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## Improving Vitamins C and D<sub>3</sub> Content of Medicinal Pumpkin Plant Grown under Organic Farming Programs

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### ABSTRACT

This study aimed to evaluate the application of different farming programs. Three organic farming (P<sub>2</sub>, P<sub>3</sub>, and P<sub>4</sub>) were used and compared with traditional program P<sub>1</sub> (chemical). Their effect in improving medicinal pumpkin seeds quality was examined. As a quality addition of supplying chain, the quantitative and qualitative content of vitamins C and D<sub>3</sub> were assayed. This plant seeds are considered of healthy and medical importance, and have their marketing placement. A big amount of the seeds has been consumed directly by human year round. Using the seeds in food production processes increased their nutritional value. The field experiment was conducted on two dates. The design used was the randomized complete block design. All data were collected and analyzed according to ANOVA table using Duncan's multiple range test at P=0.05. The results obtained showed significant distinguish of the organic farming program (P<sub>3</sub>) compared to the rest the programs used. This treatment had significant increase of vitamins C and D<sub>3</sub> content in the seeds in the two dates (0.0883 mg l<sup>-1</sup>, 0.08671 mg l<sup>-1</sup>, 12.38 mg l<sup>-1</sup> and 12.32 mg l<sup>-1</sup> respectively). The other two organic programs (P<sub>2</sub>, P<sub>4</sub>) were significantly distinct compared to the traditional one (P<sub>1</sub>). The study concluded that these studied farming programs can be an effective choice to improve seed quality of this crop. The healthy and nutritional value are enhanced, and the side effects of traditional agriculture on the environment are reduced.

### KEY WORDS:

organic fertilizers, organic farming, farming programs, spent mushroom substrate, vitamins C and D<sub>3</sub>.

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# تحسين محتوى فيتاميني C وD<sub>3</sub> في نبات القرع الطبي المزروع تحت برامج الزراعية العضوية

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## الخلاصة

هدفت هذه الدراسة إلى تقييم مدى تطبيق برامج زراعية مختلفة. تم استخدام ثلاثة برامج للزراعة العضوية (P<sub>2</sub> و P<sub>3</sub> و P<sub>4</sub>) ومقارنتها ببرنامج للزراعة التقليدية (P<sub>1</sub> الكيمائي). تم دراسة تأثيرها في تحسين جودة بذور القرع الطبي. وكإضافة نوعية لسلسلة التوريد الغذائي، تم تقدير المحتويات الكمية والنوعية لفيتاميني C وD<sub>3</sub>، تعد بذور هذا النبات ذات أهمية صحية وطبية، ولها مكانتها التسويقية. يتم استهلاك كمية كبيرة من البذور مباشرة من قبل الإنسان على مدار السنة. وقد أدى استخدام البذور في عمليات الانتاج الغذائي إلى زيادة قيمتها الغذائية. أجريت التجربة الحقلية في مواعدين اثنتين. وكان التصميم المستخدم هو تصميم القطاعات العشوائية الكاملة. جمعت البيانات وحللت وفقاً لجدول ANOVA باستخدام اختبار Duncan متعدد المدى عند مستوى احتمالية P = 0.05. أظهرت النتائج تميزاً معنوياً لبرنامج الزراعة العضوية (P<sub>3</sub>) مقارنة ببقية البرامج المستخدمة. أدت هذه المعاملة إلى زيادة معنوية في محتوى فيتاميني C وD<sub>3</sub> في البذور عند المواعدين المستخدمين (0.0883 ملغم لتر<sup>-1</sup>، و0.08671 ملغم لتر<sup>-1</sup> وكذلك 12.38 ملغم لتر<sup>-1</sup> و12.32 ملغم لتر<sup>-1</sup> على التوالي). كان البرنامجين العضويين الآخرين (P<sub>2</sub> و P<sub>4</sub>) مختلفين بشكل كبير مقارنة بالبرنامج التقليدي (P<sub>1</sub>). وخلصت الدراسة إلى أن هذه البرامج العضوية المدروسة يمكن أن تكون خياراً فعالاً لتحسين نوعية بذور هذا المحصول. حيث تبرز القيمة الصحية والغذائية، وتقلل الآثار الجانبية للزراعة التقليدية على البيئة.

