 100kg/fed P(13.2%) and inoculation with *Azot+ pseu* (12.4%) treatments while protein concentration was lower especially in control (12.02%) and non- inoculation (11.8%) treatments . Table (3). Of course using 50, 75 and 100 kg/fed recommended phosphorus fertilizers hadn't significant difference. . Dual inoculation with *Azotobacter+ Pseudomonas* had highest protein content. Results were in agreement with the findings of most of the workers/coworkers like:[24]. [18]also demonstrated bio-fertilizers are nitrogen fixing bacteria and nitrogen is basic matter to forming protein

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