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ORIGINAL PAPER

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EFFECTS OF ENRICHED ZEOLITE AND POULTRY MANURE ON QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF GREEN BEAN (*Phaseolus vulgaris* L.)

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ABSTRACT

In order to investigate the effects of enriched zeolite and poultry manure on quantitative and qualitative characteristics of green beans (*Phaseolus vulgaris* L.), a field experiment was conducted as a factorial in a randomized complete block design with three replications at Amol, Mazandaran province, Iran in 2017 and 2018. Zeolite in five levels including Z1 (control), Z2 – zeolite application, Z3 – enriched zeolite with zinc, Z4 – enriched zeolite with copper and Z5 – enriched zeolite with zinc + copper as well as poultry manure in five levels including P1 (control), P2 – poultry manure application, P3 – fortified poultry manure with zinc, P4 – fortified poultry manure with copper and P5 – fortified poultry manure with zinc + copper were considered as experimental factors. The results showed that the application of enriched poultry manure and zeolite with zinc and copper caused a significant increase in yield components, yield, photosynthetic pigments and nutrient concentrations in green beans compared to separate application of poultry manure or zeolite and control. In terms of poultry manure application, maximum grain yield (1.74 t/ha) by P5 treatment and in case of zeolite application, highest grain yield (1.71 t/ha) was achieved by Z5 treatment, and by not using fertilizer treatments, yield decreased by about 27% and 23.3%, respectively. Therefore, considering the positive response of green bean to the application of enriched poultry manure and zeolite, it seems that the use of these fertilizers while reducing the application of chemical fertilizers is a suitable method for improving plant yield along with healthy and sustainable production of agricultural products.

Key words: nutrient uptake, photosynthetic pigments, poultry manure, yield, zeolite

INTRODUCTION

Green bean (*Phaseolus vulgaris* L.) ranks first among legumes in terms of area under cultivation and economic value, and with 20–25% protein and 50–56% carbohydrates, is the second most important legume after chickpeas in the world [Calvo-Polanco et al. 2014].

The arid and semi-arid climate of the Iran is characterized by high temperatures, low unpredictable precipitation and high evaporation values [Keshavarz Mirzamohammadi et al. 2021a]. It is well documented that such these conditions, the content of micro and macro nutrients in the soil affects the germination and early growth [Zhang et al. 2017]. Successful establishment of crop plant is obtained through rapid germination and when seeds are intimately in contact with the soil on a well-prepared seedbed [Keshavarz and

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Khodabin 2019]. Rapid and uniform field emergence and seedling growth are essential for further plant establishment and to reach the yield potential. Proper Zn and Cu management is essential in better seedling growth and achieving higher biomass yields [Keshavarz 2020]. Excessive use of chemical fertilizers has polluted soil and water resources in many parts of the world [Liu et al. 2014]. Increasing the use efficiency of chemical fertilizers is one of the important methods to reduce the problem of environmental pollution. One of the new technologies that have been used to prevent the loss of chemical fertilizers is the application of natural soil remediation compounds such as zeolite in agricultural fields [Mahesh et al. 2018]. Davari et al. [2017] found out that the combination of zeolite with NPK elements causes gradual and slow release of nutrients and ultimately increases the efficiency of nutrient uptake by the plants [Davari et al. 2017]. The use of zeolite through improving the physical and chemical structure of soil leads to increasing soil water holding capacity, saving in application of chemical fertilizers and preventing environmental pollution [Baghbani-Arani et al. 2017, Keshavarz-Mirzamohammadi et al. 2021b]. Khodaei-Joghan and Asilan [2012] stated that the zeolite application improved the use efficiency of nitrogen and potassium fertilizers, and ultimately increased canola yield by increasing the storage capacity of nutrients in the root surface of the plant. Increased yields of other crop such as corn [Sembiring et al. 2017], rice [Zheng et al. 2018] and wheat [Movahedi Naini et al. 2017] using zeolite have been reported in the researchers' results.

