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Assess Effect of Rhizobium Bacteria (*Rhizobium leguminosarum* L.) and Iron Nano-chelate on Seed yield and its Components of Pinto Bean (*phaseolus vulgaris* L.) Cultivars

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: One of the best management methods to preserve soil quality is the use of biofertilizers and the use of Nano-fertilizers. Nano-fertilizers are the most effective and at the same time the most independent method of fertilizers, which are aimed at reducing the loss of nutrients and increasing the efficiency of fertilizer consumption.
OBJECTIVES: This study was aimed to investigate the effect of foliar application of iron Nano chelate and rhizobium bacteria on crop production of pinto bean genotypes.
METHODS: Current research was done according factorial experiment based on randomized completely block design (RCBD) with three replications in Ilam city in 2014. The investigated treatments include rhizobium bacteria at two levels (inoculated and non-inoculated), the foliar application agent of iron Nano-chelate at two levels (no foliar application and foliar application with iron Nano-chelate with a concentration of two per thousand) and pinto bean cultivars (including three cultivars Talash, Khomein and local).
RESULT: The simple and reciprocal effects of treatments on seed yield were statistically significant. The average comparison results showed that Khomein had the highest number of pods per plant in the inoculation treatment with Rhizobium bacteria. Also, in the foliar treatment with iron Nano-chelate, Khomein cultivar had the highest number of pods per plant. Using iron Nano-chelate increased the number of pods per plant by increasing the durability of flowers and turning them into pods. Khomein cultivar had the highest seed yield (with an average of 4759 $kg.ha^{-1}$) in the treatment of inoculation with rhizobium bacteria and foliar application with iron Nano-chelate.
CONCLUSION: Based on the results, the use of Khomein cultivar with foliar application, in all cases replace of iron Nano-chelate and inoculation with Rhizobial bacteria can be effective in increasing the yield of pinto beans in the region.

KEYWORDS: *Foliar application, Micro elements, Nutrition, Pod length, Pulse.*

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1. BACKGROUND

Pinto beans with the scientific name *Phaselous vulgaris* is one of the most desirable and widely consumed types of beans, which is important due to its good digestibility, palatability and quick cooking (Majnoun Hosseini, 2015). Application of nano fertilizers to feed plants is one of the most important applications of nano technology in various fields and trends of agriculture in water and soil sector. Nano-fertilizers are the most effective and at the same time the most independent method of fertilizers, which are aimed at reducing the loss of nutrients and increasing the efficiency of fertilizer consumption. Iron nano-chelate fertilizer is a safe fertilizer base for iron release due to its proper stability and controlled releasing power. Also, iron nano- chelate fertilizer has a unique complex and has nine percent iron soluble in water in the pH range of 3 to 11 (Sabeki *et al.*, 2017). Iron is one of the essential elements, but it is not very active. Among the low consumption elements, plants need iron the most. Therefore, iron nano-chelate fertilizer, in addition to meeting the plants' need for these elements, increases plant yield (Baghai *et al*., 2012). The application of iron nano- chelate fertilizer in rainfed peas caused a significant increase in the number of sub-branches and the number of pods per plant (Mir *et al*., 2016). Foliar application of iron nano- chelate caused a significant increase in the height of the lentil plant (Nadri, 2013). On the other hand, nowadays, the use of biological fertilizers is necessary to reduce the consumption of chemical fertilizers in the production of healthy food products. Such a replacement can be considered economically and environmentally. The symbiosis of beans and rhizobium bacteria can increase the plant's need for nitrogen element and replace chemical nitrogen fertilizers (Bashan and Levanony, 1990). The researchers found that it is necessary to inoculate plants of the leguminous family using rhizobium inoculum, and the reasons for its use were the lack of plant-specific rhizobium due to the cultivation of a new species or cultivar (non-native) and the lack of rhizobium due to environmental stress (Khosravi and Asadi Rahmani, 2022.). In addition to their very important role in the nitrogen balance of the biosphere, rhizobial bacteria can also increase the growth and yield of plants in different ways, so that they increase the absorption of other elements such as potassium and phosphorus from the soil (Mehrpoyan *et al*., 2010).