**الكلمات المفتاحية:** الأسمدة العضوية، الزراعة العضوية، البرامج الزراعية، ركيزة الفطر المستهلك، فيتامينات C وD<sub>3</sub>.

## INTRODUCTION

Styrian pumpkin variety was first appeared in Southeastern Austria Styria (Fruhvirth and Hermetter, 2008). Common pumpkin (*Cucurbita pepo* convar. *pepo* var. *styriaca*) is annual plant related to the family of Cucurbitaceae. It has extended sprawling prickly stem, nearly 300 - 500 cm, with female flowers on stalks and male flowers appeared in leaf axil. Also, the plant has fibrous root and fruits colored green to orange (Aroiee and Omidbaigi, 2002). Styria is an economically significant horticultural plant. It is widely grown for its valuable seeds that contain protein and minerals. It is well known that oil extracted from the seeds is low in cholesterol. This material, in men, has an effect in reducing symptoms of benign prostatic hyperplasia. In women, it is decreasing hot flashes, reliving hormone-related headaches, and easing blood pressure. Fighting heart diseases, treating overactive bladder, and reducing hair loss are other benefits (Ebrahimzadeh, et al., 2021 and Al-Jaf et al., 2023).

Seed of pumpkin is rich source of oils essential for human body. Namely, they contain several nutraceuticals such as  $\beta$ -sitosterol, amino acids, unsaturated fatty acids, tocopherols, phenolic compounds, and cucurbitacins as active substances (Kim et al., 2012). Therefore, they are used in manufacturing various drugs such as Grunfig, Pepostrin, and Peponen. Medications for gastrointestinal regulation, atherosclerosis, and urinary tract inflammation are also included (Fadaei and Akbarpour, 2023). In addition, they are rich in omega-3, 6 and 9. Seed oil can promote wellness of human immunodeficiency virus\ acquired immunodeficiency syndrome, HIV\ AIDS (Najafi et al., 2021). Moreover, it has antioxidant

properties due to presence of some vitamins such as C and  $\beta$ -carotene included in vitamin A (Jeznach *et al.*, 2012).

Recently, vitamin D<sub>3</sub> deficiency has been one of the important issues people face. The vitamin is mostly derived from animal sources. Thus, using nutritional supplements and chemical medications by vegetarian people is increased, especially during the COVID-19 period. Researchers have attempted to discover different sources other than animal or chemical to obtain this vitamin. Some research reported that D<sub>3</sub> can be available in some plants belonging to Solanaceae and Cucurbitaceae families (Aburjai, *et al.*, 1998, Japelt, 2011, and Black, *et al.*, 2017; Al-Fhdawi *et al.*, 2023). Vitamin C is an effective safe nutrients. Vitamin C an importance role in human health such as protection against immune system deficiencies, prenatal health problems, cardiovascular disease. Pumpkin seed content of vitamin C 15.76-26.02 mg 100g<sup>-1</sup> (Barros *et al.*, 2024).

Lately, several studies have been conducted on secondary metabolites of medicinal plants to increase their natural components and yield. In the farming of medicinal plants management, applying materials of minimal damaging side effects on human health and environment is highly recommended (Fadaei and Akbarpour, 2023). This can be achieved under organic farming programs depending on the use of organic and bio fertilizers, ash, bio-controlling, and plant extraction. This technique protects the environment and supports sustainability. Furthermore, Raw materials of such plants are of high quality and free from harmful chemical residues including pesticides, fertilizers, and heavy metals (Ahmed, *et al.*, 2022).