Among the types of organic fertilizers, the application of poultry manure improves the physicochemical and microbial properties of soil, decomposes organic matter and thus leads to increased photosynthesis, growth and crop yield [Diacono and Montemurro 2010, Keshavarz-Mirzamohammadi et al. 2021a]. Unfortunately, some of these wastes are not dealt with properly and are causing considerable harm to the environment. Poultry manure contains plant nutrients and also some trace elements or heavy metals. Soremi et al. [2017] reported that poultry manure application significantly increased NPK concentrations in plant tissues and ultimately improved bean dry matter yield. Increased yield as well as uptake of macro and micronutrients in other crops such as corn [Fallah et al. 2007] and potatoes [Yazdanpanah and Motalebifard 2016] using poultry manure have been reported in the researchers' results.

Due to the fact that limited studies have been conducted on the response of green bean plant to the use of enriched zeolite and poultry manure, and this technique could potentially reduce the cost of exporting the plant nutrients off the farm and allow for them to be transported further more economically. So, this study was conducted to investigate the effects of fortified zeolite and poultry manure on the quantitative and qualitative characteristics of green beans.

MATERIALS AND METHODS

The experiment was carried out in the seasons of 2017 and 2018 at the research farm of Amol Islamic Azad University (Amol, Mazandaran, Iran; 36°40'N, 52°41'E; 128 m a.s.l.). The climate of the experimental site is temperate and humid. Soil physical and chemical properties of the experimental site are presented in Table 1. In this research green bean of sunray variety was used for cultivation.

Table 1. Soil physical and chemical properties of the experiment site

Properties	
pH	7.28
EC (ds/m)	1.36
Organic matter (%)	2.23
Sand (%)	13
Silt (%)	42
Clay (%)	45
Total N (%)	0.21
Available P (mg/kg)	13
Available K (mg/kg)	253

EC – electrical conductivity

The experiment was carried out as a factorial in a randomized complete block design with three replications. Treatments included zeoilte at 5 levels (Z1 – control or non-use of zeolite, Z2 – use of zeolite,

Table 2. Analysis of variance and mean comparison of yield components and yield of green bean

Treatments	Seed yield (t/ha)	Fresh pod yield (t/ha)	100 seed weight (g)	Pod length (cm)	Plant height (cm)
P1 (control)	1.53 e	10.74 e	38.00 c	12.75 e	56.49 e
P2	1.65 d	11.59 d	38.48 bc	13.00 d	58.38 d
P3	1.75 c	12.30 c	38.88 bc	13.22 c	59.89 c
P4	1.92 b	13.51 b	39.58 ab	13.64 b	62.29 b
P5	2.06 a	14.45 a	40.12 a	13.97 a	64.17 a
Z1 (control)	1.59 e	11.21 e	38.26 b	12.57 e	59.12 c
Z2	1.67 d	11.79 d	38.59 b	12.90 d	59.62 bc
Z3	1.77 c	12.46 c	38.98 ab	13.28 c	60.19 bc
Z4	1.85 b	13.03 b	39.31 ab	13.61 b	60.69 ab
Z5	2.00 a	14.10 a	39.91 a	14.21 a	61.59 a
Poultry manure (P)	**	**	**	**	**
Zeolite (Z)	**	**	**	**	**
P × Z	ns	ns	ns	**	ns

The means within a column with the same letter are not significantly different by LSD test at $p < 0.05$.
ns means non-significant, * means significant at 5% probability level, ** means significant at 1% probability level

