African Journal of Biotechnology Vol. 10(32), pp. 6011-6020, 4 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.415
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Introducing a sustainable soil fertility system for chickpea (*Cicer arietinum* L.)

Khosro Mohammadi[1]*, Amir Ghalavand[2], Majid Aghaalikhani[2], Gholamreza Heidari[3] and Yousef Sohrabi[3]

[1]Department of Agronomy, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.
[2]Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.
[3]Department of Agronomy, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran.

Accepted 19 May, 2011

In order to introduce a sustainable soil fertility system for chickpea, field experiments were carried out in 2007 and 2008 growing seasons. Experiments were arranged in split-split plot arrangements with three replications. Main plots consisted of (G_1): establishing a mixed vegetation of vetch and barley, (G_2): without green manure. Also, five strategies for obtaining the base fertilizer requirement including (N_1): farm manure; (N_2): compost; (N_3): triple super phosphate; (N_4): farm manure + compost and (N_5): farm manure + compost + triple super phosphate. Four levels of biofertilizers consisted of (B_1): Phosphate solubilizing bacteria; (B_2): *Trichoderma harzianum*; (B_3): Phosphate solubilizing bacteria + *T. harzianum*; and (B_4): without biofertilizers were arranged in sub-sub plots. Results showed that green manure increased pod number and number of fertile pods per plant. Integrating biofertilizers (B_3) and green manure (G_1) produced the highest pod number per plant and grain yield. The highest amounts of yield and yield components were obtained in G_1N_5 treatment. Comparison of 3-way interactions showed that $G_1N_5B_3$ was the best treatment. Significant increase of N, P, K, Fe and Mg content in leaves and grains emphasized on superiority of mentioned treatment because each one of these nutrients has an approved role in chlorophyll synthesis and photosynthesis ability of the crop. The N_5 treatment in addition to having the highest yield had the best grain quality due to high protein, starch and total sugar contents, low crude fiber and reduced cooking time.

Key words: Biofertilizer, chickpea, compost, farm manure, soil fertility system.

INTRODUCTION

The chickpea (*Cicer arietinum* L.) as a healthy vegetarian food has an important role in human food and domestic animal feed in Iran. It is a cheap source of high quality protein in the diets of millions of people in developing countries, who cannot afford animal protein for balanced nutrition (Zia Ul-Haq et al., 2007). In addition to proteins, it is a good source of carbohydrates, minerals and trace elements (Huisman and Van der Poel, 1994). Also, chickpea plays a key role in organic cropping systems. In such agro-ecosystems, with limited availability of nitrogen, chickpea potentially constitutes both a cash crop and a source of N incorporation into system via biological nitrogen fixation. The growing chickpea in crop rotation increases crop productivity and sustainability under semi-arid climate. Maintenance and management of soil fertility is the core of development of sustainable food production systems (Doran et al., 1988). To be sustainable, organic farming needs to be self-sufficient in nitrogen (N); through the fixation of atmospheric nitrogen (N_2) by legumes (Berry et al., 2002), recycling of crop residues and/or green manures (Elfstrand et al., 2007) and the application of farm manure, compost and biofertilizer (Ravindra et al., 2007).

Green manure application to the soil is considered a good management practice in all agricultural production systems because of increasing sustainability of the cropping system by reducing soil erosion, improving soil properties and increasing soil organic matter and fertility

*Corresponding author. E-mail: khosromohammadi60@yahoo.com. Tel: +98 918 8732916. Fax: +98 871 6387100.

Table 1. Physical and chemical soil characteristics before starting the experiment.

EC (dSm^{-1})	pH	Sand	Silt	Clay	Total nitrogen	Saturated humidity	Available phosphorus	Available potassium
		(%)					(ppm)	
1.2	7.4	36.8	35.7	27.5	0.09	38	9.2	255

levels (Doran and Smith, 1987; Power, 1990; Tejada et al., 2008; Sarwar et al., 2010). A major benefit attributed to the green manure and organic fertilizer is the increased organic matter of the soil (Iqbal et al., 2008). Weed suppression derived from the allelopathic effect of green manure has become an important method to control weeds in sustainable agriculture (Liebman et al., 2004). Nitrogen accumulations by leguminous green manure range from 45 to 225 kg ha^{-1} (McLeod, 1982).

Forage legumes are valuable members in crop rotations because they generate income from grazing or hay making and still contribute nitrogen from regrowth and root residues. Furthermore, they help recycling of on farm nutrients. Phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and other nutrients are accumulated by green manure during a growing season and increased fertility of agroecosystms. During decomposition of organic matter in soil, carbonic and other organic acids are formed as a byproduct of microbial activity. These organic acids react with insoluble mineral rocks, phosphate precipitates and releasing phosphates and exchangeable nutrients (McLeod, 1982).

Phosphorus is present as mineral deposits, which are a non-renewable natural resource. There is global concern about the energy and costs involved in mining the phosphate rock and its transport to manufacturing sites, as well as in the manufacture of different fertilizers and their transport to farm fields and application to the crops. Photosynthesis and stomatal conductance are reduced by P deficiency (Guidi et al., 1994) and, conversely, increased phosphorus increase photosynthesis (Gao et al., 1989). Phosphate solubilizing bacteria are also known to increase phosphorus uptake resulting in better growth and higher yield of crop plants (Alagawadi and Gaur, 1988; Rudresh et al., 2005). The combined inoculation of Rhizobium and phosphate solubilizing bacteria has increased nodulation, growth and yield parameters in chickpea (Alagawadi and Gaur, 1988; Jain et al., 1999; Rudresh et al., 2005; Sattar and Gaur, 1987).

Trichoderma sp. has long been known as effective antagonists against soil-borne plant pathogenic fungi (Bennett and Lane, 1992; Kumar and Mukerji, 1996) and promotes vegetative growth in plant. The study of combining these organisms and organic manures is of great potential value to organic agriculture in order to avoid chemical fertilizers and pesticides. This study was carried out to introduced a sustainable soil fertility system, evaluates the combined effect of biofertilizers and organic manure such green manure, compost and farm manure on chickpea.

