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Increasing the efficiency of *Glomus mosseae* and ACC-deaminase producing bacteria in chickpea by plant residue management

Reza Soleimani *[a]

[a] Soil and Water Research Department, Ilam Agricultural and Natural Resources Research and Education Center, AREEO, Ilam, Iran

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ABSTRACT

Nowadays, managing the production of crops under rainfed conditions for achieving maximum growth potential and yield of plants for food supply is an undeniable necessity. Under rainfed condition, one of important variability in plant physiology is increase of ethylene biosynthesis up to stress ethylene that reduces plant growth. Stress ethylene cause to decline in vegetative period and decrease of yield. Mycorrhiza and ACC-deaminase producing bacteria decline the destructive effects of water deficit stress under rainfed condition. In order to determine the effectiveness of residue conservation and application of *Glomus mosseae* and ACC-deaminase producing bacteria in reducing the effects of water deficit stress in rainfed conditions, this study was conducted as split plots in a randomized complete block design (RCBD) in three replications from 2017 for two cropping years. The main plots include three different residue conservation treatments including residue removal, half residue conservation and total residue conservation and sub-plots at four levels including 1- control (no use of *Glomus mosseae* and ACC-deaminase producing bacteria), 2- application of *Glomus mosseae* 3- inoculation of ACC-deaminase producing bacteria (preparation of Bacillus simplex UT1 inoculation with 107 CFU ml-1 population or colony unit formed in ml and method of seed coating application 4- concomitant use of *Glomus mosseae* and ACC-deaminase-producing bacteria. Quantitative traits of chickpea such as grain and biological yield, 100-seed weight and number of seeds per square meter and quality traits as relative leaf water content (RWC), concentration and total uptake of potassium and zinc as two important elements in water relations were measured in plants. Data were analyzed by SAS v.9.2 software and the means were compared by LSD test. The results showed by keeping half of the residues, 1224 kg/ha of seeds of chickpea was obtained, which showed an increase of 7.81% compared to the control. The yield of chickpea seeds in the treatment of without *Glomus mosseae* and ACC-deaminase producing bacteria was 1097 kg ha^{-1}, while the highest yield of chickpea in the treatment of *Glomus mosseae* and ACC-producing bacteria at the amount of 1294 kg ha^{-1}, which was a statistically significant increase of 17.9% compared to the control. Under residue preservation condition, even the application of ACC-deaminase-producing bacteria alone and the application of *Glomus mosseae* alone showed an increase of 10.3% and 13.1%, respectively, compared to the control. In general, the combined application of *Glomus mosseae* and ACC-deaminase producing bacteria increased yield, yield components and improved nutrient concentration compared to the control treatment. Based on the results of this study, in treatments of residue preservation, the use of *Glomus mosseae* is recommended, especially in combination with inoculation of ACC-deaminase producing bacteria in order to stabilize the production of chickpeas.

Highlights

- The half plant residues improved mycorrhiza and bacteria inoculation efficiency
- Deletion of plant residues reduced microbial inoculation efficiency

* Corresponding author.
E-mail address: soleimanir@ut.ac.ir
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- Plant residue favored nutrient enrichment in plant tissue
- The maintenance of half of plant residues is recommended.

1. Introduction

The researchers reported that the change in plant residue management has a great impact on the soil characteristics and finally the plant yield (Xu et al., 2022). Minimum tillage, at least by preserving the residues, leads to the improvement of the physical characteristics of the soil and the increase of soil moisture (Popolizio et al., 2022). Optimum preservation of plant residues in rainfed condition, through better moisture retention, can have a positive effect on plant yield (Sarangi et al., 2022). Cultivation systems without proper management of residues, creating unfavorable biological and chemical conditions in the soil, reduce the amount of organic carbon and nutrients in the soil (Srinivasarao et al., 2023). The results of various experiments indicate a significant effect of crop rotation on the amount of inorganic and organic nitrogen in the soil and the mineralization process of soil organic nitrogen and residual nitrogen (Singh et al., 2021). In this regard, researchers reported that the use of deep plowing leads to a faster loss of soil moisture and ultimately to a decrease in grain yield, and in contrast to systems without tillage and surface plowing, it leads to a decrease in evaporation (Du and Effah, 2022). Therefore, it can be stated that crop management and tillage methods play an important role in the physical properties and water movement in the soil. By preserving part of the plant residues, conservation agriculture effects on some soil properties such as temperature, storage and distribution of moisture in the soil and soil compaction (Garcia-Tejero c, 2020). In the long term, addition of plant residues compared to burning of the residues increased the yield of the plant by 50%, and this yield increase was the result of improving the dynamics of nutrient elements and biological characteristics of the soil (Korav et al., 2024).

