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Original Article

Study of the Response of *Leucojum aestivum* L. Bulbs Collected from Different Regions to Organic and Biological Fertilizers under Field Conditions

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Abstract

Leucojum aestivum L. is a perennial and bulbous plant. Its bulbs are used in the medical industry because of having alkaloids. This study was conducted as split plot in a completely randomized design with 3 replications in 2018 at the University of Gilan in field conditions. The main factor included bulbs collected from three regions of Lahijan, Tonekabon and Langrood. The sub-factor included 10 levels of organic fertilizers, biochar (3, 6 and 12 tons per hectare), inoculation of *Glomus intraradices*, *Glomus mosseae* and *Glomus intraradices* + *Glomus mosseae* fungi, inoculation of Azospirillum, Azotobacter and Pseudomonas bacteria and the control. Data collected and analysed for morphological traits and absorbed macro and micro as elements by plants. There was significant difference between genotypes in terms of the plant height, leaf area per plant, leaf weight per plant, bulb weight, flowering stem weight, root weight and total plant weight ($p<0.01$). A significant difference was observed in the types of fertilizers used for all morphological traits and yield ($p<0.01$) and for phosphorus, potassium and iron content ($p<0.05$). The genotype by treatments interaction effect was significant for leaf number, leaf area per plant, leaf weight per plant, bulb weight, flowering stem weight, root weight and total weight showed a difference ($p<0.01$). Lahijan genotype plants had the highest plant height (57.6 cm), leaf length (52.5 cm) and plant leaf weight (27.2 g/per plant). The highest root weight (9.5 g) and total plant weight (74.1 g/per plant) was for biochar treatment of 12 ton/h. The study results indicated that *Leucojum aestivum* L. had good adaptation to crop conditions, and using biochar fertilizer and some biofertilizers improved various crop traits of this plant.

Keywords: *Leucojum aestivum* L., Biochar, Gilan, Biological fertilizer.

Introduction

Leucojum aestivum L. from Amaryllidaceae family is a perennial and bulbous plant. It is distributed in different countries, including Ireland, Czechoslovakia, the eastern of Turkey and Iran [1]. This plant is grown in the east to west of Iran, south of the Caspian Sea and north of Iran [2]. In Europe, its bulbs are used in the medical industry due to having alkaloids. Germination tests showed that the optimum germination temperature was between 20 and 25 °C. This plant is well grown in alluvial soils with high levels of nitrogen and the size of plants increased with soil water content. The application of chemical fertilizers leads to environmental pollution and ecological problems [3]. Therefore, in order to reduce these risks, the use of organic resources and inputs in addition to meeting the plant nutritional needs will help sustain the sustainability of agricultural systems in the long term [4]. The organic matter is used by soil organisms, and as a result, some polysaccharide mucilages, proteins and cyclic carbonated

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compounds are produced that play a positive role in the formation and stability of soil aggregates and structure [5] and increase soil resistance to types of erosion [6]. Biochar is one of the carbon stabilizers that has high chemical stability, high carbon and long term ability to remain in the soil, and about 50% of the initial carbon is stabilized in biochar [7], but the remaining burning carbon is only 3% that has been reported about 10-20% in the biodegradation process after 5-10 years [7]. Biochar contains carbon-rich materials [8]. Biochar by balancing soil pH, cation exchange capacity, nutrient storage capacity, carbon content, soil biological factors and water storage capacity in soil improves soil fertility [9]. An increase in pH of soil was reported as a result of biochar consumption [10,11]. Using 1 ton per hectare of biochar in wheat field improved wheat yield but showed no increase in higher level treatments [12]. The biological fertilizers contain a variety of preservatives that stimulate the host plant growth [13]. Auxin, cytokinin and gibberellin are produced by several microorganisms, including Azospirillum, Agrobacterium and Pseudomonas, and are able to increase the density and length of capillary roots as well as the extension of lateral roots [14,15] leading to stimulation of systemic resistance [16]. Azotobacter inoculation improves the morphological properties and dry matter of *Matricaria chamomilla* [17]. Azospirillum increased the yield, absorption of nutrients, plant height, leaf size and root length [18]. Pseudomonas family has a wide range of plant growth promoting traits such as auxin production [19]. The biological fertilizer solubilizing phosphate increased growth, quality and quantity indicators of nitrogen and phosphorus of *Rosmarinus officinalis* [20]. Phosphate solubilizers have a positive effect on the number of umbels, plant height and grain yield of *Pimpinella anisum* [21]. In *Caharanthus roseus*, the seedling inoculation with Pseudomonas flurescence caused the increase in the plant dry matter content [22]. It was observed in *Origanum majorana* that inoculation with Pseudomonas flurescence increased the stem length and leaf number compared to the control [23]. Artemisia squamosa mycorrhiza has a significant effect on the plant height, fresh and dry weight of the root and stem compared to the control plants [24]. *Glomus mosseae* was reported in phosphorus absorption and increased dry matter yield in fennel [25]. The use of biological fertilizers increased the yield and yield components of stevia [26]. Considering the importance of using organic and biological fertilizers in sustainable agriculture and the necessity of using them in crop systems, this study was first conducted in Iran and in order to study the response of *Leucojum aestivum* L. to different types and levels of biological fertilizers in field conditions.

