



РАСТЕНИЕВОДСТВО CROP PRODUCTION

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Effect of combined use of fertilizer and plant growth stimulating bacteria *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas* on the quality and components of corn forage in Iran

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Abstract. *Zea mays* variety 704 (single cross) was studied to investigate effect of chemical fertilizers and growth-promoting bacteria on yield and yield components of corn (*Zea mays*). A factorial experiment was conducted in a completely randomized block design with three replications at Tehran-Varamin Research Farm (Iran) in 2017. The treatments were as follows: inoculation of the seeds with growth promoters in four levels: *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas*; *Rhizobium*, *Azospirillum* and *Pseudomonas*; *Rhizobium*, *Azotobacter* and *Pseudomonas*; *Azospirillum*, *Azotobacter* and *Pseudomonas* and use of nitrogen (N) and phosphorus (P) fertilizers at four levels: no use, 1/3, 2/3, and 100 % recommended were applied. The results showed that the use of fertilizer was significant on the traits such as several leaves per plant, number of seeds per row, number of seeds per ear, plant height and forage yield at 1 % level. The results indicated that the highest forage yield of 33.78 t ha⁻¹ was obtained from the interaction between the use of fertilizers and biological fertilizers, *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas*, which was 42 % higher than control.

Key words: growth promoting bacteria, forage corn, fertilizer, Varamin Plain

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Introduction

Application of chemical fertilizers increases plant yields, however, promotes quickly availability of nutrients to plants [1, 2]. A huge amount of mineral nutrients accumulates the soil due to use of synthetic fertilizers and in the long run, causes environmental hazards such as leaching, resulting in groundwater contamination [3, 4]. To meet global demands for crops, farming systems in industrialized countries have undergone profound transformations. On the one hand, high application rates of synthetic fertilizers and manure together with the use of pesticides, irrigation, and short crop rotations have increased yields and have helped to reduce hunger in these countries [5]. Sorghum is one of the most important crop plants whose seeds are used for feeding poultry and its aerial parts after harvest are used for production of silage forage. Highest absorption of nitrogen in corn occurs at the stages of male and female organ formation. Corn requires urgent N uptake during one to two weeks before flowering, and 3-4 weeks of flowering [6, 7]. These soil bacterial species burgeoning in plant rhizosphere, which grow in, on or around the plant stimulate plant growth by a plethora of mechanisms that are collectively known as plant growth-promoting rhizobacteria (PGPR) [8]. Today, due to the untapped use of chemical fertilizers, organic matter of agricultural land has declined in the world and soil composition has become hard and undesirable [9, 10]. Researchers have reported that use of growth promoters, while reducing their intake and increasing efficiency of chemical fertilizers, increases plant growth by increasing N and P absorption [9]. In sustainable agricultural systems, the use of biological fertilizers is important in increasing product production and maintaining sustainable soil fertility. Today, bio-fertilizers are considered as an alternative to chemical fertilizers to increase soil fertility and production of products in sustainable agriculture [11, 12]. Biological fertilizers increase the effects of organic and chemical fertilizers on agricultural production by increasing the activity of growth-promoting bacteria [13]. *Azotobacter*, *Azospirillum*, *Pseudomonas*, and *Rhizobium* bacteria are some of the most important plants' growth promoters. In addition to nitrogen biomass and phosphorus solubilization, the production of significant amounts of growth-stimulating hormones, especially auxin, gibberellin and cytokines during growth and development of plants affects the crop [14]. Several reports on the ability to produce phytohormones by *D-isotropy* PGPR bacteria, including *Azotobacter* bacteria [9], *Azospirillum* [15], as well as *Rhizobium* bacteria [16]. In some cases, it has been observed that levels of nitrogen fertilizers inoculant plants with di-isotropy bacteria have increased growth and development of plants, in which case there are other mechanisms other than nitrogen fixation, including the production of regulating agents such as indoleacetic acid, the reason for the increase in plant growth for this particular study. Many *Rhizobia* species have shown the ability to produce indoleacetic acid (IAA). Increasing concentration of IAA in rhizosphere also leads to an increase in growth and development of plant root system. This, in turn, increases the number of radionuclides, including signals (IAA),

