



Effect of Organic Substrate and Phosphorus Fertilization on Seed, Oil Yields and Composition of Medicinal Pumpkin (*Cucurbita pepo* var. *styriaca*)

R. Pajooeshgar*, M. Azizi**, H. Nemati*** and R. Khorasani****

*Ph.D. Student of Horticultural Science, Department of Horticulture, Faculty of Agriculture, Ferdowsi University of Mashhad, International Campus, Mashhad, IRAN

**Professor, Department of Horticulture, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, IRAN

***Assistant Professor, Department of Horticulture, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, IRAN

****Associate Professor, Department of Soil Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, IRAN

(Corresponding author: M. Azizi)

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ABSTRACT: Intensive cropping systems with fertilizer responsive crops that rely on high input of inorganic fertilizers often lead to nonsustainability in production and also pose a serious threat to soil health. Application of organic sources of nutrients with no or very little use of fossil fuel-based inorganic fertilizers is rapidly gaining favor. However, considering economics and also physiological potential of varieties, entire dependence on organic sources of nutrients may not be adequate to attain the most productivity. Integrated nutrient management with both organic and inorganic fertilizers was investigated. A field trial was conducted with different fertilizer combinations of organic substrates (cow manure, spent mushroom substrates (SMS) and vermicomposts) and phosphorus fertilization (0, 75, 100 and 125 kg ha⁻¹) to study their effects on seed number, seed phosphorus and zinc content, oil yield and oil quality in medicinal medicinal pumpkin seed (*Cucurbita pepo* var. *styraca*). For lonely application of organic substrates and phosphorus concentrations; vermicomposts and 125 P (kg.ha⁻¹) treatments had the highest seed number, oil percentage with high value of oleic acid in medicinal medicinal pumpkin seeds. Results from the experiment revealed that among the sixteen treatments compared, the combination of vermicomposts and all levels of phosphorus applications had the maximum of seed number with high oil percentage and qualities. Perhaps in some combine vermicomposts and phosphorus fertilizer applications, linoleic decreased but because of effective role of these combinations on seed number and oil percentage, can help to increase the main important fatty acids.

Key words: Organic substrates, Phosphorus fertilizer, Linoleic acid

INTRODUCTION

Medicinal medicinal pumpkin (*Cucurbita pepo* var. *styraca*) seed oil is a common salad oil in Austria. Due to its colour, the foaming and the strong aroma, it has only limited application for cooking. The seeds themselves are eaten and show good results in curing several prostate diseases (Murkovic *et al.*, 2004). The main characteristic of the Styrian Oil Medicinal pumpkin is the dark green colour of the thin-coated seeds (Teppner, 2000). The colour of the oil, which is pressed from the seeds, is dark green to red ochre and has a strong red fluorescence. The oil content of the medicinal medicinal pumpkin seed varies from 42-54% and the composition of fatty acids is dependent on several factors (variety, area in which the plants are

grown, climate, state of ripeness). The dominant fatty acids comprise palmitic acid (C16:0, 9.5-14.5%), stearic acid (C18:0, 3.1- 7.4%), oleic acid (C18:1, 21.0-46.9%) and linoleic acid (C18:2, 35.6-60.8%). These four fatty acids make up 98±0.13% of the total. Other fatty acids are well below 0.5% (Murkovic *et al.*, 2004). The content of vitamin E in medicinal medicinal pumpkin seeds is very high (Murkovic *et al.*, 1996). The main vitamin E isomers occurring in medicinal medicinal pumpkin seeds are - and -tocopherols with concentration of n.d.-91 mg/kg and 41-620 mg/kg, respectively. The other two tocopherols occur at very low concentrations. Additionally, a- and g-tocotrienol are found in significant amounts but have not yet been quantified (Murkovic *et al.*, 2004).

Chemical fertilizers resulted in overall growth and enhancement of active constituents of medicinal and crop plants, but their overuse and unbalanced application without prior analysis of soil nutrient status coupled with intensive farming operations and inadequate land management often lead to non sustainability in production, posing a serious threat to underground water resources, destroying microorganisms and insects, and deteriorating soil health in terms of decline in soil organic matter (Qazi *et al.*, 2009).

