

Effect of Different Fertilization Systems on Yield and Seed Mineral Elements of Pumpkin

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ABSTRACT- To investigate the effects of bio-chemical and organic fertilizers on yield and seeds NPK contents of pumpkin (*Cucurbita pepo* var. styriaca), an experiment was conducted at the Research Farm of Kurdistan University in 2009 growing season. Treatments were included 1) control, 2) seeds inoculated with free-living N fixing bacteria (NFB), 3) seeds inoculated with phosphate-solubilizing bacteria (PSB), 4) chemical fertilizer (C), 5) organic fertilizer (O), 6) NFB + PSB, 7) NFB+ 50% O, 8) NFB+ 50% C, 9) PSB + 50% O, 10) PSB + 50% C, 11) 50% O + 50% C, 12) NFB+ PSB + 50% O, 13) NFB+ PSB + 50% C, 14) NFB+ PSB + 50% O + 50%. The highest value of nitrogen concentration was observed in the NFB+ 50% C and NFB+ PSB+ 50% C treatments. The highest potassium concentration was found in the NFB+ 50% C treatment. The maximum phosphorous concentration was observed in PSB+ 50% C. The maximum seed yield was obtained from the treatment that pumpkin seeds inoculated with free-living N (NFB) and phosphate-solubilizing bacteria (PSB) + 50% organic fertilizer. The highest fruit yield was obtained from NFB+ PSB+ 50% C+ 50% O. It was concluded that application of biofertilizers in combination with 50% chemical and organic fertilizers, reduced the use of chemical fertilizers and produced higher seed and fruit yield.

Keywords: Bio Fertilizer, Compost, Medicinal Pumpkin, Nitrogen, Potassium, Yield

INTRODUCTION

Medicinal pumpkin, called Styrian oil pumpkin or *Cucurbita pepo*, has been formed by an accidental natural mutation that led to tremendous morphological changes of the seed architecture (9). Nitrogen (N) and phosphorus (P) are the most important nutrients for

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plant growth (4). Large quantities of chemical fertilizers are used to replenish soil N and P, resulting in high costs and severe environmental contamination (29). Biofertilizers contain a variety of beneficial microorganisms and enzymes which accelerates and improves plant growth and protects plants from pests and diseases (8). P bio-fertilizers in the form of microorganisms, especially phosphate-solubilizing bacteria in rhizosphere, can help increase the availability of accumulated phosphates for plant growth by solubilization (18). Important genera of phosphate solubilizing bacteria are *Bacillus* and *Pseudomonas* (22). Phosphate solubilizing microorganisms secrete organic acids and phosphatase enzyme that act on insoluble phosphate and convert it into plant available form (20, 28). This process not only reduces higher cost of manufacturing fertilizers but also mobilizes the fertilizers added to soil (27). Biological nitrogen fixation by free-living nitrogen fixing microorganisms occurs in soils by several aerobic (*Azotobacter*, *Beijerinickia*) or microaerobic (*Azospirillum*) prokaryotes (12). These bacteria contain nitrogenase enzyme responsible for fixing atmospheric di-nitrogen into soil, thus improving the soil fertility (11). The addition of organic material of various origins to soil has been one of the most common practices to improve soil properties (7). Compost enhances the environmental sustainability of agriculture by decreasing chemical inputs and increasing soil organic matter (16). The benefits of animal manure application to soil organic matter quality, nutrient availability, soil aggregation and other soil functions are well known (14). Application of organic manure combined with chemical fertilizer is an important approach to maintaining and improving the soil fertility, and increasing fertilizer use efficiency (15). Therefore, the present investigation was undertaken to study the effects of N-fixing and P-solubilizing bacteria in the presence of chemical and organic fertilizers on yield, mineral elements of pumpkin seeds.

MATERIALS AND METHODS

This study was carried out at the Research Farm of Kurdistan University, Sanandaj, Iran in 2009. This area is located at latitude of 35°15' N and longitude of 47°1' E at the altitude of 1300 m above the sea level. Soil samples were taken from the upper 15 cm layer of the soil profile of each plot and were analyzed for physical and chemical characteristics for fertilizer recommendation. Soil properties are presented in Table 1. The experiment was laid out in a randomized complete-block design with three replications and 14 treatments. Treatments included: 1) control, 2) NFB - seeds inoculated with free-living N fixing bacteria, 3) PSB - seeds inoculated with phosphate-solubilizing bacteria, 4) C - chemical fertilizer (urea + mono super-phosphate), 5) O - organic fertilizer (animal manure + compost), 6) NFB + PSB, 7) NFB+ 50% O, 8) NFB+ 50% C, 9) PSB + 50% O, 10) PSB + 50% C, 11) 50% O + 50% C, 12) NFB+ PSB + 50% O, 13) NFB+ PSB + 50% C, 14) NFB+ PSB + 50% O + 50% C. After land preparation, including plowing, disking and ridging, medicinal pumpkin was planted in late May 2009. Each plot had five rows with 1 m inter - row spacing and 50 cm between plants in each row. Three to four seeds per hole were placed at 3-4 cm planting depth. The plants were thinned to one at the 3- 4 leaf stage. Single super phosphate fertilizer (phosphorous source which comprises 16% water soluble phosphate, 150 kg