2. OBJECTIVES

Due to the fact that no study has been conducted on the effects of combined application of iron nano-chelate and rhizobium bacteria on bean genotypes in Ilam region, therefore, the present experiment aims to investigate the effect of foliar application of iron nano chelate in the presence of rhizobium bacteria (*Rhizobium leguminosarum*) on seed yield and yield components of pinto bean genotypes.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was carried out in the research farm at a distance of 35 km from the northwest of Ilam province, with a location of longitude 46 degrees and 9 minutes east and latitude 33 degrees and 57 minutes north and an altitude of 1114 from the sea level in the spring of 2013. The climate of the region has a warm Mediterranean climate and an average annual rainfall of 450 mm per year with irregular distribution. The analysis of physical and chemical properties of field soil at a depth of 0 to 30 cm is given in table 1.

Table 1. Physical and chemical characteristics of experimental field

Soil depth (cm)	Soil texture	Clay (%)
0-30	Sandy loam	42.5
pH	**OC (%)**	**N (%)**
7.82	1.38	0.13
Silt (%)	**Sand (%)**	**EC ($dS.m^{-1}$)**
39.5	18	1.35
K (ppm)	**P (ppm)**	**SP (%)**
450	4.2	45

The experiment was carried out as a factorial using randomized completelyblock design(RCBD) with three replications. The test factors include rhizobium bacteria at two levels (inoculated and non-inoculated), foliar application of iron nano-chelate at two levels (no foliar application and application of foliar application with iron nano-chelate at concentration of two per thousand) and bean cultivars including three cultivars (Talash, Khomein and local). Each experimental plot consisted of five planting lines with distance of 40 cm and length of 4 meters. The distance between the plants in the row was 15 cm, and the distance between the plots and repetitions was 1 and 2 meters, respectively in the. Between both plots, a row of nonplanting was considered. The distance between two repetitions was determined to be 1 meter.

3.2. Farm Management

For experimental treatments, at the time of planting, the seeds were first treated with 20% sugar solution and then inoculated with *Rhizobium leguminosarum* bivar phaseoli strain 177 with density of 2 x 108 cells per gram of inoculum. Then, the inoculated seeds were kept in the shade on a clean surface for less than a day, and finally, after drying, they were cultivated at a depth of 4 to 5 cm. Iron nano-chelate fertilizer was obtained from Ahrar Sharq Export Company and was foliar-sprayed at the rate of 2% in the middle of flowering. The amount of foliar application was such that the bean plants were completely covered with the solution. During the growing season, necessary care for the plant, such as weeding, was done manually, and no pesticides were used before and after cultivation. The first drip irrigation was done immediately after planting and subsequent irrigations were done once every 6-7 days according to the climatic and soil moisture condition.

3.3. Measured Traits

In the physiological ripening stage, five pinto beans plants were randomly selected from each plot and the characteristics of plant height, number of pods per plant, pod length, number of seeds per pod and 100 seed weight were measured. Seed yield was calculated by harvesting from the 3 middle lines of each plot by removing the half-meter margin from the beginning and end of each plot (2.4 square meters).

3.4. Statistical Analysis

All statistical calculations, variance analysis and mean comparisons were done by SAS (Ver.6.12) statistical software and the means were compared with Duncan's test at the 5% of probability level.

4. RESULT AND DISCUSSION

4.1. *Plant height*

The results of analysis of variance indicated that the effects of seed inoculation with rhizobium bacteria, cultivar, and interaction effect of treatments had a significant effect on plant height, although the effects of other treatments on this trait were not significant (Table 2).

Table 2. Result of analysis of variance effect of treatment on studied traits

S.O.V	df	Plant height	No. pod per plant	Pod length
Replication	2	788.513	11.36	0.375
Rhizobium bacteria	1	258.06*	42.25*	32.30**
Iron Nano-chelate	1	868.3^{ns}	12.25 ^{ns}	2.40 ^{ns}
Cultivar	2	2162.25**	56.25**	1.30 ^{ns}
Rhizobium × Iron Nano-chelate	1	56.04^{ns}	6.56 ^{ns}	0.95 ^{ns}
Rhizobium × Cultivar	2	749.14*	27.3**	7.3*
Iron Nano-chelate × Cultivar	2	15.45^{ns}	21.2*	1.45 ^{ns}
Rhizobium × Nano-chelate × Cultivar	2	284.45^{ns}	3.24 ^{ns}	0.74 ^{ns}
Error	22	47.52	3.27	0.88
CV (%)	-	8.9	4.5	6.3

^{ns}, * and **: no significant, significant at 5% and 1% of probability level, respectively.

