

EFFECTS OF DRESSING FERTILIZER AND DIFFERENT NITROGEN DOSES TOGETHER WITH BACTERIA (*Rhizobium spp.*) INOCULATION TREATMENTS ON NODULATION AND SOME PARAMETERS OF CHICKPEA (*Cicer arietinum* L.)

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Abstract

The present study was conducted to investigate the effects of dressing fertilizers and different nitrogen doses on nodule and root parameters of chickpea (*Cicer arietinum* L.) plants. Nitrogen doses were arranged as 0, 30, 60, 90 and 120 kg N ha⁻¹ and dressing fertilizers were applied as 4 kg N da⁻¹. According to the results dressing fertilizer treatments significantly increased average nodule dry weights and root nitrogen concentrations. Number of nodules, nodule weight per plant, nodule nitrogen concentration and root nitrogen concentrations increased with increasing nitrogen doses. Bacteria treatments generally increased nodule weight per plant until a certain nitrogen dose (60 kg N ha⁻¹). Considering all treatments together, it was observed that increasing nitrogen doses together with dressing fertilizer and bacteria treatments yielded remarkable increases especially in average nodule weights and root nitrogen concentrations.

Key words: bacteria inoculation, dressing fertilizer, nitrogen dose, chickpea, nodule

1. INTRODUCTION

Chickpea is an annual crop belonging to legumes (Fabaceae) family. It has been cultivated for thousands of years. Plant origin was reported as Southeastern Anatolia. World chickpea production is commonly intensified in Middle East and South-west sections of Asia. World annual production is 7.807.891 tons from 9.893.672 hectares land area with an average yield of 789 kg ha⁻¹ (FAO 2002). About 80-85% of this total production comes from 4 countries (India, Turkey, Pakistan and Iran). India is the leading chickpea producer with an annual production of 5.320.000 tons (68% of world production). Turkey is the second country with an annual production of 590 000 tons (7.6%).

Chickpea is the among the most significant legume crops of Turkey. While chickpea cultivated lands were 270.000 hectares in 1980s (Anonymous 1996), the value reached to 760.000 hectares in recent years. Such values have made Turkey a significant chickpea exporter country. While chickpea cultivated lands in Turkey are intensified in transition zones and Southeastern Anatolia region, eastern Anatolia region has about 10% of chickpea cultivated lands. With recently developed high-yield and disease and pest-resistant cultivars, chickpea cultivated lands highly increased especially in Eastern Anatolia region including Sivas province.

Chickpea is a quite significant foodstuff for human nutrition. The kernels contain 15-31% protein, 40-60% carbohydrate, 4.5-5.5% oil, phosphorus and calcium (Sehirali 1988). Therefore, chickpea has a great place in human nutrition. Considering the upcoming hunger problems with ever-increasing world population, it was thought that chickpea will have significant contributions for the prevention of possible hunger problems in Turkey. Sufficient nutrition is a great factor in improving yield levels in chickpea cultivation.

Nitrogen and phosphorus are the major plant nutrients limiting the yields in chickpea cultivation. Turkish soils are mostly poor in nitrogen and phosphorus. Therefore, nitrogenous and phosphorus fertilizers should definitely be included in fertilization programs in chickpea cultivation.

Nitrogen is an essential nutrient for almost all plants and it is the greatest yield-limiting factor in plant production activities. Population is ever-increasing both in the world and in Turkey and the energy required to produce nitrogenous fertilizers is decreasing. Therefore, microorganisms should be used more to meet the nitrogen needs of plants in agricultural activities (Porter 1979). It is estimated that 175×10^6 tons nitrogen was biologically fixated ever year. Of such an amount, 50% is supplied by legume-*Rhizobium* partnership (Anonymous 1983). With the sufficient *Rhizobium* bacteria levels in soils, the greatest benefit is achieved from such a partnership of legume-*Rhizobium*. The nitrogen supplied to soil through this partnership is an available and the most economical source of nitrogen for both legume and non-legume crops (Date 1982).

Green fertilizers, incorporation of plants into the soil, improve N mineralization and various microbiological activities of the soils (Vigil et al. 1991; Frazer et al. 1998). Several previous studies investigating the effects of organic and mineral fertilization on nitrogen mineralization revealed that especially the legume crops significantly improved soil N_{min} gains, green fertilizer plants supplied significant amounts of nitrogen to soils and significant portion (80-95%) of mineralized nitrogen transformed into nitrate form (Gök et al. 2004; Pamiralan 2011). In a similar study with broad bean and trefoil, Gök & Sağlamtimur (1991) indicated that $100-230 \text{ kg N ha}^{-1}$ was fixated to soils under non-inoculated conditions and significant portion of this fixated nitrogen mineralized in 2-3 months under Çukurova conditions.

