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Effect of bio-fertilizers on the yield and yield components of *Cucurbita pepo* L.

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ABSTRACT

In order to evaluate the influence of bio-fertilizers on the yield, yield components, oil percentage and protein of Pumpkin medicinal plant, an experiment was conducted at the Research Farm of Agriculture and Natural Resources Research Center of West Azarbaijan based on randomized complete block design with 4 replications in 2008. Treatments were biological phosphorus (*Bacillus lentus* P5 and *Pseudomonas putida* P13)+chemical phosphorus, biological nitrogen (*Azospirillum*), *Thiobacillus*, NPK, Livestock Manure, Livestock Manure+ biological phosphorus (*Bacillus lentusP5* and *Pseudomonas putidaP13*), Livestock Manure+biological nitrogen, Livestock Manure+*Thiobacillus*, Livestock Manure+biological nitrogen+biological phosphorus, Livestock Manure+*Thiobacillus*+biological phosphorus, Livestock Manure+biological nitrogen+*Thiobacillus*, and Livestock Manure+*Thiobacillus*+biological nitrogen+ biological phosphorus. The highest oil percent (61%) and oil yield (2634 kg/ha) were obtained from fertilizer treatment 13. The highest yield of protein (1976 kg/ha) belonged to the treatment 13 as well as the lowest yield of protein (375 kg/ha) was obtained from treatment 4 (control). According to the results of this study fertilizer treatment 13 (30 tons/ha of Livestock Manure+ 100 g/ha phosphate barvar 2 + 1 liter/ha Nitroxin fertilizer + 1.5 kg/ha *Thiobacillus*) is recommended for increase yield quality and quantity of cucurbit.

KEYWORDS

Cucurbita;
Bio- fertilizers;
Oil yield;
Protein and seed.

INTRODUCTION

Medicinal Pumpkin (*Cucurbita pepo convar. pepo var styrica*) an annual and herbaceous plant, is native to tropical and subtropical regions and from the America has spread to other parts of the world[15,24]. Seeds of these plants are rich source of protein, oil and valuable active ingredients such as fatty acids, Phytosterols and Vitamin E. And from the active ingredients of that, drugs such as Peponen, Prostaliquid and irritation urine is made[9]. The amount of oil in seed is about 40 to 60 percent and most important fatty acid constituent the oil of this plant is linoleic acid 45 to 50 percent[15]. Use of biological fertilizers in agricultural ecosystems with the purpose of eliminating or substantially reducing the use of chemical inputs is important[19]. Bashan et al[3] showed that the use of *Azotobacter* causes to increase nitrogen content in grains. Ratti et al[17] observed that application of phosphate solubilizing bacteria (PSB) on lemon grass plant biomass increased relative to control. Gardezi et al[6] and Hameeda et al[8] reported that the usage of dilatory compost (through the processing of organic wastes such as manure, crop residue) can be obtained by earthworms on the pearl millet and *Sesbania emerus* plant were observed significantly increased plant height. There are clear and positive reports on the use of biological fertilizers for *Azosprillum* and *azotobacter* on *Sorghum*[22], onion[14], wheat and mustard[7]. Rezvani Moghaddam et al[18] with applying manure, compost and fertilizers, phosphorus and nitrogen in castor oil plant showed that the highest oil percent and grain yield was obtained in compost fertilizer treatments and combined nitrogen and phosphorus fertilizer. Eghball et al[5] stated that the usage of manure or compost can increase the nutrient concentration and organic matter of soil, and therefore may affect the yield of cultivated plants. The maximum oil content was obtained in peanuts respectively by using 75% NPK + 25% of the composition of manure and biofertilizaer, and the highest oil yield obtained from the use of 25% of nitrogen fertilizer and 75% organic fertilizer[4]. They also showed that the highest percentage and protein yield with 75% NPK + 25% of the organic and biological fertilizers. The environment protection and reduce the consumption of fertilizers is one of the main targets of sustainable production in agricultural ecosystems. In this regard, the main objective of this study was to evaluate the effect of different biological fertilizers on morphological traits and yield of *Cucurbita pepo var. sterica*.