Continue table 2.

S.O.V	df	100 seed weight	No. seed per pod	Seed yield
Replication	2	15.47	0.25	15127.38
Rhizobium bacteria	1	204.49*	4.23**	8044942.83**
Iron nano-chelate	1	39.52**	6.94**	567729.48**
Cultivar	2	41.05**	8.15**	11702476.25**
Rhizobium × Iron Nano-chelate	1	3.11 ^{ns}	0.33 ^{ns}	61299.48 ^{ns}
Rhizobium × Cultivar	2	26.90**	3.24**	147090.14**
Iron Nano-chelate × Cultivar	2	28.41**	1.75**	1795053.86**
Rhizobium × Nano-chelate × Cultivar	2	1.02 ^{ns}	0.152 ^{ns}	370400.42**
Error	22	2.10	0.189	21344.52
CV (%)	-	4.7	3.1	10.5

^{ns}, * and **: no significant, significant at 5% and 1% of probability level, respectively.

By examining the results of the mean comparison of interaction effects of treatments on plant height, it was determined that the highest plant height in the inoculation treatment with Rhizobium bacteria was obtained for Khomein cultivar with an average of 82.27 cm and the lowest plant height was obtained in the absence of inoculation treatment attributed (Fig. 1). Some other researchers have also reported the positive effects of inoculation with different strains of rhizobium symbiotic bacteria on plant height (Ansari *et al.*, 2018). Since the height of the plant is almost fixed in the flowering stage, therefore, the foliar application of iron nano-chelate fertilizer in the flowering stage did not have a significant effect on the height of the common bean plant. The results of this research are consistent with the findings of Badaghi (2008).

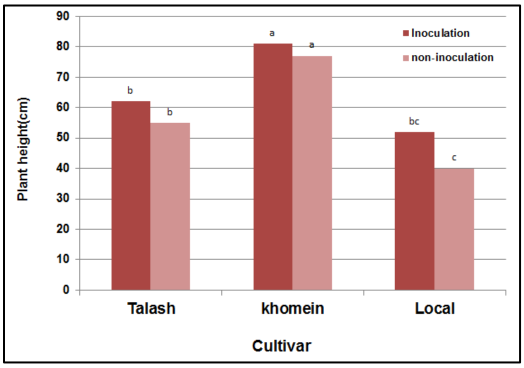

Fig. 1. Interaction effect of Rhizobium bacteria and cultivar on plant height by Duncan's test at the 5% of probability level.

The plant height is not an important factor in determining the seed yield, but probably the cultivars with a higher height have a higher dry matter yield. Height is usually influenced by genetic factors, but the environment also affects plant height (Harris *et al.*, 2008).

4.2. *Number of pods per plant*

The results of analysis of variance showed that the effect of rhizobium bacteria and cultivar had a significant effect on the number of pods per plant, and the interaction effects of rhizobium bacteria and the cultivar and iron nano-chelate in the cultivar also had a significant effect on this trait. While other treatments had not significant effect on this trait (Table 2). The highest number of pods per plant was obtained in the inoculation treatment with Rhizobium bacteria and Khomein cultivar with an average of 18.1 cm. While the use of rhizobium bacteria in the local cultivar caused a significant decrease in the number of pods per plant compared to the non-inoculation treatment in the same cultivar (Fig. 2). In a field experiment on the effect of toxins produced by rhizobium bacteria, researchers concluded that some types of bacteria, the amount of toxins they produce, have an effect on plant activity and reduce the number of pods per plant (Jahanara *et al.*, 2013). This result can be the reason for the decrease in the number of pods per plant in the local cultivar after inoculation with rhizobium bacteria. Also, the number of pods per plant was affected by inoculation with rhizobium in the genotypes of Talash and Khomein. In this connection, Fathi amirkhiz *et al.* (2011) reported that soybean inoculation with rhizobium Japonicum bacteria has been able to increase the yield components by increasing the growth and development of the plant and allocating more photosynthetic materials to the seed.