Integrated supply of nutrient to plants through planned combinations of organic and inorganic sources is becoming an increasingly important aspect of environmentally sound agriculture. Little research has been reported on the influence of organic manure and conjoint application of manure and inorganic fertilizer on yield and quality of medicinal medicinal pumpkin oil.

Moreover, the high cost and demand for chemical fertilizers necessitate fine tuning of their requirement and management. Application of organic sources of nutrients with no or very little use of inorganic fertilizers and use of bio-fertilizers for crop production are gaining momentum as they are environmentally safe compared to chemical fertilizers. The purpose of this study was to compare the effect of chemical (Phosphorus) and Organic (vermicomposts, cow manure and spent mushroom substrates) treatments on the chemical composition of seeds oil from medicinal medicinal pumpkin seed (*Cucurbita pepo* var. *styraca*).

MATERIALS AND METHODS

The present study was conducted at Agricultural Research Farm of Kohnekan, Iran during the 2014 growing season. The experimental units were designed as split plots based on randomized complete block design (RCBD) in four replications and sixteen fertilizers combinations. Organic substrates consisted of 4 substrates (control, cow manure (25 T ha⁻¹), SMS (10 T ha⁻¹) and vermicomposts (10 T ha⁻¹)), which allotted to the main plots. Nutrition analysis of each substrate was done before the seed sowing and their results presented in Table 1.

Sub-plots were comprised of different levels of phosphorus application: 0, 75, 100 and 125 (kg ha⁻¹). Each plot comprised two rows with 100 cm spacing between them and each row had 4 plants with 40 cm space between plants. After the land preparation (such as plowing, disking and ridging), sowing was carried out on April 10th in the soil directly. The plants were thinned to one at 4-6 true leaf stages. In general, weather condition during all trial period was favorable for the growth and development of pumpkin. Obviously, when more than 75% of the fruits became yellowish orange in color and stem and leaves began to dry and the seeds became dark green and well rounded, manual harvesting was done. The seeds were manually extracted from the fruits and then naturally dried by sun light until constant weight. The traits consisting of seed number per fruit, phosphorus and zinc content of seeds were measured. The sun-dried seeds were transferred to laboratory. The Murphy and Riley method (1962) and Mortvedt (1999) were employed to determine the P and Zn values of medicinal medicinal pumpkin seeds, respectively.

FAME was prepared according to the standard method (Nakic *et al.*, 2006). The fatty acid profile was determined by gas chromatographic separation of their methyl esters (Nakic *et al.*, 2006) on a capillary column (J&W Scientific DB-23, 30m × 0.25mm × 0.25 μm). The temperatures of the injector and detector were set at 250 °C. The initial oven temperature was 170 °C. This temperature was maintained for 8 min and then increased at a rate of 2 °C/min to 190°C, which was held for 7 min. Helium was used as the carrier gas at a flow rate of 0.87 mL/min and the injection volume was 0.3 μL. The FAME peaks were identified using FAME standards. The fatty acid composition is expressed as weight percentage of total (internal normalization method). Chromatography software (Unicam 4880 chromatography data system) was employed for data collection and processing.

Data on seed quantity and quality were analyzed using MSTAT C program and treatments means were compared using Duncan's Multiple Range test at 5% level of probability.

Table 1: Chemical properties of experimental substrates before sowing.

Substrates	N (ppm)	P (ppm)	K (ppm)
Control (Soil)	0.057	10.8	273
SMS	14530	76.40	19290
Coe manure	14170	6120	19880
Vermicomposts	15970	7120	5090

RESULTS AND DISCUSSION

A. Organic substrates effects

Data presented in Table (2) revealed that application of organic fertilizer significantly increased the seed number, seed phosphorus content and oil yield in medicinal medicinal pumpkin over control (no manure). Seed number per fruit increased by 41, 29 and 46% over control with SMS, cow manure and

vermicomposts, respectively. The highest value of seed phosphorus content was observed in vermicomposts substrates and increased 13% over control. Among the four treatments compared, the lowest increase over control (0.13%) was with SMS applied, and the highest increase in oil yield was with Vermicomposts (16%), followed by cow manure (13%).