Z3 – use of enriched zeolite with zinc, Z4 – use of enriched zeolite with copper and Z5 – use of enriched zeolite with zinc and copper) and poultry manure at five levels (P1 – control or non-use of poultry manure, P2 – use of poultry manure, P3 – use of fortified poultry manure with zinc, P4 – use of fortified poultry manure with copper and P5 – use of fortified poultry manure with zinc and copper). The Zn treatment was 5.7 kg/ha of Zn (as 25 kg/ha of $ZnSO_4$ $7H_2O$) and the Cu treatment also received 15 kg h^{-1} $CuSO_4 \cdot 5H_2O$ (net Cu concentration was 25.5%). The experimental field was divided into three equal replications, each replication consisting of 25 experimental treatments (5 × 5 × 3 = 75). The plots dimension was 15 m^2 (5 × 3 m) with 100 cm spacing between plots. Each experimental plot consisted of 6 rows with 50 cm row spacing and 20 cm plant spacing in each row. So, there were 18 plants per 1 m^2 and 270 (18 × 15 m^2 = 270) plants in each experimental plot. Nitrogen, phosphorus and potassium fertilizers were applied uniformly in all test plots according to the soil test results. After planting, the initial irrigation was carried out immediately and then the subsequent regular irrigations were done every nine days. Weed control was performed by hand weeding during three stages of plant growth.

At the end of the growing season, plant height and pod length were determined randomly by measuring the height of 15 plants and length of 15 pods from each experimental plot, then the means were recorded as plant height and pod length, respectively. To measure the 100 seed weight, 100 seeds from each experimental plot were counted and weighed using a digital scale. Fresh pod yield was determined by harvesting an area of five square meters from each experimental plot and weighing it with an accurate digital scale. To determine the grain yield, an area of five square meters was taken from each experimental plot and after drying at 72°C for 48 h in the oven, the grains were manually separated from the pods, then the grain yield was weighed with a digital scale. The concentration of chlorophyll a, b and carotenoids [Arnon 1949] and anthocyanin [Teow et al. 2007] was measured at the flowering stage. To measure nutrients concentrations in plant, after sampling and grinding, nitrogen concentration by Kjeldahl method, phosphorus by colorimetric method, potassium by flame photometric method and micronutrient nutrients by atomic absorption spectrophotometer method were obtained [Emami 1996].

The present data are the means value of two independent experiments. Bartlett test was used to examine the variances congruity for two years. Pooled data were subjected to two-way analysis of variance (ANOVA) and expressed as the means because the results followed a similar trend and the variances were homogenous. The data were analyzed using SAS 9.2 software and significant differences ($P < 0.05$) among treatments were determined by ANOVA followed by least significant difference test (LSD). The graphs were constructed using Microsoft Excel (Version 2007).

RESULTS AND DISCUSSION

Yield components and grain yield (GY)

The results of analysis of variance showed that the main effects of poultry manure and zeolite were significant on plant height, pod length, 100 seed weight, pod fresh yield and grain yield (GY). Among the studied traits, only pod length was significantly affected by the interaction of P × Z (Tab. 2).

The results of mean comparison of the main effects of poultry manure showed that the highest plant height (64.17 cm), pod length (13.97 cm), 100-seed weight (40.12 g), fresh pod yield (14.45 t/ha) and GY (2.06 t/ha) were obtained by applying P5 treatment and they were higher than control treatment (P1), by average of 11.9%, 8.7%, 5.2%, 25.6% and 25.7%, respectively. Also, no significant difference was observed between P4 and P5 treatments in terms of 100 seed weight (Tab. 2).

The results of mean comparison of the simple effects of zeolite indicated that the highest plant height (61.59 cm), pod length (14.21 cm), 100-seed weight (39.91 g), fresh pod yield (14.10 t/ha) and GY (2 t/ha) were obtained by applying Z5 treatment. In addition, the non-zeolite treatment obtained the lowest content of those traits (Tab. 2).

The results of interaction between zeolite and poultry manure showed that the highest pod length (15.08 cm) was obtained by applying P5Z5 treatment, while control treatment (p1), the pod length was reduced by average of 19.6% (Fig. 1).