Nitrogen is supplementary component of several plant compounds. Nitrogen constitutes basic building stones of almost all proteins including enzymes controlling biological processes and also constitutes the primary component of all amino acids (Güzel & Gülüt 2010). Nitrogen has also quite significant effects on quality. Previous studies revealed direct relationships between nitrogen and product quality. Increasing grain N concentrations and thus improved yields were reported with increasing nitrogen doses applied to plants (Marschner 1997). High N treatments generally increase grain N contents (Blair 1993).

Farmers should be more conscious in chickpea fertilization. As it was in several field crops, nitrogen is applied in split fashion in chickpea cultivation. Such split nitrogen treatments affect the yields significantly. Growers usually do not apply dressing fertilizers in legume crops and the literature about dressing fertilizer treatments in chickpea is quite limited. Therefore, the present study was conducted to determine the effects of dressing fertilizers and nitrogen treatments together with bacteria inoculation on some nodule and root parameters of chickpea plants.

2. MATERIAL AND METHOD

2.1. Material

Experiments were conducted in greenhouses of Plant and Animal Production Department of Cumhuriyet University Sivas Vocational Collage. Pot experiments were carried out in randomized plots design in 3 replications. Soils taken from 0-30 cm profile of experimental fields of the vocational collage were used as the growth medium. Soil physical and chemical characteristics are provided in Table 1. Soils were air dried; pass through 2 mm sieve and 3 kg soil was placed into each pot. Experimental soils were silty-clay-loam in texture. They were slightly alkaline ($\text{pH} = 7.39$). Lime content was low (18.4%), salinity level was low (0.026 %), nitrogen content was sufficient (0.095 %), and phosphorus content was low (38.9 kg ha^{-1}). Half of the pots had dressing fertilizer treatments (40 kg N ha^{-1}) and the other half did not have dressing fertilizer treatments. Half of the treatments had bacteria inoculation (*Rhizobium* spp. recommended for chickpea) and the other half did not. Bacteria treatments were applied (as to have 1 kg bacteria for 100 kg soil) beneath the root zone (about 4-5 cm beneath the seed) after immersing into 5% sugar solution and treated through with the bacteria in peat soil. Nitrogen doses were arranged as; 0 kg N ha^{-1} , 30 kg N ha^{-1} , 60 kg N ha^{-1} , 90 kg N ha^{-1} and 120 kg N ha^{-1} (in $\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$ form). As the basic fertilizers, $100 \text{ mg kg}^{-1} \text{ P}$ and $125 \text{ mg kg}^{-1} \text{ K}$ (in the form of KH_2PO_4), $2.5 \text{ mg kg}^{-1} \text{ Zn}$ (in the form of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) and $2.5 \text{ mg kg}^{-1} \text{ Fe}$ (in the form of Fe-EDTA) were applied to each pot. Experiments were initiated on 30.06.2016 and harvest was performed on 17.08.2016. Seçkin chickpea cultivar was used as the plant material of the study. Initially 5 seeds were sown to each pot and following the

emergence, number of plants was thinned to 2 through removing the rest from the roots. Chickpea plants were harvested at pod-set period. Root and leaf samples were initially washed with tap water, then with 0.1% HCl, again with tap water and rinsed twice with distilled water. Then samples were dried at 65 °C for 48 hours until a constant weight (Kacar 1972). Root dry weights were measured and samples were ground.

Table 1. Some physical and chemical properties of the soil

Soil Property	Depth (0-30cm)
pH	7.39
Lime (%)	18.4
Salt (%)	0.026
Organic matter (%)	1.59
Texture	SiCL
Total N (%)	0.095
Available P (kg ha ⁻¹)	38.9
Available K (kg ha ⁻¹)	735.4
Available Fe (mg kg ⁻¹)	4.21
Available Mn (mg kg ⁻¹)	1.92
Available Zn (mg kg ⁻¹)	0.39
Available Cu (mg kg ⁻¹)	1.04

2.2. Method

2.2.1. Measurements and Analyses

Number of Nodules

Roots were removed from the pots without creating any damages on them. Then they were washed initially with tap water, then with distilled water again without creating any damages on roots and nodules. Number of nodules were counted and recorded.

Nodule Dry Weight

The nodules washed initially with tap water and then with distilled water roughly were dried at 65 °C for 48 hours until a constant weight and dry nodule weight was determined.

Average Nodule Weight

Nodule dry weight was divided by number of nodules to get average nodule weight.

Root and Nodule N Contents

Root and nodule N contents were determined with Kjeldahl method as specified in Bremner (1965).

