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Effects of plant density, *Rhizobium* inoculation and microelements on nodolation, chlorophyll cotent and yield of chickpea (*Cicer arietinum* L.)

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ABSTRACT

The objective of this study was to determine the effects of seed inoculation with Rhizobium and microelements application in different plant densities on nodulation, seedling emergence, chlorophyll content, seed protein and grain yield of chickpea (Cicer arietinum). Field experiment was done in Research farm at University of Mohaghegh Ardabili in 2009, A complete blocks design in a 3x2x2 factorial arrangement, with 4 replications was used to test two levels of seed inoculation (with and without inoculation), two levels of microelements application (with and without microelements spraying) and different plant densities in three levels at 25, 35 and 45 plants m^{-2} (D_1, D_2 and D_3). Seed inoculation had significant effects on nodulation, emergence percentage, time of maturity, chlorophyll content, plant height, seed protein, pods per plant, hollow pod and grain yield. The highest values of these treats were observed in the inoculation treatments, except time of maturity and hollow pods percentage. Microelements application showed significant effects on nodulation, time of maturity, relative chlorophyll content, plant height, pod per plant, hollow pods percentage and grain yield. The highest grain yield was recorded in microelements spray application. The effects of plant densities on maturity time, plant height, pods per plant, hollow pods percentage and grain yield were found significant. The lowest values for time of maturity and the highest values for plant height were observed at D_3. The maximum pods per plant and hollow pads percentage were obtained at D_1.The highest grain yield was recorded at D3. The interaction effect of Rhizobium inoculation and microelements spraying on nodulation, seed protein and grain yield were significant. The highest protein content was achieved in seed inoculation with microelements application and the lowest of it was in non-inoculation and without of spraying microelements.

Keywords: Chickpea, Microelement, Nodulation, Plant density, *Rhizobium* inoculation.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) belongs to the family leguminosae. It is one of the important grain legume cultivated in the world. Chickpea seeds contain essential amino acids like isoleucine, leucine, lysine, phenylalanine and valine [12]. The protein in chickpea is highly digestible (70-90%) [32].

Plant density has more effect on yield and yield components of chickpea. Many researchers reported the effect of plant population on grain yield and the some of agronomic characteristics of chickpea. Ali and Singh [4] reported that the effect of plant densities i.e. 33 and 40 plants m^{-2} do not differ significantly in yield and yield attributes. Furthermore, these researchers pointed that, the sowing density used depends principally on soil water availability. Yigitoglu [34] reported that the highest pads (n ha^{-1}) and seed yield of chickpea was obtained in high plant density (45 plants m^{-2}) and suggested that planting density depends to environmental conditions, seed size, plant type and way of sowing. Valimohammadi et al. [30] and Sing et al. [24] reported that, the optimum planting density for chickpea is 35 plants m^{-2} with consider to environment conditions. However, Ahmadi and Kanoni [1] reported that the optimum planting density of chickpea is 25 plants m^{-2}.

Leguminous plants are able to utilize nitrogen derived from the symbiotic relationship they form with root nodule bacteria. This phenomenon is extremely important and the value of this “free” fertilizer N, can be placed in global perspective if one considers that an estimated 50 million tons of nitrogen are manufactured industrially each year against an estimated 90 million tons fixed by plant processes [18]. Legume inoculation is a way of assuring that the strain of *Rhizobium* appropriate for the cultivar being seeded is present in the soil at the proper time and in numbers sufficient to assure a quick and effective infection and subsequent nitrogen fixation [2; 29].

The inoculation of seeds with *Rhizobium* increase nodulation, nitrogen uptake, seed protein [21; 25]. The combined inoculation of *Rhizobium* and phosphate solubilizing bacteria has increased nodulation, plant height, seed protein and yield parameters in chickpea [15; 16].

Mineral nutrient deficiencies limit nitrogen fixation by the legume-*rhizobium* symbiosis and decrease yield of legumes. Nutrient limitations in legume production result from deficiencies of not only major nutrients but also micronutrients such as molybdenum (Mo), zinc (Zn), boron (B) and iron (Fe) [6]. Inadequate nodulation of pigeon pea can be associated with low plant available Mo. Increase in flower numbers, pod set improvement, and reduction in days to flowering are influenced by Mo [19]. Application of recommended doses of fertilizers (RDF) is essential to obtain higher seed protein and grain yield in legume crops [13; 17].