MATERIALS AND METHODS

Field experiments were conducted at Agricultural Research Center of Sanandaj (11º45′ Lat. N; 30º47′ Long. E, 1400 m above sea level) in Kurdistan province of Iran, during 2007 and 2008 growing seasons. This farm had been sown by wheat last year. Experiments were arranged in split-split plot based on randomized complete block design with three replications. Main plots consisted of using green manure (G_1) and not-using green manure (G_2). The green manure used was a combination of hairy vetch (*Vicia panunica*) and barley (*Hordeum vulgare*). Sub-plots were five strategies of supplying the base fertilizer requirements of chickpea including (N_1): 20 Mg farm manure ha^{-1}; (N_2): 10 Mg compost ha^{-1}; (N_3): 75 kg triple super phosphate ha^{-1}; (N_4): 10 Mg farm manure ha^{-1} + 5 Mg compost ha^{-1} and (N_5): 10 Mg farm manure ha^{-1} + 5 Mg compost ha^{-1} + 50 kg triple super phosphate ha^{-1}. Four levels of biofertilizers including (B_1): *Bacillus lentus* + *Pseudomonas putida*; (B_2): *Trichoderma harzianum*; (B_3): *B. lentus* + *P. putida* and *T. harzianum*; and (B_4): not using biofertilizers as control were arranged in sub-sub plots. Expectation values of base fertilizers were determined according to soil test analysis. The G_1 plots were planted on October 15, 2007 with equal proportions of hairy vetch (*V. panunica*) and barley (*H. vulgare*) in rows 10 cm apart. On April 10, 2008, the green manures were incorporated into the soil by hand-hoeing. The plots without green manure were plowed twice. Three soil samples were taken from the upper 15 cm layer of the soil profile of each plot and analyzed for physical and chemical characteristics (Carter, 1993; Dahnke and Olsen, 1990) (Table 1). The farm manure and compost were also analyzed for chemical and nutrients properties (Table 2).

The chickpea seeds, according to arrangement of sub-sub plots were treated with *T. harzianum* isolate T_{39}, *B. lentus* isolate P5 and *P. putida* isolate P_{13}. Also, *Mesorhizobium* sp. cicer strain SW_7 was added to all the treatments. Phosphate solubilizing bacteria (PSB), *T. harzianum* and *Mesorhizobium* sp. were obtained from Iranian Soil and Water Research Institute. Farm manure, compost and chemical fertilizer were added to plots before sowing chickpea. Chickpea seeds were planted on April 25, 2008 and harvested on the 75th day after sowing. Main plot size was of 6 × 12 m and spaces between main plots were three meter. Weeds were removed manually in all plots.

The phosphorus and nitrogen content of shoot and matured seeds was determined by vanado molybdate phosphoric acid yellow colour method and Microkjeldahl method, respectively (Jackson, 1973). Also, the potassium content was determined by Flame Photometer model-EEL (AOAC, 1990). The other minerals, such as calcium, manganese, magnesium and iron, were determined with an atomic absorption spectrophotometer (Perkin-Elmer Model 5000) (AOAC, 1990). Seed protein content was determined by measuring the N content with the Microkjeldhal method and multiplying it by 6.25 to express to total protein content (Bremner, 1996). Crude fiber and starch were determined using the methods described by Rong et al. (1996). The separation and quantification of sugar compounds from seeds were carried out by

Table 2. Chemical characteristics of farm manure and compost applied to the soil.

Characteristic	pH	N	P	K	Ca	Mg	Zn	Cu
		(%)			(ppm)			
Farm manure	7.45	0.47	0.49	0.31	745	1100	2	25
Compost	7.2	0.7	1.15	0.51	1950	1890	12	295

an Agilent 1100 series HPLC system (Agilent, USA), (Zeng et al., 2007). Seed protein contents were determined by near infrared reflectance spectroscopy (Bran Luebbe Infra Alyzer 350). Chlorophyll readings were taken with a hand-held dual wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan) at the flowering stage. At harvest time harvest, grain yield and yield components were evaluated from an area of 2 × 2.5 m^2 in each sub-sub plot. One hundred grams of harvested mature seeds of chickpea from different treatment were taken in beakers fitted with condensers to avoid evaporation losses during boiling. Distilled water was added in the ratio of 1:4 (w/v) to the beakers and cooking time was determined by the method of Williams et al. (1983). The data collected in this study was subjected to analysis of variance (ANOVA) and means comparison was done using Duncan's multiple range test (DMRT) (Little and Hills, 1978).

RESULTS AND DISCUSSION

Leaf chlorophyll

According to the analysis of variance, leaf chlorophyll was significantly affected by different soil fertility methods, in such a manner means comparisons showed that green manure significantly increased leaf chlorophyll (Table 3). Adding leguminous green manures to the soil produced improved soil nitrogen content through symbiotic associations with Rhizobium bacteria and increased other nutrients during decomposition of organic matter (Tejada et al., 2008). Regarding the key role of elements such as nitrogen, iron, and magnesium in chlorophyll structure, it seems that supply of these elements by green manure is the main reason for increasing leaf chlorophyll. Means comparison also revealed that simultaneous application of bacterium and fungus to the soil increased leaf chlorophyll significantly (Table 3). Co-inoculation of some *Trichoderma* strains and PSB along with effective *Rhizobium* spp. stimulates chickpea nodulation and nitrogen fixation and provides more nitrogen offered to the crop (Parmar and Dadarwal, 1999; Rudresh et al., 2005; Verma et al., 2007). Correlation between nitrogen and chlorophyll content has been reported in many studies (Pandey et al., 2000; Dordas and Sioulas, 2008). Rajendran et al. (2008) reported that the amount of chlorophyll increased when the co-inoculation with *Rhizobium* strains and PSB. Comparisons of base fertilizer levels showed that the highest chlorophyll content was obtained from N_5 treatment. Followed by N_5 treatment and the least was N_4 treatment (Table 3). Increasing leaf chlorophyll content in these treatments is related to more mineral elements such as iron, magnesium, and manganese provided by simultaneous application of compost and farm manure.