MATERIALS AND METHODS

This experiment was conducted at Research Center of Agriculture and Natural Resources of West Azarbaijan with latitude 37 º, 53´ North and longitude 45º, 10´ East, and a height of 1325 meters above sea level in 2008. The average annual precipitation of 237 mm and mean annual temperature is about 13.1 ° C. Some physico-chemical characteristics of soil were shown in TABLE 1.

TABLE 1 : Analysis of some physical and chemical characteristics of soil and manure.

	Saturation S.P	Electrical conductivity	pH	Neutral Materials T.N.V	Organic matter	Nitrogen	Phosphorus	Potassium	Sand	Silt	Clay	Soil texture
	%	$Ds.m^{-1}$	-	%	%	%	ppm	ppm	%	%	%	-
Soil	56	0.117	7.91	29.8	1.5	_	16.14	222	13	46	41	loam Clay
Manure	_	1.92	8.5	_	_	1.54	0.75	2.8	_	_	_	_

Before sowing, moldboard plow land with deep plowing in autumn and tillage for the final seed bed preparation was done. Chemical analysis of manure was given in TABLE 1. Seeds of Pumpkin (*Cucurbita pepo* convar. pepo *var* styriaca) have been diluted by adding water 3-5 times of bio-fertilizers weight and then have been mixed up totally. Treated seeds were immediately sown in soil at depth of 2 cm. Treatments included 13 fertilizer treatments listed in TABLE 2, were arranged in a randomized complete block design with four replications. Experimental units in each replication composed of five lines. Inter-row and inter-plant spacing was 1.0 and 0.4 m, respectively. After emergence, one of four seedlings was remained in each hole of 1.0×0.4 m planted seeds containing 25000 plants per hectare. The field was kept weed free by hand weeding.

Fruits were harvested in 27th September, and main stem and sub stem length were measured by 10 samples per experimental unit. The 1000 seeds weight was determined by weighing 4 samples of 100 seed for each plot. We harvest 2 m2 of each plot to obtain biological and seed yield, and then harvest index (ratio of seed yield to biological yield) was calculated. The oil of seeds was determined by Soxhlet extraction for 6 hours. The Seed protein content was calculated by multiplying total nitrogen content with factor 6.25. Total nitrogen content was determined by the micro-Kjeldahl method[11]. Data analysis of variance was done by SAS 9.1 software. The means differences among the treatments were compared by LSD Multiple Comparison Test at 0.05 level of probability.

TABLE 2 : Fertilizer treatments

Number	Treatment	Amount of Fertilizer per unit area
1	Phosphate fertilizer 2 (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5) +Phosphorus fertilizer	Phosphate barvar 2 (100 g/ha) +Phosphorus Fertilizer (60 kg/ha)
2	Nitroxin (*Azotobacter* and *Azospirillum*)	Nitroxin Fertilizer (1 liter/ha)
3	*Thiobacillus*	Fertilizer (1.5kg/ha) *Thiobacillus*
4	Control	without biological and chemical Fertilizers
5	NPK	PhosphorusFertilizer (120 kg/ha)+ Potassium Fertilizer (100 kg/ha) + Nitrogen Fertilizer (60 kg/ha)
6	Livestock Manure	Livestock Manure (30 t/ha)
7	Livestock Manure+Phosphate barvar 2	Livestock Manure (30 t/ha)+ Phosphate barvar 2 (100 g/ha)
8	Livestock Manure+Nitroxin	Livestock Manure (30 ton/ha)+ Nitroxin Fertilizer (Azotobacter) (1 liter/ha)
9	Livestock Manure+ Thiobacillus	Livestock Manure (30 ton/ha)+ Thiobacillus Fertilizer (1.5kg/ha)
10	Livestock Manure+ Nitroxin+Phosphate barvar 2	Livestock Manure (30 ton/ha)+ Phosphate barvar 2 (100gr/ha)+ Nitroxin Fertilizer (Azotobacter) (1 liter/ha)
11	Livestock Manure + Thiobacillus+Phosphate barvar 2	Livestock Manure (30 ton/ha)+ Phosphate barvar 2 (100gr/ha)+ Thiobacillus Fertilizer (1.5kg/ha)
12	Livestock Manure + Nitroxin+ Thiobacillus	Livestock Manure (30 ton/ha)+ Nitroxin Fertilizer (Azotobacter) (1 liter/ha) + Thiobacillus Fertilizer (1.5kg/ha)
13	Livestock Manure+ Thiobacillus+Nitroxin+Phosphate barvar 2	Livestock Manure (30 ton/ha)+ Phosphate barvar 2 (100gr/ha) + Nitroxin Fertilizer (Azotobacter) (1 liter/ha) + Thiobacillus Fertilizer (1.5kg/ha)

