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Study of Effects of Different Compost Application Levels and Seed Priming on Biological Nitrogen Fixation in two Chickpea Cultivars

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ABSTRACT: *In order to study the effects of different compost application levels and seed priming on biological nitrogen fixation in two chickpea cultivars an experiment was carried out at Garizeh Agricultural Research Station, Sanandaj. The experiment design was factorial with three factors set up in RCB design. The factors were: cultivar (ILC and Hashem), seed priming (check, KH_2PO_4, distilled water, KNO_3), compost (check, 10 and 20 t/ha). Results showed that only in terms of % root nitrogen there was significant difference among the cultivars. Also, the main effects of priming type and compost level were significantly different on the percentage of root and stem (shoot) nitrogen, number of root nodes, and percentage of root and stem (shoot) protein. The interactions of cultivar×priming and cultivar×compost were significantly different in terms of all the evaluated traits except for number of root nodes and % root nitrogen, respectively. The interaction of priming and compost was significant concerning all the evaluated traits. The means comparison showed that seed priming with distilled water resulted in the highest root protein and nitrogen fixation and seed priming with KNO_3 resulted in the highest shoot nitrogen and protein. Also, the highest percentage of root nitrogen and protein content obtained from the application of 20t/ha of compost. Among the combined levels of seed priming type and compost the treatment of priming with distilled water and no-compost yielded the highest % root nitrogen, number of root nodes and % root protein and the combined treatment of priming with KNO_3 and no-compost yielded the highest % shoot protein and nitrogen.*

Key words: *chickpea; compost; seed priming; nitrogen fixation*

INTRODUCTION

Chickpea (*Cicerarietinum* L.) is a self-pollinated and diploid plant and after pea and bean is in the third rank of the world's most important legumes (Cho *et al.*, 2004).

The area under cultivation and annual production of chickpea in Iran are 650000ha and 400000 t/ha, respectively (Kanouni *et al.*, 2008).

Although the use of chemical fertilizers is the fastest and most reliable way of supplying soil fertility but their huge cost, contamination and destruction of life environment have persuaded farmers to use biological and organic fertilizers with a suitable use of chemical ones.

Organic fertilizers especially municipal solid waste compost and manures have a high content of organic matter compared with chemical fertilizers and they can be accessible to the plants as a rich source of nutrients (Abdel-Mawgoud, 2006).

Unfortunately, the rate of organic matters in farming soils of our country is less than 1% which is due to overuse of chemical fertilizers, especially nitrogen fertilizers, and lack of use of organic fertilizers.

It is necessary to increase the soil organic matters by adding organic fertilizers to the soil and prevent the soil structure from destruction (Malakooti and Homaei, 2005).

Nowadays, the application of compost on the farmlands is noticeably under consideration and has been addressed as the most economical source of nitrogen and the best environmental strategy.

Compost has favorable effects on physicochemical properties of the soil such as pH, nutrients absorption capacity and increasing the exchange of macro and micro elements and it also increases the plant growth and yield and makes less pollution in the life environment becauseits nutrients are released slowly and become available to the plant (Hector Santos, 2011).

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Eriksen *et al*. (1999) studied the effects of compost on soil properties in a field of corn and reported the increase in soil organic matters as a result of applying municipal solid waste compost.

Giusquiani *et al*. (1988) studied the soil amendedwith municipal solid waste compost and reported the increase in organic matters of the soil containing compost relative to the check.

In the experiment of Singer *et al*. (2004) the application of compost increased soil pH, organic carbon and potassium content.

Also these materials improve the physical properties of the soil including apparent density, field capacity and soil structure.

Researches in arid and semi-arid areas and farmer's sayings indicate that seed's poor establishment is the main cause of yield loss of field crops (Harris *et al*., 2008).

Increase in seed emergenceand seedlings establishment in the field could cause increase in absorbing water and nutrients and the plants wouldbe able to use the sunlight more efficiently (Finch-Savage and Clark, 2004).

Germination is the first developmental stage of plants which is also a sensitive stage of plant growth and a key process in plant emergence.

Of the most important treatments for enhancing seed germination vigor is seed priming.