ha⁻¹) and organic fertilizers (10 t ha⁻¹) were applied before sowing. It was applied 100 kg ha⁻¹ potassium sulfate based on soil analyses for all treatments. Organic fertilizers were analyzed for chemical and nutrients properties (Table 2). Urea fertilizer (nitrogen source containing 46% nitrogen) was added to plants at the early flowering stage (120 kg ha⁻¹). Two kinds of phosphate -solubilizing bacteria (*Pseudomonas putida* strain p 13, *Bacillus lentus* strain p 5) and two kinds of nitrogen fixing bacteria (*Azotobacter*, *Azospirillum*) were used as the biofertilizers. These bacteria strains were originally isolated from farm soils in Iran and were obtained from Iranian Soil and Water Research Institute. For seed inoculation, 100 g PSB and 1 lit NFB for 8 kg seed per hectare were used before being immediately planted.

Table 1. Some physicochemical characteristics of soil in research field

Soil texture	Clay %	Sand %	Silt %	pH	EC (dSm ⁻¹)	OC (%)	N %	K (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Cu (mg kg ⁻¹)	P (mg kg ⁻¹)
Sandy loam	16	58	26	7.9	0.61	1.46	0.21	82.95	5.56	12.19	2.3	5.14

Table 2. Chemical characteristics of organic fertilizers applied in the soil

characteristics	pH	N (%)	P (%)	K (%)	Ca (mg kg ⁻¹)	Mg (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cu (mg kg ⁻¹)
Animal manure	7.51	0.52	0.45	0.32	832	1205	3	28
Compost	7.18	0.72	1.21	0.55	1871	1951	14	302

Fruit and seed yield

Fruits were harvested from three central rows of each plot on September 2009. Ripe fruits were harvested, weighted and then the seeds of each fruit were manually extracted and dried in shade and room temperature. All dried seeds were weighted separately. Grain harvest index was calculated by the following Eq.

Grain harvest index= Seed yield/ Fruit yield

Chemical composition of pumpkin seed

The seed N content was measured by the micro-kjeldahl method as described by Peach and Tracey(19).

Potassium (K) and P were determined using a flame photometer and spectro photometric method, respectively, as described by Skroch et al. (26). P and K uptake per unit area were determined by the following Eq. (24):

P or K Uptake = seed yield × P or K concentration

Statistical analysis

The SAS – GLM procedure was used for data analysis. Treatment means were compared using LSD test (24).

RESULTS AND DISCUSSION

significantly affected by the treatments. The results indicated that all the measured traits, except for the harvest index were ($p < 0.01$) (Tables 3 and 4).

Table 3. Analysis of variance for different fertilizer treatments on fruit and seed yield and harvest index of pumpkin

SOV	df	Mean square		
		Fruit yield	Seed yield	Harvest index
Block	2	600880.07*	645.23 ^{ns}	0.097 ^{ns}
Treatment	13	511288.42**	1447.22**	0.064 ^{ns}
Error	26	143778.45	185.61	0.042
CV		8.57	17.21	12.01

ns: not significant, * Significant at $P < 0.05$, ** Significant at $P < 0.01$

Table 4. Analysis of variance for different fertilizer treatments on chemical composition of pumpkin seed

SOV	df	Mean square				
		N	K	K uptake	P	P uptake
Block	2	35.45**	71446.41**	88.63 ^{ns}	12139.93 ^{ns}	282.05 ^{ns}
Treatment	13	0.718**	51987.49**	1545.53**	113551.37**	3534.77**
Error	26	0.205	5274.04	343.74	27153.18	490.18
CV		8.22	7.62	26.02	13.34	24.08