Organic fertilizers improve soil texture and regulate pH value. Eventually, yield components, active substances, and mineral composition of the plant are influenced. Nitrogen (N) is one of the components of poultry manure. This element improves soil productivity by increasing organic carbon and microorganisms (Hialy, 2018). Poultry manure also contains nutrients essential for plant's growth, such as calcium, magnesium, sulphur, manganese, copper, zinc, iron, boron and molybdenum (Suhartini, *et al.*, 2020). Spent mushroom substrate (SMS) is one of these fertilizers used in plant production. It has essential role through amending soil properties; especially supplying minerals and organic materials. Consequently, leads to an increase in plant growth, quantitative and qualitative yield characteristics (Ahmed, *et al.*, 2023).

Compost tea has a great importance in sustainable agriculture as well. It directly provides mineral nutrients to the plant (Ros, *et al.*, 2020). Bio fertilizers comprise variety of useful microorganisms such as *Azotobacter* and *Trichoderma*. This type improves and accelerates plant growth and protects plants from diseases (Habibi, *et al.*, 2015). The high quantity and quality medicinal plants can be produced under ideal growth requirements, particularly environmental conditions. Planting date is one crucial factor; as early sowing during spring may expose seedlings to cold and possible frost damage. On the other hand, delaying planting may negatively affect plant growth and development. Exposure to unsuitable environmental conditions causes lack growth and less accumulation of active compounds in plants (Omidbeigi, 2004 and Latifi, *et al.*, 2012). To achieve best value of active compound such as antioxidant, phenolic substances, and flavonoids in pumpkin, planting time is strongly considered (Oloyede, *et al.* 2014 and Ahmed, *et al.* 2022). According to the importance of Styrian pumpkin and farming programs, this study was proposed. The aim of the study was to evaluate the efficiency of organic farming programs on

vitamins C and D<sub>3</sub> in this plant. In addition, investigating the effect of sowing date was examined.

## MATERIALS AND METHODS

This experiment was conducted in the research center at the Department of Horticulture and landscape, Tikrit University. Medicinal pumpkin plants (*Cucurbita pepo* Subsp. *pepo* Var. *Styriaca*) was examined in this experiment. Analysis of experiment soil was implemented (table 1). Four treatments (terraces) were applied with three replications. On both sides of each terrace, ten plants were planted for each treatment. Each experimental unit was 2.5\*0.7 m dimensions. This study was one-way experiment. The treatments included four farming programs; chemical farming program as a control P<sub>1</sub>, organic P<sub>2</sub>, organic P<sub>3</sub>, and organic P<sub>4</sub>. Each of which was implemented on March 10,2019 and April 1, 2019 (table 2). Properties of the organic fertilizers used in the programs 2,3, and 4 are shown in table 3.

Table 1. Physical and chemical properties of field soil

Measurements	Value	Measurements	Value %
N	6 ppm	Gypsum	16.3
K	40.05 ppm	Lime	21.4
Na	134.5 ppm	O.M	1.2
P	9.5 ppm	Sand	58
EC	4.475 ms cm <sup>-1</sup>	Loam	24
pH	8	Clay	18
Texture	sandy loam		

Table 2. Weather data for the experiment location (2019)

Date Month	Solar Mj/m2/m	Min. Humidity %	Max. Humidity %	Daily low Temp. °C	Daily high Temp. °C	Temp. average °C	Min. Temp. °C	Max. Temp. °C	Rain mm
March	15.43	40.21	91.70	2.39	24.86	14.04	7.57	20.50	46.40
April	18.27	23.49	75.68	7.74	30.20	17.81	11.36	24.25	33.00
May	24.61	18.20	57.72	11.57	45.92	28.43	19.45	37.41	6.40
Jun	27.28	10.51	28.63	22.26	48.83	35.47	26.86	44.09	0.00
July	27.94	15.96	30.58	22.68	46.33	35.42	26.80	44.03	0.00

Table 3. The properties of organic fertilizers

Measurement	Unit	Organic fertilizer		Measurement	Unit	Organic fertilizer	
		SMS	poultry manure			SMS	poultry manure
N	ppm	9016	17710	EC	mS.cm <sup>-1</sup>	5.2	4.85
K	ppm	1550	3200	pH	—	8.1	7.8
Na	ppm	7350	2700	O.M	%	24.6	19.2
P	ppm	23.5	11	O.C	%	14.3	11.2
C/N ratio		1/16	1/6				

**The studied programs:**

1- Program 1 (P<sub>1</sub>) included ground addition of compound (NPK 12-8-16 + TE) and foliar addition of compound 20-20-20 + TE + Amino acid. Chemical controlling with Malathion - 50 wp (g L<sup>-1</sup>) was used. This treatment was applied as a control.