and ultimately, as an expanding ring or loop, generates more amounts of indoleacetic acid and increases growth and yield of the product [17]. The research on sunflower plant showed that simultaneous use of *Azotobacter*, *Azospirillum*, *Pseudomonas* and U.S. cadmium increased the grain yield [18]. A study [19] stated that indigenous *Rhizobium* bacteria can produce the auxin hormone and that this ability is not the same among different species of rhizobia. The most important mechanism of stimulating plant growth by *Rhizobium* strains is the production of Indo-phytonutrient, which results in better root growth, followed by increased water absorption and nutrient uptake, resulting in increased plant growth [20]. In a laboratory study, the researchers stated that inoculation of sorghum seeds with *Rhizobium* bacteria did not fix the nitrogen in the roots, but the bacterium could naturally increase growth hormones such as auxin, cytokine and riboflavin molecules, oligosaccharides and vitamins, which increased root development and increased adsorption of phosphorus [21]. Plant height, dry weight and dry leaves of corn plants increased by inoculation with *Azospirillum* bacteria [20], fresh weight of the aerial part of the plant, leaf number and corn plant height increased by the inoculation of its seeds with the bacteria of the genus *Pseudomonas* [22]. The dry weight of corn (biomass) was increased, with the seeds inoculated with bacteria *A. chroococcum* and *A. brasilense* [23]. The beneficial and plant growth-enhancing effects of PGPR are well reported and explained. PGPR inoculation has increased different crop yields in normal and stress conditions. From the recent literature, PGPR inoculation increased the stress resistance and production of the crops, including tomato [24], lettuce [25], wheat [26]. The authors of [11] reported in a corn study that the use of phosphorus-soluble mycorrhiza and microorganisms reduced consumption of fertilizers by at least 50 %. The purpose of this study was to investigate the effect of combination of chemical fertilizers and plant growth-stimulating bacteria on yield and its components. The yield of corn fodder was recorded in Iran (Tehran, Varamin City) [11].

Materials and methods

In order to investigate the effect of chemical fertilizers and growth-enhancing bacteria on growth stages of corn, forage hybrids of a single cross 704 cultivar were tested at the Tehran-Varamin Research Farm University of Varamin located in the southern region of Tehran City in 2017, using a factorial design in the form of completely randomized blocks and was replicated three times. Geographically, this training farm is located at 51 degrees and 38 minutes north latitude and 35 degrees and 19 minutes east longitude with a height of 920 meters above sea level. The area has warm summers and semi-cold winters. The treatments were as follows: inoculation of the seeds with growth promoters in four levels: B1 = *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas*, B2 = *Rhizobium*, *Azospirillum* and *Pseudomonas*, B3 = *Rhizobium*, *Azotobacter* and *Pseudomonas*, B4 = *Azospirillum*, *Azotobacter* and *Pseudomonas*. Use of nitrogen and phosphorus fertilizers was at four levels: A1 = no use, A2 = 1/3 recommended, A3 = 2/3 recommended, A4 = 100 % recommended. Before the beginning of the experiment and applying the seedlings, soil samples were taken from the soil and, based on the results of the soil test, chemical component treatments were based on 100 % fertilizer recommendation of 230.4 kg N, 69 kg of P and 100 kg of potassium (K) as a pure element, was applied per hectare (Table 1). The cultivar used in this study was a single-