Moreover, Bhuiyan et al. [6] reported that using micronutrient (Zn) fertilizer increase grain yield and protein percentage up to 25 and 40%, respectively. The most fundamental effects of micronutrients on protein metabolism are through its involvement in the stability and function of genetic material [16].

However, little work has been done on the combined effects of *Rhizobium* inoculation, microelements application and plant density on nodulation, seedling emergence, chlorophyll content, seed protein and grain yield of chickpea (*Cicer arietinum* L.). The objectives of this study were to determinate the effects of seed inoculation, microelements application and different plant densities on chickpea nodulation, emergence rate, maturity time, plant height, chlorophyll content and grain yield.

MATERIALS AND METHODS

Field experiment was done in Ardabil (38º15" N; long 48º15" E; Altitude of 1350 m), northwest of Iran, at the Research farm of University of Mohaghegh Ardabili, in 2010. Climatically, the experimental area is in the country's semiarid temperate zone, with cold winter and moderate summer. Average annual rainfall is about 400 mm, and the most of rainfall is concentrated between winter and spring (January to June). The soil texture is silty loam with electrical conductivity (EC) about 3.61 ds m^{-1}, pH about 8.2 and saturation percentage (SP) about 46%. The soil was classified as entisol type.

The experimental design was a randomized complete blocks, in a factorial arrangement with four replications. Treatments were: plant densities at 25, 35 and 45 plants m^{-2} as D_1, D_2 and D_3, respectively, with 10, 7 and 5.5 cm intra-row spacing respectively, and 40 cm inter-row spacing, Inoculation with *Rhizobium* bacteria at two levels (with and without inoculation) and microelement application at two levels (with and without spraying).

Rhizobium legominosarum bv. *Ciceri* was grown in yeast extract mannitol broth in flask, shaken at 125 rpm at 28°C for 6 days to obtain cell density about 4×10^7 cells per ml [5]. Inoculation was done by soaking the surface sterilized seeds of chickpea (*C. arietinum* L.) in the liquid culture medium for 1 h. Seeds were sown in May 2010 by hand. Each plot consisted of 6 rows with 4 m long. Plots sizes were 8.4 m^{-2}. Six pots were sown in the end of three middle rows for study the nodulation traits. Two Seeds were sown in each heap in planting rows and six seeds were sown in each pot (high pot 20 cm and internal diameter according to density). Seed was placed at 5cm deep. In stage of 4-6 leafy, the plants were thinned to obtain the suitable plant density. Weeds were controlled by hand weeding. No insecticide or fungicide was used to control insects, pests or diseases. Microelements were: Fe (1600 ppm), Zn (1200 ppm), Mn (1500 ppm) and B (200 ppm) that spread in two stages (stage of three leaves and before flowering), amounting to 3/1000.

Daily emergence counts for seedlings that were visible above the soil level with a minimum height of 1.0 cm. This counting was taken for 28 days. Chlorophyll content was estimated in 30 leaf blades counting them from the top, i.e. in the youngest, but already fully developed leaves. Leaves relative chlorophyll content was measured with a portable chlorophyll meter (Minolta, SPAD-502, Japan).

Maturity time was measured in days to 70 % physiological maturity. Seed protein was measured by seed analyzer (Zeltex, ZX9500, Japan).

At 90 days after sowing, six pots were deputed and the adhering soil particles were carefully removed by gentle shaking under running tap water and then the number of nodules per plant counted. Dry and fresh weight, nodule number and active nodule from nodules total were also recorded by methods suggested by Almas et al. [5]. Samples were dried at 70 °C in air oven for 72 hours. The data from border rows were not taken. Remaining rows were harvested after complete maturity. After harvest grain yield were recorded.

RESULTS AND DISCUSSION

Seed inoculation with *rhizobium cicerea* produced significantly highest nodule number, nodule fresh weight, nodule dry weight and active nodule per plant than control (Table1). Favorable effects of inoculation with *rhizobium* spp. and significant increase in nodulation and yield of legume crops have been reported by many researchers such as Stancheva et al. [26] and Ogutcu et al. [17]. Microelements spraying significantly increased nodule characteristics. Maximum nodule number, nodule fresh weight, nodule dry weight and active nodule per plant were produced by Microelements spraying as compared to control (Table 1). Tenywa [28] reported that Co and Mo application increased nodulation and number of active nodules in common bean plants. Interaction effects of *rhizobium* inoculation × microelements spraying on nodule characteristics were significant. Maximum nodule number, nodule fresh weight, nodule dry weight and active nodule per plant were recorded with seed inoculation × microelements spraying (Table 1). The conducive effect of dual inoculation of seeds with *Rhizobium* and microelements application on nodulation and N_2 fixation in legumes has been established [35]. Daza et al [8] in bean reported that seed inoculation and microelement application in vetch increased nodule dry weight and nodule number per plant.