Seed priming treatment shortens the time period from planting to emergence and protects the seeds against biotic and abiotic factors during the critical stage of plant establishment.

These treatments also result in uniform emergence and establishment and improve crop yield (Basra *et al*., 2004).

Seed priming is a technique by which the seeds prepare physiologically and biochemically for germination before planting and facing ecological conditions of the environment.

This technique can cause variousbiological and physiological appearances in the primed seed and the obtained plant so that the mentioned traits can be observed in germination quality, initial plant establishment, utilizing the environmental inputs, early maturity and qualitative and quantitative increase in crop yield (Peel *et al*., 2004).

Of major effects of seed priming those on germination, initial plant establishment, increase in root and shoot dry weight, increase in velocity and percentage of germination can be addressed.

Mansouri *et al*. (2013) studied the effects of priming on the yield and yield components of seed of two chickpea cultivars (ILC and Hashem) and reported that the seed priming increased germination velocity by 31% in both cultivars and also resulted in considerable increase in germination percentage, number of seeds per pod, number pods per square meter, 1000-kernel weight and biological yield.

Besides improving germination and plant growth and increasing their resistance to the environmental stresses, the positive influences of priming have been proved on node-making and rate of nitrogen fixation in root nodes of chickpea.

Kaur *et al*. (2006) reported increase in biomass and number of nodes of chickpea seedlings obtained from hydro and osmo-primed seeds.

Finally, according to what mentioned above, the most important aim of the present study is evaluating the effects of different levels of compost and seed priming type on biological fixation of nitrogen in two cultivars of chickpea (ILCline andHashem).

MATERIALS AND METHODS

The trial was conducted on the Garizeh Agricultural Research Farm located in Sanangaj (latitude 11°35′N, longitude 30°47′E) in 2013.

The experimental design was factorial arranged in RCB with three factors and three replications. The first factor contained two chickpea cultivars (Hashem and ILC line received from Maragheh dry farming research Center), the second factor contained four seed priming (check, KH_2PO_4, distilled water and KNO_3) and the third factor contained three compost levels (check, 10t/ha and 20t/ha).

Before planting, the soil of the experiment's land was analyzed (Table 1). Also the compost used in this study was analyzed before being applied to measure the content of its nutrients. The cultivars were planted on April 14, 2013in 8m rows at a density of 35 plants/m^2.

Appropriate to each level of seed priming the seeds were pre-treated and then planted.

During the growth period all of the farming (management) practicessuch asirrigation and weeding were done similarly for all experiment's treatments.

The most important traits studied in this experiment were: %root nitrogen, %shoot nitrogen, number of root nodes and %shoot protein. The percentage of shoot and root nitrogen was calculated according to the Kajeldal method and using the following equation:

%N= [(amount of acid consumed in titration×0.0014)/ sample's weight]×100

Table 1
Results of soil test at a depth of 30cm.

K (mg\kg)	*P (mg\kg)*	*Soil tissue typy*	*Sand%*	*Silt%*	*Clay%*	*T.N.V%*	*OC%*	*Sp%*	*pH*	*Ec*10-3*
331	2.40	ClayLoam	22	40	38	11	1.22	47.44	8.09	0.80

In order to estimate the shoot and root protein content the nitrogen rate of the sample was multiplied by 6.25.

The data analysis was done according to the statistical model of the design and with the use of the SAS9.2 program. The means comparison of each trait was made using Duncan's multi-range test at $p \leq 0.05$. Graphs were drawn with the Excel program.

RESULTS AND DISCUSSION

Percentage of Root Nitrogen Content

According to the ANOVA (Table 2), the effects of cultivar, seed priming and compost and the interactions of cultivar×priming and priming × compost were significantly different at $p \leq 0.01$ in terms of the %root nitrogen content.

The results of means comparison from point of view of the effects of cultivar on the %root N content showed that the cultivar Hashemwith an average root nitrogen of 1.60% was superior to ILC.

Also, the means comparison of the effects of seed priming type on the %root nitrogen indicated that the treatment of seeds with KNO_3resulted in less %root nitrogen than the rest of seed primings.