Statistical Analyses

Experimental data were subjected to ANOVA with SPSS 22.0 for Windows software. Differences between treatment means were tested with Tukey's test at $p < 0.05$. Correlation analyses were also performed in this study.

3. RESULTS AND DISCUSSION

Effects of dressing fertilizer, different nitrogen doses and bacteria inoculation on number of nodules, nodule nitrogen concentrations, nodule dry weights, average nodule weights, root nitrogen concentrations and root weights are provided in following tables.

In general, the plants without dressing fertilizer treatments had higher number of nodules and nodule nitrogen concentrations than the plants with dressing fertilizer treatments (Table 2). The greatest nodule nitrogen concentration (7.01%) was obtained from plants without dressing fertilizer treatments and with bacteria inoculation and 30 kg N ha⁻¹ treatments. These plants were followed the ones with dressing fertilizer, bacteria inoculation and 60 kg N ha⁻¹ treatments (6.60%) and the ones with dressing fertilizer and 60 kg N ha⁻¹ treatments and without bacteria inoculation treatments (6.52%). Generally in both dressing fertilizer treated and untreated plants, bacteria inoculations increased nodule nitrogen concentrations. Graham and Halliday (1977) indicated that legumes met their nitrogen requirements through symbiotic rhizobium bacteria and thus nitrogenous fertilizers reduced symbiotic nitrogen fixation. Considering the entire treatments together, it was observed that nodule nitrogen concentrations generally increased until 60 kg N ha⁻¹ dose and decreased at further nitrogen doses. Thusly, Akdağ (1990) in a study investigating the effects of bacteria inoculation and four different nitrogen doses (0, 25, 50 and 75 kg N ha⁻¹) on yield and other plant parameters of Spanish chickpea cultivar reported that nitrogen treatments had positive effects on number of pods per plant, number of kernels per plant, plant kernel and biological yields, kernel and protein yield per decare.

Table 2. Effects of dressing fertilizer, nitrogen treatments and bacteria inoculations on nodule nitrogen concentration (%) and number of nodules

Dress Fertilization	Bacteria Inoculation	N Dose (kg/ha ⁻¹)	Nodule N (%)	Number of Nodules (piece)
(-) Dress Fertilization	(-) Bacteria	0	6.08 ±0.41 a-d	62 ±51.6 fg
		30	6.21 ±0.07 a-d	82 ±34.6 e-g
		60	6.35 ±0.05 a-c	317 ±106.8 a-c
		90	6.50 ±0.44 ab	307 ±65.1 a-c
		120	6.30 ±0.60 a-c	410 ±28.3 a
	(+) Bacteria	0	4.67 ±0.45 ef	157 ±4.2 d-f
		30	7.01 ±0.22 a	174 ±5.7 de
		60	5.67 ±0.13 a-f	351 ±41.7 ab
		90	6.51 ±0.21 ab	297 ±58.7 bc
		120	5.99 ±0.00 a-e	345 ±4.2 ab
Average			6.13 ±0.26 A	250 ±40.1 A
(+) Dress Fertilization	(-) Bacteria	0	4.89 ±2.06 d-f	48 ±53.7 g
		30	5.00 ±0.09 c-f	100 ±16.3 e-g
		60	6.52 ±0.08 ab	166 ±58.0 d-f
		90	6.38 ±0.15 a-b	228 ±21.9 cd
		120	6.29 ±0.77 a-c	392 ±101.1 ab
	(+) Bacteria	0	4.35 ±0.23 f	62 ±45.3 fg
		30	6.12 ±0.72 a-d	216 ±40.3 cd
		60	6.60 ±0.19 ab	68 ±36.8 e-g
		90	5.63 ±0.93 b-f	124 ±9.2 d-g
		120	5.51 ±0.65 b-f	140 ±5.7 d-g
Average			5.73 ±0.6 B	154 ±38.8 B

P<0.0

Average number of nodules revealed that the plants without dressing fertilizer treatments formed higher number of nodules than the plants with dressing fertilizer treatments (Table 2). Number of nodules generally increased with increasing nitrogen doses. Among the entire treatments, the greatest number of nodules (410 nodules) was observed in plants without dressing fertilizer and bacteria treatments and with 120 kg N ha⁻¹ N treatments and the lowest number of nodules (48 nodules) was observed in control plants with dressing fertilizer and without bacteria inoculation treatments. Thusly, previous studies revealed that since nodule-forming rhizobium bacteria were autochthonous in Anatolian soils, efficient nodule formation was observed without bacteria inoculation treatments (Özdemir & Engin 1991; Keatinge et al. 1995; Erdoğan 1997). In plants without bacteria inoculation, number of nodules distinctively increased after the nitrogen dose of 60 kg N ha⁻¹. Number of nodules also increased generally in plants without dressing fertilizer and with bacteria inoculation treatments. Additionally, bacteria inoculations together with dressing fertilizer treatments did not create any increases in number of nodules. Previous studies reported that bacteria inoculations had positive effects on nodule formation and yield of chickpea plants (Gürbüz 1980; Akdağ & Şehirali 1994; Erdoğan 1997).