Table 1. Chickpea studied traits at different plant density, microelements application and seed inoculation [(1)]

Treatments	Variable			
	Nodule number (No. plant^{-1})	Nodule fresh weight (g plant^{-1})	Nodule dry weight (g plant^{-1})	Active nodule (%)
Rhizobium cic.				
inoculation	24.44^{a}	131.99^{a}	35.63^{a}	83.5^{a}
Non-inoculation	0^{b}	0^{b}	0^{b}	0^{b}
Microelements				
spraying	13.33^{a}	76.43^{a}	18.69^{a}	89^{a}
Non-spraying	11.11^{b}	55.56^{b}	16.94^{b}	78^{b}
Density				
D1	12.83^{a}	69.85^{a}	18.84^{a}	81.5^{a}
D2	12.08^{a}	63.74^{a}	17.61^{a}	84^{a}
D3	11.75^{a}	64.39^{a}	16.99^{a}	85^{a}
c.v (%)	16.29	14.20	11.42	14.5

[(1)]*Means followed by equal do not differ by Duncan's multiple range test, at 5% of probability.*

Effect of seed inoculation on emergence percentage was significant (Table 2). The highest emergence percentage was obtained in seed inoculation, than control treatment (Table 2). Rhizobial inoculation can also stimulate other microorganisms, seedling emergence, and grain and straw yields of lowland rice (*Oryza sativa* L.) [7; 16; 31].

The results of the variance analysis showed that the effects of inoculation, microelements and plant density on maturity time were significant. Maturity time were the highest in non-inoculation, microelement spraying, D_2 and D_3 than inoculation, non-spraying and D_1 (Table 2). Albayrak [3] and Togay et la. [29] Reported that the earliest harvest was done in the inoculated and the latest harvest was realized in the non-inoculated. In the case of planting densities, minimum days to maturity were found in the D_3. A steady increase in number of days to maturity tack place when decreased plant density (Table 2). Khan et al. [14] in soybean reported that with decrease plant density, increased number of days to maturity. These results are in agreement with valimohammadi et al. [30] in chickpea. They reported that low plant density have a maximum days to maturity.

Plants which treated with *Rhizobium* and microelements scored the highest chlorophyll content (Table 2). Gwata et al [10] reported that chlorotic plants with yellow leaves were visually distinguishable from the vigorous plants with dark green leaves. Because the plants were grown in a nitrogen-free medium, the available nitrogen as indicated by the dark green leaves was derived from the N_2- fixation process. Nitrogenous compounds resulting from N_2-fixation are exported from root nodules in the form of ureides (allantoin and allantoic acids) and translocated to the leaves where they are catabolized [33] and used for the biosynthesis of chlorophyll and other proteins essential for photosynthesis. Concentration of chlorophyll dyes is a reliable index of physiological plant condition [27], though frequently it is not duly appreciated in agricultural sciences. Results presented in table 2, point to its higher vigor under inoculation conditions. Plants from combinations inoculated with Rhyzobium cicerea were characterized by a higher chlorophyll concentration. Fujiwara and Tsutsumi [9] demonstrated that fertilizer with microelements increases the chlorophyll content in barley leaves.

The main effect of different methods of Rhizobium inoculation, microelements spraying and plant density on plant height was found significant (Table 2). Plant receiving the treatment, seed inoculation with Rhizobium, microelements spraying and D3 recorded the highest plant height and the lowest it was noted in treatment under non-inoculation, non-microelements spraying and D1. Hoque and Haq [11] reported that inoculation of seed with Rhizobium significantly increase plant height of lentil. Shinde and Bhilare [23] and Togay et al. [29] found similar result in chickpea.