Table 2. Effects of organic substrates on medicinal medicinal pumpkin seed properties

Organic Substrates	Seed number/fruit	Seed phosphorus content (mg 100g ⁻¹)	Seed Zinc content (mg 100g ⁻¹)	Oil (%)
Control	185.6 d	0.2236 d	86.12 a	38.44 b
SMS	261.3 b	0.2451 b	87.08 a	38.49 b
Cow manure	239.4 c	0.2422 c	79.50 b	43.41 a
Vermicomposts	271.6 a	0.2534 a	73.31 c	44.57 a
LSD 5%	9.08	0.01	3.77	1.48

*LSD (0.05) represents least significant difference at 5% probability.

Animal manure is a valuable resource as a soil fertilizer because it provides large amounts of macro- and micronutrients for crop growth and is a low-cost, environmentally friendly alternative to mineral fertilizers (Haghighat *et al.*, 2013). Sarhan *et al.*, (2011) worked on organic manures in tomato crop and reported that organic manures significantly affected tomato plant height, leaf area and fruit number per plant. Abd El-Rahman and Hosny (2001) stated that using organic manure improved the yield and yield components of egg-plant fruits.

Shahmohammadi *et al.*, (2014) reported that highest seed yield were obtained in compost substrates (10 ton ha⁻¹) compared to control treatments. Same results were reported by Rezvanimoghadam *et al.*, (2012). The use of organic amendments, such as traditional thermophilic composts, has long been recognized as an effective means of improving soil structure, enhancing soil fertility, increasing microbial diversity and populations, microbial activity, improving the moisture-holding capacity of soils and increasing crop yields (Arancon *et al.*, 2004). Vermicompost contains most nutrients in plant-available forms such as nitrates, phosphates, and exchangeable calcium and soluble potassium. Vermicomposts are rich in microbial populations and diversity, particularly fungi, bacteria and actinomycetes. Researchers have shown that vermicomposts consistently promote biological activity which can cause plants to germinate, flower and grow and yield better than in commercial container media, independent of nutrient availability (Atiyeh *et al.*,

2000a, b). Vermicomposts contain plant growth regulators and other plant growth influencing materials produced by microorganisms including humates (Atiyeh *et al.*, 2002).

Other studies on organic fertilizers showed that in compare of cow manure and vermicomposts, vermicomposts have more positive effects on seed yield and oil content than cow manure (Jahan *et al.*, 2013). The results in Table 2 confirmed the effective role of vermicomposts compared to other organic substrates on medicinal pumpkin seed properties.

The highest values for zinc content of seeds were obtained in SMS (86.12 mg 100g⁻¹), followed by control (86.12 mg 100g⁻¹) treatments and lowest value were observed in vermicomposts (73.31 mg 100g⁻¹). There are some informations about the antagonistic effects of phosphorus on zinc absorption by plants (Das *et al.*, 2005; Karamanos *et al.*, 2008). Das *et al.*, (2005) showed that application of phosphorus and zinc in a same time caused to have negative effects on Zn availability in soil and decreased the Zn absorption. On the other hand, data in Table (1) showed the highest phosphorus values between four substrates were obtained in vermicomposts. Thus, our results are consistent with other investigators.

B. Phosphorus levels effects

Data presented in Table (3) revealed that application of phosphorus fertilizer significantly influenced the seed phosphorus and zinc content and oil percentage in medicinal pumpkin seed over control (no manure), except seed number.

There were no significant differences between four phosphorus levels for seed number per fruit and slight positive effects were observed in increasing P levels. Phosphorus content of seed was increased about 24% (from 0 up to 125 kg ha⁻¹). The highest zinc values in seed were obtained from control treatments and the

lowest values (81.89, 80.09 and 78.85 mg 100g⁻¹) were observed in 125, 75 and 100 (kg ha⁻¹) phosphorus application levels, respectively. Seed oil percentage increased by 14, 18 and 18.2% over control with SMS, cow manure and vermicomposts, respectively (Table 3).

Table 3. Effects of different phosphorus levels on medicinal pumpkin seed properties.

Seed number/fruit	Seed phosphorus content (mg 100g ⁻¹)	Seed Zinc content (mg 100g ⁻¹)	Oil (%)
236.2 a	0.2216 c	85.18 a	36.63 c
241.4 a	0.2503 b	80.09 b	41.79 ab
239.6 a	0.2171 d	78.85 b	43.19 a
240.7 a	0.2754 a	81.89 ab	43.29 a
9.08	0.01	3.77	1.48

*LSD (0.05) represents least significant difference at 5% probability.

