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Effect of Organic Matter and Biofertilizers on Chickpea Quality and Biological Nitrogen Fixation

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Abstract**—**In order to evaluation the effects of soil organic matter and biofertilizer on chickpea quality and biological nitrogen fixation, field experiments were carried out in 2007 and 2008 growing seasons. In this research the effects of different strategies for soil fertilization were investigated on grain yield and yield component, minerals, organic compounds and cooking time of chickpea. Experimental units were arranged in split-split plots based on randomized complete blocks with three replications. Main plots consisted of (G1): establishing a mixed vegetation of *Vicia panunica* and *Hordeum vulgare* and (G2): control, as green manure levels. Also, five strategies for obtaining the base fertilizer requirement including (N1): 20 $t.ha^{-1}$ farmyard manure; (N2): 10 $t.ha^{-1}$ compost; (N3): 75 $kg.ha^{-1}$ triple super phosphate; (N4): 10 $t.ha^{-1}$ farmyard manure + 5 $t.ha^{-1}$ compost and (N5): 10 $t.ha^{-1}$ farmyard manure + 5 $t.ha^{-1}$ compost + 50 $kg.ha^{-1}$ triple super phosphate were considered in sub plots. Furthermoree four levels of biofertilizers consisted of (B1): *Bacillus lentus* + *Pseudomonas putida*; (B2): *Trichoderma harzianum*; (B3): *Bacillus lentus* + *Pseudomonas putida* + *Trichoderma harzianum*; and (B4): control (without biofertilizers) were arranged in sub-sub plots. Results showed that integrating biofertilizers (B3) and green manure (G1) produced the highest grain yield. The highest amounts of yield were obtained in G1×N5 interaction. Comparison of all 2-way and 3-way interactions showed that G1N5B3 was determined as the superior treatment. Significant increasing of N, P_2O_5, K_2O, 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 abilities of the crops. The combined application of compost, farmyard manure and chemical phosphorus (N5) 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.

Keywords**—**chickpea, biofertilizer, nitrogen fixation.

I. INTRODUCTION

THE chickpea (*Cicer arietinum* L.) as a healthy vegetarian food has an important role in human food and domestic animal feed in Iran.

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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 [10]. Also chickpea play a key role in organic cropping systems. In such agro ecosystem with limited availability of nitrogen, chickpea potentially constitute both a cash crops and a source of N incorporation into the system via biological nitrogen fixation. To be sustainable, organic farming needs to be self-sufficient in nitrogen (N) through the fixation of atmospheric di-nitrogen (N2) by legumes, recycling of crop residues (green manures) [6] and the application of farmyard manure, compost and biofertilizer [18]. Green manures application to the soil is considered a good management practice in all agricultural production system because of increasing cropping system via sustainability by reducing soil erosion, improving soil physical properties and increasing soil organic matter and fertility levels [16]. Phosphorus is present as mineral deposits, which are a nonrenewable 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 [8] and, conversely, increases P increased photosynthesis [7]. Phosphate solubilizing bacteria are also known to increase phosphorus uptake resulting in better growth and higher yield of crop plants [20]. The combined inoculation of Rhizobium and phosphate solubilizing bacteria has increased nodulation, growth and yield parameters in chickpea [12, 20]. *Trichoderma* sp. have long been known as effective antagonists against soil borne plant pathogenic fungi [2] and promote 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.

Supplementation of soil and inoculants with glutamate, glycerol, and organic matter has been shown to enhance the survival and numbers of rhizobia in soils and increase both early nodulation and N2 fixation [21]. This result indicates that, although rhizobia can surely persist in soils, their efficacy can be enhanced by carbon addition in organic matter.

The present research is going to introduce a sustainable soil fertility system, evaluates the combined effect of biofertilizers and organic manure such green manure, compost and farmyard manure on chickpea quality and biological nitrogen fixation.