Table 3. Effects of dressing fertilizer, nitrogen treatments and bacteria inoculations on nodule dry weights (g) and average nodule weights (mg)

Dress Fertilization	Bacteria Inoculation	N Dose (kg/ha ⁻¹)	Nodule Dry Weight (g)		Average Nodule Weight (mg)	
(-) Dress Fertilization	(-) Bacteria	0	0.43	±0.20 f-h	6.99	±3.8 b
		30	0.48	±0.41 d-g	5.93	±11.7 b
		60	0.44	±0.26 e-h	1.40	±2.5 gh
		90	0.33	±0.28 hi	1.09	±4.3 h
		120	0.57	±0.39 cd	1.40	±13.9 gh
	(+) Bacteria	0	0.31	±0.07 i	2.00	±15.7 fg
		30	0.51	±0.22 d-f	2.93	±23.0 ef
		60	0.69	±0.29 ab	1.96	±7.0 fg
		90	0.50	±0.41 d-f	1.68	±7.0 g
		120	0.74	±0.28 ab	2.14	±45.5 f
Average		0.50	±0.28 B	2.75	±13.4 B	
(+) Dress Fertilization	(-) Bacteria	0	0.19	±0.01 j	3.85	±0.1 d
		30	0.37	±0.14 g-i	3.69	±8.8 de
		60	0.54	±0.04 d-f	3.22	±0.6 e
		90	0.55	±0.28 de	2.42	±12.9 e-g
		120	0.72	±0.11 ab	1.84	±1.1 g
	(+) Bacteria	0	0.43	±0.03 f-h	6.94	±0.6 ab
		30	0.47	±0.01 d-g	2.18	±0.4 f
		60	0.52	±0.04 d-f	7.65	±1.2 a
		90	0.68	±0.04 bc	5.51	±4.6 c
		120	0.80	±0.06 a	5.68	±11.3 c
Average		0.53	±0.08 A	4.30	±4.2 A	

P<0.0

Considering the effects of dressing fertilizer, nitrogen treatments and bacteria inoculations on nodule dry weights (g) and average nodule weights (mg) (Table 3), it was observed that the plants with dressing fertilizer treatments had higher nodule dry weights and average nodule weights than the plants without dressing fertilizer treatments. The greatest nodule dry weight (0.80 g) was obtained from the plants with dressing fertilizer, bacteria inoculation and 120 kg N ha⁻¹ N treatments and the lowest value (0.19 g)

was obtained from the plants without dressing fertilizer and bacteria treatments and with 0 kg N ha⁻¹ N treatment. Present findings revealed for both bacteria inoculated and non-inoculated plant that increasing nitrogen doses resulted in distinctive increases in number of nodules. Present data also revealed that bacteria inoculation increased nodule dry weights in all nitrogen doses. Similarly, Tippanavar et al. (1990) in a study carried out in India to investigate the effects of bacteria inoculations on some plant parameters reported that bacteria inoculations increased plant kernel yield, number of nodules and nodule weights.

Considering the average nodule weights, it was observed that average nodule weights in non-inoculated plants decreased with increasing nitrogen doses (Table 3). The greatest average nodule weight (7.65 mg) was obtained from the plants with dressing fertilizer, bacteria inoculation and 60 kg N ha⁻¹ treatments and the lowest value (1.09 mg) was obtained from the plants without dressing fertilizer and bacteria inoculation treatments and with 90 kg N ha⁻¹ treatments. In general in all nitrogen doses, average nodule weights were distinctively high in plants with dressing fertilizer and bacteria inoculation treatments.