Leaf and grain mineral elements

Green manure had a significant effect on leaf and grain nitrogen content. Means comparisons specified that incorporating vetch and barley biomass into the soil before chickpea cultivation, increased leaf and grain nitrogen contents by 18% and 7% respectively (Tables 3 and 4). Nitrogen fixation by vetch increasing soil organic matter, and optimizing conditions for *Rhizobium* bacteria are the main reasons for increasing nitrogen uptake. Elfstrand et al. (2007) reported that green manure application has increased nitrogen content of plant. Moreover, findings of Ryan et al. (2008) indicated that application of vetch as green manure enriched N in grain and straw. Basal fertilizers had a significant effect on leaf and grain nitrogen content (Tables 3 and 4); the highest leaf nitrogen (5.26%) and N containing grain (2744 mg 100 g^{-1}) was obtained from N5 treatment.

The main reason is that compost and farm manure can increase N availability to plant due to more nitrogen offered to plant. Hatch et al. (2007) reported that incorporation of farm manure to the soil had beneficial effects of increasing biological nitrogen fixation, dry matter, and N yields in red clover. In addition, biofertilizers had significant effect on leaf and grain nitrogen contents. The highest leaf and grain nitrogen contents were obtained from B_3 treatment (Tables 3 and 4). In fact, the positive interaction between biofertilizers and rhizobium bacterium caused an increase in biological nitrogen fixation. Rosas et al. (2006) reported that Phosphate-solubilizing *P. putida* can influence the rhizobia-legume symbiosis and increased the number and dry weight of nodules in alfalfa and soybean. El-Komy (2005) demonstrated the beneficial influence of co-inoculation of biofertilizers in providing balanced nitrogen and phosphorus nutrients in wheat plants. There are evidences that some *Pseudomonas* species, not only increase nutrient absorption, as N, P and K, but also act as biocontrol agents of phytopathogenic fungi and produce phyto-hormones in the rhizosphere, which promote plant growth (O'Sullivan and O'Gara, 1992).

The results showed that different methods of soil fertility had a significant effect on leaf and grain phosphorus contents. The highest leaf and grain P content was obtained from N_5 treatment (Tables 3 and 4). Increa-

Table 3. Effect of soil fertility systems on chlorophyll and nutrient accumulation in chickpea seed.

Treatment	Chlorophyll (Spad reading)	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Manganese	Iron
		(mg/100g)						
Green manure								
Vetch + barley (G_1)	44.11 [a]	2283 [a]	273.8 [a]	1208.2 [a]	184.9 [a]	4.3 [a]	2.64 [a]	4.42 [a]
No green manure (G_2)	41.05 [b]	2140 [b]	268.2 [b]	1196.4 [a]	182.9 [a]	4.2 [b]	2.55 [b]	4.38 [a]
Basal fertilizers								
Farm manure (N_1)	39.18 [e]	2045 [c]	271.6 [b]	1190.2 [b]	184.1 [a]	4.1 [c]	2.45 [c]	4.39 [c]
Compost (N_2)	43.06 [c]	2071 [c]	264.7 [c]	1159.3 [c]	184.6 [a]	4.1 [c]	2.72 [a]	4.09 [e]
Chemical fertilizer (N_3)	41.50 [d]	1868 [c]	273.2 [b]	1073.7 [d]	183.4 [a]	4.1 [c]	2.44 [c]	4.14 [d]
Farm + Compost (N_4)	46.25 [b]	2579 [b]	256.1 [d]	1290.2 [a]	183.8 [a]	4.4 [b]	2.61 [b]	4.57 [b]
Farm+Compost+Chemical (N_5)	47.06 [a]	2744 [a]	289.6 [a]	1298.1 [a]	183.5 [a]	4.6 [a]	2.75 [a]	4.80 [a]
Biofertilizers								
PSB (B_1)	43.41 [b]	2269 [b]	273.5 [b]	1201.1 [b]	184.3 [a]	4.3 [a]	2.63 [a]	4.42 [a]
Trichoderma fungi (B_2)	43.35 [b]	2295 [b]	266.2 [c]	1176.3 [c]	183.7 [ab]	4.2 [b]	2.56 [b]	4.35 [c]
PSB + fungi (B_3)	44.12 [a]	2315 [a]	279.8 [a]	1232.1 [a]	183.2 [b]	4.3 [a]	2.62 [a]	4.47 [a]
Control (B_4)	43.22 [b]	2167 [c]	264.9 [c]	1199.8 [b]	184.4 [a]	4.2 [b]	2.57 [b]	4.36 [c]

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (P = 0.05).

Table 4. Effect of soil fertility systems on seed cooking time, mineral and organic compounds uptake by chickpea.

Treatment	Leaf nitrogen (%)	Leaf phosphorus (%)	Leaf potassium (%)	Grain protein (%)	Grain crude fiber (%)	Grain starch ($mg.kg^{-1}$)	Cooking time (minute)
Green manure							
Vetch + barley (G_1)	4.93 [a]	0.31 [a]	2.02 [a]	14.26 [a]	8.19 [a]	155.6 [a]	66.35 [a]
No green manure (G_2)	4.18 [b]	0.24 [b]	2.00 [a]	13.37 [b]	7.85 [a]	153.6 [a]	65.81 [a]
Basal fertilizers							
Farm manure (N_1)	3.87 [d]	0.24 [c]	1.82 [d]	12.59 [c]	7.78 [c]	156.3 [a]	64.43 [b]
Compost (N_2)	4.15 [c]	0.21 [d]	1.80 [d]	15.42 [b]	7.43 [d]	153.3 [b]	64.31 [b]
Chemical fertilizer (N_3)	3.17 [e]	0.31 [b]	1.98 [c]	12.38 [c]	9.55 [a]	153.2 [b]	66.01 [a]
Farm + Compost (N_4)	4.76 [b]	0.30 [b]	2.03 [b]	16.11 [b]	7.07 [e]	157.2 [a]	62.18 [c]
Farm+Compost+Chemical (N_5)	5.26 [a]	0.43 [a]	2.41 [a]	17.15 [a]	8.28 [b]	157.5 [a]	62.68 [c]
Biofertilizers							
PSB (B_1)	4.22 [b]	0.31 [a]	1.91 [b]	14.18 [b]	8.12 [a]	154.1 [a]	65.01 [b]
Trichoderma fungi (B_2)	4.24 [b]	0.28 [b]	2.00 [a]	14.30 [b]	8.07 [a]	154.2 [a]	66.71 [a]
PSB + fungi (B_3)	4.53 [a]	0.33 [a]	2.03 [a]	15.06 [a]	7.99 [a]	153.6 [a]	66.80 [a]
Control (B_4)	4.22 [b]	0.27 [b]	2.02 [a]	13.54 [c]	8.00 [a]	152.6 [a]	65.06 [b]