The interaction effect of Rhizobium inoculation and microelements spraying on protein content of seeds of chickpea was significant (Table 2). The highest protein content was achieved in seed inoculation with microelements application and the lowest in non-inoculation with non-spraying microelements (Table 2). Solaiman and Rabbani [25] found that the performance of Rhizobium inoculant alone was superior to uninoculated control in protein content in green and mature seeds of pea. Kazemi poshtmasari et al. [13] reported that the highest protein content was obtained in microelements application and the lowest of it was achieved in control treatment.

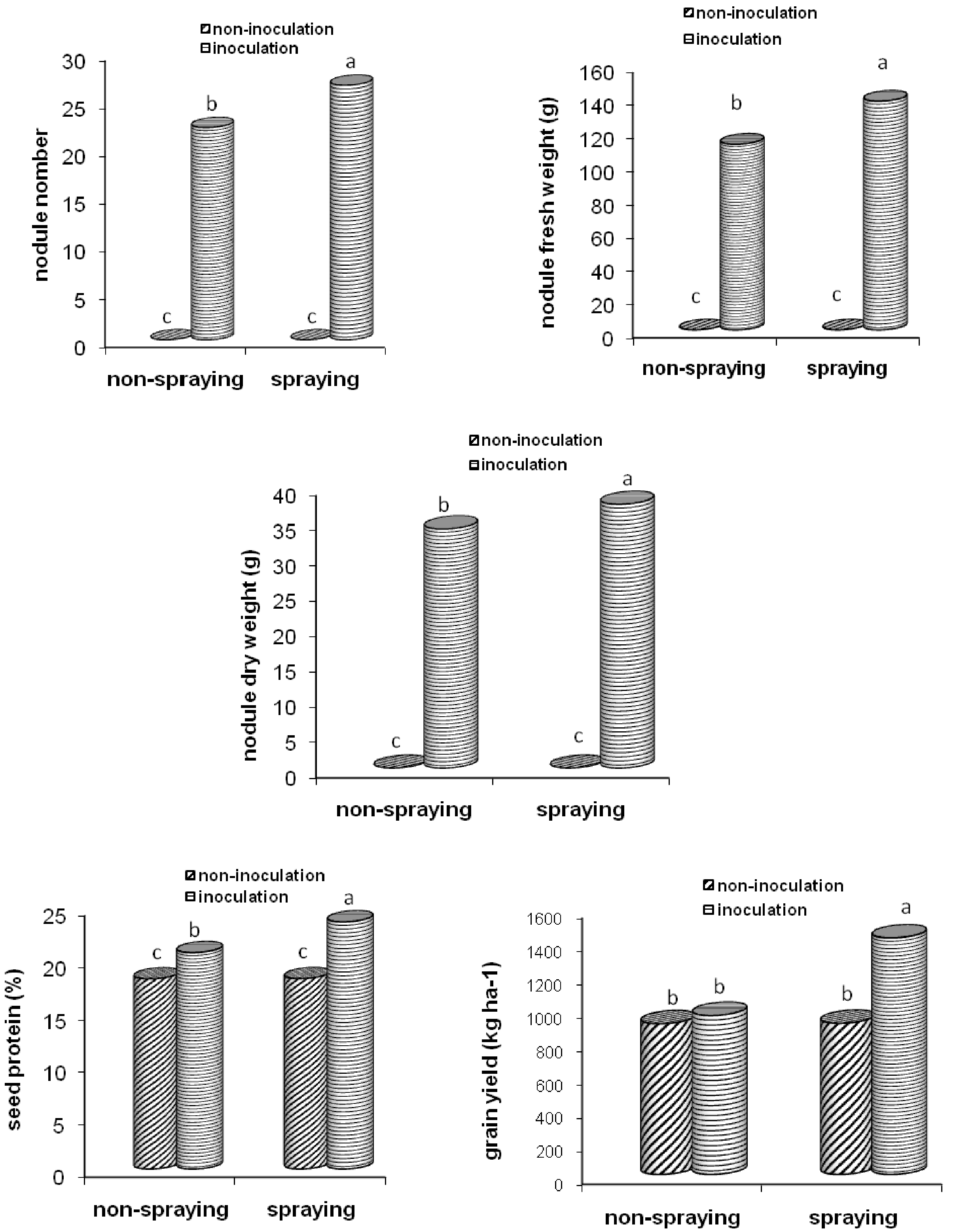
Seed inoculation with rhizobium, microelements application and 25 plants m^{-2} density produced the highest number of pods per plant. The lowest number of pods per plant was noted in non-inoculated, non-spraying, 35 and 45 plants m^{-2} density. Seed inoculation with rhizobium, microelements application, D2 and D3 produced the lowest percentage of hollow pod (Table 2). The contribution of biologically fixed N on increasing the pod number and hollow pod were remarkable. This result was in agreement with the findings of Solaiman [25]on chickpea. He reported that Rhizobium inoculant significantly increased number of pods compared to uninoculated control. Rabbani et al. [20] also found that the number of pods per plant was increased with Rhizobium inoculant. Togay et al. [29] reported that number of pods of chickpea increased due to Rhizobium inoculation and micronutrients application.