Some non-biological factors, including drought stress, affect the growth and performance of plants, so that minimum bioproductivity is considered a fundamental problem, at least in regions that affected by drouth stress (Anbukkani et al., 2017), so that drought stress in rainfed fields is the most important abiotic factor limiting the growth and production of crops in Iran. Drought is a multi-dimensional stress and is effective at different levels. At the plant level, the response to water deficit stress has a special complexity and is a reflection of the combination of stress effects caused by the lack of water available to the root and the responses at physiological levels of the plant (Tranker et al., 2016). Drought stress induces cell wall destruction and electrolyte leakage from the cell wall (Alzahrani et al., 2018). This stress has an effect on hormonal and physiological changes, and the resulting oxidative damage as one of the important factors limiting plant growth and production (Khan et al., 2020). Oxidative damages and disruption in the production of free oxygen radicals resulted in alteration and destruction of proteins, membrane lipids and other cellular components (Rahman et al., 2021). Reactive oxygen species (ROS), which are an important factor in inducing drought stress, unlike atmospheric oxygen, have unlimited ability to oxidize different cellular components and can lead to oxidative destruction of cells (Puthur, 2016). Therefore, in the process of tolerance to drought stress, these reactive oxygen species should be inhibited, which is possible to some extent with changes in plant physiology (Caverzan et al., 2016).

One of the most important changes in plant physiology affected by drought stress is the increase in ethylene biosynthesis (stress ethylene). Stress ethylene reduces the vegetative period and ultimately decreases of yield. Ethylene produced by the plant in normal conditions helps to seed germination, development of initial roots, stem elongation and fruit ripening. Ethylene is involved in the stages of seed germination, photosynthesis, transpiration and root development (Danish and Zafar -ul-Hye, 2020). But in their studies, the researchers found the fluctuation in production and contradiction in ethylene function in plants. Low ethylene (at concentrations of 0.05 micrograms per liter of plant extract) is required for seed germination, but further increase in ethylene levels due to environmental stresses reduces plant growth (Glick, 2005). By examining the water stress in plants, it concluded that any type of water stress increased the synthesis of ACC and thus stimulates ethylene biosynthesis. By reducing the ACC concentration on the outer surface of the plant's root tissues, to balance the ACC level inside and outside the plant's root cells, more of the plant's ACC outdoors (in the soil) secreted and created a slope. Concentration from inside the plant tissue reduces the concentration of ACC and thus the ethylene concentration within the plant's root tissues.

With this process, ACC-deaminase-producing bacteria reduce stress ethylene production and its adverse effects on plant growth, so plant plants decrease, especially in early growth (Reed and Glick, 2023). Water stress increased the synthesis of ACC and thus stimulates ethylene biosynthesis. By reducing the ACC concentration on the outer surface of the plant's root tissues, to balance the ACC level, more ACC secreted to the soil. ACC-deaminase-producing bacteria consume ACC and reduce stress ethylene production by above mechanism (Reed and Glick, 2023). Water stress decreases the relative content of water in the leaves by increasing ethylene biosynthesis and reducing the activity of some enzymes (Gowtham et al., 2022). Some fungi and bacteria affect the process of stress ethylene in the plant. Some soil bacteria produce the ACC-deaminase, as they are called ACC-deaminase producing bacteria (Gamalero et al., 2023). This enzyme converts ACC (pre -production of ethylene) to alpha ketobotyrate and ammonium. By adjusting ACC production in plants by ACC-deaminase enzyme producing bacteria, the amount of ethylene production in the plant is prevented. By reducing the ACC concentration at the outer surface of the plant's root tissues, to balance the ACC level inside and outside the plant's root cells, more than the plant's ACC is released out of the root (in the soil), and is also secreted. Creating a concentration slope from the inside of the plant to the outside reduces the ACC concentration and consequently the ethylene concentration within the plant's root tissues. With this process, ACC-deaminase enzyme producing bacteria reduce stress ethylene production and its adverse effects on plant growth so that plant plants decrease (Reed and Glick, 2023). Studies have shown that the percentage of mycorrhiza colonization and dehydrogenase enzyme increased in 0- 30 cm deep as affected by biofertilizer treatment (Wilkes et al., 2021). Mycorrhiza increase the dry weight of the chickpea aerial parts significantly compared to control (Laranjeira et al., 2021). The concentration and absorption of nutrient elements increase in the presence of mycorrhiza. Therefore, improving nutritional conditions along with other beneficial effects of mycorrhiza can moderate the negative effects of drought and increase plant growth and performance. Plants with mycorrhizal inoculation increased their tolerance to water deficit (Ezzati Lotfabadi et al., 2022).

In both greenhouse and field conditions, AM fungi significantly increase the concentration of nutrients such as nitrogen, potassium, phosphorus, calcium, magnesium, manganese, copper, iron, zinc, sodium and chlorine, which it increases plant growth and also has an inhibitory effect on the absorbed sodium to chlorine ratio (Chandrasekaran, 2022). In the study of the effect of inoculation of ACC-deaminase producing bacteria on the growth and yield of wheat, it was found that the bacteria with this enzyme improved grain yield, straw, root weight, root length and absorption of nitrogen, phosphorus and potassium in straw and grain compared to the control. They considered all these effects due to ACC reduction of ethylene level in the plant. As a result of inoculation with bacteria containing ACC-deaminase enzyme, they announced that the activity of the enzyme is variable in different isolates. ACC-deaminase enzyme is directly responsible for different behaviors in plant response to water stress and the use of endophytes with ACC-deaminase activity has the potential to facilitate plant growth (Etesami et al., 2020).