RESULTS AND DISCUSSION

Results according to analysis of variance showed significant effect of treatments on the seed yield, oil percentage, oil yield, protein yield, the length of main stem, sub stem length, fruit yield and harvest index of seed ($P \leq 0.01$). But the effect of fertilizer treatments on the number of sub stem per plant, biological yield and percent of protein was non-significant (TABLE 3).

TABLE 3 : The analysis of variance (ANOVA) of fertilizer treatments effects on the agronomic characters and yield of medicinal pumpkin (*Cucurbita pepo convar. pepo var styrica*).

Source of variation	df	Mean of square					
		Seed Yield	Oil %	Oil Yield	Protein%	Protein Yield	Length of main Stem
Replication	3	7963.218**	0.019 ns	2543.808**	0.00001 ns	1334.890**	0.006 ns
Treatment	12	1658609.698**	163.269**	1000720.665**	242.410 ns	849795.282**	3.116**
Error	36	1566.640	0.047	387.706	0.001	269.662	0.003
Coefficient of variation (%)		1.42	0.41	1.30	0.01	1.37	1.56

ns, non-significant **, significant in 1% probability level.

Means comparison showed that the highest yield of seed (3875 kg/ha) was obtained from T13 (Livestock Manure+ *Thiobacillus*+Nitroxin+Phosphate barvar 2) and the lowest yield (500.1 kg/ha) belonged to control treatment. All biofertilizer treatments produced the higher yield of seed than control (without fertilizer). But there were significant reductions in treatments 2 (Nitroxin included *Azotobacter* and *Azospirillum* species), 3 (*Thiobacillus*) and 5 (NPK) as same as control (Figure 1).

The lowest percent of seed oil (40 %) was obtained from control treatment. All experimental treatments increased the oil percent, while the minimum increase belonged to chemical originated NPK. The highest percent of seed oil (61 %) was obtained from 13 (Livestock Manure+ Thiobacillus+Nitroxin+Phosphate barvar 2)

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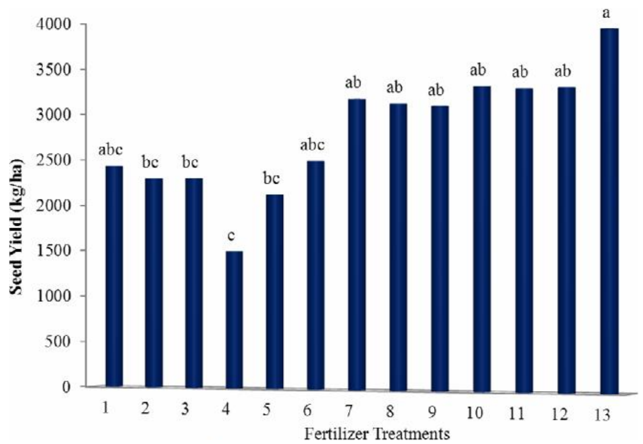

Treatments:

1: Phosphate fertilizer 2 (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5) +Phosphorus fertilizer
2: Nitroxin (*Azotobacter* and *Azospirillum*)
3: *Thiobacillus*
4: Control
5: NPK
6: Livestock Manure
7: Livestock Manure+Phosphate barvar 2 (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5)
8: Livestock Manure+Nitroxin (*Azotobacter* and *Azospirillum*)
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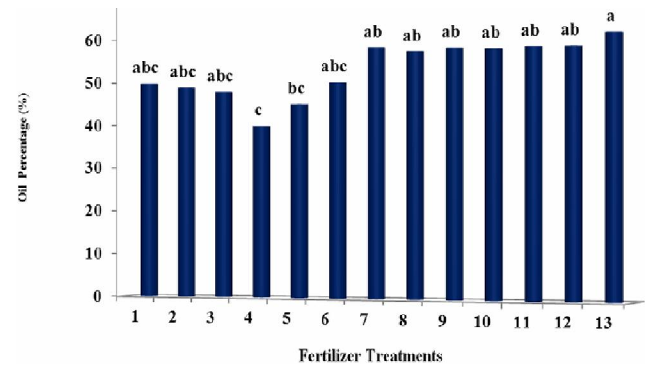
12: Livestock Manure + Nitroxin (*Azotobacter* and *Azospirillum*)+ *Thiobacillus*
13: Livestock Manure+ *Thiobacillus*+Nitroxin (*Azotobacter* and *Azospirillum*)+Phosphate barvar 2 (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5)