Table 2. Chickpea studied traits at different plant density, microelements application and seed inoculation [(1)]

Treatments	Variable							
	Emergence percentage	**Maturity time (day)**	**Chlorophyll content**	**Plant height (cm)**	**Pod per plant**	**Hollow pod (%)**	**Seed protein (%)**	**Grain yield (kg ha^{-1})**
Rhizobium cic.								
inoculation	88^{a}	109^{b}	33.14^{a}	34.23^{a}	32^{a}	7^{b}	22.15^{a}	1163.06^{a}
Non-inoculation	79^{b}	117^{a}	28.21^{b}	30.55^{b}	23.32^{b}	14^{a}	18.17^{b}	906.82^{b}
Microelements								
spraying	85^{a}	119^{a}	34.34^{a}	35.40^{a}	30.25^{a}	8^{b}	20.89^{a}	1138.05^{a}
Non-spraying	82^{a}	111^{b}	27.01^{b}	29.38^{b}	25.07^{b}	13^{a}	19.43^{a}	931.83^{b}
Density								
D1	83^{a}	117^{a}	30.21^{a}	27.91^{c}	33^{a}	14^{a}	19.90^{a}	785.38^{c}
D2	85^{a}	117^{a}	31.05^{a}	32.65^{b}	26^{b}	10^{b}	19.73^{a}	1056.68^{b}
D3	82.5^{a}	109^{b}	30.76^{a}	37.14^{a}	24^{b}	10^{b}	20.85^{a}	1262.76^{a}
c.v.(%)	18.31	16	9.1	15.12	11.25	12	10.61	18.95

[(1)]*Means followed by equal do not differ by Duncan's multiple range test, at 5% of probability*

Figure 1. Nodulation, seed protein and grain yield of chickpea submitted to two inoculation treatments and two microelements application rates (1)



(1)Means followed by equal do not differ by Duncan,s multiple range test, at 5% of probability.

Interaction between seed inoculation and microelements application was observed in grain yield (Figure 1). The highest grain yield was observed in treatment compounds of between seed inoculation and microelements spraying. The lowest grain yield was achieved in non-inoculation and non-spraying (Figure 1). Effect of plant density on grain yield was significant (Table 2). The highest grain yield was achieved at D3 and the lowest of it was observed at D1. The sowing density used depends principally on soil water availability. Valimohammadi et al. [22; 30] reported that highest grain yield of chickpea was obtained in high plant density (45Plant m^{-2}). Planting density depends to environmental condition, seed size, plant type and way of sowing. Singh et al. [24] reported that optimum plant density for chickpea is 35 plants m^{-2} with consider to environment conditions. Daza et al. [8] reported that the highest grain yield obtained in microelements application and lowest of it was achieved in control treatment. Togay et al [29] reported that the highest grain yield in chickpea was obtained in seed inoculation with *Rhizobium*.

CONCLUSION

Cicer arietinum L. is the third most widely grown legume in the world and even through rhizobia nodulating chickpeas are thought to be highly host specific, *C. arietinum* and its nodule bacterial should be more carefully studied. Sufficient information, generated from work on several temperate legumes, is available to indicate the tremendous potential of adequate inoculation technology. Site- and region-specific data concerning chickpea Rhizobia are often lacking or unfortunately of dubious quality. It is now necessary in the Mediterranean area to provide appropriate information about symbiosis, inoculation and the need for inoculation as support for the development of chickpea crop. On the basis of this study, it is concluded that inoculation of seed with *Rhizobium* in combination with microelement fertilizer significantly affected the yield and nodules formation of chickpea. Optimum plant density of chickpea was 45 plants m^{-2}. In field experiments it was important to improve biological nitrogen fixation by application of microelements. Limitation of microelements is the main cause of poor nodulation and biological fixation presented by the *rhizobium* chickpea association in our field conditions.

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