In terms of water resources, Iran is one of the countries with deficit water conditions. On the other hand, agriculture in the country has several challenges. The problem of soil erosion and decreasing its fertility will significantly reduce the yield of crops and will increase production costs. The benefits of conservation agriculture are not limited to increasing the yield of crops. The use of conservation agriculture improves the physical, chemical and biological characteristics of the soil, which can be important in achieving sustainable agriculture. Mycorrhiza and ACC-deaminase producing bacteria increase plants' tolerance to drought stress. Although some researches have been presented regarding the physical and chemical changes of soil under different types of tillage systems, but limited information is available on the changes in the preservation of residues in rainfed conditions and especially its relationship with the inoculation of mycorrhiza and beneficial bacteria. On the other hand, cereals and legumes are the most important plants in the rotations of the rainfed regions. Therefore, the need to implement the current project was strongly recommended. The low performance of crops such as chickpea requires the use of different agricultural management. The use of biological methods, including the use of mycorrhiza and beneficial bacteria, especially in the conditions of water deficit stress prevailing in rainfed farms, are considered as methods of improving the conditions of growth and development of plants in conditions of water deficit stress. By causing changes in the rhizosphere of the plant and in the morphology of the root, as well as the ACC-deaminase producing bacteria, by reducing the stress ethylene in the plant, mycorrhiza increase the tolerance of the plant to the stress conditions in dry conditions.

Due to the low efficiency of inoculation of fungi and beneficial bacteria due to the lack of usable water and low organic matter in the dry conditions, in conservation agriculture due to the preservation of residues, better conditions are expected to increase the efficiency of the inoculation. Therefore, this research was carried out in order to investigate the effect of preserving residues, mycorrhiza and ACC-deaminase producing bacteria under rainfed conditions.

2. Materials and methods

2.1. Experimental location and design

The location of this research was in Ilam province with geographical coordinates of 33 degrees 45 minutes and 36 second as north latitude and 46 degrees 35 minutes and 59 seconds as east longitude. First, in the fall (before any agricultural operation), soil samplings were done to measure physical and chemical properties including electrical conductivity, acidity, organic carbon, nitrogen, phosphorus, potassium, iron, zinc, copper, manganese, lime and texture. The results of the analysis of soil at the experimental site are shown in Table 1. Then the land was plowed when the soil moisture was at the level of field capacity (FC) and immediately prepared with a disk. In order to investigate the effectiveness of the mycorrhiza (*Glomus mosseae*) and ACC-deaminase producing bacteria (*Bacillus simplex* UT1) in different residue management (without residues and with residues) this research was carried out in Cherdavel research station of Ilam province. Experiment was done in the form of split plot in the design of randomized complete blocks. Experimental treatments include management of residues in the main plots (1- no residues, 2- keeping half of the residues, 3- keeping all the residues) and the use of biological fertilizers in the sub-plots (1- *Glomus mosseae* fungus, 2- ACC-deaminase producing bacteria (*Bacillus simplex* UT1) 3- *Glomus mosseae* and ACC-deaminase producing bacteria (*Bacillus simplex* UT1) 4- No use of fungi and bacteria) with three repetitions.

2.2. Preparation of materials and samples

The bacterial inoculants were obtained from the Tehran University with a population of 10^7 CFU ml^{-1} (colony formed per milliliter) and the application method was the seed coating. The steps for preparing the bacteria used were as follows. Using a global positioning system (GPS), location and sampling of rhizosphere soils under wheat cultivation in the conditions of water deficit stress in Ilam province were carried out. In the separation stage, a homogeneous soil suspension was prepared. In this way, 10 grams of soil from the rhizosphere soil obtained by vigorously shaking the roots in a sterile solution of 0.9% sodium chloride to 250 ml Erlenmeyer flasks. These flasks containing 90 ml of sterile distilled water. (Barillot et al., 2013). Then, dilution series (from 10^{-2} to 10^{-9}) were prepared and 0.1 ml of it was spread on petridishes containing culture medium. In order to purify the isolates, the colonies formed from the highest dilutions of each cultured sample were selected and recultured up to three times on the culture medium (Harrigan and McKean, 2004).

The method of Penrose and Glick (2003) was used for the quantitative measurement of alpha-ketobutyrate as an indicator of ACC-deaminase enzyme activity. To evaluate the tolerance of isolates to different levels of dehydration stress, their growth ability in NB culture medium containing different concentrations of polyethylene glycol 6000 was used. Before carrying out this research, by using several stages of testing and comparing the isolates, a bacterium belonging to the *Bacillus simplex* was isolated from the soils of farms with rainfed conditions of Ilam. This bacterium had the characteristics of a superior bacterium for water deficit conditions. The results showed that this bacterium showed a growth trend similar to ammonium consumption due to the decomposition of ACC. The ratio of the colony diameter of *Bacillus simplex* bacteria with ACC decomposition to its diameter with ammonium consumption was equal to one, and also this bacterium was able to produce 380 nanomoles per milligram of protein per hour of alpha-ketobutyrate as a measure of ACC-deaminase enzyme activity and ACC decomposition.

The relative water content (RWC) was measured by selecting the youngest developed leaves from each replicate. In the laboratory, the fresh weight was determined and then the leaves were placed in distilled water for 24 hours at room temperature and in the dark condition, then the turgor weight was determined. In the next step, the leaves were placed in an oven at 70 ºC for 72 hours and dried. The relative amount of water was measured by selecting the youngest developed leaves from each replication. In the laboratory, the fresh weight was determined and then the leaves were placed in distilled water for 24 hours at room temperature and in the dark, and then the turgor weight was determined. In the next step, using dry weight and complete turgor (after 24 hours of leaf floating in distilled water), the relative weight of water was calculated. (Ritchie et al., 1990).