Figure 1 : Means comparison of seed yield (kg/ha) of medicinal pumpkin (*Cucurbita pepo convar*) affected by bio-fertilizer treatments. The same letters show non-significant differences between at *P*≤0.05

followed by other biological manures (Figure 2).

Results of means comparison (Figure 3) revealed that the highest yield of oil (2364 kg/ha) was produced by plants treated with T13 (Livestock Manure+ *Thiobacillus*+Nitroxin+Phosphate barvar 2) followed by biological manure treatments. But the significant reduction was observed in chemical NPK, 2 (Nitroxin included *Azotobacter* and *Azospirillum* species) and 3 (*Thiobacillus*). While the minimum yield of seed oil belonged to control treatment (600 kg/ha). The chemical fertilizer (NPK) showed the minimum increase in oil yield compared with control. Despite the higher increase of oil yield by biofertilizer nutrients than control, this yield was less than combination of biofertilizer and chemical nutrient (Treatment 1) (Figure 3).



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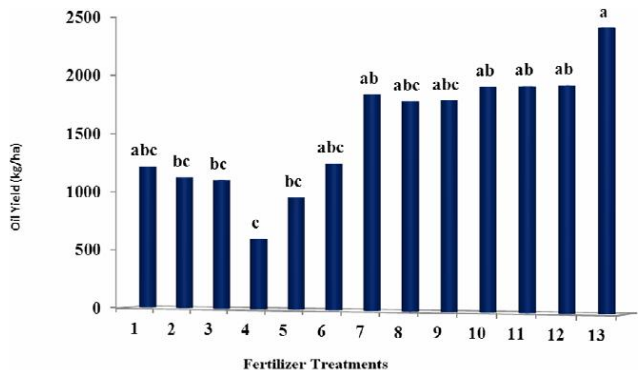
13: Livestock Manure+ *Thiobacillus*+Nitroxin (*Azotobacter* and *Azospirillum*)+Phosphate barvar 2 (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5)

Figure 2 : Means comparison of seed oil percentage (%) of medicinal pumpkin *(Cucurbita pepo convar)* affected by fertilizer treatments. The same letters show non-significant differences between at *P*≤0.05.

Changes of protein yield (Figure 4), like oil yield indicated that the highest (1976 kg/ha) and lowest (375 kg/ha) yield of protein were respectively obtained from T13 (Livestock Manure+ *Thiobacillus*+Nitroxin+Phosphate barvar 2) and control treatment. Chemical NPK with lowest increase in protein yield compared to other biological treatments, showed the enhancement of these organic treatment to produce protein in pumpkin plants (Figure 4).

The longest stem (500 cm) belonged to Livestock Manure +*Thiobacillus*+Nitroxin+Phosphate barvar 2 (T13) and the shortest stem (125 cm) was observed at control treatment (without fertilizer). Chemical NPK treatment had the lowest increase in stem length in comparison with control treatment. Despite the significant increase in other biological manures, these arises were less than T13 (Figure 5).



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3: *Thiobacillus*
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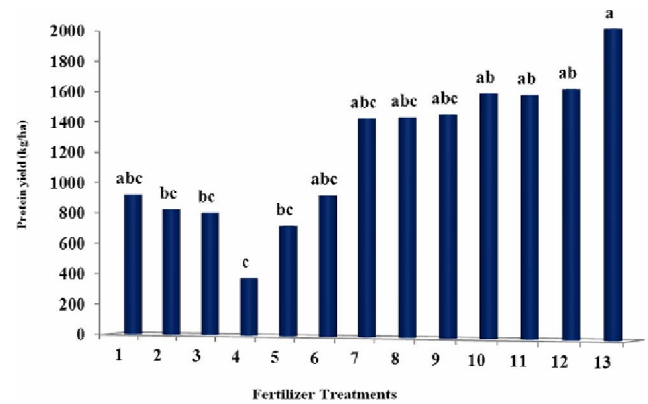
13: Livestock Manure+ *Thiobacillus*+Nitroxin (*Azotobacter* and *Azospirillum*)+Phosphate barvar 2 (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5)