grain hybrid single-grain hybrid 704 (forage) from PueblaSeed and Plant Research Institute (Iran). After creating a groove on the stack manually, non-fungicidal seeds after inoculation with *Azotobacter chroococcum* (strain 5), *Azospirillum lipoferum* (Strain OF) and *Rhizobium leguminosarum* bv. phaseoli in a value of 1 liter per 25 kg of seed and phosphate solubilizing bacterium, *Pseudomonas fluorescens* (Snain P21) in a quantity of 100 g per 25 kg of seed per hectare, based on experimental data of approximately 108 live and active bacteria per ml. All of these bacteria were natural and native to Iranian soils and were isolated and purified by inoculation by the Department of Biological Research of the Iranian Institute of Soil and Water Research, in collaboration with the Agrarian and Technological Institute of RUDN, Moscow, Russia, and inoculated to corn seed (single cross) 704 cultivar.

Table 1

Some physical and chemical characteristics of the soil

| Depth (sm) | Saturation | Neutrazing agents (%) | Electric conductivity (ds/m) | pH | Organic carbon (%) | Nitrogen (%) | Phosphorus (%) | Potassium (%) | Sand (%) | Mud (%) | Clay (%) |
|------------|------------|-----------------------|------------------------------|-----|--------------------|--------------|----------------|---------------|----------|---------|----------|
| 0.30 | 36.5 | 18 | 2.9 | 7.5 | 0.30 | 0.03 | 8 | 180 | 30 | 42 | 28 |

To mix and inoculate seeds, the seed was first applied to a broad and clean plastic, and then gradually spread the appropriate amount of the seed. The seeds were inoculated by a method of stirring. The inoculated seeds were left in the shade and after drying; they were placed at 15 cm intervals in the grooves and covered with dirt. Each experimental plot consisted of 4 cultivars with a length of 6 m. Inter-row spacing was 65 cm and intra-row spacing was 15 cm. The traits such as several leaves per plant, number of seeds per row, number of seeds per ear, plant height and forage yield were evaluated. In the end, two lateral and half-lines from the beginning and the end of each row in each plot were eliminated as marginal effects, and the sampling was performed from two midpoints. For this purpose, in corn grain pulp (R4), 10 berets were harvested from each plot and agronomic traits were measured. Forage harvesting was also taken from two rows in the middle after removing the half-meter marginal effects from the beginning and the end of each plot in one row and the forage yield was calculated. Statistical analysis of data was done by SAS software. For comparing the means, Duncan's multi-domain test was used at 5 % probability level.

Results and discussion

Plant height

Based on the results of the analysis of variance, plant height was affected by the use of chemical and biological fertilizers at 1 % level and their interaction effects were significant at 5 % level (Table 2). The results of the main effects showed that with the increasing use of fertilizer, the plant height also increased so that the highest plant height with an average of 208 cm was related to the recommended level of fertilizer and the lowest of it with an average of 185.08 cm belonged to non-fertilizer treatment. Among the different levels of biofertilizer application, the highest plant height with a mean of 201.25 cm was related to the treatment of *Rhizobium*, *Azospirillum*,

Azotobacter, and *Pseudomonas*. The concentration of *Rhizobium*, *Azospirillum*, and *Pseudomonas* was in the range of 194.87 cm (Table 3). The results of comparison of the mean effects of interaction of traits showed that the highest plant height with an average of 201.66 cm belonged to the fertilizer application based on the recommended amount of fertilizers. Bioassay, *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas*, and the lowest plant height with an average of 180.50 cm, was related to non-fertilization and biodiversity use of *Rhizobium*, *Azospirillum* and *Pseudomonas* (Table 4). It seems that the use of biofertilizers has a positive effect on plant growth (plant height). Zahir and colleagues observed an increase in the height of the 704 maize plant corn that was inoculated with *Azospirillum* bacteria [14]. In addition, an increase of 8.5 % was reported in corn plant height, which seeds were inoculated with *Azospirillum* and *Pseudomonas* [27]. In a study, Radha *et al.* reported an increase in the height of the corn plant inoculated with *Azospirillum lipopherom* [20].