Among the other seed primings used in the present study there was no significant difference.

The positive effects of priming on germination could be related to the increase in the activity of endobetamanase enzyme that weakens cell wall and helps the root emerge easily.

Different types of seed priming increase the activity of hydrolysis enzymes and due to easy availability to nutrients during germination the primed seeds can complete the germination process in a shorter period of time (Nonami *et al*., 1995).

Therefore, the roots will emerge more rapidly and uniformly and necessaryground will be prepared for quicker absorption and better fixation of nitrogen.

In the present study the compost level of 20t/ha with an average of 1.58% and the level of 10t/ha with an average of 1.34% the highest and the lowest percentage of nitrogen, respectively.

Studies show that the application of organic fertilizers due to containing phosphor (P) and most of micro-nutrients will increase the growth an expansion of the plant root.

One of the most important roles of P in plants is to increase the absorption of nitrogen through increasing the root length. So, it can be said that in the present study the compost level of 20t/ha,due to providing P and other micronutrients, will increase nitrogen fixation in the plant roots.

The reason is that the compost through supplying nitrogen for the plant and increasing microbial and enzymatic activities in the soilcauses increase innitrogen access to the plant.

Hatch *et al*. (2007) in a study on clover showed that the application of organic fertilizers increase the root nitrogen fixation.

Concerning the response to different types of seed priming, both cultivars in this study achieved the highest %nitrogen through priming with distilled water and the lowest %nitrogen in ILC was achieved with KNO_3 and in Hashem with KH_2PO_4 (Table 3).

Also, both cultivars achieved the highest percentage of nitrogen through the compost level of 20t/ha and the lowest through the level of 10t/ha (Table 4).

The means comparison of the effects ofpriming×compost level on %root nitrogen (Table 5) showed that seed priming with distilled water and no-compost level (check) with an average of 1.60% and seed priming with KH_2PO_4in combination with 10t/ha of compost with an average of 1.25% had the highest and the lowest %root nitrogen, respectively.

Percentage of Shoot Nitrogen

According to the ANOVA table the main effects of priming type and compost level and the interactions cultivar × priming, cultivar ×compost and priming × compost level were significantly different at $p \leq 0.01$ concerning %shoot nitrogen (Table 2).

In the case of the effects of seed priming type on %shoot nitrogen, KNO_3 treatment not only achieved the highest percentage of shoot nitrogen (with an average of 3.98%), but also increased the above-mentioned trait relative to the check, KH_2PO_4 and distilled water by 10, 4.7 and 16%, respectively.

In studying the effects of compost levels on %shoot nitrogen it was found that the application of compost did not have a positive effect on %shoot nitrogen so that the compost levels of 10 and 20t/hadecreased %shoot nitrogen by 1.5 and 6.6% relative to the check.

Concerning the effects of cultivar and seed priming type on %shoot nitrogen (Table 3) it was seen that ILC had the highest percentage of shoot nitrogen with KNO_3 treatment and Hashem with KH_2PO_4.

Also the two above-mentioned cultivars responded differently to different levels of seed priming so that ILC had the highest %shoot nitrogen percentage with no compost and Hashem with 10t/ha of compost.

It is worth mentioning that the both cultivars had the lowest %shoot nitrogen with the application of 20t/ha of compost (Table 4).

In this study no-seed priming in combination with no-compost (with an average of 3.66%) and seed priming with KNO3 in combination with no-compost (with an average of 4.58%) had the highest and the lowest percentage of shoot nitrogen, respectively (Table 5).

Number of Root Nodes

The analysis of variance showed that in terms of effect on the number of root nodes there was significant difference ($p \leq 0.01$) between the interactions of cultivar×compost and priming type×compost.

The means comparison of the effects of cultivar×compost level on the number of root nodes indicated that ILC at the compost application rate of 10t/ha (with an average of 14.77 number) and Hashem at 20t/ha (with an average of 11.33 number) achieved the highest number of root nodes.

In total, ILC in combination with 10t/ha of compost and Hashem with 20t/ha of compost achieved the highest and the lowest number of root nodes (Table 4).