These results showed that the use of both poultry manure and zeolite, especially the enrichment of these fertilizers with zinc and copper caused a significant improvement in yield components and GY of green beans. Application of copper and zinc improves yield components and GY by affecting leaf chlorophyll concentration and increasing photosynthesis [Li et al. 2007]. The application of both organic and inorganic fertilizer sources of plant nutrients is necessary to achieve desired yields of crop. In this study, sourcing of plant elements from organic and micro-nutrient fertilizers in different proportions enhanced biomass and yield components of green beans. The use of organic manures increased soil organic carbon and total nitrogen, provided nutrients to the plants and contributed sequestrating carbon in soil. Zinc improves the growth and GY of green bean through its impact on physiological processes and nitrogen metabolism in plants [Lack et al. 2016]. The significant effects of poultry manure [Alhrout et al. 2016] and zeolite [Turkmen and Kutuk 2017] on improving yield components and GY of bean reported by researchers, which were consistent with the results of this experiment.

One of the reasons for increasing seed weight is to improve photosynthesis and increase the transfer of photosynthetic materials to the grain due to the application of enriched zeolite and poultry manure with zinc and copper. Jasim and Mhanna [2014] demonstrated that the use of poultry manure increased the plant height, 100 seed weight and finally the seed yield of beans compared to control or not using poultry manure.

Improved plant growth in zeolite-containing treatments may be due to increased soil water holding capacity [Baghbani-Arani et al. 2017] as well as increased nutrient availability [Khodaei-Joghan and Asilan 2012]. The basic concept of zeolite is the maintenance and improvement of soil water holding for sustaining crop productivity on a long-term basis, which can be achieved through the combined use of various sources of nutrients and by managing them scientifically for optimum growth, yield and quality of crop. Zeolite, by preventing nitrogen loss, causes the availability of this element for the plant, improves growth and finally increases grain yield of the plant [Baghbani-Arani et al. 2017]. The results showed that the enrichment of each fertilizer treatments with copper had better effects than zinc in improving the yield components and GY of green beans. Copper promotes cell elongation, increases plant height, and improves GY by synthesizing proteins and regulating the hormone auxin [Kumar et al. 2009].