2- Program 2 (P<sub>2</sub>) represented applying 16 t h<sup>-1</sup> spent mushroom substrate (SMS) for all plants in this experimental unit. 1.5 g ash, 1 g *Trichoderma sp.* (19\*10<sup>7</sup> spore g<sup>-1</sup>), *Azotobacter sp.* 10 ml (2\*10<sup>9</sup> CFU ml<sup>-1</sup>) were separately added to each planting hole of this treatment. In addition, foliar spraying of compost tea processed from SMS was added on the plant shoots. Six weeks after first sowing date, 10 ml plant<sup>-1</sup> of *Trichoderma* was added. After four weeks, the process of bio-controlling was repeated. Then, extractions of garlic and eucalyptus were applied to the plants.

3- Program 3 (P<sub>3</sub>) comprised of the same applications involved in program 2 with moderation. Ground and foliar additions of SMS excluded. Instead, poultry manure was added.

4- In the program P<sub>4</sub> (1:1 SMS and poultry manure), 50% of each P<sub>3</sub> and P<sub>4</sub> were applied. All farm practices and application dates are displayed in table 4.

Table 4. Application dates of agricultural practices used in the experiment.

Agricultural practices	Applying Dates	
	Date one	Date two
Sowing date	10/3/2019	1/4/2019
Ground applying fertilizers	23/2/2019	18/3/2019
Biofertilizers	Trichoderma	24/2/2019
		19/3/2019
		24/3/2019
		19/4/2019
Foliar application	Azotobacter	3/3/2019
		25/3/2019
	First	3/4/2019
		25/4/2019
	Second	9/4/2019
		30/4/2019
Controlling applications	Third	25/4/2019
		15/5/2019
	Trichoderma	10/5/2019
		30/5/2019
		24/4/2019
		5/5/2019
	Plants	24/5/2019
	Garlic	30/5/2019
extraction eucalyptus	20/4/2019	
	10/5/2019	
	20/4/2019	
	10/5/2019	
Ash	23/2/2019	18/3/2019

The seed content of ascorbic acid (vitamin C) and vitamin D<sub>3</sub> were measured in this experiment (Kozhanova, et al., 2002). HPLC model SYKAM (Germany) provided with vitamins detector UV- 280 nm. The mobile phase was an isocratic acetonitrile: D.W: (75: 25)

at flow rate at 0.7 mL/min, column was C18 – ODS (25 cm \* 4.6 mm). Randomized Complete Block Design (RCBD) was used for this one-way experiment. All results were statistically analyzed using GenStat 12<sup>th</sup> ed. According to Payne, *et al.* (2009). Duncan's multiple range test was implemented to compare the mean values at  $P= 0.05$ .

## RESULTS AND DISCUSSION

Farming programs impacted vitamin C content positively in the date one (figure 1) and the date two (figure2). The result showed that program P<sub>3</sub> significantly higher in vitamin C content compared with P<sub>4</sub> (25%), P<sub>2</sub> (50%), and P<sub>1</sub> (59%). Farming Program P<sub>1</sub> was significantly lowest compared with the other farming programs (figure 1).