Table 1. Physical and chemical analysis in depth of 0-30 cm of soil

Year	pH	EC	Ava. P	Ava. K	OC	TN	TNV	Texture
		(dS m^{-1})	(mg kg^{-1})			(%)		
First Year	7.21	0.23	14.1	326	1.35	0.13	21.3	SiCl
Second Year	7.25	0.31	12.7	311	1.12	0.11	23.6	SiCl

Glomus mosseae inoculum includes root remains and fungal organs, sand and soil. The use of mycorrhiza will be done before planting (300 grams per planting line). Then the seeds will be placed on the inoculants. Finally, the seeds and inoculants were covered with soil. The chickpea variety, Adel, with a density of 30 plants per square meter, and all procedures were performed and plant harvesting will be done. In this research, the investigated parameters were seed yield, harvest index, total biomass, 100-grains weight and the number of pods per plant. Analysis of variance and mean comparison were performed with SAS 9.2 program and Duncan's multi-range test, respectively.

3. Results and discussion

Among the superior bacterial isolates with different abilities in terms of ACC-deaminase producing bacteria, a comparison was made. In this experiment, by creating the same conditions (with FC=60%) changes in the dry weight of wheat plants due to inoculation with different isolates were investigated. With this test, it was found that a bacterial isolate, as the superior bacteria with ACC-deaminase producing ability had a more suitable performance than other isolates. After carrying out the genetic identification steps, the results of determining the sequences were checked by Blast software and according to the degree of affinities, it was determined that there is a 99% probability that this bacterial isolate belongs to the *Bacillus simplex* species. The BankIt gene was registered with Accession Number: KT599261. Based on electronic correspondence, this bacterium was included in the mentioned gene bank as *Bacillus simplex* strain UT1.

3.1. Grain yield and biological yield

The main effect of residue management on grain yield and biological yield was significant at the level of 1% and 5% percent, respectively, and had no significant effect on the harvest index. The main effect of biofertilizer application on seed yield, biological yield and harvest index was significant at the level of 5%, 1% and 5%, respectively (Table 2). The yield of chickpea seeds increased from 1137 kg ha^{-1} in the control, with an increase of 8.53%, to 1234 kg ha^{-1} in the condition of preserving half of the residues, and this increase in biological yield was equal to 8.20%. According to these changes in grain and biological yield, the harvest index increased from 44.6% in the condition without residues to 44.8% in the preserving the residues condition (Table 3). In the same case, the results of the researches showed that in an experiment in the conditions of preservation of residues, the use of mycorrhiza increased the chickpea yield while reducing the effects of drought stress (Moradtalab et al., 2019). Other researchers showed that bacteria with the ability to ACC-deaminase producing bacteria were able to significantly increase o root length (Chandra et al., 2018). The use of *Glomus mosseae* in the condition of preservation of residues increases the activity of antioxidant enzymes (superoxide dismutase, catalase and glutathione reductase) (Gong et al., 2005).

Table 2. Variance analysis (Mean square) of chickpea yield, yield components and RWC under residue management and AM and ACC-deaminase producing bacteria

S.O.V	df	Grain yield	Biological yield	100-grains weight	Grains/m^2	RWC %
Year	1	1243 ^{ns}	1053 ^{ns}	2107 ^{ns}	1382 ^{ns}	825 ^{ns}
R(Y)	4	5107304 ^{ns}	38055 ^{ns}	1203 ^{ns}	165 ^{ns}	726 ^{ns}
(M)	2	13067949 **	120654376 *	21053647**	655427 *	6633232 *
Y*M	2	43543 ^{ns}	12074 ^{ns}	1047 ^{ns}	653 ^{ns}	638 ^{ns}
E1	4	21327 ^{ns}	14508 ^{ns}	457 ^{ns}	763 ^{ns}	736 ^{ns}
F	3	20505572*	456301239 **	13065376*	8755436 **	7665534 *
Y*F	3	35734 ^{ns}	1546 ^{ns}	126 ^{ns}	528 ^{ns}	364 ^{ns}
M*F	6	12465 *	11653142 *	45321*	7676632 *	7675534 *
Y*M*F	6	16544 ^{ns}	37644 ^{ns}	543 ^{ns}	866 ^{ns}	486 ^{ns}
E2	36	4114	3215	543	761	628
CV%**%**		16.6	18.3	15.7	15.4	17.8

ns, * and **, non-significant and significant difference at 5% and 1% probability levels, respectively.