Table 2

Results of variance analysis of traits

| Source of changes | De-grees of freedom | Average of square | | | | | | |
|------------------------------|---------------------|---------------------|---------------|------------------------------|-------------------------|-------------------------|-------------------------------------|--------------|
| | | Plant height | Stem diameter | Number of the row in per ear | Number of seeds per row | Number of seeds per ear | Number of active leaves in the bush | Fodder yield |
| Repetition | 2 | ^{ns} 203/0 | **5.02 | ^{ns} 65/0 | ^{ns} 25/2 | ^{ns} 06/3607 | ^{ns} 002/0 | **9.00 |
| Chemical fertilizer (A) | 3 | **1518.26 | **46.35 | **6.70 | **244/63 | **126256.73 | **7.16 | **2580.96 |
| Chemical fertilizer (B) | 3 | **106.14 | **29.71 | **0.97 | **38.74 | ^{ns} 00/2070 | **3.30 | **165.25 |
| B × A | 9 | *4.03 | **1.21 | **0.71 | **20.54 | ^{ns} 80/3287 | *0.02 | **7.07 |
| Error | 30 | 1.52 | 0.10 | 0.20 | 5.63 | 2091.95 | 0.006 | 0.56 |
| Coefficient of variation (%) | | 0.6 | 5.41 | 3.01 | 4.93 | 6.24 | 3.61 | 1.2 |

^{ns}, * and **: respectively, are meaningless, significant at a probability level of 5 % and 1 % respectively.

These results are also consistent with Tilak *et al.* (1982), who observed the increase in corn grain yield due to inoculation with *E. coli* and *Azospirillum* Brazilian bacteria [23].

Stem diameter

The results of variance analysis of traits showed that stem diameter was affected by chemical and biological fertilizer application as well as their interaction effects and was statistically significant ($p < 0.05$). Based on the results of the comparison of the mean of the main effects of the traits, with the increase in the use of fertilizer, the stem diameter also increased. So that the highest stem diameter with an average of 25.20 mm belonged to chemical fertilizer application based on 100 % and the lowest stem diameter with an average of 21.58 mm for treatment where no fertilizer was used (Table 3). Among the different levels of biofertilizer, the highest stem diameter with

an average of 24.87 mm was related to the treatment of *Rhizobium*, *Azospirillum*, *Azotobacter*, and *Pseudomonas* (Table 3).

Based on the results of the comparison table, average interaction effects were observed, with the highest stem diameter with an average of 27 mm belong to chemical fertilizer application based on the recommended dose of 2.3 % with *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas* and the lowest stem diameter with a mean of 19.83 mm for non-use of fertilizer and the use of bio-fertilizers of *Rhizobium*, *Azospirillum* and *Pseudomonas* (Table 4).

Number of rows per ear

The results of the table of variance analysis of traits showed that the number of rows per ear was affected by chemical fertilizer, biofertilizer, and their interaction effect, and it was statistically significant at 1 % level (Table 2). Based on the results of comparison of the main effects, the number of rows in the ear increased with increasing the use of chemical fertilizer, so that the highest number of rows in ear with a mean of 15.97 rows belonged to chemical fertilizer application based on 100 %, and the lowest with a mean of 14.24 rows related to non-fertilizer treatment. Among the different levels of biofertilizers, the highest number of rows in the ear with a mean of 15.40 rows was related to *Rhizobium*, *Azotobacter* and *Pseudomonas* consumption and the lowest number of rows in the ear with a mean of 14.77 rows related to *Azospirillum*, *Azotobacter* and *Pseudomonas* consumption (Table 3).