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (P = 0.05).

sing effect of combined application of compost and farm manure on soil enzymatic activity such as phosphatase and increasing P availability for plant has been reported by El-baruni and Olsen (1979). Triple super phosphate fertilizer (N_3) in comparison with compost and farm manure significantly increased leaf and grain P contents. Moreover, application of green manure significantly increased leaf and grain P contents. Adding phosphorus of green manure to soil and appropriate conditions prepared for PSB are the main reasons for increasing leaf and grain P content in this treatment. Mean comparison showed that combined application of biofertilizers (B_3) produced the highest leaf P content (0.33%) and grain P content (279 mg 100 g^{-1}). Ability of *Bacillus* sp. to produce organic acid such as gluconic, citric and fumaric acids under P-limiting conditions may increase the solubility of poorly soluble phosphorus (Veneklaas et al., 2003). *Pseudomonas* strain inoculation increased phosphatase activity. Phosphatase could contribute to the mineralization of phytate (Wang et al., 2007). Since phosphatases play an important role in nutrient P availability of organic manures, crop residue, and phosphates activity, soil P availability appear to complement each other (El-Baruni and Olsen, 1979), therefore, providing P in rhizosphere can increase P uptake by plant. In addition, regarding to the importance of this element, the increase of P causes stimulating growth and increasing grain yield. Similar report of increase in phosphorus uptake by combined inoculation of *Trichoderma* sp. and PSB were reported by Rudresh et al. (2005).

Basal fertilizers and biofertilizers had significant effect on leaf and grain potassium contents, however, green manure had no significant effect on K content (Sarwar et al., 2008a). Combined application of basal fertilizers improved plant nutrition conditions. The highest leaf and grain K contents were obtained from N_5 treatment. There is evidence that compost application increases potassium absorption in chickpea seeds (Sahni et al., 2008). Combined application of biofertilizers produced the highest leaf and grain K contents (Tables 3 and 4). The combined application of compost and seed inoculation with *Pseudomonas* increased the availability and uptake of minerals like P, Mn, and K in chickpea plants (Sahni et al., 2008).

Green manure had significant effect on magnesium and manganese contents, but there was no significant effect on grain calcium and iron content. The highest Mg, Mn, and Fe contents were obtained from N_5 treatment and the highest Ca content was obtained from N_2 treatment (Table 4). It seems that application of compost causes increasing availability of nutrition elements to plant. Sahni et al. (2008) reported that compost application increased the availability and uptake of minerals like Zn, Mn and Fe in chickpea plants. Combined application of biofertilizers increased content of nutrition elements contents of grain except calcium (Table 4). These findings are agreed with the report of Sahni et al. (2008). They have stated that *Pseudomonas* increased the availability and uptake of minerals like Fe and Mn in chickpea plants.

Grain organic compounds

There was a significant effect of green manure on grain protein content, but no significant difference was observed in starch and crude fiber. The results showed that application of green manure increased protein content of seed (Table 4). Biologically, nitrogen fixed by legumes is a main benefit of growing green manures. The application of green manures to soil is considered as an effective management practice in any agricultural system due to stimulating soil microbial growth and activity with subsequent mineralization of plant nutrients (Eriksen, 2005). This microbial activity increases biological nitrogen fixation in soil (Chen et al., 2003) and nitrogen uptake by plant. Correlation between nitrogen and protein content has been reported (Al-Jaloud et al., 1996; Dordas and Sioulas, 2008). Other studies have demonstrated that application of green manure increased grain protein content (Tejada et al., 2008).

There was a significant effect of base fertilizers on protein, crude fiber and starch content of chickpea grain. Protein and starch content of grain were found to be enhanced by the combined application of triple base fertilizer (N_5) compared to individual one (Table 4). Co-application of compost and farm manure decreased crude fiber and enhanced chickpea seed quality. Result showed that biofertilizers had no significant effect on crude fiber and starch content of grain. Chickpea inoculated with biofertilizers have significantly higher grain protein content. Maximum protein content (15.06%) was observed in the treatment that received a combined inoculation of PSB and *T. harzianum*. Vinale et al. (2008) reported that *Trichoderma* sp. induced genes were associated with protein metabolism. Jutur and Reddy (2007) have also reported positive correlation between PSB and protein content.

Green manure had no significant effect on grain sugars content while both biofertilizer and base fertilizers influenced sugar content significantly. Combined application of triple base fertilizer (N5) increased sucrose, stachyose, verbascose, ciceritol and total sugar content of grain. Combined inoculation of PSB and *T. harzianum* increased the ciceritol and raffinose (Table 5).

Cooking time

One of the main drawbacks that limit the utilization of legumes is their long cooking time (Williams et al., 1983). In this study, combined application of compost and farm manure (N_4) decreased cooking time of chickpea grain (Table 4). Also combined inoculation of biofertilizers has increase the effect on cooking time. The longer cooking time requirement could be attributed to its larger seed weight, since seed size governs the distance to which, water must penetrate in order to reach the innermost portion of seeds. A significant positive correlation of cooking time with grain weight has been reported by Kaur et al. (2005). Individual application of chemical fertilizer has a longer cooking time (66 min) and seed weight (20.72 g) compared to individual application of compost and farm manure. Chickpea has been observed to be the

Table 5. Effect of soil fertility systems on sugars content of chickpea seed.