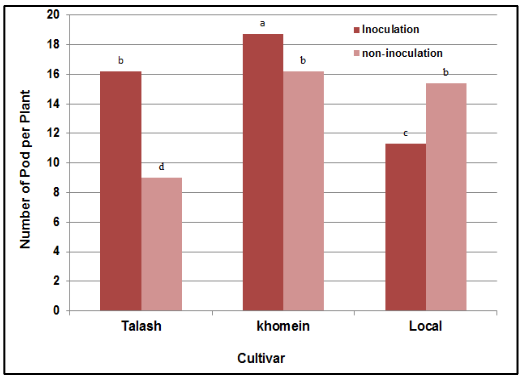

Fig. 2. Interaction effect of Rhizobium bacteria and cultivar on number of Pod per Plant by Duncan's test at the 5% of probability level.

The results of mean comparisons of interaction effect of nano-iron chelate and cultivar on the number of pods per plant t showed that the highest number of pods per plant belonged to Khomein cultivar in foliar application with iron nano-chelate. In general, in all investigated cultivars, number of pods per plant increased as a result of foliar application by iron nano-chelate (Fig. 3).

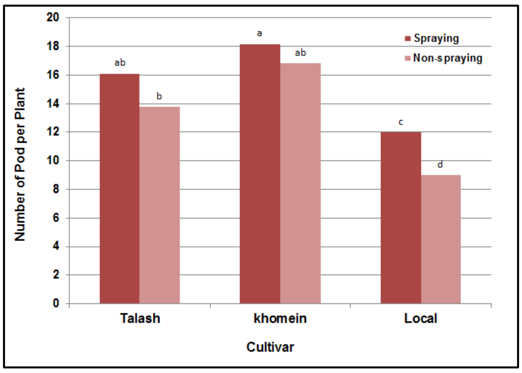

Fig. 3. Interaction effect of Iron Nano-chelate and cultivar on number of pod per plant by Duncan's test at the 5% of probability level.

Therefore, in the present experiment, it seems that foliar application with iron nano-chelate increases the durability of the flower and turns it into pods, through the increase of assimilates, due to the role of this element in photosynthesis, it increases the number of pods per plant (Marshner, 1995). Some researchers also stated that the use of iron nano- chelate fertilizer during the flowering stage has a positive effect on the quantitative and qualitative traits of Pinto bean (Hamzai *et al.*, 2014).

4.3. *Pod length*

The interaction effect of seed inoculation in cultivars was significant at the level of 1% on pod length (Table 2). Mean comparisons of interaction and rhizobium bacteria and cultivars on pod length revealed that the longest pod length was related to inoculation treatment with rhizobium bacteria in Khomein cultivar with an average of 9.75 cm. The combination of the local cultivar and the absence of inoculation the lowest pod length. Also, inoculation with rhizbiome increased the pod length in local and talash cultivars compared to not being inoculated. The results showed that pod length is affected by inoculation with rhizobium bacteria (Fig. 4).

4.4. *Number of seed per pod*

Based on the results of analysis of variance, there was a significant difference between different levels of inoculation and genotype in terms of the number of seeds per pods (Table 2). The interaction effects of rhizobium bacteria and bean cultivars, as well as the interaction effect of iron nano-chelate and bean cultivars on the number of seed per pod were significant (Table 2).

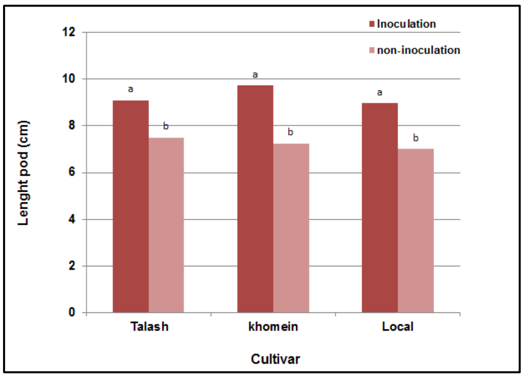

Fig. 4. Interaction effect of Rhizobium bacteria and cultivar on pod length by Duncan's test at the 5% of probability level.