Table 3

Comparison of the mean of the main effects

| Treatment | Plant height (cm) | Stem diameter (mm) | Number of rows per ear | Number of seed in the row | Number of seed in the ear | Number of leaves per plant | Forage yield (t/ha) |
|------------------------------|-------------------|--------------------|------------------------|---------------------------|---------------------------|----------------------------|---------------------|
| Chemical Fertilizer A | | | | | | | |
| Control (a1) | c185.08 | c21.58 | 14.24 d | c41.97 | c597.24 | c12.60 | b47.62 |
| 1/3 Recommended dose (a2) | b193.25 | b21.87 | c15.01 | b47.36 | b710.35 | b13.20 | c51.75 |
| 2/3 Recommended dose (a3) | a208.00 | a25.04 | b15.55 | a51.35 | a797.66 | a14.17 | b74.58 |
| 100 % Recommended dose1 (a4) | a207.54 | a25.20 | a15.97 | a51.64 | a824.88 | a14.17 | a75.25 |
| Bio-Fertilizer B | | | | | | | |
| B1=RZ+AS+AZ+PS | a201.25 | a24.87 | a15.30 | b47.23 | a723.77 | a14.00 | a64.95 |
| B2=RZ+AS+PS | c194.87 | b21.45 | 15.30 a | b47.54 | a730.81 | c12.95 | c56.91 |
| B3=RZ+AZ+PS | b197.20 | c22.87 | a15.40 | b46.81 | a723.94 | b13.23 | b62.79 |
| B4=AS+AZ+PS | a200.54 | b24.50 | b14.77 | a50.47 | a751.61 | a13.95 | a64.54 |

The meanings of at least one letter do not have a significant statistical difference in the Duncan multi-scope test at the 5 % probability level.

The authors of [28] stated that *Rhizobium* bacteria increased root contact in soil by increasing root length and increasing root system in cereals and eventually increasing absorption of nutrients by production of hormones that increased production of photosynthetic material in vegetative stage and its allocation to reproductive organs resulting in an increase in number of rows in the ear. Based on the results of the comparison of the mean interactions effects showed that the highest number of rows per ear

with a mean of 16.8 rows was related to chemical fertilizer application based on 100 % recommended dose plus *Rhizobium*, *Azospirillum* and *Pseudomonas*, and the lowest value was 13.88 rows belonged to the non-fertilization treatment and the use of biological fertilizers *Azospirillum*, *Azotobacter* and *Pseudomonas* (Table 4).

The authors of [29] concluded that combined use of nitrogen fertilizers and inoculum with *Azotobacter*, in addition to increasing soil fertility, improves yield and yield components in plants. Increasing number of ear bean seeds by inoculation of corn seed with *Azospirillum* bacteria also increased airborne dry weight of 42.6 and 67.4 % increase in corn root weight, which seeds were inoculated with growth-enhancing bacteria [19, 30].

Number of seeds per row

Based on the results of the analysis of variance of traits, number of seeds per row was affected by biofertilizer and biological effects as well as their interactions at the level of 1 % (Table 2). The results of the comparison of the mean of the main effects showed that with increase in fertilizer application, the number of seeds per row also increased, so that the highest number of seeds per row with an average of 51.64 belonged to chemical fertilizer application based on 100 % recommended and the lowest with a mean of 41.97 % of seeds belonging to the non-use of chemical fertilizers (Table 3). Among the different levels of biofertilizer use, the highest number of seeds in the row with a mean of 50.74 % belonged to *Azospirillum*, *Azotobacter* and *Pseudomonas*, and the lowest with a mean of 46.81 seeds was related to treatment with *Rhizobium*, *Azotobacter* and *Pseudomonas* (Table 3).