Treatment	Sucrose	Raffinose	Stachyose	Verbascose	Ciceritol	Total sugar
	(%)					
Green manure						
Vetch + barley (G_1)	2.57[a]	0.64 [a]	1.49 [a]	0.47 [a]	2.39 [a]	7.46 [a]
No green manure (G_2)	2.48[a]	0.62 [a]	1.18 [b]	0.46 [a]	2.35 [a]	7.33 [a]
Basal fertilizers						
Farm manure (N_1)	1.56[d]	0.62 [b]	1.35 [a]	0.41 [b]	1.97 [d]	5.94 [e]
Compost (N_2)	1.73[c]	0.61 [b]	1.45 [a]	0.49 [ab]	2.02 [d]	6.37 [d]
Chemical fertilizer (N_3)	3.29[ab]	0.64 [a]	1.35 [a]	0.53 [a]	2.60 [b]	8.19 [b]
Farm + Compost (N_4)	2.97[b]	0.61 [b]	1.45 [a]	0.48 [ab]	2.28 [c]	7.71 [c]
Farm+Compost+Chemical (N_5)	3.42[a]	0.64 [a]	1.45 [a]	0.54 [a]	2.95 [a]	8.77 [a]
Biofertilizers						
PSB (B_1)	2.50	0.61 [b]	1.36 [a]	0.46 [a]	2.36 [ab]	7.31 [a]
Trichoderma fungi (B_2)	2.54	0.64 [a]	1.39 [a]	0.46 [a]	2.32 [b]	7.38 [a]
PSB + fungi (B_3)	2.54	0.64 [a]	1.37 [a]	0.46 [a]	2.41 [a]	7.44 [a]
Control (B_4)	2.52	0.62 [b]	1.41 [a]	0.48 [a]	2.39 [a]	7.46 [a]

Men values in each column with the same superscript(s) do not differ significantly by DMRT (P = 0.05).

most difficult to cook among the commonly consumed legumes due to its larger seed size and chemical composition (Singh, 1999). Differences in gelatinization pattern of the starch and the susceptibility of the cell constituents, notably the protein, to softening may be contributed to the overall textural characteristics of the legume (Sefa-Dedah and Stanley, 1979). Green manure had no significant effect on cooking time.

Yield and yield components

Analysis of variance showed that various soil fertility methods and their interactions had significant effects on pod number per plant and number of fertile pods. The comparison of treatments means indicated that green manure increased pod number per plant and number of fertile pods (Table 6). Simultaneous application of compost, farm manure and chemical fertilizer produced higher amounts of pod number and fertile pods per plant. Base fertilizers × green manure interaction identified that green manure cultivation along with simultaneous application of compost; farm manure, and chemical fertilizer (G_1N_5) produced 72.83 pods per plant and 39.75 fertile pods. Moreover, it showed a significant difference with other treatments (Table 7). Evaluating the green manure × biofertilizers interaction revealed that G_1B_3 treatment produced the highest pod number per plant (Table 8). The existence of appropriate amount of moisture, nutrients, and the lack of pathogens are the most important factors for pods fertility and seed production. Simultaneous application of compost, farm manure, and chemical fertilizer significantly increased fertile pod number which is attributed more nutrients provision. It seems that existence of PSB along with green manure and existence of *Trichoderma* fungus have increased fertile pod number because of more availability of nutrients and decreasing pathogens and stress factors, respectively. Report of Rudresh et al. (2005) indicated increasing fertile pods per plant under combined application of phosphate solubilizing bacterium and *Trichoderma* spp., agreed with the study results. Base fertilizer and biofertilizer and their interaction had a significant effect on seed number per pod and 100 grain weight. Highest seed number per pod (1.168) and 100 grain weight (21.69 g) were observed in N_5B_3 treatment (data not shown). Comparison of green manure × base fertilizer interactions showed that the highest seed number per pod was obtained in G_1N_5 treatment. Although, green manure had no significant effect on seed number, existence of green manure along with integrated application of fertilizers increased seed number per plant. There is a statistically significant correlation between seed number per plant pod fertility percentage. Rudresh et al. (2005) emphasized that nutrients availability plays an impartment role in increasing seed number per pod. Despite the increase in 100 grain weight in N_3 compared to N_4, there was no significant difference between them. However, combined application of compost and farm manure in comparison with individual application of them increased 100 grain weight. Application of super phosphate chemical fertilizer and PSB had no significant effect on increasing 100 grain weight. Nitrogen plays an important role in grain filling; however, it seems that despite phosphorus high role in plant metabolism; this element has less effect on 100 grain weight than nitrogen.

Table 6. Effect of soil fertility systems on grain yield and yield components of chickpea.

Treatment	Grain yield (kg ha^{-1})	Pod number per plant	Fertile pods per plant	Grain number per pod	100 grain weight (g)
Green manure					
Vetch + barley (G_1)	1961.1^a	45.63^a	28.53^a	1.090^a	20.93^a
No green manure (G_2)	1785.6^b	38.78^b	26.01^b	1.073^a	20.54^a
Basal fertilizer					
Farm manure (N_1)	969.3^d	21.83^e	14.88^e	1.002^c	20.34^b
Compost (N_2)	1521.1^c	32.22^d	21.37^d	1.100^b	20.42^b
Chemical fertilizer (N_3)	2119.4^b	44.87^c	29.41^c	1.022^c	20.72^b
Farm + Compost (N_4)	2147.5^b	52.60^b	34.02^b	1.031^{ab}	20.28^b
Farm + Compost + Chemical (N_5)	2609.2^a	59.72^a	36.65^a	1.151^a	21.31^a
Biofertilizer					
PSB (B_1)	1756.1^c	39.72^b	25.84^c	1.083^b	20.79^a
Trichoderma fungi (B_2)	1866.2^b	40.79^b	27.41^b	1.072^b	21.15^a
PSB + fungi (B_3)	2560.3^b	57.66^a	35.07^a	1.144^a	21.19^a
Control (B_4)	1310.7^d	30.83^c	20.73^d	1.028^c	19.52^b

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (P = 0.05).

Table 7. Interactive effect of basal fertilizers and green manure on grain yield and yield components of chickpea.