Yazdani *et al.* (2008) reported that the application of compost increased the number of N fixing nodes of soybean roots.

Also in studying the effects of the interaction seed priming × compost level concerning number of root nodes, it was seen that this interaction did not significantly influence the number of root nodes so that the treatment of no-priming×no-compost (with an average of 14.11 nodes) had the highest number of root nodes and the treatment

Percentage of Root Protein

According to the analysis of variance table the main effects of seed priming type and compost levels and the interactions cultivar×seed priming and priming×compost level were significant on the %root protein at $p \leq 0.01$ (Table 2).

The means comparison of the effects of seed priming type on %root protein showed that there was no significant difference among the check, KH_2PO_4 and distilled water. The only significant difference seen was between the above-mentioned treatments and KNO_3.

In this study the seed priming with KNO3 decreased %root protein compared with the check, KH2PO4 and distilled water by 11, 9.9 and 12%, respectively.

In the present study the application of 20t/ha of compost with an average of 9.53% not only yielded the highest %root protein but also increased it by 1.2 and 9.8% compared with the check and 10t/ha levels, respectively.

Both cultivars in this study showed the highest %root protein in response to no application of compost, although there was not significant difference between the mentioned level and 20t/ha of compost (Table 3).

One of the advantages of applying compost is the amendment of soil structure and addition of organic matters to the soil. In contrast to chemical fertilizers that quickly supply the nutrients to the plants, the organic fertilizers such as compost release the nutrients slowly and supply them to the plants.

Therefore, there is no surprise that the application of compost in comparison with the check has not increased the root protein content significantly.

Khosrowjerdi *et. al.* (2013) studied the effects of bio-fertilizers on %root protein of chickpea and reported that the bio-fertilizer did not, in comparison with the check, increase the %root protein significantly whose findingsarein line with those of the present study.

In this study the highest %root protein was seen in the interaction of seed priming with distilled water and no-compost level and the lowest %root protein was seen at the check level of both treatments (Table 5).

Percentage of Shoot Protein

Regarding %shoot protein, the analysis of variance revealed that there was no significant difference among the seed priming type, compost levels and the interactions:cultivar×seed priming,cultivar×compost, seed priming×compost (Table 2).

The means comparison of the effects of priming type on %shoot protein indicated that seed priming

Table 2
Means Square of the Studied Traits

MS

SOV	Df	%Root N	%Shoot N	No. of Root Nodes	%Root Protein	%Shoot Protein
Replication	2	0/01	0/06	5/31	3/08	**1/68**
Cultivar	1	0/04*	ns0/02	ns15/31	ns1/1	**ns0/13**
Seed Priming	3	0/07**	1/35**	ns3/19	4/30**	**44/49****
Compost	2	0/19**	0/39**	ns0/78	6/29**	**28/31****
Cultivar×Priming	3	0/06**	2/76**	ns5/35	3/19**	**84/82****
Cultivar×Compost	2	ns0/009	0/54**	34/63**	ns0/27	**28/37****
Priming×Compost	6	0/06**	0/98**	6/10**	3/23**	**35/97****
Priming×Compost×Cultivar	6	0/10**	1/39**	7/24**	4/59**	**40/88****
Error	46	0/008	0/07	2/13	0/44	**0/55**
CV%	–	6/15	7/31	11/14	7/26	**3/27**

* and **: significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. ns: no significant difference

Table 3
Means Comparison of Mixed Levels of Cultivar and Seed Priming Concerning Effect on the Studied Traits

Cultivar	*Seed Priming*	*%Root N*	*%Shoot N*	*No. of Root Nodes*	*%Root Protein*	*%Shoot Protein*
ILC	Check	1.42bc	3.89ab	14.18a	8.94ab	24.33b
	KH2PO4	1.53ab	3.30cd	13.18a	9.58a	20.64d
	Distilled Water	1.55ab	3.15d	14.25a	9.76a	19.72d
	KNO3	1.33c	4.26a	13.52a	7.96b	26.67a
Hashem	Check	1.60a	3.25d	12.59a	9.89a	20.53d
	KH2PO4	1.44bc	4.28a	13.48a	9.08a	25.38b
	Distilled Water	1.54ab	3.54bd	12.33a	9.33a	22.14c
	KNO3	1.47ac	3.69bc	11.48a	8.95ab	23.16c