Material and Methods

In order to study the effect of different levels of organic and biological fertilizers on *Leucojum aestivum* L. collected from different regions, this study was conducted as split plot in a completely randomized design with three replications in 2018 at the University of Gilan in field conditions. The main factor included bulbs from three regions of Lahijan, Tonekabon and Langrood. The sub-factor included 10 levels of organic fertilizers, biochar (3, 6 and 12 tons per hectare), inoculation of *Glomus intraradices*, *Glomus mosseae* and *Glomus intraradices + Glomus mosseae* fungi, inoculation with bacteria (Azospirillum, Azotobacter and Pseudomonas) and the control. The bulbs collected from the above mentioned regions were cultivated in pots. For planting the bulbs in pots, we used pot soil containing 70% of the field soil and 30% of sand. The amount of biochar treatments was calculated and applied based on the area of the opening of the pots. Each gram of mycorrhiza included at least 100 active fungal organs (including spores, hyphae, and roots). In each pot of the biological fungal treatment, 50 grams of strain was added in the soil around the bulb. In order to apply the bacterial treatment, before planting the bulbs, we poured the strains' inoculation inoculum prepared from Soil and Water Institute with a small amount of sugar in a container to create adhesion, and bulbs, according to the instructions of the Institute of Soil and Water, were put there for 20 minutes in order to be inoculated. Four pots were used for each treatment in each replication. Irrigation was done after planting of bulbs. During the growing season, the plants' irrigation and weeding were taken on a regular basis. After full flowering, flowering traits such as the number of flowers (counting), flower diameter (caliper) and flower size (m) were measured. The traits of the number of stems (counting), number of leaves (counting), leaf length (cm), leaf width (mm), leaf chlorophyll [27], and absorbed elements of the plant such as nitrogen, phosphorus, calcium, potassium, magnesium, zinc, iron, copper, boron and manganese were measured. After collecting, drying, and powdering the leaves, the amount of some macro and micro elements were measured. Nitrogen was measured by Kjeldahl method [28], phosphorus was measured by spectrophotometer and iron, copper, zinc, and manganese mincronutrients were measured by atomic absorption spectroscopy [29,30]. In order to measure potassium we used digestion method [31], the plant dried sample was burnt in a furnace with a temperature of 550 °C for 8 hours, and for the reaction we used 2 molar chloride acid and then it was calculated using a photometer. The data were analyzed via software Excel and analyzed via software SAS. The mean comparison was performed using Duncan's multiple range test.

Results

The results of analysis of variance showed significant difference between the genotypes in terms of the plant height, large leaf area, plant leaf area, large leaf weight, leaf weight per plant, bulb weight, flowering stem weight, root weight and plant total weight ($p<0.01$) (Table 1). A significant difference was found between types of fertilizers for all of morphological traits and yield ($p<0.01$) (Table 1). The genotype by treatments interaction effect were significant for plant height and leaf width ($p<0.05$). Also The genotype by treatments interaction showed a significant difference for large leaf area, leaf number, leaf area per plant, large leaf weight, leaf weight per plant, bulb weight, flowering stem weight, root weight and total weight ($p<0.01$). The mean comparison of genotypes showed that Lahijan genotype had the highest plant height (57.6 cm), leaf length (52.5 cm), large leaf weight (5.9 g) and plant leaf weight (27.2 g/plant) (Table 2). The highest leaf width (13.2 mm), the lowest bulb weight (10.06 g), root weight (8.09 g), total plant weight (44.2 g per plant) belonged to Tonekabon genotype. The highest large leaf area (5.4 cm), leaf area per plant (25.8 cm²) belonged to Langrood genotype. The mean comparison of fertilizer treatments showed that the lowest plant height (46.5 cm) was for Pseudomonas, the lowest length (46.7 cm) was for the control and fungal inoculation treatments and the lowest leaf width (11.7 mm) was for the control and fungal inoculation treatments using Azotobacter (Table 3).

The highest large leaf area (5.4 cm), leaf area per plant (25.8 cm²) belonged to Langrood region. The mean comparison of fertilizer treatments showed that the lowest height (46.5 cm) was for Pseudomonas, the lowest length (46.7 cm) was for the control and fungal inoculation treatments and the lowest leaf width (11.7 mm) was for the control and fungal inoculation treatments using Azotobacter (Table 3). The highest large leaf area (6.8 cm²), number of leaves (5 per plant), plant leaf area (39.5 cm²), large leaf weight (7.2 g), leaf weights per plant (39.7 g), flowering stem (11.1 g), root weight (9.5 g) and total plant weight (74.1 g per plant) belonged to biochar treatment of 12 tons per hectare (Table 3).