Table 3. The effect of residue maintenance on grain yield and yield components

Residue management	Grain yield (Kg/ha)	100-grains weight (gr)	Grains/m^2	RWC (%)
Without residue	839b	21.2c	436b	59.5c
Semi-residue maintenance	934a	a 25.5	473a	68.7a
Total residue maintenance	897b	b 24.3	451b	63.5b

In each column, the similar letter/letters indicated non-significant differences among means

3.2. The effect of application of *Glomus mosseae* and ACC-deaminase producing bacteria on changes in grain yield and relative leaf water content

The main effect of biofertilizer application on grain yield was significant ($p<0.05$) (Table 2). By comparing the mean of the data using the LSD test method, it was determined that the treatment with the use of mycorrhiza and ACC-deaminase producing bacteria with a seed yield of 945 kg ha^{-1} was in the superior statistical group and the yield increase in comparison with the control was equal to 12.4% (Table 3). The researchers stated that mycorrhiza indirectly affects the physiological activities and the growth of the plant and increases the yield (Dorairaj et al., 2020). Under rainfed condition, drought stress naturally causes a significant decrease in chickpea yield and yield components. Since the decrease in water availability for the plant caused a decrease in yield, the use of *Glomus mosseae* and ACC-deaminase producing bacteria could increase seed yield by expanding the root system (Danish and Zafar-ul-Hye, 2019). According to researches, *Glomus mosseae* helps to create drought tolerance in plants by modulating changes in radial hydraulic conductivity (Quiroga et al., 2020). Without residues maintenance, the combined application of *Glomus mosseae* and ACC-deaminase producing bacteria had the greatest effect on increasing yield, followed by the sole application of ACC-deaminase-producing bacteria. In conditions with half of the residues preserved, the combined use of *Glomus mosseae* and ACC-deaminase producing bacteria is about twice more effective than the use of *Glomus mosseae* and ACC-deaminase producing bacteria alone, and in such conditions, this treatment can be recommended. In the conditions with preservation of all the residues, the combined application of *Glomus mosseae* and ACC-deaminase producing bacteria and then the application of *Glomus mosseae* had the best results. The combined application of *Glomus mosseae* and ACC-deaminase producing bacteria showed the greatest effect with an increase of 12.9% in grain yield. In both years, keeping half of the residues caused a significant increase in chickpea yield and yield components. The increase in grain yield as a result of preserving residues has been reported by researchers, and the main reasons for the increase in plant yield in the condition of preserving residues are the increase in the area of the main photosynthesizing organ (leaf), the increase in the number of flowers (the main reproductive organs), the increase in transfer assimilate and photosynthesis and the weight of seeds due to the improvement of humidity conditions (Shu et al., 2022).

3.3. The weight of one hundred seeds and the number of seeds per square meter

Variance analysis of the data showed that the main effect of preserving residues on the weight of one hundred seeds and the number of seeds per square meter was significant at the level of 1% & 5%, respectively (Table 2). The weight of one hundred seeds in the condition of preserving half of the remains (23.2 grams) showed an increase of 9.41 percent compared to the control (22.3 grams) and this increase in the number of seeds per square meter was 8.14 percent (Table 3). Also, the variance analysis of the data showed that the main effect of biofertilizer consumption on the weight of one hundred seeds and the number of seeds per square meter was significant at the level of 5% and 1%, respectively (Table 5). Regarding the weight of 100 seeds, it was found that the combined use of mycorrhiza and ACC-deaminase producing bacteria was the best treatment by increase of 9.23% compared to the control. However, treatments of mycorrhiza alone and inoculation with ACC-deaminase-producing bacteria alone were not statistically higher than the control group (Table 3). The number of seeds per square meter reached 472 with the combined application of mycorrhiza and ACC-deaminase producing bacteria, which was in the superior statistical group compared to the non-application of biofertilizer (with an increase of 12.4%). The treatments of the sole use of *Glomus mosseae* and ACC-deaminase producing bacteria were not statistically higher than the control (Table 4). The variance analysis of the data also showed that the interaction effect of preserving residues in the use of biofertilizers on the weight of 100-grains and the number of grains per square meter was significant at 5% level and had no significant effect, respectively (Table 2). In the conditions with preservation of half of the residues and preservation of the whole residues, the combined application of ACC-deaminase producing bacteria and *Glomus mosseae* was the highest amount (Figure 1).

Table 4. The effect of AM and ACC-deaminase producing bacteria on grain yield and yield components

Residue management	Grain yield (kg ha^{-1})	100-grains weight	Grains m^{-2}	RWC (%)
Control	810b	23.5b	403b	60.2b
AM	933ab	24.6ab	441ab	71.1ab
ACC-deaminase producing bacteria	928ab	24.7ab	450ab	70.7ab
AM and ACC-deaminase producing bacteria	964a	26.4a	470a	76.5a

In each column, the similar letter/letters indicated non-significant differences among means

3.4. Relative water content (RWC)

Analysis of variance of the data showed that the effect of residue preservation on RWC was significant at 1% level (Table 2). So that the amount of RWC showed a significant increase under preserving half of the residue condition (68.7%) compared to the control (59.5) (Table 3). The variance analysis of the data also showed that the interaction effect of residue management in biofertilizer consumption on RWC was significant (Table 2). Without residues, the effect of the combined application of ACC-deaminase producing bacteria and the *Glomus mosseae* on the weight of 100-grains was superior respect to other treatments. In the conditions with preservation of half of the residues and preservation of the whole residues, the combined application of ACC-deaminase producing bacteria and *Glomus mosseae* was the highest amount (Figure 1).