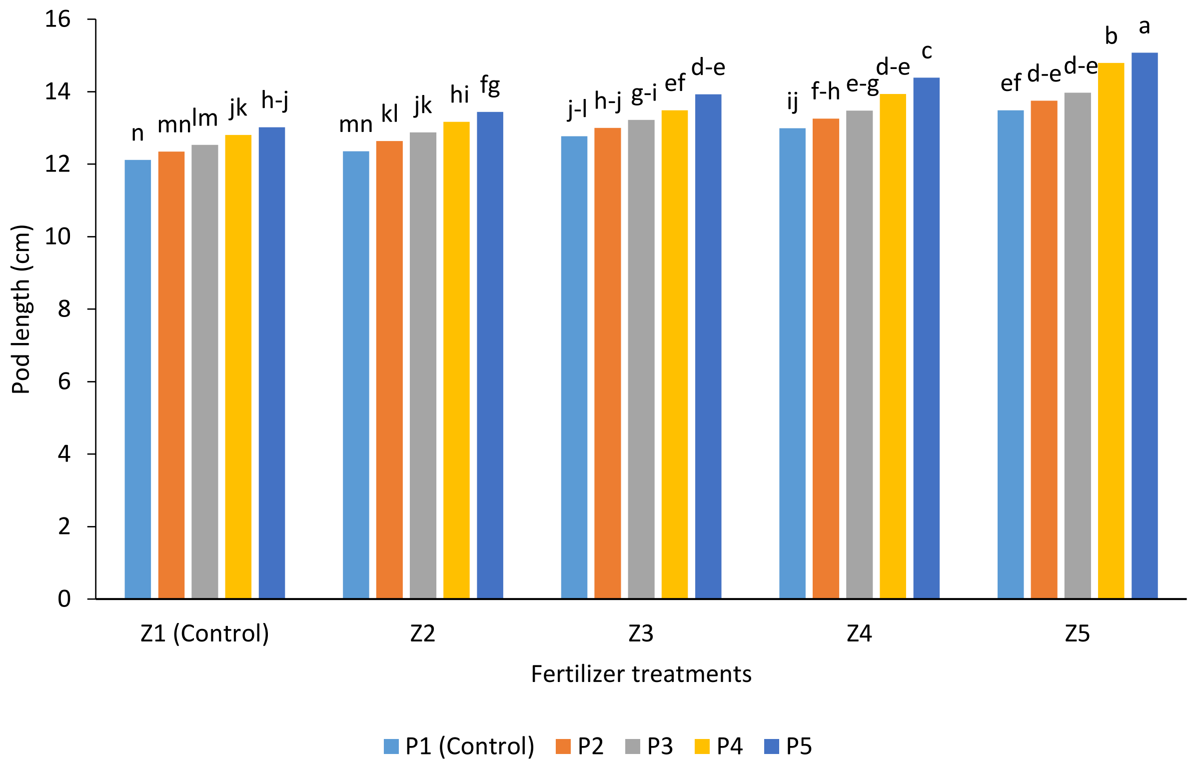

Fig. 1. Interaction effects of P × Z on pod length (P – poultry manure, Z – zeolite)

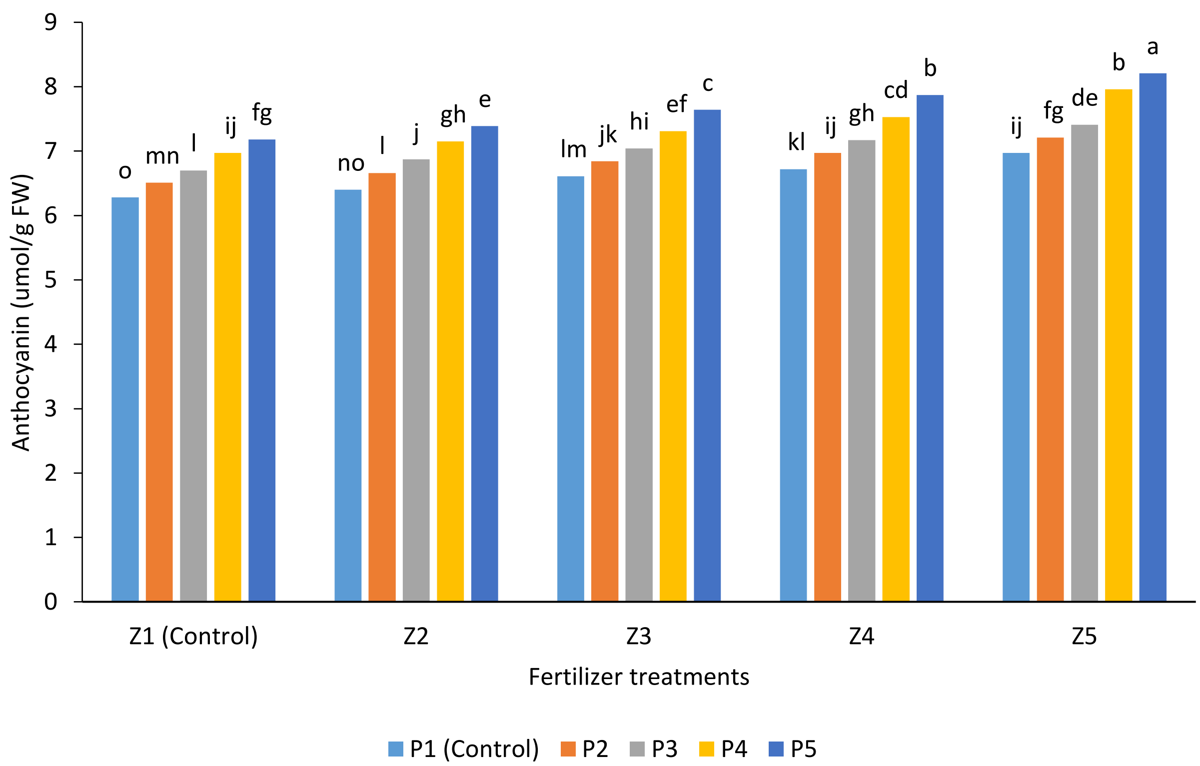

Fig. 2. Interaction effects of P × Z on anthocyanin (P – poultry manure, Z – zeolite)