The mean comparison of the interactive effect of the factors showed that the highest leaf area was observed in Langrood region and consumption of 12 tons per hectare of biochar with a mean of 7.6 cm² (Table 4). The highest number of leaves was for Langrood region, using Azospirillum bacteria with a mean of 5.9 per plant and Langrood region, using Azotobacter bacteria with a mean of 5.8 plants per plant (Table 4). The results of the mean comparison of traits showed that the highest leaf area of a plant was for 12 tons per hectare of biochar and Langrood region with a mean of 46.6 cm² (Table 4). The results of the mean comparison of the interactive effect showed that the highest single leaf weight was for Lahijan region with inoculation of two types of mosseae and intra fungi with a mean of 10.7 g, as well as Lahijan region using 12 tons per hectare with a mean of 10.7 g (Table 4). It was observed that the highest leaf weight of a plant was for Lahijan region with inoculation of two mosseae and intra fungi with a mean of 64.5 grams as well as Lahijan region using 12 tons per hectare of biochar with a mean of 54 grams (Table 4). The highest bulb weight per plant was for Lahijan region using Pseudomonas with a mean of 17.5 g. The mean comparison of the interactive effect of region * fertilizer showed that the flowering stem weight was higher in Lahijan region using Azotobacter bacteria with a mean of 16.7 g. The highest root weight was observed in Tonekabon region using Azotobacter with a mean of 12.5 g. The highest total plant weight was for Lahijan region using 12 tons per hectare of biochar with a mean of 90.8 grams.

Table 1 Variance Analysis of Effect of Region and Different Biofertilizers on Morphological Traits of *Leucojum aestivum* L.

S.O.V	df	Plant height	Bud diameter	Leaf lenght	Leaf width	One leaf area	Leaf number	Plant leaf area	Leaf weight	Plant leaves weight	Bulb weight	Flowering stem weight	Root weight	Total weight
Region	2	1560 **	0.9 ^{ns}	33.5 ^{ns}	5.00 ^{ns}	4.65 **	0.24 ^{ns}	306.2 **	45.1 **	904.3 **	30.8 **	0.28 ^{ns}	11.34 **	1020 **
Error	6	295	3.64	1145	27.5	0.25	2.65	8.52	0.28	1.39	2.84	0.32	0.49	0.23
Fertilizing	9	43 **	4.45 ^{ns}	78.9 *	6.05 **	8.05 **	2.58 **	488.8 **	12.88 **	693.6 **	25.6 **	17.42 **	11.19 **	1276 **
Region*Fertilizing	18	27.6 *	4.08 ^{ns}	18.36 ^{ns}	3.13 *	2.12 **	1.76 **	56.1 **	5.74 **	227.7 **	17.2 **	18.52 **	9.82 **	223 **
Error	54	12.58	6.21	12.74	1.79	0.21	0.69	6.16	0.15	1.26	1.35	0.16	0.41	0.23
CV%	-	7.02	12.74	6.92	10.51	9.18	18.70	10.97	7.84	4.92	10.37	4.46	8.35	0.96

^{ns}, no significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$.

Table 2 Mean comparison of effect of region on morphological traits of *Leucojum aestivum* L.

Region	Plant height (cm)	Bud diameter (mm)	Leaf length (cm)	Leaf width (mm)	One leaf area (cm^2)	Leaf number (n/p)	Plant leaf area (cm^2)	Leaf weight (g)	Plant leaves weight (g)	Bulb weight (g)	Flowering stem weight (g)	Root weight (g)	Total weight (g)
Lahijan	57.6 a	19.3 a	52.5 a	12.6 ab	4.62 c	4.36 a	19.4 c	5.94 a	27.2 a	11.7 a	9.00 a	6.96 b	54.3 a
Tonekabon	50.7 b	19.5 a	51.7 b	13.2 a	5.05 b	4.50 a	22.5 b	3.59 c	16.6 c	10.06 b	9.13 a	8.09 a	44.2 b
Langrood	43.2 c	19.7 a	50.5 b	12.4 b	5.41 a	4.53 a	25.8 a	5.36 b	24.6 b	11.9 a	9.19 a	7.94 a	54.2 a

The similar letters in each column indicating no significant difference between the means

Table 3 Mean comparison of the effect of different biofertilizers on morphological traits of *Leucojum aestivum* L.