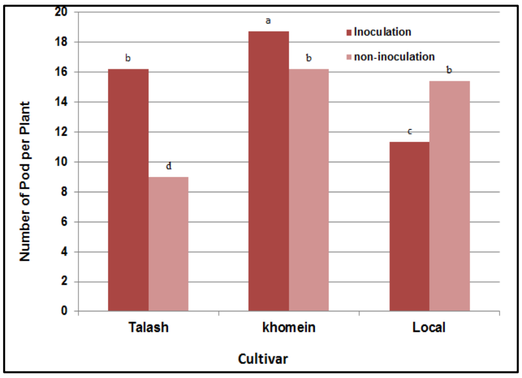

Fig. 5. Interaction effect of Rhizobium and cultivar on number of seed per Pod by Duncan's test at 5% of probability level.

The mean showed that the highest number of seed per pod with an average of 4.2 seeds belonged to Khomein cultivar and bacterial inoculation treatment. The lowest number of seed per pod (with an average of 3.67 seeds) was observed in the local cultivar and Talash (with an average of 3.7 seeds) and the non-inoculation treatment (Fig. 5). The mean comparisons of treatments showed that the highest number of seeds per pod with an average of 3.83 seeds belonged to the local cultivar in foliar application with iron nano-chelate and the lowest number of seeds per pod with an average of 2.66 seeds belonged to the Khomein cultivar in the treatment without foliar application (Fig. 6). In different environmental conditions, the number of seeds per pod is the most stable component of yield in legumes, because in a given cultivar, the number of egg cells in each ovary is almost constant. Therefore, the effect of the number of seeds per pod in different environmental conditions in yield fluctuations is not the same as the effect of number of pods per plant (Majnoun Hosseini, 2015).

4.5. *100 Seed weight*

The simple effects of the investigated treatments on the 100 seed weight were significant at the 1% of level (Table 2). The interaction effect of common bean cultivars × Rhizobium bacteria as well as the interaction effect of cultivars × iron nano-chelate had a significant effect on the 100 seed weight (Table 2). Khomein and Talash cultivars in the inoculation treatment with Rhizobium bacteria had the highest 100 seed weight with an average of 33.33 g and 32.96 g, respectively. The lowest of 100 seed weight was observed in the local genotype and inoculated with bacteria (with an average of 20.66 g) (Fig. 7). The highest 100 seed weight belonged to the talash cultivar and no foliar application treatment(with an average of 33.8 g) and Khomein cultivar and no foliar application treatment (with an average of 33.31 g). The lowest weight of 100 sedd weight was also observed in the local cultivar and the foliar treatment of iron nano-chelate (with an average of 20.55 g) (Fig. 8).

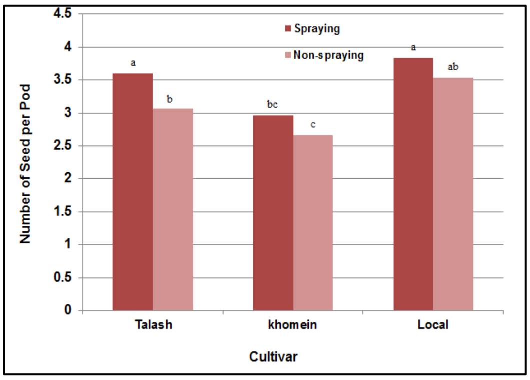

Fig. 6. Interaction effect of Iron Nano-chelate and cultivar on number of seed per pod by Duncan's test at the 5% of probability level.