Photosynthetic pigments

The results of analysis of variance showed that the simple effects of poultry manure and zeolite were significant on all photosynthetic pigments including anthocyanins, carotenoids and chlorophylls a, b and ab. Also, the interaction effect of between poultry manure and zeolite was significant only on anthocyanin (Tab. 3).

The results of mean comparison of the main effects of poultry manure showed that the maximum concentration of chlorophyll a (8.55 μmol/g FW), chlorophyll b (2.60 μmol/g FW), total chlorophyll (11.15 μmol/g FW), carotenoids (1.48 μmol/g FW) and anthocyanins (7.66 μmol/g FW) were obtained in P5 treatment which were higher than control about 12.5%, 13.4%, 12.7%, 24.3% and 13.8%, respectively (Tab. 3).

The results of mean comparison of the main effects of zeolite showed that the highest concentrations of chlorophyll a (8.44 μmol/g FW), chlorophyll b (2.56 μmol/g FW), total chlorophyll (11.01 μmol/g FW), carotenoids (1.44 μmol/g FW) and anthocyanins (7.55 μmol/g FW) were obtained by application of Z5 treatment which were than control by average of 9.7%, 10.5%, 9.9%, 18.7% and 10.8%, respectively (Tab. 3).

The results of interaction between zeolite and poultry manure showed that the maximum concentration of anthocyanin (8.21 nmol/g FW) was obtained by applying P5Z5 treatment which was higher than control by about 23.5% (Fig. 2).

The results showed that enrichment of zeolite and poultry manure with micronutrients significantly increased the concentration of photosynthetic pigments compared to control or individual application of zeolite and poultry manure. The presence of copper and zinc in poultry manure increased the amount of chlorophyll in the plant, which was consistent with the results of Saikachout et al. [2015].

Enrichment of zeolite or poultry manure with copper had better results than enrichment of these fertilizers with zinc. Optimal amounts of zinc, copper and manganese increase the amount of chlorophyll, anthocyanin and carotenoids in the plant and among these elements, the effect of copper on the accumulation of photosynthetic pigments is more than zinc and manganese [Baek et al. 2012]. Increased synthesis and accumulation of chlorophyll, anthocyanin and carotenoids in the plant by copper application has also been presented in the results of other researchers [Yadav et al. 2016, Keshavarz et al. 2018].

Madhavi [2007] reported that the plant chlorophyll content increased significantly with increasing poultry manure consumption. Application of zeolite by increasing nitrogen uptake by the plant increases chlorophyll synthesis improves photosynthesis and ultimately increases crop production [Lija et al. 2014]. Increased chlorophyll concentration of plants such as soybean [Nozari et al. 2013] and stevia [Bakhshandeh et al. 2016] by the use of zeolite has been reported in the results of other researchers.

Soil pH. The soil pH was significantly affected by main effect of zeolite or organic fertilizer treatments (Tab. 2). When compared with control, the enrichment of zeolite or organic fertilizer with Zn + Cu, decreased pH by 6.4% and 6.8%, respectively. The data indicated that the no fertilizer treatments (control) produced the highest pH with an average of 8.03 (in zeolite treatment) and 8.02 (in organic fertilizer), respectively (Tab. 4). Furthermore, no significant difference was found between P3, P4 and P5 and Z3, Z4 and Z5. The addition of Zn and Cu resulted in decreased soil pH. In contrast, control treatment leads to higher soil pH than zeolite or organic fertilizer enriched with Zn + Cu, possibly because of accumulation of cations [Keshavarz et al. 2018]. It has been demonstrated that organic amendment and ammonium-based fertilizers (NH_4^+) caused lower pH by production of H^+ ions [Singh et al. 2019]. Mineralisation of organic waste nitrogen produces organic and inorganic acids which provide H^+ ions in soil.