The values with common letters do not have significant difference ($p \leq 0.05$)

Table 4
Means Comparison of Mixed Levels of Cultivar and Compost Concerning Effect on the Studied Traits

Cultivar	*Compost*	*%Root N*	*%Shoot N*	*No. of Root Nodes*	*%Root Protein*	*%Shoot Protein*
ILC	Check	1.51ab	3.88a	14.25a	9.26a	24.28a
	10t/ha	1.34c	3.53b	14.77a	8.37b	2208b
	20t/ha	1.53ab	3.54b	12.33bc	9.52a	22.16b
Hashem	Check	1.53ab	3.66ab	12.41bc	9.57a	22/90b
	10t/ha	1.43bc	3.90a	11.36c	8.80ab	24.41a
	20t/ha	1.58a	3.51b	13.63ab	9.53a	20.96

The values with common letters do not have significant difference ($p \leq 0.05$)

Table 5
Means Comparison of Mixed Levels of Priming and Compost Concerning Effect on the Studied Traits

Seed Priming Type	Compost Level	%Root N	%Shoot N	No. of Root Nodes	%Root Protein	%Shoot Protein
Check	Check	1.43cf	3.66c	14.11a	8.95bd	22.94cd
	10t/ha	1.51ae	3.39cd	13.94a	9.27ac	21.20e
	20t/ha	1.60ac	3.66cd	13.83ab	10.02ab	22.89cd
KH2PO4	Check	1.61ab	3.34cd	13.77ab	10.09ab	20.88ef
	10t/ha	1.25f	4.22ab	13.66ab	7.81d	26.41b
	20t/ha	1.60ac	3.81bc	13.55ab	10.01ab	21.75de
Distilled Water	Check	1.66a	3.50cd	13.33ac	10.39a	21.93de
	10t/ha	1.43bf	3.40cd	12.89ac	8.82bd	21.27e
	20t/ha	1.56ad	3.13d	12.77ac	9.39ac	19.58f
KNO3	Check	1.38df	4.58a	12.05ac	8.23cd	28.63a
	10t/ha	1.35ef	3.85bc	12ac	8.46cd	24.10c
	20t/ha	1.47be	3.51cd	11.61c	8.68cd	22.02de

The values with common letters do not have significant difference ($p \leq 0.05$)

not only could not increase the %shoot protein compared with the check, but treatment with KNO_3decreased %shoot protein by 11% compared with the check.

Among the compost levels, although the 20t/ha level with an average of 9.53% showed the highest shoot protein but it did not statistically show significant difference with the check.

Mohammadi *et al.* (2009) reported that the application of compost increased raw fiber and quality of chickpea seeds and also they observed the lowest %seed protein in the compost treatment.

Regarding the interaction of cultivar and priming type, ILC and Hashem showed the highest % shoor protein by priming with KNO_3 and KH_2PO_4 (Table 3).

Also, ILC and Hashem showed the highest %shoot protein at no-compost (check) and 10t/ha compost levels, respectively (Table 4).

Finally, the study of the interaction of seed priming and compost levels showed that priming the seeds with KNO3 combined with the check (no compost) with an average of 28.63% and no seed priming combined with the 10t/ha of compost with an average of 21.2% achieved the highest and the lowest shoot protein, respectively (Table 5).

CONCLUSION

According to the results of the present study among all the seeds priming, the priming with distilled water yielded the highest rate of root N fixation and protein content and the priming with KNO_3 yielded the highest content of shoot nitrogen and protein.

Also, the highest root nitrogen and protein were obtainedbyapplying 20t/ha of compost.

Therefore, under the environmental conditions of the present study the seed priming and application of compost play an important role in increasing the N fixation in chickpea.

Therefore, by using seed priming and compost we not only canreduce the application of nitrogen fertilizers but also can step towards sustainable agriculture.

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