II. Material And Methods

The field experiments were conducted at Agricultural Research Center of Sanandaj in Kurdistan province of Iran during the 2007 and 2008 growing seasons. Experimental units were arranged in split-split plots based on randomized complete blocks with three replications. Main plots consisted of (G1): establishing a mixed vegetation of *Vicia panunica* and *Hordeum vulgare* and (G2): control, as green manure levels. Also, five strategies for obtaining the base fertilizer requirement including (N1): 20 $t.ha^{-1}$ farmyard manure; (N2): 10 $t.ha^{-1}$ compost; (N3): 75 $kg.ha^{-1}$ triple super phosphates; (N4): 10 $t.ha^{-1}$ farmyard manure + 5 $t.ha^{-1}$ compost and (N5): 10 $t.ha^{-1}$ farmyard manure + 5 $t.ha^{-1}$ compost + 50 $kg.ha^{-1}$ triple super phosphates were considered in sub plots. Four levels of biofertilizers consisted of (B1): *Bacillus lentus* + *Pseudomonas putida*; (B2): *Trichoderma harzianum*; (B3): *Bacillus lentus* + *Pseudomonas putida* and *Trichoderma harzianum*; and (B4): Control (without biofertilizers) were arranged in sub-sub plots. The G1 plots were planted with green manure comprise of vetch (*Vicia panunica*) and barley (*Hordeum vulgare*) with equation portion on 15 October 2007 (in the rows 10 cm apart). On April 10th 2008, these green manures were either incorporated into the soil with a hand-hoe in the manner of a chisel plough. Three soil cores from the tillage zone (0-15 cm) of each plot were collected and routine soil test analysis determined by the Dahnke and Olsen (1990) soil test method [4]. The farmyard manure and compost were also analyzed for chemical and nutrients properties.The chickpea seeds, according to arrangement of sub-sub plots treated with *Trichoderma harzianum* isolate T39, *Bacillus lentus* isolate P5 and *Pseudomonas putida* isolate P13. Also, *Mesorhizobium sp*. cicer strain SW7 was added to all the treatments. Chickpea seeds planted On 25 April 2008 and harvested on the 75th day after sowing. The nitrogen and phosphorus content of shoot and matured seeds was determined by vanado molybdate phosphoric acid yellow colour method and Microkjeldhal method, respectively [13]. Also, the potassium content was determined by Flame Photometer model-EEL [1]. The other minerals, such as calcium, manganese, magnesium and iron, were determined with an atomic absorption spectrophotometer (Perkin-Elmer Model 5000) [1]. Nitrogen fixation was estimated by measuring ‘‘in situ’’ the acetylene reduction activity (ARA) at the end of the vegetative stage Huss-Danell et al [9]. Nodule assessment was carried out as described by Vincent method [24].

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 [3]. Crude fiber and starch were determined using the methods described by Rong et al [19]. The separation and quantification of Sugar compounds from seeds were carried out by an Agilent 1100 series HPLC system (Agilent, USA), which consisted of a G1311A pump and a G1362A refraction index detector [27]. Seed protein contents were determined by near infrared reflectance spectroscopy, using a 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 were evaluated from an area of 2 m × 2.5 m 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. Cooking time was determined by the method of Williams et al. [26]. The data collected in this study was subjected to analysis of variance (ANOVA) and means comparison has done using Duncan’s Multiple Range Test.