Table 4

Comparison of mean effects of traits

| Treatment | Bush height (cm) | Stem diameter (mm) | Number of rows per ear | Number of seeds per row | Number of seeds per ear | Number of active leaves per pant | Forage yield (ton.ha ⁻¹) |
|--|------------------|--------------------|------------------------|-------------------------|-------------------------|----------------------------------|--------------------------------------|
| (A×B)Bio-fertilizer ×Chemical fertilizer | | | | | | | |
| A ₁ B ₁ | 188.00 E | 22.50 d-e | 14.70 d-g | 40.70 ef | 598.30 fg | 13.00 f | f 49.83 |
| A ₁ B ₂ | f 180.50 | 19.83 h | 14.40 e-g | f 38.16 | 549.60 g | i 11.96 | 44.66 h |
| A ₁ B ₃ | f 182.50 | 21.50 F | 14.00 fg | 42.50 de | 595.00 fg | 12.30 h | g47.00 |
| A ₁ B ₄ | 189.33 e | 22.50 d-e | g13.86 | 46.53 b-d | 646.07 ef | 13.16 e | f49.00 |
| A ₂ B ₁ | c196.33 | c23.50 | 15.56 cd | de42.60 | d-f663.10 | 13.76 bc | 54.33 d |
| A ₂ B ₂ | 189.66 e | 20.83 g | d-f14.83 | 50.46 ab | 749.03 bc | g12.60 | g47.66 |
| A ₂ B ₃ | 193.16 d | 21.00 fg | de15.13 | 45.66 cd | 690.93 c-e | f 12.93 | e01.33 |
| A ₂ B ₄ | 193.83 d | e22.16 | g14.53 e-g | ab50.73 | 738.33 b-d | 13.50 d | d53.66 |
| A ₃ B ₁ | a210.66 | a27.00 | 15.46 cd | a53.03 | 820.20 ab | a14.63 | ab77.33 |
| A ₃ B ₂ | 204.83 b | 22.33 d-e | 15.16 de | ab50.86 | 771.00 a-c | 13.63 cd | 67.83 c |
| A ₃ B ₃ | b206.66 | b24.33 | ab16.33 | 48.93 a-c | ab798.60 | 13.83 b | b76.00 |
| A ₃ B ₄ | a209.83 | a26.50 | 15.23 de | a52.56 | 800.83 ab | 14.60 a | ab77.16 |
| A ₄ B ₁ | a210.00 | a26.50 | cd15.46 | a52.60 | ab813.47 | 14.63 a | a78.33 |
| A ₄ B ₂ | b204.50 | 22.83 d | 16.80 a | ab50.66 | 853.60 a | cd13.63 | c67.50 |
| A ₄ B ₃ | b206.50 | b206.50 | a-b16.16 | ab50.16 | 811.23 ab | b13.86 | b76.33 |
| A ₄ B ₄ | a209.16 | a26.83 | cd15.46 | a53.13 | ab821.20 | a14.56 | a78.33 |

The meanings of at least one letter do not have a significant statistical difference in the Duncan multi-scope test at the 5% probability level.

Based on the results of the comparison of the mean interactions effects, the highest number of seeds per row with an average of 53.13 seeds belonged to chemical fertilizer application 100 % recommended dose with *Azospirillum*, *Azotobacter* and

Pseudomonas, and the lowest number of seeds per row with an average of 38.16 seeds related to non-use of fertilizer and consumption of *Rhizobium*, *Azospirillum* and *Pseudomonas* (Table 4). The increase of 19.8 % of grain yield due to inoculation of maize seeds with *Azotobacter*, and *Pseudomonas* bacteria reported by [27] is consistent with the findings of this research.

Number of seeds per ear

Based on the results of the analysis of variance of traits, number of seeds per ear was affected by the use of fertilizer and was significant at 1 % level, but the use of bio-fertilizer, as well as the effects of biological and chemical fertilizer, had a significant difference in grain number ear did not show up (Table 2). The results of the comparison of the mean of the main effects showed that with an increase in fertilizer, number of seeds per ear also increased so that the highest number of seeds per ear with a mean of 824.88 seeds belonged to the treatment. The use of chemical fertilizer was based on 100 % recommended and the lowest with a mean of 597.24 seeds belonging to non-fertilizer treatment (Table 3). Among the different levels of consumption of biofertilizer also the highest the number of grains per ear with an average of 61.751 grains belonged to *Azospirillum*, *Azotobacter* and *Pseudomonas* treatments and the lowest with 77.732 grains belonged to *Rhizobium*, *Azotobacter* and *Pseudomonas* treatments (Table 3).