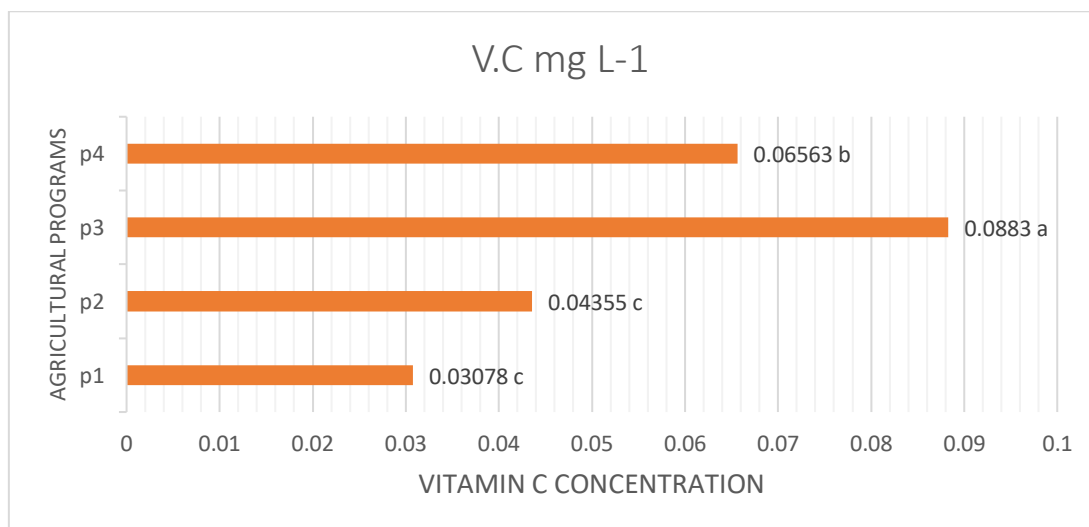


Figure 1. Effect of farming programs on vitamin C in medicinal pumpkin plant under date 1

Also, the farming program P<sub>3</sub> responded similarly in the date two. Significantly the highest vitamin C was observed in P<sub>3</sub>. Vitamin C reduced in P<sub>4</sub> (37%), P<sub>2</sub> (53%), and P<sub>1</sub> (50%) compared with P<sub>3</sub> (figure 2). According to statically analysis, there were no significant different between P<sub>2</sub> (0.04355 mg l<sup>-1</sup>) and P<sub>1</sub> (0.03078 mg l<sup>-1</sup>). Vitamin C content was inconstant between the date one and the date two.

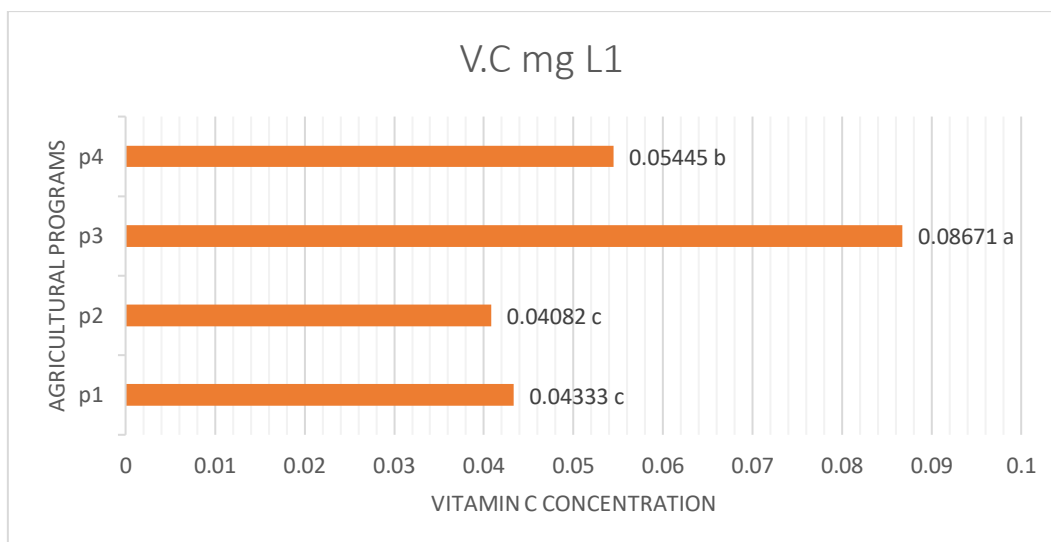


Figure 2. Effect of farming programs on vitamin C in medicinal pumpkin plant under date 2

Figure (3) explained the effect of farming programs on vitamin D<sub>3</sub> on date 1. The result showed that program P<sub>3</sub> caused significant effect on the D<sub>3</sub>. This treatment gave the highest value (12.38 mg l<sup>-1</sup>), compared to the lowest (7.77 mg l<sup>-1</sup>) under the treatment of program P<sub>2</sub>.