3.5. Concentration and absorption of total potassium and zinc

Variance analysis of the data showed that the effect of plant residue management on potassium concentration, total potassium absorption and total zinc absorption was significant at 5%, 1% and 5% levels, respectively, and there was no significant difference on zinc concentration (Table 5). The results showed that the concentration of potassium in the conditions of preservation of half of the residues (1.75%) showed a significant increase compared to the control (Table 6). The total absorbed potassium in the condition of maintaining half of the residues (48.2 kg ha^{-1}) showed an increase of 18.1% compared to the control (40.8 kg ha^{-1}) (Table 6). Variance analysis of data showed that the main effect of biofertilizer application on potassium concentration, total absorbed potassium and total absorbed zinc was significant at 5%, 1% and 1%, respectively, and there was no significant difference on zinc concentration (Table 5). Regarding the concentration of potassium, it was found that the combined application of *Glomus mosseae* and ACC-deaminase producing bacteria was superior to the treatment control. However, the treatments of applying silicon alone and inoculation with ACC-deaminase producing bacteria alone were not in a higher statistical group than the control (Table 7). The concentration of potassium in the aerial parts of chickpea plants with the combined application of *Glomus mosseae* and ACC-deaminase producing bacteria reached 1.78%, which was in the superior statistical group compared to the non-application of biological materials (1.60%) (Table 7). Total potassium absorption showed a 23.1% increase compared to the control with the combined use of *Glomus mosseae* and ACC-deaminase producing bacteria, and this treatment was superior. In the inoculation treatments of ACC-deaminase-producing bacteria alone and the use of *Glomus mosseae* alone compared to the control, the differences were significant and an increase of 15.2% and 14.1% was observed, respectively (Table 7). The absorption of total zinc in the aerial parts of the chickpea plant with the combined application of Glomus mouse fungus and ACC-deaminase producing bacteria reached 7.18 kg/ha, which was in the superior statistical group compared to the non-application of biological materials (26.1%) (Table 7). The treatments using bacteria producing ACC-deaminase enzyme and *Glomus mosseae* showed an increase of 19.5% and 15.9%, respectively, compared to the control. The variance analysis of the data also showed that the interaction effect of residue management in biofertilizer application was significant on total absorption of potassium and zinc at the level of five percent and had no significant effect on the concentration of potassium and zinc (Table 5). In the study of changes in total potassium absorption in the treatment of without plant residues, it was found that the combined application of *Glomus mosseae* and ACC-deaminase-producing bacteria produced the highest amount of total potassium absorption (51.1 kg/ha) (Table 7). The researchers stated that the increase in potassium absorption under water stress conditions improves the water relations in the plant (Akhtyamova et al., 2023). In the treatments for preserving plant residues, *Glomus mosseae* and ACC-deaminase-producing bacteria increase water absorption while stimulating root development. Regarding the total absorbed zinc, in conditions without residues, the combined application of ACC-deaminase producing bacteria *Glomus mosseae* obtained the highest amount of absorbed zinc compared to the control. In the conditions with preservation of residues, the treatment of the combined application of ACC-deaminase producing bacteria and *Glomus mosseae* was superior, and in the conditions of preservation of half of the residues and preservation of all residues, the total zinc absorbed increased by 10.8% and 23.4%, respectively, compared to the control. In the condition of preserving half of the residues, the use of ACC-deaminase-producing bacteria alone and the use of *Glomus mosseae* alone also showed a significant increase in the total absorbed zinc compared to the control (Table 8).