Nutrients uptake. The results of analysis of variance showed that the simple effects of poultry manure and zeolite were significant on nutrient concentrations in green bean pods. The interaction effect of P × Z was significant only on nitrogen and potassium concentrations (Tab. 4).

The results of mean comparison of the main effects of poultry manure showed that the greatest content of nitrogen, phosphorus and potassium (by average of 3.55, 0.49 and 4.56%, respectively,) and the maximum content of copper, zinc, iron and manganese (by aver-

Table 3. Analysis of variance and mean comparison of photosynthetic pigments of green bean

Treatments	Chl a (μmol/g FW)	Chl b (μmol/g FW)	Chl a + b (μmol/g FW)	Carotenoid (μmol/g FW)	Antocianin (μmol/g FW)
P1 (control)	7.48 e	2.25 e	9.73 e	1.12 e	6.60 e
P2	7.73 d	2.33 d	10.05 d	1.21 d	6.84 d
P3	7.93 c	2.39 c	10.32 c	1.27 c	7.04 c
P4	8.27 b	2.51 b	10.78 b	1.39 b	7.39 b
P5	8.55 a	2.60 a	11.15 a	1.48 a	7.66 a
Z1 (control)	7.62 e	2.29 c	9.91 e	1.17 e	6.73 e
Z2	7.78 d	2.35 c	10.13 d	1.22 d	6.89 d
Z3	7.97 c	2.41 b	10.38 c	1.29 c	7.09 c
Z4	8.14 b	2.46 b	10.61 b	1.34 b	7.25 b
Z5	8.44 a	2.56 a	11.01 a	1.44 a	7.55 a
Poultry manure (P)	**	**	**	**	**
Zeolite (Z)	**	**	**	**	**
P × Z	ns	ns	ns	ns	**

Explanations as in Table 2.

Table 4. Analysis of variance and mean comparison of nutrients uptake of green bean

Treatments	Soil pH	N (%)	P (%)	K (%)	Cu (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Mn (mg/kg)
P1 (control)	8.02a	2.48 e	0.42 e	3.49 e	23.32 e	30.58 d	77.74 d	62.41 d
P2	7.41c	2.73 d	0.44 d	3.74 d	24.05 d	31.31 c	78.95 c	63.66 c
P3	7.54b	2.93 c	0.45 c	3.94 c	24.65 c	31.91 c	79.95 c	64.60 c
P4	7.49b	3.27 b	0.47 b	4.28 b	25.69 b	32.95 b	81.69 b	66.31 b
P5	7.49b	3.55 a	0.49 a	4.56 a	26.51 a	33.77 a	83.05 a	67.68 a
Z1 (control)	8.03a	2.62 e	0.43 e	3.63 e	23.72 e	30.98 d	78.41 d	63.11 d
Z2	7.38c	2.78 d	0.44 d	3.79 d	24.22 d	31.48 cd	79.23 cd	63.91 cd
Z3	7.51b	2.98 c	0.45 c	3.99 c	24.79 c	32.06 bc	80.19 bc	64.84 bc
Z4	7.45b	3.14 b	0.46 b	4.15 b	25.29 b	32.55 b	81.02 b	65.62 b
Z5	7.44b	3.44 a	0.48 a	4.45 a	26.19 a	33.46 a	82.53 a	67.17 a
Poultry manure (P)	**	**	**	**	**	**	**	**
Zeolite (Z)	**	**	**	**	**	**	**	**
P × Z	ns	**	ns	**	ns	ns	ns	ns

Explanations as in Table 2.

age of 26.51, 33.77, 83.05 and 67.68 mg·kg^{-1}, respectively,) were obtained in P5 treatment (Tab. 4).