Fertilizers	Plant height (cm)	Bud diameter (mm)	Leaf length (cm)	Leaf width (mm)	One leaf area (cm^2)	Leaf number (n/p)	Plant leaf area (cm^2)	Leaf weight (g)	Plant leaves weight (g)	Bulb weight (g)	Flowering stem weight (g)	Root weight (g)	Total weight (g)
control	53.4 a	20.4 a	46.7 d	11.7 c	3.77 f	3.90 d	12.7 e	3.48 g	11.6 h	7.88 g	7.12 g	6.27 e	32.7 j
G. mosseae(1)	51.7 ab	18.5 a	47.3 cd	12.5 bc	3.94 ef	3.75 d	14.6 e	4.58 de	17.3 g	9.44 f	8.00 f	6.12 e	41 i
G. Intraradices(2)	53.2 a	18.4 a	50.5 bc	12.9 abc	4.37 de	4.05 cd	19.3 d	4.64 de	19.1 f	10.3 ef	8.42 e	8.02 bc	46.1 g
(1)+(2)	50.3 ab	19.6 a	50.3 bc	13.3 ab	4.43 d	4.44 bcd	22.2 c	6.84 b	35.9 b	11.5 bcd	9.07 d	8.10 bc	64.8 b
Biochar 3	48.4 bc	18.9 a	51.4 b	14.1 a	5.14 c	4.86 abc	24 c	4.96 cd	23.3 d	13.6 a	9.99 c	7.21 d	54.2 d
Biochar 6	51.9 ab	20 a	54 ab	12.9 abc	5.91 b	4.52 bcd	27.6 b	5.09 c	25.2 c	12.5 ab	10.29 bc	8.55 b	55.5 c
Biochar 12	50.2 ab	20 a	53.5 ab	13.2 ab	6.87 a	5.06 ab	39.5 a	7.27 a	39.7 a	12.5 ab	11.16 a	9.54 a	74.1 a
Azospirilium	48.7bc	20a	56.1a	13.8ab	5.42c	5.42a	22c	4.43e	20.9e	10.9de	9.12d	7.74cd	48.7e
Azotobacter	50.8ab	19.4a	52.9ab	11.7c	5.45c	4.54bcd	22c	4.32ef	17.4g	11.1cde	10.58b	8.55b	47.4f
Pseudomonas	46.5c	20.4a	52.9ab	11.7c	4.97c	4.08cd	22c	4.01f	17.4g	12.2bc	7.32g	6.54e	44.4h

The similar letters in each column indicating no significant difference between the means

Table 4 Mean comparison of interactions of different biofertilizers on different regions bulbs of *Leucojum aestivum* L.