ns: not significant, * Significant at $P < 0.05$, ** Significant at $P < 0.01$

Fruit and seed yield

Fruit yield varied from 6640.4 to 1933.7 g m⁻², the highest fruit yield being obtained from using the NFB+ PSB+ 50% C+ 50% O; however, this treatment did not differ significantly from NFB+ PSB+ 50% C and NFB+ PSB+ 50% O treatments (Table 5). The lowest fruit yield was obtained from the control treatment. The highest seed yield (1185 g m⁻²) was recorded with the application of NFB+PSB+50%O, which did not differ from the NFB+ PSB+ 50% C+ 50% O treatment. The lowest seed yield (13.26 g m⁻²) was again noted in the control. No statistical differences were observed among the remaining treatments, but they were all higher than the control. Results showed that the highest pumpkin yield was obtained by the inoculation of seeds with a mixture of various bacteria in the presence of the chemical and/ or organic fertilizers. This may be due to the integrated application of fertilizers - nutrients from chemical fertilizers enhanced the establishment of crops while those from mineralization of organic fertilizer promoted yield (2). The promotion in yield of pumpkin by mixture of biofertilizers incubation could be attributed to the accumulation effects of these microorganisms, including enhanced supply of nutrients such as N and P to the crop and production of

growth promoting substances (23). These results were in agreement with those of Al-Harbi and Wahb-Allah (1). They found that in summer squash, the highest fruit yield was obtained by applying 72 kg N fertilizer with biofertilizer (*Azotobacter*, *Azospirillum* and *Klebsiella*) inoculation.

Table 5. The effect of fertilizer treatments on fruit yield, seed yield and harvest index of pumpkin (*Cucurbita pepo* var. *styriaca*).

Treatments				Fruit yield (g m ⁻²)	Seed yield (g m ⁻²)	Harvest index (%)
N-fixing Bacteria (NFB)	Phosphate- solubilizing Bacteria (PSB)	Chemical fertilizer (C)	Organic fertilizer (O)			
-	-	-	-	1933.7 e	31.26 e	1.89 a
+	-	-	-	3874.9 cd	66.03 cd	1.70 a
-	+	-	-	3674.6 cd	64.77 cd	1.89 a
+	+	-	-	4010.4 cd	67.27 cd	1.67 a
-	-	+	-	3823.8 cd	66.5 cd	1.73 a
-	-	-	+	3673.3 cd	61.88 cd	1.77 a
-	-	+	+	3400.6 d	58.22 cd	1.31 a
+	-	+	-	5790.5 b	74.09 c	1.67 a
+	-	-	+	4321.4 c	70.69 cd	1.75 a
-	+	+	-	4213.5 c	69.14 cd	1.74 a
-	+	-	+	4239.1 c	73.41 c	1.72 a
+	+	+	-	6272.2 ab	97.97 b	1.59 a
+	+	-	+	6056.2 ab	118.5 a	1.60 a
+	+	+	+	6640.4 a	105.49 ab	1.89 a
LSD%5				636.39	19.68	0.37

Means with a common letter are not significantly different using LSD Test at 5% probability level. Amount used for the treatments: NFB 1 lit ha⁻¹; PSB 100 g ha⁻¹; C Single super phosphate (150 kg ha⁻¹) + Urea (120 kg ha⁻¹) when applied alone and Single super phosphate (75 kg ha⁻¹) + Urea (60 kg ha⁻¹) when combined with other treatments; O 10 ton ha⁻¹ when applied alone and 5 ton ha⁻¹ when combined with other treatments.

Mineral elements

The highest nitrogen concentration (6.23%) was observed in NFB+ 50% C but it was not significantly different from the NFB+ PSB+ 50% C (6.16%) treatment (Table 6). Increased seed N content after applying nitrogen fixing bacteria might be due to the direct effects of these bacteria in N-fixation, hormone production or to more efficient N assimilation aided by the bacterial nitrate reductase (25).

The highest potassium concentration (1218 mg/100g) was obtained from using 50% chemical fertilizers + inoculated with nitrogen fixing bacteria (NFB+ 50% C). The maximum enhancement of K uptake was observed in NFB+ PSB+ 50% O treatment (Table 6). The maximum phosphorous concentration (1533.4 mg/100g) was achieved with the combination of 50% chemical fertilizer and the inoculation of phosphate solubilizing bacteria (PSB+ 50% C). This may be related to producing low seed yield in PSB+ 50% C compared with NFB+ PSB+ 50% C, NFB+ PSB+ 50% O and NFB+ PSB+ 50% C+ 50% O treatments (Tables 5 and 6). Also the highest P uptake occurred with NFB+ PSB+ 50% C+ 50% O. There were no significant differences between this

treatment and NFB+ PSB+ 50% C and NFB+ PSB+ 50% O treatments (Table 7). Sahravat (23) reported that significant linear relationships were observed between grain yield and total P uptake in upland rice.

