

ROLE OF PHOSPHORUS, SILICON, AND CITRIC ACID IN ANATOMICAL TRAITS OF PEPPER PLANT CULTIVATED IN PLASTIC GREENHOUSE

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ABSTRACT

This study was aimed to investigate effect of phosphorus, silicon, and citric acid on anatomical traits of pepper, This research was conducted at research stations, College of Agricultural Engineering Sciences, University of Baghdad, Jadiriya Campus, during 2021-2022 season. The study employed factorial experiment within randomized complete block design (RCBD) with three replicates. The first factor included three levels of phosphorus (p) (0, 160, and 320 kg Ha⁻¹ P₂O₅), the second factor included three levels of potassium silicate (s) (0, 75, and 100 kg Ha⁻¹), while the third factor included four levels of citric acid (c) (0, 2, 4, and 6 kg Ha⁻¹). The results revealed that the P3S2C3 treatment had a significant increase in lateral root diameter (10.10 mm), P1S3C4 increased epidermis root thickness (30.93 µm), P3S3C2 led to increased root vascular bundle and stem, leaf cuticle thickness (284.9 µm, 6.63 µm, and 5.63 µm, respectively), the P1S2C4 treatment exhibited increased stem vascular bundle thickness (279.9 µm), The P1S2C2 showed increases in mesophyll thickness (106.03 µm), The P2S3C showed increases in leaf area (631.3 dcm² plant⁻¹) and P3S3C4 showed increases yield of plant (3.48 kg plant⁻¹).

Keywords: lateral roots; epidermis; vascular bundle; mesophyll; mineral nutrition

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دور الفسفور والسليكون وحامض الستريك في الصفات التشريحية لنبات الفلفل المزروع في البيت البلاستيكي

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المستخلص

هدفت هذه الدراسة الى معرفة تأثير الفسفور والسليكون وحامض الستريك في الصفات التشريحية لنبات الفلفل، نُفذ البحث في المحطة البحثية، كلية علوم الهندسة الزراعية، جامعة بغداد - مجمع الجادرية للموسم 2021-2022 بتجربة عاملية وفق تصميم القطاعات الكاملة المعشاة بثلاث مكررات. إشتمل العامل الاول على ثلاث مستويات من الفسفور P (0 و 160 و 320 كغم P₂O₅ هكتار⁻¹) والعامل الثاني على ثلاث مستويات من سليكات البوتاسيوم S (0 و 75 و 100 كغم هكتار⁻¹) والعامل الثالث على اربع مستويات من حامض الستريك C (0 و 2 و 4 و 6 كغم هكتار⁻¹). أظهرت نتائج التحليل الإحصائي تفوق المعاملة P3S2C3 في زيادة قطر الجذور الجانبية (10.10 ملم) والمعاملة P1S3C4 في زيادة سمك بشرة الجذر (30.93 µm) والمعاملة P3S3C2 في زيادة سمك الحزمة الوعائية للجذر وطبقة الكيوتكل في الساق والورقة (284.9 µm و 6.63 µm و 5.63 µm) والمعاملة P1S2C4 في زيادة سمك الحزمة الوعائية للساق (279.9 µm) والمعاملة P1S2C2 في زيادة سمك النسيج المتوسط (الميزوفيل) في الورقة (106.03 µm) والمعاملة P2S3C3 في زيادة المساحة الورقية (631.3 دسم² نبات⁻¹) والمعاملة P3S3C4 في زيادة حاصل النبات (3.48 كغم نبات⁻¹).

الكلمات المفتاحية: جذور جانبية، بشرة، حزمة وعائية، ميزوفيل، تغذية معدنية.

مستل من اطروحة دكتوراه للباحث الاول.



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INTRODUCTION

Pepper (*Capsicum annuum* L.) is an important summer vegetable crop belonging to the Solanaceae family. Its fruits are consumed due to their high content of various chemical compounds, some of which directly enter our diet, such as vitamin C, vitamin A, iron, calcium, as well as other compounds like capsaicinoids, carotenoids, and phenolic compounds. These compounds are used to extend the shelf life of industrial products, protect them from oxidation, and synthesizing certain therapeutic compounds for inflammation, in addition to the production of some cosmetic products (8, 27, 30). Effective management of the nutritional process ensures the development of plants with a strong anatomical structure, which in turn plays a role in improving both the quantity and quality of production. Such management includes the type of chemical elements added, their chemical form, the method and timing of their application. Some of these elements contribute to the structural changes in plants by increasing the thickness of the cuticle layer, especially silicon, with its role in increasing vascular system diameters in the root, stem, leaf, and finally fruit (16, 20). Silicon also plays a role in the absorption of certain elements in the soil solution, including phosphorus. Orthosilicic acid (H_4SiO_4) increases the availability of phosphorus due to its high affinity for phosphate adsorption in the soil solution, even at very low phosphorus concentrations (13,36). Additionally, silicon directly contributes in increasing the stem diameter, shoot dry weight, and concentration of chlorophyll, elements in leaves of pepper (24,33,35). Therefore, It is need to increase the level of available silicon by adding available fertilizers such as potassium silicates, which also leads to stimulating vegetative growth and increasing fruit production (1, 19, 25). Phosphorus is one of the essential elements that determine the productivity of plants, although 58-89% of its total content is fixed in the soil (17). It affects the formation of plant cells and tissues as it is involved in phospholipids composition in cell membranes, as well as in nucleic acids synthesis. However, plants also suffer from a lack of available phosphorus, as its concentration in the soil