Region	Fertilizer	Plant height (cm)	Bud diameter (mm)	Leaf length (cm)	Leaf width (mm)	One leaf area (cm^2)	Leaf number (n/p)	Plant leaf area (cm^2)	Leaf weight (g)	Plant leaves weight (g)	Bulb weight (g)	Flowering stem weight (g)	Root weight (g)
Lahijan	Control	56.5 b-e	49.3 b-f	10.8 g	3.7 jkl	4.5 a-g	14.6 h-k	3.4 hi	14 nop	7.9 lm	6 o	5.3 o	32.7 u
	G. mosseae(1)	57.8 bcd	49.1 b-f	13 a-g	2.9 l	4.7 a-f	11.8 k	4.1 fgh	20.03 hij	8.2 klm	6.7 mn	7 h-m	41.8 r
	G. Intraradices(2)	62.8 ab	54.4 a-d	12 b-g	3.8 jk	4.5 a-g	13.8 ijk	5.5 d	21.2 gh	9.4 i-l	6.5 no	6.7 h-n	43.8 pq
	(1)+(2)	54.9 c-g	50.05 a-f	11.1 fg	4.4 h-k	4.5 a-g	24.9 d	10.7 a	64.5 a	9.8 h-l	7.2l mn	7.3 g-k	89.4 b
	Biochar 3	52 d-h	50.3 a-f	14.3 abc	4.5 hij	5.3 a-d	17.8 f-i	5.5 d	23 fg	14.4 bc	9.7 g	7.5 g-j	54.5 h
	Biochar 6	64.3 a	55 abc	12.3 a-g	5.5 efg	3.6 e-g	22.4 def	5.1 de	23.6 f	10.5 g-k	7.4 lm	9 def	47.3 lm
	Biochar 12	59.7 abc	51.6 a-f	13 a-g	6.8 bc	4.5 a-g	34.8 bc	10.7 a	54 b	16.2 ab	12.7 b	7.8 f-i	90.8 a
	Azospirilium	54.8 c-g	56.6 a	14.6 ab	5.5 efg	4.8 a-f	22.4 def	5.1 de	20.5 hi	11.8 d-h	9.5 gh	6 l-o	47.4 lm
	Azotobacter	56.05 c-f	54.6 a-d	11.8 c-g	4.2 ijk	3.6 d-g	12.8 jk	4.5 efg	13.2 op	11.2 e-i	16.7 a	6.1 k-o	46.6 mn
	pseudomonas	57 bcd	54.3 a-d	12.6 a-g	4.7 ghi	3.3 fg	19.3 efg	4.3 fg	17.6 kl	17.5 a	7.4 lm	6.7 i-n	49.3 k
Tonekabon	Control	56.6 b-e	45.3 f	12.1 b-g	3.8 jk	3.5 efg	12.1 k	2.5 j	7.5 q	6.5 m	7.7 kl	7.2 g-l	28.8 v
	G. mosseae(1)	52.3 d-h	47 ef	12.5 a-g	4.2 ijk	3.5 efg	17.2 g-j	3.1 ij	12.4 p	9.5 i-l	8.4 jk	5.7 mno	36.5 t
	G. Intraradices(2)	51.6 d-i	48.3 c-f	13.5 a-g	3.6 kl	4.3 a-g	19.03 e-h	3.5 hi	17.5 kl	8.7 jkl	9 hij	9 def	44.2 op
	(1)+(2)	51.6 d-i	50.6 a-f	14.3 abc	4.3 ijk	5.1 a-e	23.5 de	3.3 i	17.1 lm	11.2 e-h	9.5 ghi	10 cd	47.5 l
	Biochar 3	50 e-j	50.3 a-f	14.1 a-d	4.5 hij	4.8 a-f	23.1 de	3.5 hi	18 jkl	12.5 c-g	8.6 j	7.3 g-k	46.7 lmn
	Biochar 6	48.6 g-l	55 abc	14.8 a	5 f-i	4.9 a-f	25.03 d	4.3 fg	22.5 fg	12.7 c-f	10.7 ef	5.6 no	51.7 i
	Biochar 12	49.3 f-k	51.6 a-f	13.9 a-e	6.2 cde	5.3 a-d	37.23 b	4.6 ef	27.5 de	13 cde	12 c	8.8 ef	61.5 e
	Azospirilium	49.3 f-k	56.6 a	13.6 a-f	6.3 b-e	5.5 ab	17.8 f-i	3.4 i	13.7 nop	9.2 i-l	11.2 de	9.2 de	43.2 q
	Azotobacter	54.6 c-g	54.6 a-d	11.4 d-g	6.7 bc	4.06 b-g	26.7 d	3.8 ghi	15.5 mn	8.6 jkl	7.2 lmn	12.5 a	44 opq
	pseudomonas	43 k-n	54.3 a-d	11.4 efg	5.6 d-g	3.9 b-g	23.4 de	3.8 ghi	14.8 no	8.5 j-m	6.7 mn	5.5 o	37.7 s
Langrood	Control	47 h-m	45.3 f	11.9 c-g	3.7 jkl	3.6 d-g	11.3 k	4.5 efg	13.5 nop	9.2 i-l	7.6 l	6.3 j-o	36.8 t
	G. mosseae(1)	45 i-m	45.6 f	11.9 c-g	4.6 hij	3 g	14.7 g-k	6.5 b	19.4 h-k	10.5 g-j	8.8 ij	5.6 no	44.7 o
	G. Intraradices(2)	45 i-n	48.66 b-f	13.2 a-g	5.7 def	3.3 fg	25.1 d	4.8 ef	18.7 i-l	12.7c-f	9.76 g	8.3 fgh	50.2 j
	(1)+(2)	44.3 j-n	50.3 a-f	14.2 abc	4.5 hij	3.7 c-g	18.4 f-i	6.5 b	26 e	13.6 dc	10.5 f	7 h-m	57.4 f
	Biochar 3	43.3 j-n	51.6 a-f	13.7 a-f	6.4 bcd	4.4 a-g	31 c	5.7 d	29.1 d	14 cd	11.6 cd	6.8 h-n	61.5 e
	Biochar 6	42.6 k-n	54.6 a-d	11.4 d-g	7.2 ab	5 a-f	35.5 b	5.8 bcd	29.5 d	14.3 bc	12.7 b	11 bc	67.5 d
	Biochar 12	41.6 mn	55.6 ab	12 a-g	7.6 a	5.3 abc	46.6 a	6.4 bc	37.7 c	8.4 j-m	8.7 j	12 ab	70.2 c
	Azospirilium	42 lmn	56.6 a	11.8 c-g	4.4 h-k	5.9 a	25.8 d	4.7 ef	28.6 d	12 d-h	6.6 no	8 fgh	55.5 g
	Azotobacter	41.6 mn	48.3 c-f	11.7 c-g	5.2 fgh	5.8 a	26.5 d	4.6 ef	23.5 f	13.5 dc	7.8 kl	7 h-m	15.8 i
	pseudomonas	39.3 n	47.6 def	11.2 fg	4.5 hij	5 a-f	35.5 de	3.8 ghi	20 hij	10.7 f-j	7.8 kl	7.4 g-j	46.2 n

The similar letters in each column indicating no significant difference between the means

The results of analysis of variance of adsorbed elements showed that a significant difference was observed in the regions in terms of calcium and zinc adsorption.

A significant difference was found in different fertilizer treatments in terms of phosphorus, potassium and iron content at 5% level. Also, the interactive effect of region * fertilizer showed a significant difference in levels of absorbed phosphorus and potassium (Table 5). The mean comparison of elements of regions showed that the lowest potassium (1.4%), and the highest calcium (0.5%) and zinc (30.3 ppm) were related to Lahijan region (Table 6). The mean comparison of elements of fertilizer treatments showed that using Pseudomonas showed the lowest amount of nitrogen absorbed (1.4%) (Table 7). The lowest amount of phosphorus (0.17%) was for *G. mosseae* and the highest phosphorus absorption was for the treatment of 6 t / ha of biochar with a mean of 0.24%. The lowest potassium (1.3%) was related to Azotobacter treatment and consumption of 6 ton / ha biochar had the highest absorption of potassium with a mean of 1.4%. The highest calcium absorption was obtained from Lahijan region with a mean of 0.5%. The highest iron (1880 ppm) was for 3 tons per hectare of biochar. The highest manganese (35.2 ppm) was absorbed by Azotobacter. The results of the interactive effect of region*fertilizer showed that Langrood region bulbs and 6 tons per hectare of biochar had the highest phosphorus absorption (0.33%) (Table 8). Langrood region and consumption of 6 tons per hectare of biochar had the highest percentage of adsorption of potassium among the treatments of the interactive effect of region*fertilizer with a mean of 1.47% (Table 8).