According to the results of comparison of mean interaction effects, it was observed that the highest number of kernels per ear with 60.853 seed was related to 100 % recommended fertilizer treatment along with *Rhizobium*, *Azospirillum* and *Pseudomonas* and the lowest with 60.549. Seeds belonged to non-fertilizer treatment and biofertilizer application of *Rhizobium*, *Azospirillum* and *Pseudomonas* (Table 4). This study is consistent with the results of [23], which shows that corn grain yield increased by inoculation with *Azotobacter chroococcus* and *Azospirillum brasilense*. Also, growth of dry weight of plant in millet stage of corn seeds whose seeds were inoculated with *Azospirillum brasilense* bacteria [28].

Number of leaves per plant

Based on the results of the analysis of variance, the number of leaves in the ear was affected by the use of fertilizer and biofertilizers at 1 % level and the effect of chemical and biological fertilizer interaction at the 5 % statistical level (Table 2). Based on the results of the comparison, the average of the main effects with the increase in the use of chemical fertilizer is the number of active leaves in plants. The highest number of leaves per plant with an average of 14.17 leaves belonged to chemical fertilizer application based on 100 % recommended and the lowest with a mean of 12.60 % belonged to non-fertilizer treatment (Table 3). Among the application of different levels of biofertilizers, the highest number of leaves per plant with an average of 14 leaves belonged to *Rhizobium*, *Azospirillum*, *Azotobacter*, *Pseudomonas* and the lowest value was 12/95 for *Rhizobium*, *Azospirillum* and *Pseudomonas* use (Table 3). Based on the results of the comparison, the average interaction effects, the highest number of leaves in the plant with an average of 14.63 leaves for fertilizer application based on 100 % recommended dose with combined use of *Rhizobium*, *Azospirillum*, *Azotobacter*, *Pseudomonas* and the lowest number of leaves per plant with a mean of 11.96 leaves for

non-fertilizer application (Table 4). Treatment with the bio-fertilizers increases the fresh air mass, the number of leaves and height. The corn plants, which were inoculated with *Pseudomonas* bacteria, as reported authors of [31], had an increase in the number of leaves. The authors of the study [18] reported an increase in fresh weight, height and number of sunflower leaves that were inoculated with *Azotobacter*, *Azospirillum* and *Pseudomonas* biodiversity, which is consistent with the results of this research.