Phosphorus fertilizers can have varied effects on crop responses, depending on the crop and environmental conditions. Its application increased root development, early flowering, fruit set and fruit ripening, seed-formation and yield (Imran and Gurmani, 2011). Increasing of P caused to nitrogen availability and resulted to increase growth factors and yield. Rezvanimoghadam *et al.*, (2013) reported that usage of chemical fertilizers containing phosphorus was increased oil yield in sesame plants. Usage of phosphate solubilizing bacteria (PSB) caused to increase the phosphorus availability, resulting number of seed per fruit and oil yield increased by 84% and 83% over control in medicinal pumpkin seed (Habibi *et al.*, 2011).

But high value of P may have antagonistic effects on nutrition availability in soil and decrease nutrition absorption (Das *et al.*, 2005; Karamanos *et al.*, 2008) and caused to decrease morphological and biological factors in plants (Rehim *et al.*, 2014; Boroomand and Hosseini Grouh, 2012; Imran and Gurmani, 2011; Ronan, 2007; Das *et al.*, 2005; Sawan *et al.*, 2001). According to data presented in Table (3), increasing in P levels caused to decrease the zinc content of seed. Thus, our results are consistent with other researchers.

C. Combine application effects of organic substrates and different phosphorus levels

Application of different phosphorus levels alone had no significant effects on seed number, but in combine applications of phosphorus fertilizers and organic substrates, the maximum seed number were obtained in SMS with all phosphorus levels and vermicomposts with/ without phosphorus fertilizers (Table 4).

The highest and lowest value of seed phosphorus levels were observed in vermicomposts × 125 (kg ha⁻¹) and cow manure × control fertilizer combinations. Because of antagonistic effects of phosphorus on zinc availability and absorption by plants (Das *et al.*, 2005; Karamanos *et al.*, 2008), the lowest values of seed zinc content were observed in vermicomposts × 125 (kg ha⁻¹) fertilizer combinations. The highest zinc content values were obtained in control × 100 (kg ha⁻¹) and SMS × 125 (kg ha⁻¹) fertilizer combinations. Oil yield is the main purpose of oil seeds cultivation of medicinal pumpkin. The vermicomposts and cow manure with all phosphorus fertilizer levels had highest values of oil percentage with no significant differences. Minimum of oil percentage were obtained in SMS × control.

The effect of organic matter on plant growth and yield was reported similar to chemical synthetic fertilizer and even better (Efthimiadou *et al.*, 2009; Zahradnik and Petrikova, 2007). Because of variety of micro and macro elements in organic substrates and elements availability or solubility in the presence of different phosphorus levels, combine application of these fertilizers may have different response in quality and quantity of medicinal pumpkin seed properties.

The composition of fatty acids varies depending on several factors including variety, growing area, climate and ripeness (Murkovic *et al.*, 1999). As can be seen in Table (5), four major fatty acids, namely, linoleic (C18:2), oleic (C18:1), palmitic (C16:0), and stearic (C18:0) were found in the medicinal pumpkin seed oil and they constituted more than 97% of the total amount.

Table 4. Effects of combine application of organic substrates and different phosphorus levels on medicinal pumpkin seed properties

Organic Substrates	P Levels (kg ha ⁻¹)	Seed number/fruit	Seed phosphorus content (mg 100g ⁻¹)	Seed Zinc content (mg 100g ⁻¹)	Oil (%)
Control	Control	186.0 d	0.2206 i	90.66 b	30.77 de
	75	187.3 d	0.2291 h	73.18 def	41.35 bcd
	100	184.3 d	0.2829 b	100.1 a	41.95 bcd
	125	185.0 d	0.1616 I	80.56 cd	39.67 e
SMS	Control	248.3 bc	0.2537 e	74.99 def	29.85 f
	75	268.3 a	0.2544 e	84.20 bc	38.22 bc
	100	264.5 ab	0.1930 j	83.40 bc	41.68 bc
	125	264.0 ab	0.2797 c	105.7 a	44.22 cde
Cow manure	Control	237.3 c	0.1789 k	89.76 b	42.72 de
	75	239.8 c	0.2702 d	78.07 cdef	43.20 abc
	100	239.8 c	0.2486 f	79.18 cde	43.58 ab
	125	240.8 c	0.2710 d	70.97 ef	44.13 ab
Vermicomposts	Control	273.3 a	0.2331 g	85.29 bc	43.17 bcd
	75	270.3 a	0.2473 f	84.93 bc	44.38 a
	100	270.0 a	0.1440 m	52.71 g	45.58 ab
	125	273.0 a	0.3895 a	70.30 f	45.15 ab
LSD 5%	-	18.16	0.01	7.55	2.97

*LSD (0.05) represents least significant difference at 5% probability.