III. Results And Discussion

Green manure had a significant effect on grain nitrogen content, nodule number and nodule activity. Means comparisons specified that incorporating vetch and barley biomass into the soil before chickpea cultivation, increased grain nitrogen contents by 7% (Table I). Nitrogen fixation by vetch, increased soil organic matter and optimized conditions for *Rhizobium* bacteria are the main reasons for increasing nitrogen uptake due to application of green manure. Elfstrand et al. (2007) reported that green manure application increased nitrogen content of plant [6]. Also findings of Ryan et al. (2008) indicated that application of vetch as green manure enriched the N in grain and straw [21]. Basal fertilizers had a significant effect on grain nitrogen content, nodule number and nodule activity (Tables I&II), in such a manner the highest grain contents nitrogen (2744 mg / 100 g) obtained from N5 treatment. The main reason is that compost and farmyard manure can increase N availability to plant due to more nitrogen offered to plant. Grain nitrogen content in N2 treatment are significantly more than of those in N1 and N3 treatments (Table I). Also, biofertilizers had significant effect on grain nitrogen contents, nodule number and nodule activity. The highest grain nitrogen contents were obtained from B1 (Table I). In fact, the positive interaction between biofertilizers and

Rhizobium bacterium caused increasing in biological nitrogen fixation. There is evidence that some *Pseudomonas* species increase nutrient absorption, as N, P and K, in addition to act as bio control agents of phyto pathogenic fungi and produce phyto hormones in the rhizosphere, which promote plant growth [15].

The result showed that different methods of soil fertility had a significant effect on grain phosphorus contents. The highest grain P contents were obtained from N5 treatment (Table I). Increasing effect of combined application of compost and farmyard manure on soil enzymic activity such as phosphatase and increase P availability for plant has been reported by El-baruni and Olsen [5]. Triple super phosphate fertilizer (N3) in comparison with compost and farmyard manure significantly increased grain P contents. Also, application of green manure significantly increased grain P contents. Adding phosphorus of green manure to soil and appropriate condition prepared for PSB are the main reasons for increase of grain P content in this treatment. Mean comparison showed that combined application of biofertilizers (B3) produced the highest grain P content (279 $mg.100g^{-1}$). Since phosphatases play an important role in nutrient P availability of organic manures and crop residue and phosphates activity and soil P availability appear to complement each other [5], therefore providing P in rhizosphere can increase P uptake by plant. Similar report of increase in phosphorus uptake by combined inoculation of *Trichoderma* sp. and PSB were reported by Rudresh et al [20].

According to the analysis of variance leaf chlorophyll significantly affected by different soil fertility methods, in such a manner means comparisons showed that green manure significantly increased leaf chlorophyll (Table I). 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 [20]. Regarding to 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 I). Rajendran et al. (2008) reported that the amount of chlorophyll increased when the co-inoculation with *Rhizobium* strains and PSB [17]. Comparisons of base fertilizer levels showed that the highest chlorophyll content was obtained from N5 treatment. After N5 treatment, followed by N4 treatment (Table I).

Basal fertilizers and biofertilizers had significant effect on grain potassium contents, but green manure had no significant effect on K content. Combined application of basal fertilizers improved plant nutrition conditions. The highest grain K contents were obtained from N5 treatment (Table I). There is evidence that compost application increase potassium absorption in chickpea seeds [22]. Combined application of biofertilizers produced the highest grain K contents (Table I). 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 [22]. Addition of organic matter through farmyard manure and compost with concomitantly rise in pH, higher N fixation and improved growth could explain the increase in the concentration of N, P, K, Ca and Mg in kernels under the plot receiving integrated nutrient management comprising farmyard manure, compost and chemical fertilizers. During the course of decomposition farmyard manure produced organic acids, which increased the P solubility and holding capacity of K in soil as well as helped in formation of soluble complex agents with micronutrients thereby decreased their fixation, resulting in greater uptake by chickpea plant and thereby higher concentration in the grains.

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 N5 treatment and the highest Ca content was obtained from N2 treatment (Table I). It seems that application of compost causes increased 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 [22]. Combined application of biofertilizers increased nutrition elements contents of grain except calcium (Table I).

There was a significant effect of green manure on grain protein content, but no significant differences observed in starch and crude fiber. Result showed that application of green manure increased protein content of seed (Table II). Biological nitrogen fixed by legumes is a main benefit of growing green manures. Other studies have demonstrated that application of green manure increased grain protein content [19, 20]. 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 (N5) compared to individual one (Table II). Co-application of compost and farmyard manure enhanced crude fiber and 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 [25]. Jutur and Reddy (2007) have also reported positive correlation between PSB and protein content [14]. 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.