Green manure	Basal fertilizer	Grain yield (kg ha^{-1})	Pod number per plant	Fertile pods per plant	Grain number per pod	100 grain weight (g)
Vetch + barley (G1)	Farm manure (N_1)	885.7^d	22.41^c	13.83^d	1.014^c	20.37^a
	Compost (N_2)	1523.7^c	32.33^c	22.01^c	1.114^b	21.43^a
	Chemical fertilizer (N_3)	2214.2^b	47.01^b	31.83^b	1.020^c	20.75^a
	Farm +Compost (N_4)	2293.3^b	53.58^b	35.25^{ab}	1.128^{ab}	20.18^a
	Farm+Compost+Chemical (N_5)	2888.5^a	72.83^a	39.75^a	1.193^a	20.93^a
No green manure (G2)	Farm manure (N_1)	1053.3^c	21.25^c	15.93^d	1.005^c	19.91^a
	Compost (N_2)	1518.4^b	32.12^b	20.75^c	1.087^b	20.41^a
	Chemical fertilizer (N_3)	2080.8^a	51.62^a	32.75^{ab}	1.025^{bc}	20.70^a
	Farm +Compost (N_4)	1945.5^{ab}	42.75^a	29.00^b	1.143^a	20.07^a
	Farm+Compost+Chemical (N_5)	2330.1^a	46.62^a	33.56^a	1.112^a	20.29^a

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (P = 0.05).

Application of biofertilizer increased 100 grain weight in comparison with control treatment. Our results indicated an interaction between biofertilizers and nitrogen fixation bacteria in chickpea roots. A greater number of nodules and dry weight was registered in soy-bean and alfalfa under co-inoculation with Rhizobia strains and phosphate solubilizing *Pseudomonas* strains (Rosas et al., 2006).

Chickpea grain yield was affected by different soil fertility systems. All two-way interactions significantly made effect on grain yield. An increase of 9% in the grain yield of chickpea was recorded under effective application of green manure (Table 6). Since the highest amounts of grain yield components were obtained from N_5 and B_3 treatments, it produced the highest grain yield. Combined inoculation of PSB and *T. harzianum* (B_3) significantly increased grain yield. Microorganisms' activity to excrete organic acids and phosphates could release elements from complexes presently in soil and increase nutrient availability to plants (Rudresh et al., 2005; Jutur and Reddy, 2007).

Similar reports of increasing yield and nutrient uptake by combined inoculation of Rhizobium and PSB have

Table 8. Interactive effect of biofertilizers and green manure on grain yield and yield components of chickpea.

Green manure	Biofertilizer	Grain yield (kg ha^{-1})	Pod number per plant	Fertile pods per plant	Grain number per pod	100 grain weight (g)
Vetch + barley (G1)	PSB (B_1)	1920.5 ^{b}	45.4 ^{b}	28.13 ^{b}	1.092 ^{a}	20.55 ^{b}
	Trichoderma fungi (B_2)	2001.6 ^{b}	44.1 ^{b}	28.53 ^{b}	1.109 ^{a}	21.60 ^{a}
	PSB + fungi (B_3)	2600.6 ^{a}	60.9 ^{a}	36.80 ^{a}	1.118 ^{a}	21.65 ^{a}
	Control (B_4)	1321.1 ^{c}	42.0 ^{b}	20.66 ^{b}	1.041 ^{a}	19.95 ^{b}
No green manure (G2)	PSB (B_1)	1591.5 ^{b}	34.05 ^{b}	23.55 ^{b}	1.075 ^{b}	20.83 ^{a}
	Trichoderma fungi (B_2)	1730.7 ^{b}	37.4 ^{b}	26.30 ^{b}	1.035 ^{b}	21.50 ^{a}
	PSB + fungi (B_3)	2519.9 ^{a}	54.4 ^{a}	33.35 ^{a}	1.170 ^{a}	21.13 ^{a}
	Control (B_4)	1300.2 ^{b}	29.6 ^{b}	20.80 ^{b}	1.015 ^{b}	19.10 ^{b}

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (P = 0.05).

been reported by Alagawadi and Gaur (1988) and Rudresh et al., (2005). The increase in growth and yield components of chickpea by combined inoculation of Rhizobium, PSB and *T. harzianum* may be attributed to cumulative effects, such as enhanced supply of N and P to the crop in spite of growth promoting substances produced by these organisms. In addition to biocontrol activity of *T. harzianum* against soil borne fungal pathogens (Windham et al., 1986; Vinale et al., 2008), the increase in grain yield can be attributed to pathogens reduced. The increase in growth and yield of chickpea could be related to nutrient supplementation among the organisms inoculated, which might have enhanced their efficiencies like N fixation by *Rhizobium*, P-solubilization by PSB and effective pathogen suppression by *T. harzianum*. Similarly, increasing nitrogen fixation by *Rhizobium* sp. due to phosphorus supplementation has been reported (Manjunath and Bagyaraj, 1984).

Base fertilizers comparison revealed that N_5 treatment had a significant difference with other treatments (Table 6). For justification of this difference, it could be stated that along with meeting plant need to phosphorus, adding compost and farm manure to soil could provide micro elements for plant. Compost applied in this study has been shown to contain elevated concentrations of micro elements including zinc (Zn). Zinc is one of the elements that chickpea shows positive response to it (Siavashi et al., 2004). Therefore, with view to the negative interaction between phosphorus and zinc, providing phosphorus by chemical fertilizer decreases zinc availability but compost offers zinc to plant gradually. Moreover, it seems that green manure causes improving soil structure and optimizing root growth conditions by providing organic matter and nutrients. Comparisons of interactions (Table 7) showed that in treatment having green manure, adding farm manure and compost to chemical fertilizer significantly increased grain yield compared to chemical fertilizer, however, in the absence of green manure no significant increase occurred in grain yield. Simultaneous application of biofertilizer and green manure also significantly increased grain yield (Sarwar et al., 2008b). Finally, $G_1N_5B_3$ treatment is introduced as the superior treatment regarding to grain yield.

Conclusion

According to the results of various quantitative and qualitative characteristics of chickpea like as yield, yield components and minerals and organic compound of grain, the $G_1N_5B_3$ treatment could be suggested as superior treatment in this study. This treatment seems to be cost effective and environmentally sound; therefore, it could allocate our agro-ecosystems into sustainable agriculture. The more ecological approach to soil management has come from the sustainable development agenda in which the main concern with the maintenance of yield is closely associated with desires to conserve natural resources, including a greater value accorded to maintenance of biodiversity.