The different in planting date with different environment condition (table 2) might be play an important role for changing the amount of vitamin D<sub>3</sub>.

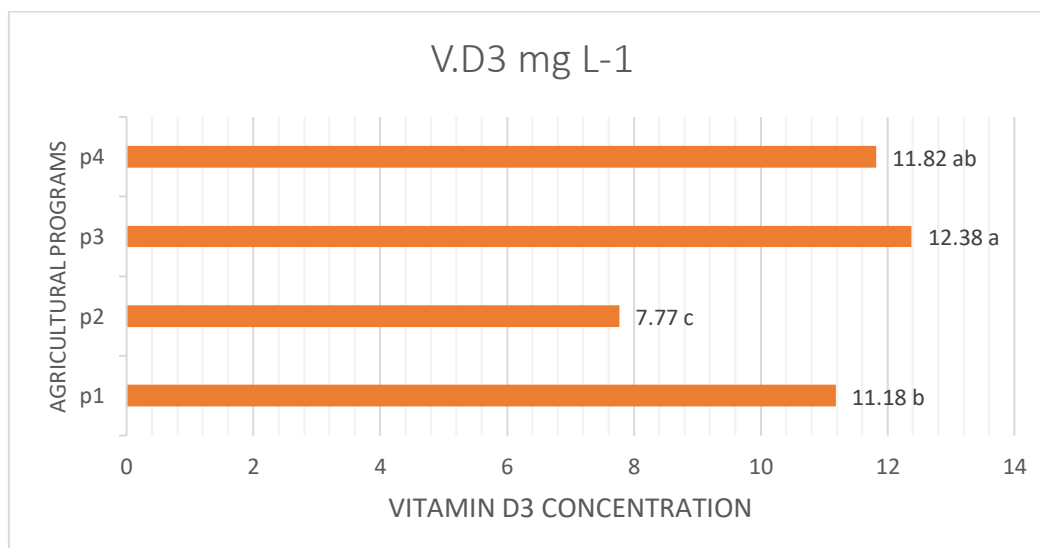


Figure 3. Effect of farming programs on vitamin D<sub>3</sub> in medicinal pumpkin plant under date 1

The results in figure (4) exhibited the effect of farming programs on vitamin D<sub>3</sub> under date 2. The program P<sub>3</sub> significantly influenced the concentration of vitamin D<sub>3</sub>. This parameter reached the highest record (12.32 mg l<sup>-1</sup>) in program P<sub>3</sub>. In comparison, the

parameter reached the lowest value (6.71 mg l<sup>-1</sup>) in the which scored when compared to program P<sub>2</sub>.