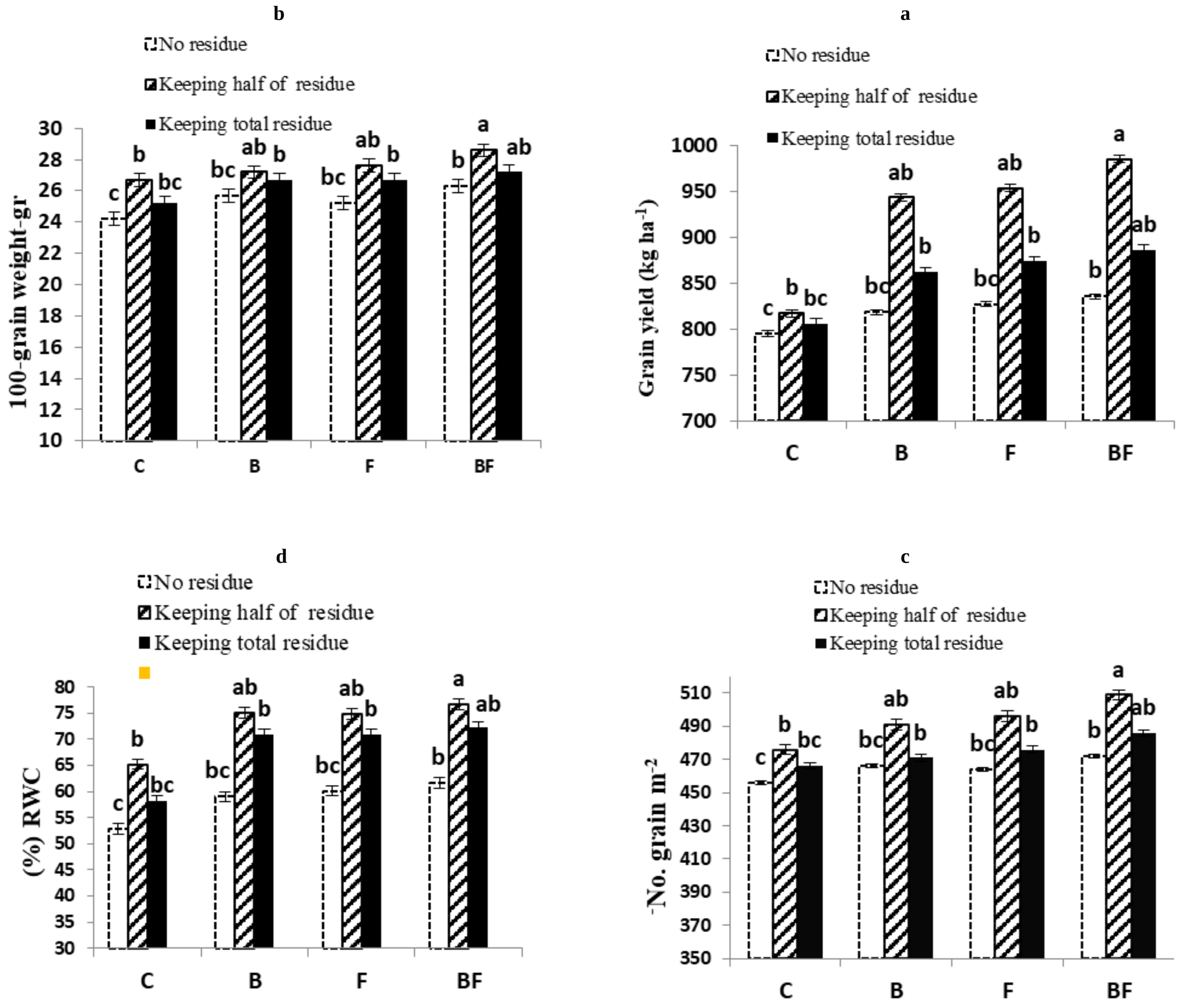

Figure 1. Mean Comparison of the effects of residue management and biofertilizer application on (a) grain yield, (b) 100-grains weight, (c) grains/m^2 and (d) RWC
C: Control; B: ACC-deaminase producing bacteria; F: *Glomus mosseae*; BF: Combined application of ACC-deaminase producing bacteria and *Glomus mosseae*

Since the decrease in water availability for the plant causes a decrease in the absorption of nutrients, the result is a decrease in dry matter yield (Yildirim et al., 2021), but in this stress condition, the use of *Glomus mosseae* and ACC-deaminase-producing bacteria could improve the absorption of nutrients. The results of this research were consistent with the researches of others. In their research, the researchers found that plant growth-promoting bacteria have a positive effect on the plant's antioxidant activity, increasing the absorption of nutrients and plant performance (Kuzmicheva et al., 2017). Researchers showed that *Glomus mosseae* increases the absorption of some elements and increases the plant's tolerance to stress (Greger et al., 2018). In explanation of the effect of *Glomus mosseae* on chickpea yield, it is possible to refer to the results of another research which stated that potassium increases flexibility in stomatal exchanges and reduces transpiration in the conditions of plant residue preservation (Raghavendra et al., 2020). Experiments have shown that bacteria with the ability to produce ACC-deaminase enzyme were able to significantly increase root length (Chandra et al., 2018). The morphological features of the root and the growth of the roots and the ratio of the root to the shoot are necessary in order to maintain the overall balance of the plant physiology and can have a high effect on tolerance to drought stress. Also, Khan et al. (2020) reported that in the condition of preserving residues, with the use of *Glomus mosseae*, the grain yield and biomass of chickpea increased (Tawfiq and Muslim, 2020).

The researches showed that the inoculation of plant growth stimulating bacteria had an effect on the concentration of nutrients in the plant (Billah et al., 2019). In sustainable production, stability of performance in different environmental conditions is considered as the main selection criterion for stress tolerance. Performance stability means a small difference between the potential performance and actual performance (in different environmental conditions).

The effect of the *Glomus mosseae* and the studied bacteria was to reduce these differences, so that the effect of plant growth stimulating bacteria on increasing the growth of chickpea plants has been reported (Mazumdar et al., 2020). Research results have shown that the plant absorbs more elements such as potassium to increase the concentration of the solution inside the cells (Zahoor et al., 217). Also, aligned with this research, other researchers reported that the amount of potassium in leaves has increased significantly due to the application of drought stress and the use of potassium and mycorrhiza (Samadi et al., 2024). The researchers concluded that in the presence of *Glomus mosseae*, elements such as potassium are transferred from the root to the shoot through the transpiration flow in the xylem (Zhang et al., 2023).

Therefore, the intensity of transpiration in the plant determines the distribution of potassium in the plant organs. Potassium absorbed in the plant causes the formation of a cuticle layer, which can affect transpiration and increase the efficiency of water consumption (Park et al., 2019).

On the other hand, the root architecture has changed with the use of *Glomus mosseae* (Liu et al., 2024) and since the root architecture is an important factor in the colonization efficiency of bacteria and fungi, therefore, the synergistic effects of bacteria and *Glomus mosseae* it leads under rainfed conditions. Also, aligned with this research, in the conditions of not using biological fertilizers, due to the lower absorption of nutrients and water by the plant, it is reflected in the form of a decrease in the number of pods and a decrease in 100-grain weight.