ACKNOWLEDGEMENTS

We would like to thank Saeid Sofizadeh (Shahid Beheshti University, Iran) and Ali Mokhtasi Bidgoli (Tarbiat Modares University, Iran) for valuable comments on the manuscript and Ali Mohammad Modarres Sanavi (Tarbiat Modares University, Iran) for his help in analyzing data. The Sanandaj Agriculture Research Center for farm support and Tarbiat Modares University is acknowledged for financial support.

REFERENCES

Al-Jaloud AA, Hussian G, Karimulla S, Al-Hamidi AH (1996). Effect of

irrigation and nitrogen on yield and yield components of two rapeseed cultivars. Agric. Water Manage. 30: 57-68.
Alagawadi AR, Gaur AC (1988). Associative effect of rhizobium and phosphate-solubilizing bacteria on the yield and nutrient uptake of chickpea. Plant Soil, 105: 241-246.
AOAC (1990). In Helrich K (Ed.). Official methods of analysis (15th ed.). Arlington, VA/Washington, DC, USA: Association of Official Analytical Chemists.
Bennett JW, Lane SD (1992). The potential role of *Trichoderma viride* in the integrated control of *Botrytis fabae*. Mycologia, 6: 199-201.
Berry PM, Sylvester-Bradley R, Phillips L, Hatch DJ, Cuttle S, Raynes F, Gosling F (2002). Is the productivity of organic farms restricted by the supply of available nitrogen? Soil Use Manage. 15: 137-143.
Bremner JM (1996). Nitrogen-total. In: Sparks, D.L., et al., (Eds.), Chemical Methods. Part 3. SSSA BOOK Series. SSSA, ASA, Madison, WI, USA, pp. 1085-1121.
Carter MR (1993). Soil sampling and methods of analysis. Canadian Society of Soil Science. Lewis Publisher. p. 1240.
Chen G, Zhu H, Zhang Y (2003). Soil microbial activities and carbon and nitrogen fixation. Res Microbiol. 154: 393-398.
Dahnke WC, Olsen, RA (1990). Soil test correlation, calibration, and recommendation. pp. 45-72. In Westerman RL (ed.) Soil testing and plant analysis, 3rd ed. SSSA Book Ser. 3. SSSA, Madison, WI.
Doran JW, Smith MS (1987). Organic matter management and utilization of soil and fertilizer nutrients. In: Follett RF, Stewart JWB, Cole CV (Eds.), Soil Fertility and Organic Matter as Critical Components of Production Systems. SSSA Spec. Pub. 9. SSSA, Madison, WI, pp. 53-72.
Doran JW, Fraser DG, Culik MN, Liebhardt WC (1988). Influence of alternative and conventional agricultural management on soil microbial process and nitrogen availability. Am. J. Alt. Agric. 2: 99-106.
Dordas CA, Sioulas C (2008). Safflower yield, chlorophyll content, photosynthesis, and water use efficiency response to nitrogen fertilization under rainfed conditions. Ind. Crop Prod. 27: 75-85.
El-Baruni B, Olsen SR (1979). Effect of manure on solubility of phosphorus in calcareous soils. Soil Sci. 128: 219-225.
El-Komy HMA (2005). Coimmobilization of A. lipoferum and B. megaterium for plant nutrition. Food Technol. Biotechnol. 43(1): 19-27.
Elfstrand S, Ba B, Rtensson M (2007). Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. Appl. Soil Ecol. 36: 70-82.
Eriksen J (2005). Gross sulphur mineralization-immobilization turnover in soil amended with plant residues. Soil Biol Biochem. 37: 2216-2224.
Gao SJ, Chen SS, Li MQ (1989). Effects of phosphorus nutrition on photosynthesis and photorespiration in tobacco leaves. Acta Phytophysio Sinica. 15: 281-287.
Guidi L, Pallini M, Soldatini GF (1994). Influence of phosphorus deficiency on photosynthesis in sunflower and soybean plants. Agrochimica, 38: 211-223.
Hatch DJ, Goodlass G, Joynes A, Shepherd MA (2007). The effect of cutting, mulching and applications of farmyard manure on nitrogen fixation in a red clover grass sward. Bioresour. Technol. 98: 3243-3248.
Huisman J, Vander Poel AF (1994). Aspects of the nutritional quality and use of cool season food legumes in animal feed. In Muehlbauer FJ and Kaiser WJ (Eds.). Expanding the production and use of cool season food legumes. Dordrecht: Kluwer Academic Publishers. pp. 53-76.
Iqbal M, Hassan A, Ibrahim M (2008). Effects of tillage systems and mulch on soil physical quality parameters and maize (*Zea mays* L.) yield in semi-arid Pakistan. Biol. Agric. Hortic. 25: 311-325.
Jackson ML (1973). Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi. pp. 134-149.
Jain PC, Kushawaha PS, Dhakal US, Khan H, Trivedi SM (1999). Response of chickpea (*Cicer arietinum* L.) to phosphorus and biofertilzier. Legume Res. 22: 241-244.
Jutur PP, Reddy AR (2007). Isolation, purification and properties of new restriction endonucleases from *Bacillus badius* and *Bacillus lentus*. Microbiol. Res. 162: 378-383.
Kaur M, Singh N, Sodhi NS (2005). Physicochemical, cooking, textural and roasting characteristics of chickpea (*Cicer arietinum* L.) cultivars. J. Food Eng. 69: 511-517.
Kumar RN, Mukerji KG (1996). Integrated disease management future perspectives. In: Mukerji KG, Mathur B, Chamola BP, Chitralekha C (Eds.). Adv. Bot., APH publishing Corp, New Delhi, pp. 335-347.
Liebman M, Mohler CL, Staver CP (2004). Ecological Management of Agricultural Weeds. Cambridge University Press. p. 545.
Little TM, Hills FC (1978). Agricultural experimentation. John Wiley and Sons Inc, USA, p. 476.
Manjunath A, Bagyaraj DJ (1984). Response of pigeon pea and cowpea to phosphorus and dual inoculation with vesicular arbuscular Mycorrhiza and Rhizobium. Trop. Agric. (Trinidad). 61: 48-52.
McLeod E (1982). Feed the Soil. Organic Agriculture Research Institute, Graton, California. p. 209.
O'Sullivan DJ, O'Gara D (1992). Trails of fluorescent Pseudomonas spp. involved in suppression of plant root pathogens. Microbiol. Rev., 56: 662-676.
Pandey RK, Maranville JW, Chetima MM (2000). Deficit irrigation and nitrogen effects on maize in a Sahelian environment II. Shoot growth, nitrogen uptake and water extraction. Agric. Water Manage. 46: 15-27.
Parmar N, Dadarwal KR (1999). Stimulation of nitrogen fixation and induction of flavonoid-like compounds by rhizobacteria. J. Appl. Microbiol. 86: 36-64.
Power JF (1990). Use of green manures in the Great Plains. In: Havlin JL, Jacobsen JS (Eds.). Proceedings of the Great Plains Soil Fertility Conference, Denver, CO. 6-7 March. Kansas State University, Manhattan, KS, pp. 1-18.
Rajendran G, Sing F, Desai AJ, Archana V (2008). Enhanced growth and nodulation of pigeon pea by co-inoculation of Bacillus strains with Rhizobium spp. Bioresour. Technol. 99: 4544-4550.
Ravindra KC, Venkatesan K, Balasubramanian T, Balakrishnan, V (2007). Effect of halophytic compost along with farmyard manure and phosphor bacteria on growth characteristics of *Arachis hypogaea* Linn. Sci. Total Environ. 38(4): 333-341.
Rong L, Volenec JJ, Joern BC, Cunningham SM (1996). Seasonal changes in nonstructural carbohydrates, protein, and macronutrient in roots of alfalfa, red clover, sweet clover, and birds foot trefoil. Crop Sci. 36: 617-623.
Rosas SB, Andres GA, Rovera M, Correa NS (2006). Phosphate-solubilizing *Pseudomonas putida* can influence the Rhizobia legume symbiosis. Soil Biol. Biochem. 38: 3502-3505.
Rudresh DL, Shivaprakash MK, Prasad RD (2005). Effect of combined application of rhizobium, phosphate solubilizing bacterium and *Trichoderma spp*. on growth, nutrient uptake and yield of chickpea (*Cicer aritenium* L.). Appl. Soil Ecol. 28: 139-146.
Ryan J, Pala M, Masri S, Singh M, Harris H (2008). Rainfed wheat-based rotations under Mediterranean conditions: Crop sequences, nitrogen fertilization, and stubble grazing in relation to grain and straw quality. Eur. J. Agron. 28: 112-118.
Sarwar G, Hussain N, Schmeisky H, Muhammad S, Ibrahim M, Ahmad S (2008a). Efficiency of various organic residues for enhancing rice-wheat production under normal soil conditions. Pak .J. Bot. 40(5): 2107-2113.
Sarwar G, Schmeisky H, Hussain N, Muhammad S, Ibrahim M, Safdar E (2008b). Improvement of soil physical and chemical properties with compost application in rice-wheat cropping system. Pak. J. Bot. 40: 275-282.
Sarwar MA, Ibrahim M, Tahir M, Ahmad K, Khan ZI, Valeem EE (2010). Appraisal of pressmud and inorganic fertilizers on soil properties, yield and sugarcane quality. Pak. J. Bot. 42: 1361-1367.
Sahni S, Sarma BK, Singh DP, Singh HP, Singh KP (2008). Vermicompost enhances performance of plant growth-promoting rhizobacteria in *Cicer arietinum* rhizosphere against *Sclerotium rolfsii*. Crop Prot. 27: 369-376.
Sattar MA, Gaur AC (1987). Production of Auxins and Gibberellins by phosphate dissolving microorganisms. Zentralbl Mikrobiol. 142: 393-395.
Sefa-Dedah S, Stanley DW (1979). Textural implications of the microstructure of legumes. Food Technol. 33: 77-83.
Singh U (1999). Cooking quality of pulses. J. Food Sci Technol. 36: 1-14.