Figure 3 : Means comparison of seed oil yield (kg/ha) of medicinal pumpkin *(Cucurbita pepo convar)* affected by fertilizer treatments. The same letters show non-significant differences between at *P*≤0.05.



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3: *Thiobacillus*
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13: Livestock Manure+ *Thiobacillus*+Nitroxin (*Azotobacter* and *Azospirillum*)+Phosphate barvar 2 (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5)

Figure 4 : Means comparison of yield of protein (kg/ha) of medicinal pumpkin *(Cucurbita pepo convar)* affected by fertilizer treatments. The same letters show non-significant differences between at *P*≤0.05.

Like stem length, the longest (400 cm) and shortest (100 cm) lateral stem were obtained from T13 and control treatments, respectively. The descending trend was occurred from T13 to T7 (with 400 cm to 330 cm) (Figure 6).

The harvest index, ratio of seed yield to biological yield showed in Figure 7, indicated that the maximum reduction of photosyntate allocation to seed was occurred at control treatment with 12.27 %. Similarity between seed yield and harvest index (Figures 1 and 7) indicated non-significant differences of biological yield among treatments (TABLE 3).

Results from this study showed that biological fertilizers plus livestock manure treatments (Treatments 7, 8, 9, 10, 11, 12 and 13) led to the optimal values of seed oil percent, seed protein content, yield of protein, stem length, the number of lateral branches, 1000 seed weight, fruit yield (fresh weight), biological yield, seed yield and harvest index of medicinal pumpkin plant.

Rotten livestock manure thoroughly supply the needed food of plants, also has a very important role in improving soil physical characteristics and is very effective in enhancing soil fertility, with increasing soil humus. Use of this fertilizers improving gas exchange in soil, maintain water and nutrients in the soil, lightening the heavy soils and enhance the adhesion properties sandy soils and an increase of yield is efficient. Yield increase in livestock manure treatments (Figures 1, 3 and 4), perhaps due to increased activity of microorganisms, and release of some CO_2 in plants and in result that causes to photosynthesis of plant[20]. Matsi et al[13] showed that use of livestock manure can increase high consumption elements nitrogen, phosphorus and

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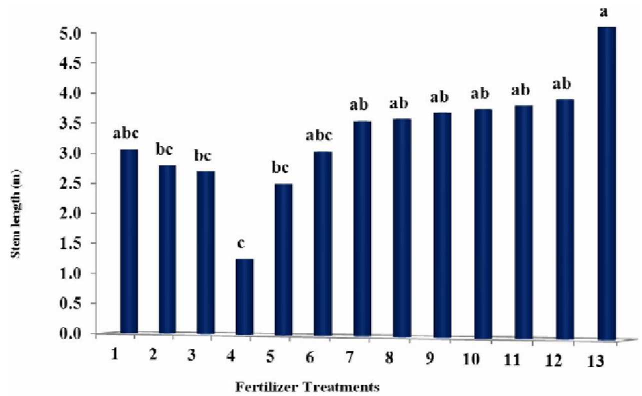

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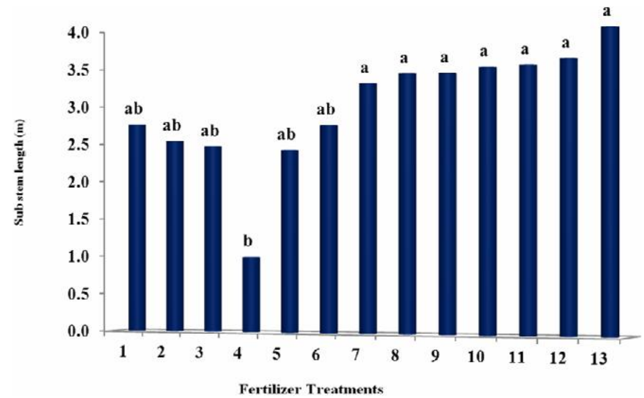
Figure 5 : Means comparison of stem length (m) of medicinal pumpkin *(Cucurbita pepo convar)* affected by fertilizer treatments. The same letters show non-significant differences between at *P*≤0.05.