Table 6. The effect of fertilizer treatments on seed N and K contents and K uptake composition of pumpkin (*Cucurbita pepo* var. *styriaca*).

Treatments				N (%)	K (mg/100g)	K uptake (g m ⁻²)
N-fixing Bacteria (NFB)	Phosphate-solubilizing Bacteria (PSB)	Chemical fertilizer (C)	Organic fertilizer (O)			
-	-	-	-	4.36 d	615.3 d	19.61 e
+	-	-	-	5.45 bc	1004.8 bc	66.77 cd
-	+	-	-	5.59 abc	978.5 c	64.96 cd
+	+	-	-	5.43 bc	1034.3 b	73.28 bcd
-	-	+	-	5.57 abc	946.2 c	62.96 d
-	-	-	+	5.21 c	864 c	53.57d
-	-	+	+	5.73 abc	871.8 c	50.92d
+	-	+	-	6.23 a	1218 a	94.54 abc
+	-	-	+	5.36 bc	1056.2 b	75.23 abcd
-	+	+	-	5.91 abc	930 c	63.6 cd
-	+	-	+	5.62 abc	995.8 bc	72.99 bcd
+	+	+	-	6.16 a	961 c	94.17 abc
+	+	-	+	5.65 abc	897 c	104.72 a
+	+	+	+	6.01 ab	970.7 c	101.28 ab
LSD%5				0.75	121.9	31.12

Means with a common letter are not significantly different using LSD Test at 5% probability level. Amount used for the treatments: NFB 1 lit ha⁻¹; PSB 100 g ha⁻¹; C Single super phosphate (150 kg ha⁻¹) + Urea (120 kg ha⁻¹) when applied alone and Single super phosphate (75 kg ha⁻¹) + Urea (60 kg ha⁻¹) when combined with other treatments; O 10 ton ha⁻¹ when applied alone and 5 ton ha⁻¹ when combined with other treatments.

The highest seed K concentration (1218 mg/100g) was observed with the application of nitrogen fixing bacteria combined with 50% chemical fertilizer. The effect of these microorganisms (*Azotobacter* and *Azospirillum*) on K uptake might be attributed to their more efficiency in supplying biologically fixed nitrogen and enhancing plant growth by producing phytohormones, which stimulate nutrients absorption (6). According to Table 6, applying organic fertilizer alone or in combination with other fertilizers increased the K uptake, compared with the control treatment. This may be due to the positive effects of organic fertilizers on soil structure that lead to increasing the area of the roots per unit of soil volume, increasing the nutrient uptake, slowing the release of nutrients and enhancing fertilizer use efficiency (17).

Despite the maximum K uptake per unit area related to NFB+ PSB+ 50%O treatment, this treatment had the lowest content of K in seeds (Table 6). Therefore, we can conclude that K content of seeds decreased because of high seed yield production and dilution of K concentration in the NFB+ PSB+ 50%O treatment.

The lowest N, K and P concentrations (4.36%, 615.35 mg/100gr and 19.61 mg/100g) were observed in unfertilized plot (control treatment). The phosphate-

dissolving bacteria can increase the availability of phosphorous. These bacteria secrete organic acids, which lead to a transfer of fixed phosphate to available phosphate (13). Also applying nitrogen fixing bacteria, compared with other treatments, increased P absorption (Table 7).

Table 7. The effect of fertilizer treatments on seed P content and P uptake composition of pumpkin seed (*Cucurbita pepo* var. *styriaca*).

Treatments					
N-fixing Bacteria (NFB)	Phosphate-solubilizing Bacteria (PSB)	Chemical fertilizer (C)	Organic fertilizer (O)	P (mg/100g)	P uptake (g m ⁻²)
-	-	-	-	779.5 f	23.34 e
+	-	-	-	991.3 ef	65.74 d
-	+	-	-	1211.9 cde	78.42 cd
+	+	-	-	1303.7 abcd	86.25 bcd
-	-	+	-	1184.2 cde	79.28 cd
-	-	-	+	1186.7 cde	73.04 cd
-	-	+	+	1270.4 abcd	74.43 cd
+	-	+	-	1194.4 cde	88.46 bcd
+	-	-	+	1172 de	82.29 cd
-	+	+	-	1533.4 a	108.63 bc
-	+	-	+	1460.3 abc	98.88 bcd
+	+	+	-	1232 abcd	120.79 ab
+	+	-	+	1273.4 abcd	150.84 a
+	+	+	+	1493.6 ab	156.37 a
LSD%5				276.6	37.16

Means with a common letter are not significantly different using LSD Test at 5% probability level. Amount used for the treatments: NFB 1 lit ha-1; PSB 100 g ha-1; C Single super phosphate (150 kg ha-1) + Urea (120 kg ha-1) when applied alone and Single super phosphate (75 kg ha-1) + Urea (60 kg ha-1) when combined with other treatments; O 10 ton ha-1 when applied alone and 5 ton ha-1 when combined with other treatments.