Table 5. Analysis of variance of grain yield, biological yield and harvest index

Variable Sources	df	Potassium concentration	Total absorbed potassium	Zinc concentration	Total absorbed zinc
Year	1	835 ns	361 ns	2134 ns	5732 ns
T(Y)	4	734 ns	102 ns	1732 ns	6382 ns
Residue	2	406431*	2530421**	5163 ns	90754327*
Y×R	2	805 ns	1578 ns	1043 ns	1472 ns
E1	4	535 ns	7427۷ ns	6321 ns	7038 ns
Fertilizer	3	164206 *	4576481 **	1852 ns	6404943 **
Y×F	3	1763 ns	7592 ns	1091 ns	1063 ns
R×F	6	323 ns	354821 *	4302 ns	7504783 *
Y×R×F	6	434ns	1024 ns	2185 ns	8049 ns
E2	36	905	7523	5427	4853
CV%		17.2	15.8	13.8	16.3

ns, * and **, non-significant and significant difference at 5% and 1% probability levels, respectively.

Table 6. The effect of residue management on concentration and total absorption potassium and zinc in areal parts of chickpea

Residue Management	Potassium concentration of areal parts (%)	Total absorbed potassium (kg ha^{-1})	Zinc concentration of areal parts (mg kg^{-1})	Total absorbed zinc (kg ha^{-1})
Residue Deletion	1.61b	40.8c	21.6a	5.51b
Maintenance of semi-residue	1.75a	48.2a	22.7a	6.26a
Maintenance of total residue	1.67ab	44.7b	22.7a	6.07ab

In each column, the similar letter/letters indicated non-significant differences among means

Table 7. The effect of silicon and ACC-deaminase producing bacteria application on potassium and zinc concentration, total absorbed potassium and zinc in areal parts of chickpea

Biological Fertilizer	Potassium concentration of areal parts (%)	Total absorbed potassium (kg ha^{-1})	Zinc concentration of areal parts (mg kg^{-1})	Total absorbed zinc (kg ha^{-1})
Control	1.61b	42.3c	21.4a	5.69c
Mycorrhiza	1.73ab	50.1b	22.3a	6.61b
ACC-deaminase producing bacteria	1.69ab	50.4b	22.4a	6.81b
Mycorrhiza and ACC-deaminase producing bacteria	1.79a	56.3a	22.7a	7.18a

In each column, the similar letter/letters indicated non-significant differences among means

Table 8. Interaction effect of water deficit stress and fertilizer application on total absorbed potassium and zinc in chickpea

Residue management	Control	Mycorrhiza	ACC-deaminase producing bacteria	Mycorrhiza and ACC-deaminase producing bacteria
	Total absorbed potassium (kg ha^{-1})			
Residue removal	41.5c	45.5bc	46.6b	51.1ab
Maintenance of semi-residue	44.8c	49.6b	51.8b	57.1a
Maintenance of total residue	43.5c	47.5b	48.6b	52.2ab
	Total absorbed zinc (kg ha^{-1})			
Residue removal	5.52c	6.41b	6.45b	6.89ab
Maintenance of semi-residue	5.57c	6.63ab	6.74ab	7.34a
Maintenance of total residue	5.71c	6.54bc	6.43b	6.91ab

In each column, the similar letter/letters indicated non-significant differences among means

4. Conclusion

In the condition of drought stress (rainfed condition) with the removal of residues, the grain yield (1137 kg/ha) showed a decrease of 7.86% compared to the condition of maintaining half of the residues (1234 kg/ha) and this decrease in biological yield is equal to 7.58%. Due to these changes in grain and biological yield, the harvest index reached from 44.6% in the condition without preserving residues to 44.8% in the condition of preserving half of the residues. The results of this research showed that in such conditions of residue management, the application of *Glomus mosseae* and ACC-deaminase producing bacteria had positive effects on the yield and yield components of the chickpea. The combined application of *Glomus mosseae* and ACC-deaminase producing bacteria had a 12.4% increase in grain yield per hectare compared to control. In addition to the combined application of *Glomus mosseae* and inoculation of ACC-deaminase producing bacteria, in conditions without residues, inoculation of ACC-deaminase producing bacteria and in the condition of maintaining residues, the use of *Glomus mosseae* had a greatest effect. It had effects on yield, yield components and nutrient concentration. Among the yield components, the number of seeds per square meter received the greatest effect from ACC-deaminase producing bacteria. Since part of the decrease in plant growth under rainfed conditions is related to the increase in the level of ethylene and it's reaching the stress ethylene concentration in the plant tissue. The results also showed that as a result of the use of *Glomus mosseae* and ACC-deaminase producing bacteria, the status of potassium and zinc elements in the aerial parts of chickpea improved significantly. Therefore, there is a requirement to pay more attention to chickpea plant nutrition in soils with low absorption capacity of these elements. In this research, it was found that the differences in performance characteristics and performance components between the use and non-use of *Glomus mosseae* and ACC-deaminase producing bacteria in the condition of non- preserving the plant residues are less and without significant effect. From this point of view, by keeping at least half of the residues, the use of *Glomus mosseae*, especially when the plant is exposed to different stresses, should be considered. Also, according to the obtained results, inoculation of seeds with ACC-deaminase producing bacteria (*Bacillus simplex* UT1) is suggested as a stress-modulating bacteria in rainfed conditions. In general, the combined use of *Glomus mosseae* and ACC-deaminase producing bacteria compared to the control treatment improved the yield, yield components and changed the concentration of nutrients in chickpeas. Although under residue removal condition, the use of *Glomus mosseae* alone had low efficiency, but it is recommended to use it in the conditions of preservation of residues, especially with inoculation of ACC-deaminase producing bacteria.

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