The results of mean comparison of the simple effects of zeolite showed that the highest content of nitrogen, phosphorus and potassium (by averages of 3.44, 0.48 and 4.45%, respectively,) and the highest content of copper, zinc, iron and manganese (by average of 26.19, 33.46, 82.53 and 67.17 mg·kg^{-1}, respectively,) were obtained in Z5 treatment (Tab. 4).

The results of interaction between zeolite and poultry manure showed that the maximum content of N (4.10%) and K (5.11%) were obtained in P5Z5 treatment which were higher than control treatment about 47% and 37.7%, respectively (Tab. 5).

The results showed that enrichment of zeolite and poultry manure with micronutrients significantly increased the nutrients uptake in the plant compared to control or individual application of zeolite and poultry manure. Turkmen and Kutuk [2017] reported that the combined application of poultry manure and chemical fertilizers, while reducing the use of chemical fertilizers, increases soil fertility, improves nutrient uptake and increases seed yield of bean. Poultry manure improves the nutrients uptake in plants by increasing organic matter and improving soil structure [Boateng et al. 2006]. Significant increase of NPK concentrations in the plant tissue and finally improvement of bean dry matter yield by the application of poultry manure has also been re-

Table 5. Interaction effects of P × Z on N and K concentrations

Treatments		N (%)	K (%)
P1 (control)	Z1 (control)	2.17 o	3.18 o
	Z2	2.29 no	3.30 no
	Z3	2.49 lm	3.50 lm
	Z4	2.61 kl	3.62 kl
	Z5	2.86 ij	3.87 ij
P2	Z1 (control)	2.40 mn	3.41 mn
	Z2	2.55 l	3.56 l
	Z3	2.73 jk	3.74 jk
	Z4	2.86 ij	3.87 ij
	Z5	3.10 fg	4.11 fg
P3	Z1 (control)	2.59 l	3.60 l
	Z2	2.76 j	3.77 j
	Z3	2.93 hi	3.94 hi
	Z4	3.06 gh	4.07 gh
	Z5	3.30 de	4.31 de
P4	Z1 (control)	2.86 ij	3.87 ij
	Z2	3.04 gh	4.05 gh
	Z3	3.20 ef	4.21 ef
	Z4	3.42 cd	4.43 cd
	Z5	3.85 b	4.86 b
P5	Z1 (control)	3.07 fg	4.08 fg
	Z2	3.28 e	4.29 e
	Z3	3.52 c	4.53 c
	Z4	3.76 b	4.77 b
	Z5	4.10 a	5.11 a

The means within a column with the same letter are not significantly different by LSD test at $p < 0.05$.

ported in the results of Soremi et al. [2017]. Studies have shown that the use of organic fertilizers has improved the nitrogen and potassium concentrations, and finally green bean yield compared to the control [Aslani and Souri 2018]. High cation exchange capacity and porous structure of zeolite lead to better absorption of nutrients and organic matter from the soil [Gholamhoseini et al. 2013]. It has been reported that the application of zeolite increased the concentrations of nitrogen, potassium, zinc and copper in bean [Ozbahce et al. 2015].

CONCLUSION

The results showed that the application of zeolite and poultry manure significantly increased yield components, GY, photosynthetic pigments and nutrients uptake compared to control. The highest amount of studied traits was obtained by the application of enriched zeolite and poultry manure with zinc and copper. Enrichment of zeolite or poultry manure with copper had better impacts than enrichment of these fertilizers with zinc in terms of studied traits. Therefore, considering the positive response of green bean plant to the application of enriched poultry manure and zeolite, it seems that the use of these fertilizers while reducing the application of chemical fertilizers and also reducing the adverse environmental impacts of their use is a suitable method for improving plant yield along with healthy and sustainable production of agricultural products.

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