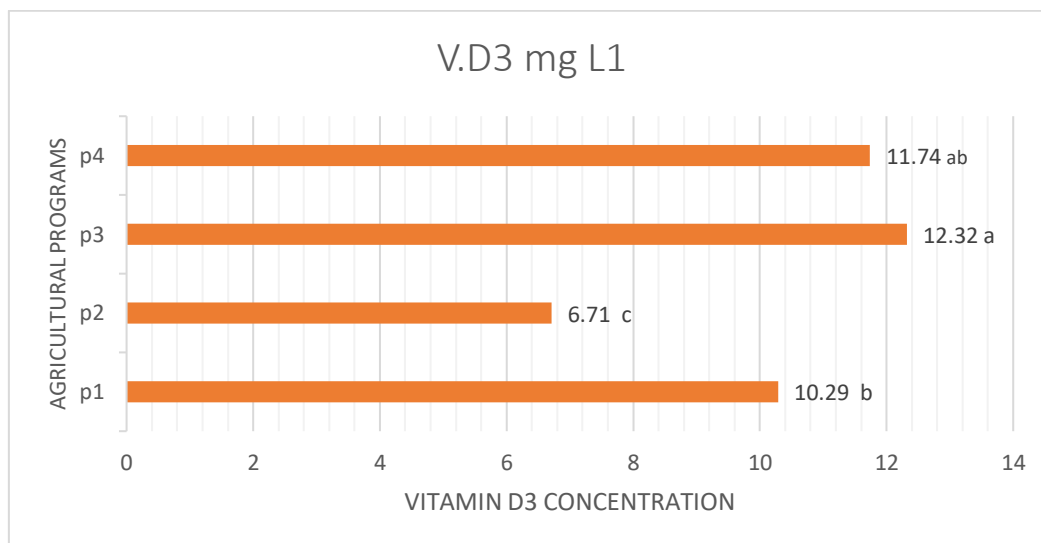


Figure 4. Effect of farming programs on vitamin D<sub>3</sub> in medicinal pumpkin plant under date 2

The result showed that program P<sub>3</sub> had significant increment on the concentration of vitamin C on the dates 1 and 2. The reason of this increase may be due to the role played by poultry manure used in this treatment. This fertilizer is rich in macro-nutrients such as nitrogen, phosphor and potassium (table 3). Nitrogen promotes plant development and improves physiological characteristics, which affects the growth, yield, and quality of plant parts (Liu *et al.*, 2023). Phosphorus plays a crucial role in the formation of cellular membranes and regulates metabolic processes, and promotes plant growth and physiological metabolism (Afzal *et al.*, 2023). Potassium activates various enzymes involved in intracellular osmotic regulation, membrane protein transport, and photosynthesis transport. These are fundamental for plant growth and development (Khandaker, *et al.*, 2022; Fadam and Hamad, 2023)

On other hand, it was confirmed that this small quantities of vitamin C, presented relatively in pumpkin seeds, are subject to plant genotype and growing conditions (Jeznach, *et al.*, 2012). This result was agreed with Isuwan (2014). Concentration of vitamin C in date 1 was increased compared to date 2. Another reason for this increment may be because of compost tea which contains mineral nutrients provided in available forms for the plant. Because it is a ready and instant resource for mineral nutrition, the compost in this program (P<sub>3</sub>), had a direct effect in plant nutrition. This caused positive effects on plant growth and productivity. Ultimately, vitamin C was positively affected (Hirzel, *et al.*, 2012).

Increase vitamin D<sub>3</sub> in program (P<sub>3</sub>) under the dates 1 and 2 (figures 3, 4) may be due to poultry manure (Baur, *et al.*, 2016). This material had effect in improving soil structure and ventilation. The fertilizer is also important for soil microorganisms. In addition, - have roles in the forming of organic acids and plant growth. The good content of N, P, K, and C\N ration in poultry manure (table 3), give the preference of P<sub>3</sub> to increase the vitamins in plant seeds. This may enhanced C and D<sub>3</sub> content (Ahmed, *et al.*, 2022).



All material included in program (P<sub>3</sub>) had an effect to rise metabolism in plant which increased concentrations of vitamins C and D<sub>3</sub>. The reason of accumulating these vitamins could be because lifespan of the plants in date 1 was longer than that of date 2. Accordingly, C and D<sub>3</sub> increased in the plants exposed to higher light intensities (Oyetunde, *et al.*, 2023). Or maybe the environmental conditions were more suitable in date 1 during plant growth stages and development (table 2).

## CONCLUSION

Styrian Pumpkin, *Cucurbita pepo* ssp. *pepo* var. *styriaca* is considered one of the important plants. Organic program P<sub>3</sub> was superior in most characteristics studied. that showed the distinctive role of organic farming programs in sustaining the health of the environment and biological diversity. Even though, there was a short period between the planting dates, there were some variation in the vitamin C and D. Accordingly, the use of organic farming program is recommended. In addition, use of other agricultural practices that contribute to improve approaches environmentally safe can be adopted.

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