More fodder yield

The results of the analysis of variance of traits showed that fresh forage yield was affected by the use of fertilizer; bio-fertilizer and their interactions were statistically significant at 1 % (Table 2). Based on the results of the comparison, the average of the main effects of traits increased with increasing fertilizer use of forage yields as well the highest forage yield (75.25 t ha⁻¹) belonged to chemical fertilizer application based on 100 % recommended and minimum forage yield with mean of 64.95 t ha⁻¹ was related to non-fertilizer treatment (Table 3). Among the different levels of biofertilizers, the highest forage yield with a mean of 64.95 t ha⁻¹ was related to the combined application of bio-fertilizers of *Rhizobium*, *Azospirillum*, *Pseudomonas* and *Azotobacter*, and the lowest forage yield with an average of 56.91 t ha⁻¹ *Rhizobium*, *Azospirillum* and *Pseudomonas* were used for treatment of biological fertilizers (Table 3). Based on the results of the comparison of the effects of the mean interaction, the highest forage yield with average of 78.33 t ha⁻¹ was chemical fertilizer application based on 100 % recommended diet plus biofertilizer, *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas*, and the lowest forage yield with 44.66 t ha⁻¹ mean of non-fertilizer application and use of biological fertilizers of *Rhizobium*, *Azospirillum* and *Pseudomonas* (Table 4), which was treated with 2/3 fertilizer with 4 types of bacteria and 2/3 of chemical fertilizer along with the *Azotobacter*, *Azospirillum* and *Pseudomonas* bacteria with a yield of 77.16 t ha⁻¹ was considered statistically significant. According to the mean comparison table, the use of 2/3 fertilizer with four species of forage increased the forage yield compared to non-fertilizer treatment, combined with *Rhizobia* bacteria, *Azospirillum*, and ascorbate 42 %. On the other hand, with application of four types of bacteria, use of chemical fertilizers decreased by 25 %, without reducing yields, which could be an effective step towards sustainable agriculture. Inoculation of corn with *Azotobacter* bacteria increased yields. In [18] it is also reported that inoculation with biological fertilizers increased the rate of crop growth. They considered the increase in crop growth rate to improve the absorption of food by the plant. Positive effects of *Azotobacter* on wheat growth and yield have been reported. The authors of [32] have reported the positive effects of this bacterium on corn. Quantitative analysis is a method for justifying and interpreting plant reactions relative to different environmental conditions during its growth stage, through which the transposition and accumulation of the products of photosynthesis in different organs can be determined by measuring the amount of dry matter produced [32]. Besides, some researchers reported an increase of 33 % in fresh weight of corn inoculated with *Pseudomonas spp.* [33]. These results are consistent with the findings of other researchers regarding the application of biological fertilizers [34].

Conclusions

In general, the application of livestock manure and biological fertilizers can increase soil organic matter and, consequently, improve soil structure, increase cation exchange capacity, microorganisms, activity, gas exchange, and water storage capacity. The positive effects of fertilizer combinations with organic and biological fertilizers on growth previously confirmed for other crops are also true for corn. In addition, the results of this study showed that growth-stimulating bacteria have a positive role in absorption and stabilization of essential elements required for plant and can significantly reduce use of synthetic fertilizers, which ultimately maintains plant performance along the lines of agriculture. Sustained when the fertilizer is completely consumed, these bacteria can be a good alternative to reducing the use of chemical fertilizers in the fields and improving the environment.

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Влияние комбинированного использования удобрений и ростостимулирующих бактерий *Rhizobium*, *Azospirillum*, *Azotobacter* и *Pseudomonas* на качество и состав кукурузного корма в Иране

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Аннотация. Для исследования влияния химических удобрений и ростостимулирующих бактерий на урожайность и качество зерна кукурузы (*Zea mays*) сорта 704 (одиночный кросс) был проведен факторный рандомизированный блочный эксперимент с тремя повторностями в 2017 г. Исследовательская ферма Варамин находится в Тегеране, Иран. Обработку семян стимулятором роста проводили в четырех комбинациях: *Rhizobium*, *Azospirillum*, *Azotobacter* и *Pseudomonas*; *Rhizobium*, *Azospirillum* и *Pseudomonas*; *Rhizobium*, *Azotobacter* и *Pseudomonas*; *Azospirillum*, *Azotobacter* и *Pseudomonas* — на фоне применения азотных N и фосфорных P удобрений в четырех вариантах: без удобрений, 1/3, 2/3 и 100 % рекомендуемой концентрации. Результаты исследований показали, что использование удобрений оказало значительный эффект на такие параметры, как количество листьев на одно растение, количество семян в ряду, количество семян на колосе, высота растения и урожайность кормов на уровне 1 %. Лучшая кормовая урожайность 33,78 т/га была получена при комбинированном использовании удобрений и биологических ростостимулирующих препаратов на основе *Rhizobium*, *Azospirillum*, *Azotobacter* и *Pseudomonas*, что оказалось на 42 % выше, чем в контроле.

Ключевые слова: ростостимулирующие бактерии, кормовая кукуруза, удобрение, Варамин

БЛАГОДАРНОСТИ

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