TABLE I
EFFECT OF DIFFERENT FERTILIZERS ON CHLOROPHYLL AND NUTRIENT ACCUMULATION IN CHICKPEA SEED

Treatment	Chlorophyll (Spad reading)	Nitrogen (mg/100g)	Phosphorus (mg/100g)	Potassium (mg/100g)	Calcium (mg/100g)	Magnesium (mg/100g)	Manganese (mg/100g)	Iron (mg/100g)
Green manure								
Vetch + barley (G1)	44.11^{a}	2283^{a}	273.8^{a}	1208.2^{a}	184.9^{a}	4.35^{a}	2.64^{a}	4.42^{a}
No green manure (G2)	41.05^{b}	2140^{b}	268.2^{b}	1196.4^{a}	182.9^{a}	4.2^{b}	2.4^{b}	4.36^{a}
Basal fertilizer								
Farmyard manure (N1)	39.18^{e}	2015^{c}	271.6^{b}	1190.2^{b}	184.1^{a}	4.1^{c}	2.67^{a}	4.39^{a}
Compost (N2)	43.06^{c}	2468^{b}	264.7^{c}	1159.3^{c}	184.6^{a}	4.1^{c}	2.61^{a}	4.09^{a}
Chemical fertilizer (N3)	41.5^{d}	1981^{c}	273.2^{b}	1073.7^{d}	183.4^{a}	4.1^{c}	2.65^{a}	4.14^{a}
Farmyard + Compost (N4)	46.25^{b}	2579^{b}	273.1^{b}	1290.2^{a}	183.8^{a}	4.48^{b}	2.66^{a}	4.57^{a}
Farmyard+Compost+Chemical (N5)	47^{a}	2744^{a}	289.6^{a}	1298.1^{a}	183.5^{a}	4.66^{a}	2.68^{a}	4.6^{a}
Biofertilizer								
PSB[a] (B1)	43.4^{b}	2269^{b}	271.5^{b}	1201^{b}	184.3^{a}	4.32^{a}	2.63^{a}	4.42^{a}
Trichoderma fungi (B2)	43.35^{b}	2289^{b}	266^{c}	1176.3^{c}	183.7^{ab}	4.27^{b}	2.56^{b}	4.35^{a}
PSB + fungi (B3)	44.2^{a}	2410^{a}	279.8^{a}	1232.1^{a}	181.2^{b}	4.34^{a}	2.65^{a}	4.47^{a}
Control (B4)	43.2^{b}	2167^{c}	264.9^{c}	1199.8^{b}	184.5^{a}	4.28^{b}	2.57^{b}	4.36^{a}

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (P = 0.05)
[a] Phosphate Solubilizing Bacteria