Siavashi K, Soleimani R, Malakouti MJ (2004). Effect of zinc sulfate application times and methods on grain yield and protein content of chickpea in rainfed conditions. Iranian J. Soil Water Sci. 18: 37-44.
Tejada M, Gonzalez JL, Garcı´a-Martı´nez AM, Parrado J (2008). Effects of different green manures on soil biological properties and maize yield. Bioresour. Technol. 99:1758-1767.
Verma M, Brar SK, Tyagi RD, Surampalli RY, Val´ero JR (2007). Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. Biochem. Eng. J. 37: 1-20.
Veneklaas EJ, Stevens J, Cawthray GR, Turner SM, Grigg AM, Lambers H (2003). Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. Plant Soil, 248: 187-197.
Vinale F, Sivasithamparam K, Ghisalberti EL, Woo SL, Lorito M (2008). *Trichoderma*-plant-pathogen interactions. Soil Biol. Biochem. 40: 1-10.
Wang D, Marschner P, Solaiman Z, Rengel Z (2007). Growth, P uptake and rhizosphere properties of intercropped wheat and chickpea in soil amended with iron phosphate or phytate. Soil Biol. Biochem. 39: 249-256.
Windham MT, Elad Y, Baker R (1986). A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology, 76: 518-521.
Williams PC, Nakoul H, Singh KB (1983). Relationship between cooking time and some physical characteristics in Chickpea (*Cicer arietinum* L.). J. Sci. Food Agric. 34: 492-496.
Zeng X, Li Y, SunY, Hong Y, Liu J, Xiang X, Wang K (2007). Determination of free sugars by high performance liquid chromatography. Chin. J. Anal. Chem. 35: 930-938.
Zia Ul-Haq M, Iqbal S, Ahmad S, Imran M, Niaz A, Bhanger MI (2007). Nutritional and compositional study of Desi chickpea (*Cicer arietinum* L.) cultivars grown in Punjab, Pakistan. Food Chem. 105: 1357-1363.