Table 4. Effects of dressing fertilizer, nitrogen treatments and bacteria inoculations on root nitrogen concentration (%) and root dry weights (g)

Dress Fertilization	Bacteria Inoculation	N Dose (kg/ha⁻¹)	Root N (%)		Dry Root Weight (g)	
(-) Dress Fertilization	(-) Bacteria	0	1.32	±0.04 f	1.05	±1.02 k
		30	1.36	±0.17 ef	1.74	±0.95 g
		60	1.61	±0.04 c-f	0.88	±0.58 l
		90	1.50	±0.02 d-f	0.91	±0.66 l
		120	1.71	±0.22 c-f	1.01	±0.92 k
	(+) Bacteria	0	1.56	±0.05 c-f	0.92	±0.57 l
		30	2.07	±0.21 ac	1.95	±1.03 d-e
		60	1.71	±0.00 c-f	1.41	±1.59 i
		90	1.96	±0.08 a-d	2.21	±2.21 b
		120	1.63	±0.02 c-f	2.48	±2.01 a
	Average		1.64	±0.09 B	1.46	±1.15 B
(+) Dress Fertilization	(-) Bacteria	0	1.52	±0.09 d-f	0.71	±0.58 m
		30	1.55	±0.15 c-f	1.70	±0.69 gh
		60	1.91	±0.05 a-d	1.75	±0.87 g
		90	1.76	±0.28 b-f	1.91	±0.66 e
		120	1.65	±0.20 c-f	1.40	±0.99 i
	(+) Bacteria	0	1.66	±0.09 c-f	1.32	±0.44 j
		30	2.27	±0.76 ab	1.82	±0.32 f
		60	2.30	±0.32 ab	1.65	±0.23 h
		90	2.44	±0.49 a	1.99	±0.67 cd
		120	1.87	±0.09 b-e	2.02	±1.95 c
	Average		1.89	±0.25 A	1.63	±0.74 A

P<0.0

Dressing fertilizer treatments increased root nitrogen concentrations and root dry weights (Table 4). Especially dressing fertilizers together with bacteria inoculation resulted in distinctive increases in all nitrogen doses. The greatest root nitrogen concentrations (2.44%, 2.30% and 2.27% N) were observed in plants with dressing fertilizer and bacteria inoculation and 90 kg N ha⁻¹, 60 kg N ha⁻¹ and 30 kg N ha⁻¹ treatments, respectively. Current findings revealed that bacteria inoculation increased root nitrogen concentration of both dressing fertilizer treated and untreated plants with increasing nitrogen doses. It

was reported in previous studies investigating the effects of bacteria inoculations on some parameters of legumes that bacteria inoculations increased plant dry matter yields (Gürbüzer, 1980; Akdağ & Şehirali, 1994; Erdoğan, 1997).

Considering the root dry weights averages, it was observed that dressing fertilizers together with increasing nitrogen doses generally increased root dry weights. The greatest root dry weight (2.84 g) was obtained from the plants without dressing fertilizer and with bacteria inoculation and 120 kg N ha⁻¹ treatments. Bacteria inoculations increased root dry weights of both dressing fertilizer treated and untreated plants. Such an effect was especially distinctive in 90 and 120 kg N ha⁻¹ treatments. It was reported in similar previous studies investigating the effects of bacteria inoculations on some parameters of legumes that bacteria inoculations increased root dry weights (Gürbüzer 1980; Akdağ & Şehirali 1994; Erdoğan 1997).

Table 5. Correlation among variables tested in the experiment

	Dress Fertilization	Bacteria Inoculation	N Dose	Number of Nodules	Nodule Dry Weight	Average Nodule Weight	Root N	Root Dry Weight
Dress Fertilization								
Bacteria Inoculation	0.894**							
N Dose	0.867**	0.969**						
Number of Nodules	-0.397**	-0.388**	-0.211					
Nodule Dry Weight	0.087	0.226	0.397**	0.398**				
Average Nodule Weight	0.372**	0.406**	0.304*	-0.792**	0.013			
Root N	0.376**	0.576**	0.611**	-0.031	0.309*	0.097		
Root Dry Weight	0.175	0.372**	0.460**	0.025	0.613**	0.098	0.453**	
Nodule N	-0.254*	-0.298*	-0.190	0.388**	0.236	-0.230	0.229	0.250

*Significant at P<0.05

**Significant at P<0.01

Correlation analyses of this study, investigating the effects of dressing fertilizer, different nitrogen doses and bacteria inoculation treatments on some root and nodule parameters of chickpea plants, revealed that dressing fertilizer treatments positively correlated with bacteria inoculation, N doses, average nodule weight and root N concentration; bacteria inoculation positively correlated with N doses, average nodule weight, root N concentration and root dry weight; N doses positively correlated with nodule dry weight, average nodule weight, root N concentration and root dry weight; number of nodules positively correlated with nodule dry weight and nodule N concentration; nodule dry weight positively correlated with root N concentration and root dry weight; root N concentration positively correlated with root dry weight (Table 5). On the other hand, dressing fertilizer treatments negatively correlated with number of nodules and nodule N concentration; bacteria inoculation negatively correlated with number of nodules and nodule N concentration.

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