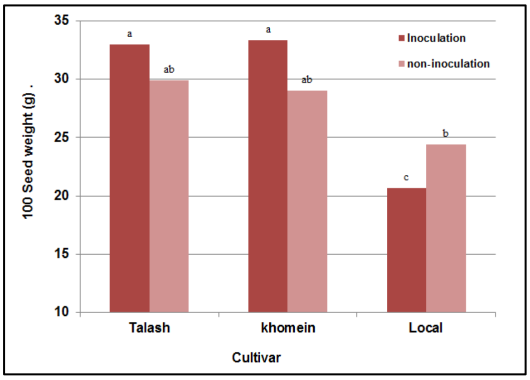

Fig. 7. Interaction effect of Rhizobium bacteria and cultivar on 100 seed weight by Duncan's test at the 5% of probability level.

In an experiment, the 100 seed weight of beans crop increased significantly (Khosravi *et al*., 2022). Some researchers have attributed the difference between cultivars in terms of seed weight to the difference in seed filling period from flowering to maturity (Bagheri and Parsa, 2013).

4.6. *Seed yield*

Based on the results of analysis of variance, the effects of inoculation with bacteria, foliar application and cultivars had a significant effect on the seed yield. Also, the interaction effects of inoculation × cultivar and foliar application × cultivar on seed yield were significant at the 1% of level (Table 2). The triple effects of rhizobium bacteria x iron nano-chelate x cultivar were also significant on seed yield (Table 2). The highest yield of Khomein cultivar was observed in rhizobium bacteria inoculation and foliar application with an average of 4759 kg.ha^{-1}. The local cultivar had the lowest seed yield in the treatment of no inoculation and no foliar application (with an average of 1839 kg.ha^{-1}) (Fig. 9).

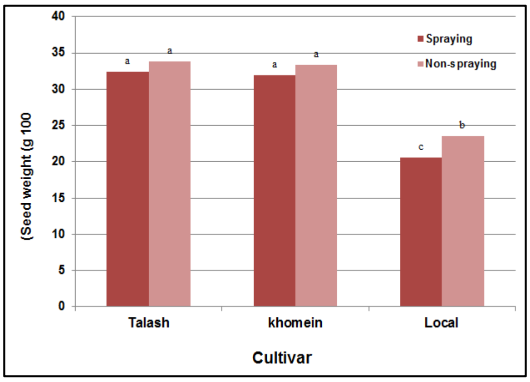

Fig. 8. Interaction Effect of Iron nano-chelate and Cultivar on 100 Seed Weight by Duncan's test at the 5% of probability level.

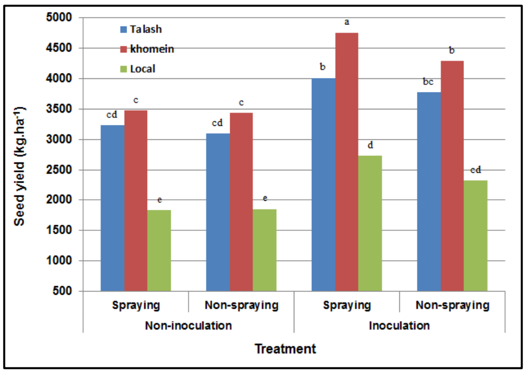

Fig. 9. Interaction Effects of Iron nano-chelate, Rhizobium Bacteria and Cultivar on Seed yield by Duncan's test at the 5% of probability level.

According to some researchers, among the yield components, the number of pods per plant is one of the most important traits in determining bean yield and has the highest correlation with seed yield (Mehrpoyan *et al*., 2013). The researchers found that although bean inoculation with nitrogen-fixing bacteria can improve rhizosphere conditions for absorption of other elements in addition to nitrogen fixation, but in this regard, there is a difference between different bean cultivars and different bacterial species (Jahanara *et al*., 2013).

5. CONCLUSION

Bacterial inoculation in three common bean cultivars increased yield. While foliar application of iron nano-chelate only caused a significant increase yield in Khomein and Talash cultivars, it did not show a significant difference in terms of seed yield in local cultivar. Considering the point that the nutrients required by plant depends on region and is affected by environmental conditions, the difference in yield in some cultivars is probably due to the genetic difference of the used cultivars in this study, and the reason for the lack of effect of iron Nano-chelate on seed yield in the local cultivar of Ilam could be the inability to absorb iron sprayed on the leaves or on the soil. A situation that may occur in some cultivars due to the difference in the adaptation of cultivars to the climate of the region.

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

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