Treatments:
1: Phosphate fertilizer 2 (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5) +Phosphorus fertilizer
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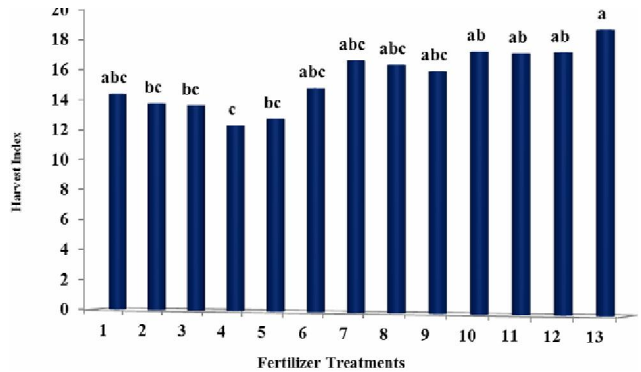
13: Livestock Manure+ *Thiobacillus*+Nitroxin (*Azotobacter* and *Azospirillum*)+Phosphate barvar 2 (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5)

Figure 6 : Means comparison of sub stem length (m) of medicinal pumpkin *(Cucurbita pepo convar)* affected by fertilizer treatments. The same letters show non-significant differences between at *P*≤0.05.

potassium accessibility. Zhang et al[25] reported that use of bio fertilizers, especially livestock manure, improve soil physical properties and this cause to more stimulate of elements and water absorption by plant roots. Use of both organic matter of livestock manure and composting municipal waste with increasing biological yield of wheat with increasing concentrations of phosphorus, potassium, iron, manganese, chloride and sodium in different organs of wheat compared to control treatment[16]. Ahmad and Jabeen[1] were observed significant enhancement in vegetative traits such as plant height, stem diameter, biological yield, oil percent and seed yield of sunflower due to application of bio fertilizers. The reason for this increase might be in relation to improving soil structure by increasing soil water holding capacity, proper ventilation and drainage. Increase in crop yield with application of biofertilizers has been reported in corn[23]. Bio fertilizers production cost is low and do not create pollution in the ecosystem. The consumption of these fertilizers not only increased yield but also decreased amount of chemical fertilizers usage[10]. The most common biofertilizers containing micro-organisms can point to nitrogen fixing bacteria (Diazotrophs) such as *Azotobacter* genus, and phosphate solubilizing microorganisms (phosphate barvar 2 manure in this research), given that the consequences of leaching nitrogen, contamination of water resources and phosphate solubilizing, calcium compounds accumulate in alkaline soils, lime with aluminum and iron in acidic soils can have a profound impact in achieving the purpose of sustainable agriculture and have increased yield[21]. Phosphorus is one of the main elements with



Treatments:

1: Phosphate fertilizer 2 (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5) +Phosphorus fertilizer

2: Nitroxin (*Azotobacter* and *Azospirillum*)

3: *Thiobacillus*

4: Control

5: NPK

6: Livestock Manure

7: Livestock Manure+Phosphate barvar 2 (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5)

8: Livestock Manure+Nitroxin (*Azotobacter* and *Azospirillum*)

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Figure 7 : Means comparison of harvest index (HI) of medicinal pumpkin *(Cucurbita pepo convar)* affected by fertilizer treatments. The same letters show non-significant differences between at *P*≤0.05.

high consumption for plant, increase the quality and quantity of the product. This element feature availability depends on many factors, because in most soils, phosphorus is in insoluble form (unusable for plants) of organic and inorganic compounds. One of the ways for solve this problem is the use of phosphatic biofertilizers that release of phosphate ions, and it puts at the disposal of plant. Phosphorus has a useful role in root development, vegetative growth, flowering, Fruit set, product ripening and increase product quality of plant[20]. In most agricultural lands, phosphorus accumulation caused irreparable damage to ecosystems, so that contamination with phosphorus and heavy metals as an environmental hazard has attracted attention of ecologists in recent decades the world[2].

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