Table 5 Variance Analysis of Effect of Region and Different Biofertilizers on nutrient uptake in *Leucojum aestivum* L.

S.O.V	df	N	P	K	Ca	Mg	Fe	Mn	Zn
Region	2	0.39 ^{ns}	0.003 ^{ns}	0.003 ^{ns}	0.28 *	0.003 ^{ns}	9728 ^{ns}	32.5 ^{ns}	484 **
Error	6	0.19	0.001	0.001	0.06	0.004	1564010	74.6	43.1
Fertilizing	9	0.35 ^{ns}	0.005 *	0.002 *	0.06 ^{ns}	0.005 ^{ns}	1300428 *	62.9 ^{ns}	38 ^{ns}
Region*Fertilizing	18	0.23 ^{ns}	0.004 *	0.002 **	0.05 ^{ns}	0.007 ^{ns}	682336^{ns}	124 ^{ns}	69 ^{ns}
Error	54	0.21	0.002	0.001	0.07	0.006	633981	70.9	76.2
CV%	-	26.1	23.9	2.4	31.7	23.9	29.1	28.7	30.4

^{ns}, no significant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 6 Mean comparison of effect of region on nutrient uptake in *Leucojum aestivum* L.

Region	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Lahijan	1.66 a	0.21 a	1.41 b	0.55 a	0.19 a	1171 a	28.1 a	30.3 a
Tonekabon	1.75 a	0.22 a	1.41 ab	0.40 b	0.17 a	1136 a	30 a	25.5 b
Langrood	1.89 a	0.20 a	1.43 a	0.38 b	0.16 a	1145 a	29.8 a	22.3 b

The similar letters in each column indicating no significant difference between the means

Table 7 Mean comparison of the effect of different biofertilizers on nutrient uptake in *Leucojum aestivum* L.

Fertilizers	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)
control	1.95 ab	0.19 abc	1.41 abc	0.35 a	0.17 a	1112 ab	27.2 ab	24.8 a
G. mosseae(1)	1.54 ab	0.17 c	1.41 abc	0.40 a	0.16 a	953 b	30.5 ab	24.5 a
G. Intraradices(2)	1.98 a	0.20 abc	1.41 abc	0.53 a	0.18 a	880 b	29.2 ab	28.4 a
(1)+(2)	1.68 ab	0.23 ab	1.41 abc	0.3 a	0.16 a	912 b	30.3 ab	23 a
Biochar 3	2.02 a	0.19 bc	1.41 bc	0.46 a	0.14 a	1880 a	28.5 ab	24.8 a
Biochar 6	1.58 ab	0.24 a	1.45 a	0.33 a	0.20 a	1075 ab	25.5 b	25.3 a
Biochar 12	1.75 ab	0.23 ab	1.41 abc	0.60 a	0.22 a	792 b	28.1 ab	27.6 a
Azospirilium	1.88 ab	0.21 abc	1.43 ab	0.42 a	0.15 a	794 b	27.5 ab	29.8 a
Azotobacter	1.85 ab	0.21 abc	1.38 c	0.50 a	0.18 a	1486 ab	35.2 a	26.5 a
Pseudomonas	1.46 b	0.25 a	1.40 abc	0.47 a	0.19 a	1623 ab	30.7 ab	25.8 a

The similar letters in each column indicating no significant difference between the means

Table 8 Mean comparison of the interaction of region in different biofertilizers on nutrient uptake in *Leucojum aestivum* L.