This fatty acid profile is confirmed by several authors (Nakic *et al.*, 2006; Gohari Ardabili *et al.*, 2011). The medicinal pumpkin seed oil contained 19.4% saturated fatty acids, with the major one being palmitic acid (10.7%) followed by stearic acid (7.7%), while it was high in unsaturated fatty acids with a total content of 80.7%.

The main unsaturated fatty acids were linoleic acid followed by oleic acid with relative amount of 30.50 -39.47 and 40.80 - 48.13 %, respectively. In most other investigations on the fatty acid composition of *C. pepo* (Lazos, 1986; El-Adawy and Taha, 2001), the percentage of linoleic acid was higher

(43.1-55.6%) than that of oleic acid (20.4- 37.8%), while, in the present study, the percentages of linoleic were lower than the oleic acids. The highest values of linoleic acid were obtained from control treatments with/without different phosphorus levels.

The relative amount of oleic acid is always negatively correlated with the relative amount of linoleic acid (Murkovic *et al.*, 1996; Schuster *et al.*, 1983; Fruhwirth and Hermetter, 2008), which is due to the substrate-product relationship of these two fatty acids (Fruhwirth and Hermetter, 2008).

Table 5. Effects of combine application of organic substrates and different phosphorus levels on oil components in medicinal pumpkin seed.

Organic substrates	P Levels (kg ha ⁻¹)	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1
		Control	0	0.18	10.13	0.12	-	7.47	40.80	39.20	0.36	0.62	0.28
	75	0.09	10.15	0.10	0.06	7.21	42.85	38.03	0.14	0.53	0.11	0.19	0.53
	100	0.07	9.89	0.15	-	7.48	42.16	38.91	0.18	0.46	0.19	0.13	0.36
	125	0.08	9.81	0.09	0.05	7.15	41.83	39.47	0.28	0.49	0.15	0.16	0.44
SMS	0	0.1	10.24	0.16	0.07	7.15	41.91	38.99	0.11	0.54	0.11	0.15	0.46
	75	0.08	10.41	0.14	0.05	7.78	48.01	32.35	0.11	0.46	0.11	0.11	0.38
	100	0.08	10.01	0.17	0.06	7.36	44.11	36.54	0.28	0.73	0.12	0.13	0.41
	125	0.1	9.46	0.13	0.09	7.33	45.16	35.94	0.21	0.61	0.16	0.22	0.59
Cow manure	0	0.11	10.15	0.10	0.14	7.28	45.87	34.86	0.17	0.61	0.17	0.13	0.40
	75	0.44	1.37	0.27	0.27	7.48	42.18	37.03	0.23	0.7	0.25	0.31	0.45
	100	0.16	10.34	0.20	0.21	7.30	42.19	37.69	0.33	0.63	0.21	0.24	0.49
	125	0.25	9.99	0.12	0.15	7.35	43.01	37.42	0.18	0.60	0.13	0.22	0.58
Vermicomposts	0	-	9.51	0.45	-	8.64	48.13	30.50	0.55	0.69	0.29	0.54	0.69
	75	0.15	10.66	0.17	0.13	7.32	40.94	38.70	0.23	0.80	0.18	0.28	0.43
	100	0.38	9.55	0.32	-	8.79	41.52	36.04	0.51	0.58	0.41	0.56	1.35
	125	0.10	9.63	0.12	0.06	7.68	45.63	34.32	0.54	0.56	0.52	0.18	0.64

The vermicomposts × control and SMS × 75 (kg ha⁻¹) had the highest oleic acid values (Fig. 1) and lowest linoleic acid (Fig. 2) and these results were in good agreement with other researcher studies.

Despite the high content of total unsaturated fatty acids in the medicinal pumpkin seed oil, linolenic acid (C18:3) was very low (< 0.6 %), which was in good agreement with all other similar studies (Fruhwrth and Hermetter, 2008; Gohari Ardabili *et al.*, 2011).