TABLE II
EFFECT OF DIFFERENT FERTILIZERS ON GRAIN YIELD, GRAIN QUALITY AND BNF

Treatment	Total sugar (%)	Grain protein (%)	Grain crude fiber (%)	Grain starch (mg.kg^{-1})	Cooking time (min)	Nodule number	Nodule activity (μmol/h)	Grain yield (kg.ha^{-1})
Green manure								
Vetch + barley (G1)	7.56^{a}	14.26^{a}	8.19^{a}	155.6^{a}	66.35^{a}	45^{a}	8.2^{a}	1961.1^{a}
No green manure (G2)	7.23^{a}	13.37^{b}	7.85^{a}	153.6^{a}	65.8^{a}	37^{b}	4.2^{b}	1785.6^{b}
Basal fertilizer								
Farmyard manure (N1)	5.94^{e}	12.59^{c}	7.78^{c}	156.3^{a}	64.43^{b}	31^{c}	7^{b}	969.3^{d}
Compost (N2)	6.37^{d}	15.42^{b}	7.43^{d}	153.3^{b}	64.31^{b}	33^{c}	6.9^{b}	1521.1^{c}
Chemical fertilizer (N3)	8.1^{b}	12.38^{c}	9.55^{a}	153.2^{b}	66^{a}	10^{d}	3.7^{d}	2119.4^{b}
Farmyard + Compost (N4)	7.71^{c}	16.11^{b}	7.07^{e}	157.2^{a}	62.18^{c}	47^{a}	8.5^{a}	2147.5^{b}
Farmyard+Compost+Chemical (N5)	8.77^{a}	17.15^{a}	8.28^{b}	157.5^{a}	62.68^{c}	32^{c}	5^{c}	2609.2^{a}
Biofertilizer								
PSB[a] (B1)	7.31^{a}	14.18^{b}	8.12^{a}	154.1^{a}	65^{b}	37^{b}	8.2^{a}	1756.1^{c}
Trichoderma fungi (B2)	7.38^{a}	14.30^{b}	8.07^{a}	154.2^{a}	66.7^{a}	34^{b}	8^{a}	1866.2^{b}
PSB + fungi (B3)	7.44^{a}	15.06^{a}	7.99^{a}	153.6^{a}	66.8^{a}	42^{a}	8.4^{a}	2560.3^{b}
Control (B4)	7.46^{a}	13.54^{c}	8^{a}	152.6^{a}	65.6^{b}	26^{c}	6^{b}	1310.7^{d}

Mean values in each column with the same superscript(s) do not differ significantly by DMRT (P = 0.05)
[a] Phosphate Solubilizing Bacteria

Combined inoculation of PSB and *T. harzianum* increased the ciceritol and raffinose. Content of phosphorus, zinc and other minerals in chickpea plant increased under application of compost, farmyard manure and biofertilizers.

Combined application of compost and farmyard manure (N4) decreased cooking time of chickpea grain (Table II). Also combined inoculation of biofertilizers has increasing effect on cooking time. The longer cooking time requirement could be attributed to its larger seed weight, as seed size governs the distance to which water must penetrate in order to reach the innermost portion of seeds. 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 farmyard manure. Green manure had no significant effect on cooking time.

Chickpea grain yield was affected by different soil fertility systems. All two-way interactions significantly influenced grain yield. An increase of 9% in the grain yield of chickpea was recorded under application of green manure was found effective and (Table II). Since the highest amounts of grain yield components were obtained from N5B3 treatment, it produced the highest grain yield. Combined inoculation of PSB and *T. harzianum* (B3) significantly increased grain yield. Microorganisms' activity to excrete organic acids and phosphates could be able to release elements from complexes existent in soil and increasing nutrient availability to plants [14, 20]. The increase in growth and yield components of chickpea by combined inoculation of Rhizobium, PSB and *T. harzianum* found here may be due to cumulative effects, such as enhanced supply of N and P to the crop in addition to growth promoting substances produced by these organisms. In addition to biocontrol activity of *T. harzianum* against soil borne fungal pathogens [25], the increase in grain yield can be attributed to reduced pathogens. Base fertilizers comparison revealed that N5 treatment had a significant difference with other treatments (Table II). For justification of this difference it could be stated that parallel to meeting plant need to phosphorus, adding compost and farmyard manure to soil can provide micro elements for plant. Compost applied in the current study has been shown to contain elevated concentrations of micro elements including zinc (Zn). Zinc is one of the elements that chickpea indicates positive response to it [23]. Also, it seems that green manure causes improving soil structure and optimizing root growth conditions by providing organic matter and nutrients. Comparisons of interactions showed that in treatment having green manure, adding farmyard manure and compost to chemical fertilizer significantly increased grain yield compared to chemical fertilizer, but 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. At last, G1N5B3 treatment is introduced as the superior treatment regarding to grain yield.

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