This could be due to the direct effect of bacteria on root growth and greater mineral uptake (10). Using organic fertilizers combined with phosphate-dissolving bacteria increased P content of pumpkin seeds (Table 7). The organic fertilizers release nutrients slowly and the application of organic fertilizers alone cannot provide essential P for plant completely (5). Therefore, the application of phosphate-dissolving bacteria can increase the available P by hydrolysis P from organic and inorganic compounds due to acidification of soil or secretion of phosphatase enzymes (21).

In general, the lowest concentration of K and P in pumpkin seeds obtained from treatments that have produced more dry matter. This is due to the dilution of K and P concentrations in seeds.

CONCLUSIONS

It was concluded that the application of biofertilizers in combination with 50% chemical and 50% organic fertilizers had a great potential to increase yield of pumpkin seeds, and improved the use efficiency of chemical fertilizers. Also, compared with conventional chemical fertilizers, integrated plant nutrition systems could ensure agricultural production and safeguard the environment. Developing a suitable nutrient management system that integrates the use of bio, organic and chemical fertilizers may be a surefire way to reach the goal of sustainable agriculture.

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تأثیر سیستم‌های مختلف حاصلخیزی بر عملکرد و محتوای عناصر معدنی دانه کدوی تخم کاغذی

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چکیده- این تحقیق به منظور بررسی تأثیر کودهای زیستی، شیمیایی و آلی بر میزان عناصر معدنی دانه و عملکرد کدوی تخم کاغذی طی سال زراعی ۱۳۸۸ در مزرعه تحقیقاتی دانشکده کشاورزی دانشگاه کردستان انجام شد. تیمارهای کودی شامل (۱) شاهد یا بدون مصرف کود (۲) باکتری‌های تثبیت کننده نیتروژن، (۳) باکتری‌های حل کننده فسفر، (۴) کود شیمیایی، (۵) کود آلی، (۶) باکتری‌های تثبیت کننده نیتروژن + باکتری‌های حل کننده فسفر، (۷) باکتری‌های تثبیت کننده نیتروژن + ۵۰٪ کود آلی، (۸) باکتری‌های تثبیت کننده نیتروژن + ۵۰٪ کود شیمیایی، (۹) باکتری‌های حل کننده فسفر + ۵۰٪ کود آلی، (۱۰) باکتری‌های حل کننده فسفر + ۵۰٪ کود شیمیایی، (۱۱) ۵۰٪ کود شیمیایی، (۱۲) کود آلی، (۱۳) باکتری‌های تثبیت کننده نیتروژن + باکتری‌های حل کننده فسفر + ۵۰٪ کود آلی و (۱۴) باکتری‌های تثبیت کننده نیتروژن + باکتری‌های حل کننده فسفر + ۵۰٪ کود شیمیایی + ۵۰٪ کود آلی بودند. نتایج نشان داد که بیشترین غلظت نیتروژن در تیمارهای باکتری‌های تثبیت کننده نیتروژن + کود شیمیایی حاصل می‌گردد. بیشترین میزان غلظت پتاسیم از تیمار باکتری‌های تثبیت کننده نیتروژن + ۵۰٪ کود شیمیایی به دست آمد. بیشترین غلظت فسفر در تیمار باکتری‌های حل کننده فسفر + ۵۰٪ کود شیمیایی مشاهده گردید. حداکثر عملکرد دانه در تیمار باکتری‌های تثبیت کننده نیتروژن + باکتری‌های حل کننده فسفر + ۵۰٪ کود آلی حاصل گردید و همچنین حداکثر عملکرد میوه در تیمار باکتری‌های تثبیت کننده نیتروژن + باکتری‌های حل کننده فسفر + ۵۰٪ کود شیمیایی + ۵۰٪ کود آلی بدست آمد. نتایج این تحقیق نشان داد در صورتی که کودهای زیستی در تلفیق با ۵۰ درصد کود شیمیایی و ۵۰ درصد کود آلی مصرف گردد، ضمن کاهش مصرف کود شیمیایی بیشترین عملکرد دانه و میوه به دست خواهد آمد.

واژه های کلیدی: ایران، بخش‌های اقتصادی، تحلیل سلسله مراتبی

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**مکاتبه کننده