Region	Fertilizer	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)
Lahijan	Control	1.79 a-d	0.17 bcd	1.41 a-f	0.38 b	0.24 abc	1758.7 abc	31.2 a-d
	G. mosseae(1)	1.47 bcd	0.18 bcd	1.42 a-f	0.41 b	0.16 bc	870.3 bc	26.2 bcd
	G. Intraradices(2)	1.76 a-d	0.18 bcd	1.38 def	0.48 b	0.15 bc	934 bc	25.9 bcd
	(1)+(2)	1.46 bcd	0.23 a-d	1.45 abc	0.51 b	0.18 abc	820.3 bc	26.1 bcd
	Biochar 3	2 abc	0.17 bcd	1.42 a-f	0.43 b	0.14 c	1727 abc	25.9 bcd
	Biochar 6	1.49 bcd	0.2 bcd	1.45 abc	0.41 b	0.15 bc	1708 abc	25.1 cd
	Biochar 12	1.72 a-d	0.24 abc	1.39 b-f	1.07 a	0.33 a	953.3 bc	36.7 a-d
	Azospirilium	1.76 a-d	0.19 bcd	1.38 c-f	0.61 ab	0.15 bc	756.7 bc	29.4 a-d
	Azotobacter	2.1 ab	0.24 abc	1.36 f	0.67 ab	0.19 abc	1550.3 abc	30.4 a-d
	pseudomonas	1.04 d	0.27 ab	1.41 a-f	0.59 ab	0.2 abc	634.3 bc	24.1 cd
Tonekabon	Control	2.45 a	0.22 bcd	1.44 a-d	0.34 b	0.14 c	658.3 bc	22.5 cd
	G. mosseae(1)	1.51 bcd	0.16 cd	1.45 ab	0.31 b	0.13 c	658.3 bc	22.9 cd
	G. Intraradices(2)	1.79 a-d	0.25 abc	1.38 c-f	0.61 ab	0.2 abc	722.3 bc	28.4 a-d
	(1)+(2)	1.79 a-d	0.21 bcd	1.37 ef	0.34 b	0.15 bc	1169 abc	37.03 a-d
	Biochar 3	1.99 abc	0.26 abc	1.4 b-f	0.46 b	0.13 c	2187.3 ab	26.9 a-d
	Biochar 6	1.14 cd	0.21 bcd	1.43 a-e	0.35 b	0.3 ab	976.3 abc	29.6 a-d
	Biochar 12	1.77 a-d	0.26 abc	1.44 a-d	0.4 b	0.18 abc	681.7 bc	26.2 bcd
	Azospirilium	1.68 a-d	0.23 abc	1.44 a-d	0.31 b	0.15 bc	749.3 bc	24.1 cd
	Azotobacter	1.61 a-d	0.2 bcd	1.38 def	0.5 b	0.19 abc	1030.7 abc	43.5 a
	pseudomonas	1.79 a-d	0.25 abc	1.41 a-f	0.37 b	0.19 abc	2531.7 a	38.7 abc
Langrood	Control	1.61 a-d	0.19 bcd	1.39 b-f	0.32 b	0.15 bc	919 bc	27.8 a-d
	G. mosseae(1)	1.75 a-d	0.16 cd	1.37 def	0.48 b	0.19 abc	1331.7 abc	42.5 ab
	G. Intraradices(2)	2.39 ab	0.18 bcd	1.45 abc	0.5 b	0.2 abc	986.3 abc	33.4 a-d
	(1)+(2)	1.79 a-d	0.23 abc	1.42 a-e	0.3 b	0.16 bc	747.3 bc	27.9 a-d
	Biochar 3	2.06 abc	0.13 d	1.41 a-f	0.48 b	0.16 bc	1728.3 abc	32.9 a-d
	Biochar 6	1.98 abc	0.33 a	1.47 a	0.24 b	0.15 bc	542.7 bc	21.9 cd
	Biochar 12	1.74 a-d	0.18 bcd	1.42 a-f	0.32 b	0.15 bc	741.7 bc	21.5 d
	Azospirilium	2.19 ab	0.22 bcd	1.45 ab	0.34 b	0.14 bc	878.3 bc	29.1 a-d
	Azotobacter	1.83 a-d	0.19 bcd	1.42 a-f	0.35 b	0.17 bc	1879 abc	31.7 a-d
	pseudomonas	1.54 a-d	0.23 bcd	1.44 a-d	0.46 b	0.18 abc	1704.3 abc	29.3 a-d

The similar letters in each column indicating no significant difference between the means

Discussion

A significant difference was observed in the bulbs of different regions in different traits (Table 1), so that Lahijan region had the highest plant height, leaf length, large leaf weight and plant leaf weight. The highest leaf width, minimum bulb weight, root weight, total plant weight belonged to Tonekabon region. This is while the highest large leaf area and leaf area of a plant belong to Langrood site. Considering the uniform environmental conditions and soil factor used in fertilizer treatments, so it can be concluded that a significant difference can be due to the plant factor including genetics, plant age and bulb size and accumulated nutrients in bulbs, so that in the study of soil factor of regions a difference was observed in the three regions in terms of macro elements such as potassium, phosphorus and organic matter. In a study by Abbaszadeh [32] a difference was reported in *Camphorosma monspliaca* of different regions in terms of morphological traits, the yield of different organs, physiological traits and percentage of elements. Also, Layeghhaghighi *et al*. [33] reported changes in morphophysiological properties, content of essential oil and some elements of *Artemisia sieberi* affected by the change in a region soil. In a study, Ardekani *et al*. [34] reported changes in different traits of *Camphorosma monspeliaca* due to the effect of a region soil, as well as in a study Tajali *et al*. [35] reported changes in the percentage of *Camphorosma monspeliaca* essential oil of different regions.

The mean comparison showed that the results obtained in field conditions were different from the results of evaluation of *Leucojum aestivum* L. in regions' conditions. Therefore, it can be concluded that under crop conditions this plant should be expected to be different from the region, and to make a wild plant as a crop, we should conclude by relying on information obtained from crop conditions such as the field and greenhouse under crop treatments and the plant response to them.

The mean comparison of fertilizer treatments showed that the lowest height was for Pseudomonas. Given that the plant height is affected by the number of cells, cell length and cell size, and the presence of nitrons stimulates the plant growth, so it seems that in other treatments due to the nature of the treatments used, the length growth of the plants is more than that of Pseudomonas treatment. Increasing the plant height has been reported using a variety of organic and biological fertilizers [36,37].