The highest values of linolenic acid were observed in vermicomposts × control, vermicomposts × 100 (kg ha⁻¹) and vermicomposts × 125 (kg ha⁻¹) fertilizer combinations.

Also, the level of other fatty acids in the medicinal pumpkin seed oil was very low, similar to the results reported in the literature (Stevenson *et al.*, 2007; Gohari Ardabili *et al.*, 2011).

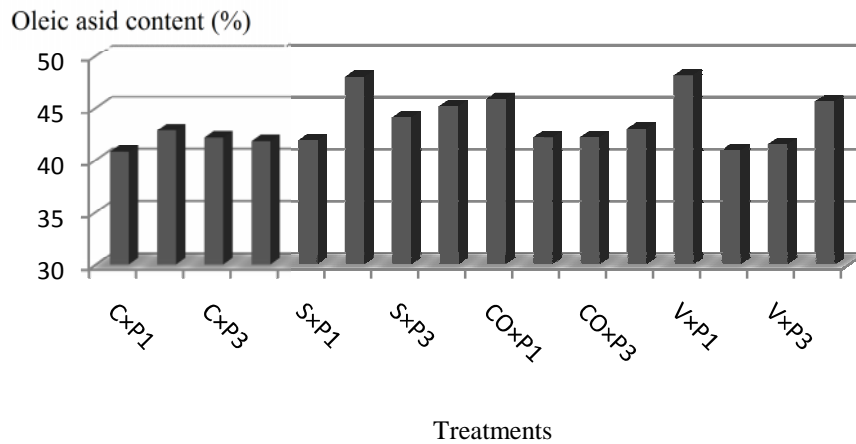


Fig. 1. Effects of combine application of organic substrates and phosphorus levels on oleic acid production in medicinal pumpkin seed oil (C: Control; Phosphorus levels contained P1:0, P2:75, P3:100, P4:125; S: SMS; CO: Cow manure and V: vermicomposts).

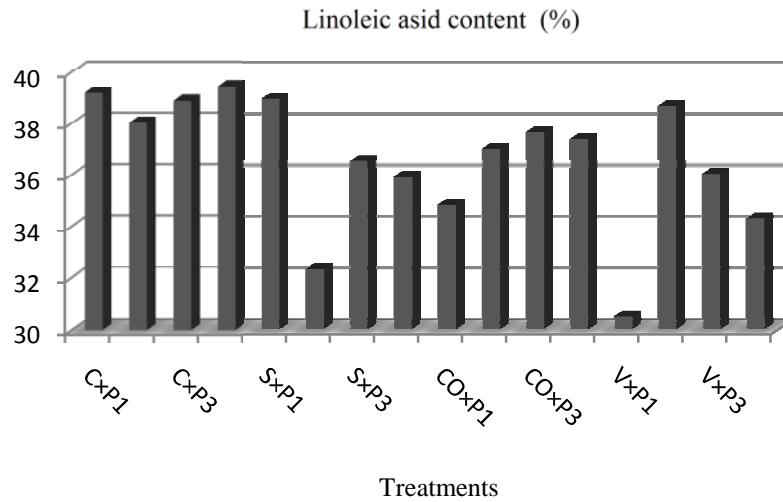


Fig. 2. Effects of combine application of organic substrates and phosphorus levels on linoleic acid production in medicinal pumpkin seed oil C: Control; Phosphorus levels contained P1:0, P2:75, P3:100, P4:125; S: SMS; CO: Cow manure and V: vermicomposts).

CONCLUSION

The most aim characters in medicinal pumpkin production concerned to seeds and oil seed components which have greatest role in pharmaceutical products. So, according to data presented in this paper, we can suggest some fertilizer to gain the maximum seed yield with high oil quality and quantity. For lonely application of organic substrates and phosphorus concentrations, vermicomposts and 125 P (kg.ha⁻¹) treatments had the highest seed number, oil percentage with high value of oleic acid in medicinal pumpkin seeds. For combining applications of organic substrates × P levels, results showed the maximum of seed number with high oil percentage and quality were found in vermicomposts × all levels of phosphorus applications. Perhaps in some combine vermicomposts and phosphorus fertilizer applications, linoleic decreased but because of effective role of these combinations in increasing seed number and oil percentage, it can help to increase the main important fatty acids, totally.

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