It was also observed that the lowest leaf length was for the control treatment with inoculum with fungi and the lowest leaf width was for the control treatment with inoculum with fungi and Azotobacter (Table 3). Although, in general, we observed the capability to produce some growth promoting hormones, especially the types of auxin, gibberellin and cytokinin [38], involvement in biological stabilization of nitrogen [39], solubility of mineral phosphorus and mineralization of organic phosphate [40], the production of phytohormones and vitamins and the development of the plant's root system in bio fertilizers, different results in different traits in different treatments i.e. in some traits, using 12 tons per hectare of biochar and in some other using bacteria compared to the rest of the treatments (Table 4) could be due to heterogeneity in weight and age of bulbs, the amount of absorbed elements and the response of different traits to fertilizers used. The positive effect of phosphate solubilizers was reported on the number of umbels, and plant height in *Pimpinella anisum* [21]. In *Origanum majorana*, it was observed that inoculation with Pseudomonas flurscence resulted in an increase in the stem length and leaf number compared to the control, which is inconsistent with the results of our study [23]. It has been reported that mycorrhiza on *Artemisia squamosa* had an increasing effect on the plant height, compared to control plants [24], which was similar to the study results.

In the study of yield of different traits it was observed that the highest leaf weight was for Lahijan region (Table 2), among fertilizer treatments, 12 tons per hectare of biochar (Table 3), and for the interactive effect of treatments, Lahijan region had the most bulbs with 12 tons per hectare of biochar and Azospirillum (Table 4). The highest bulb weight of a plant was for Langrood region (Table 2). The highest bulb weight was for biochar treatment (3 tons / ha) (Table 3). The highest total plant height was for 12 tons per hectare of biochar, but the weight of the flowering stem was higher in Azotobacter treatment than in the rest (Table 3). Also, the highest bulb weight was observed in Azotobacter treatment in Tonekabon region (Table 4). In the interactive effect treatments, except for the bulb weight, in other weights, Lahijan region had the highest bulb weight compared to different treatments. Given that, the bulb weight of Tonekabon region was higher, so it seems that the reaction of different ecotypes in the allocation of photosynthetic materials to their different behavioral organs is different. This can be used for production goals using the flower or bulb production approach. Azetobacter species are found under various climatic conditions from very hot to polar regions. Azetobacter, in addition to nitrogen fixation, improves the plant nutrition and growth through increasing the mobility and absorption of nutrients, and in particular the production of plant growth hormones. In addition, the bacterium indirectly helps control plant health through the control of pathogens [41]. Also, the level of nutrients, especially iron and phosphorus, is effective on Azotobacter growth [42]. According to the reports, Azetobacter increases the root length of plants by secretion of indoleacetic acid [14]. Due to the positive effect of Azotobacter on the flowering stem, there are several reports of the positive effect of Azotobacter on the shoot that confirms the results' consistency. In the conducted studies on the use of Azetobacter in *Rosmarinus officinalis*, the plant height, and the number of branches per plant increased significantly compared to the control [43]. For *Plantago ovata*, the application of biofertilizers such as Azetobacter increased the quantitative and qualitative yield of the plant [44]. Azospirillum, in addition to its ability to fix nitrogen, having growth promoting growth improves the root growth, consequently increases the rate of absorption of water and nutrients and thereby increases the yield [45], which is consistent with our study results. In *Curcuma longa*, the plant biological yield increased using Azospirillum lipoferum compared to the control treatment [46]. Using carbon sequestering, CEC soil promoter, soil fertility increased and a structure full of biochar porosity leads to an increase in its specific surface area, so that this property in the soil leads to increased water storage. Also, biochar improves the stability and survival capacity of the nutrient in the soil and prevents its leaching. This can be first due to the trapping of nutrients in the pores of carbon materials and, second, the slow biological oxidation by producing carboxylic groups on biochar margin and increasing nutrients' storage capacity [4]. In a pot study, Chan *et al*. [47] reported that improvement in nitrogen use efficiency in *Raphanus sativus* increased the plant growth using biochar, which is consistent with our study results. In rice cultivation, also a significant positive effect was reported on yield [48]. No significant change was observed in the bulb elements (region factor) (Table 6) and fertilizer treatments (Table 7). In studying the interactive effect of elements, most of the elements showed no very specific trend and the absorption of some elements in biochar treatments could be due to the release of elements from the disease, also, due to the effect of biochar on soil pH [49, 10, 11], the presence of lime in biochar [10], nitrogen release [50, 51], preventing leaching of elements and reducing ammonium and cationic leaching (Mg^{2+} and Ca^{2+}), and repairing soil structure and texture [51]. Also, in some of the measured elements, the control treatment is in the high group, which indicates less nutritional needs of the plant. In a study by Zakerian [52] it was reported that plants grown in natural areas have less nutritional needs than crop plants. Therefore, the results show that for the cultivation of wild plants there is a need for comprehensive study of the plants under crop conditions and we cannot rely on a region results. The seedlings have low absorption rates, which can be due to the lower fertilizer requirement of the plant or the bulb rich in elements that during the growing season supply needs for various elements of the plant, or less nutrient needs of the plant, so its production site is poor and low yield agricultural lands. No difference in some physiological traits indicates that this plant is adapted to different crop conditions.

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