solution, such as H_3PO_4 , H_2PO_4 , HPO_4 , and PO_4 , is very low, ranging from 0.1 to 1.0 micromoles which is three times lower than the phosphorus concentration within the plant (12, 37) in addition to its slow allocation from roots to vegetative growth parts (32). As a results; many researchers intensively studied phosphorus effects and bioavailability in plants (14, 18, 23, 38) since it plays an important role in stimulating root growth and increasing its surface area, as well as increasing the number and length of lateral roots, leading to an increase in roots dry weight ratio compared to vegetative growth (28, 29). Furthermore, phosphorus contributes to promoting shoot growth and increasing the yield of pepper plants (21, 31), It has a role in stimulating plants to flowering (2). Citric acid is one of the natural acids produced by plants that contribute to the absorption of mineral elements, increase the rate of photosynthesis, and reduce abiotic stress factors such as drought and heavy metal toxicity (6). This acid has an effect on increasing the availability of iron, manganese, copper, and zinc in the soil solution, as well as increasing acidity and fruit yield in pepper crops (3, 39). Many studies had proven that early application of citric acid (dipping) could increase fruit's shelf life and reduce weight loss (4). This research was aimed to investigate the effect of phosphorus, silicon, and citric acid on strengthening the internal tissues of the root and stem and its relationship with increasing fruit production.

MATERIALS AND METHODS

Field preparation and planting

This research was conducted in a plastic greenhouse located at the Research Stations of the College of Agricultural Engineering, University of Baghdad, during 2021/2022 season. The greenhouse had an area of 463.5 m^2 (9m wide and 51.5m long).The soil was prepared and underwent solar sterilization during the July and August months. Subsequently, plowing and soil alignment operations were carried out, and random soil samples were collected for laboratory analysis at the Agricultural Research Department, Ministry of Agriculture. Table 1 shows the physical and chemical characteristics of the soil. The soil was divided into five ridges, each consisting of a raised soil shoulder about 20cm

above the soil surface and 80cm wide. There was a distance of 90 cm between each ridge, creating a service pathway. Drip irrigation system was installed on the surface of each ridge. Carisma hybrid pepper (a bell-shape pepper variety approved by the National Committee of Seed Certification and Testing) was chosen for the experiment. The seedlings were translocated on October 27, 2021, after forming four true leaves. The spacing between plants was 40 cm both within and between the rows on the same ridge. Each experimental unit consisted of eight plants, including two

plants planted in a plastic pot with a capacity of ten liters (30 cm in height and 25 cm in diameter). The pots were placed at ground level. The root measurements were taken at the end of the growth season and the isolation distance between each experimental unit was 80 cm (with an experimental unit area of 1.28 m²). The side branches were pruned up to the main branching zone (Y) for all plants, and the plants were supported on both sides with special strings. The field application was completed on September 1, 2022.

Table 1. Some chemical and physical properties of the soil sample

Adjective	Units	soil content
Ph		7.1
E C (1:1)	Ms cm ⁻¹	1.63
CEC	meq100 ⁻¹ gm soil	11.7
O.M.	%	1.26
Availabl N	Ppm	49
Availabl P	Ppm	14.4
Availabl K	Ppm	341
Availabl Ca	meq L. ⁻¹	17.5
Availabl Mg	meq / L.	10
Availabl Na	meq L. ⁻¹	3.41
Availabl K ⁺	meq L. ⁻¹	0.87
Sand	%	38.8
Silt	%	46.4
Clay	%	14.8
Texture		Loam soil

Study treatments: The study included three factors as follows:

* Addition of phosphorus (symbolized as P) to the soil in three levels: 0, 160, and 320 kg ha⁻¹ as P₂O₅. The recommended fertilizer dose for pepper plants is 160 kg ha⁻¹ P₂O₅ (5).

* Addition of silicon (S) to the soil in the form of potassium silicate (52.8% SiO₂ + 32.4% K₂O) in three levels: 0, 75, and 100 kg ha⁻¹.

* Addition of citric acid (C) in four levels: 0, 2, 4, and 6 kg ha⁻¹. Each concentration was repeated in each addition. It was added one month after seeding and continued during the growing season in eight times, starting from 1/12/2021 to 5/5/2022.

The study factors (P) * (S) * (C) interacted, resulting in 36 plots in each replication. The treatments were added to the soil as dissolved in water and applied to the soil through irrigation.

Implementation of treatments: The phosphorus, silicon, and citric acid treatments, along with their interactions, were applied one month after planting and continued throughout the growth season in eight applications,

starting from December 1, 2021, to May 5, 2022, according to the predetermined concentrations, except for the citric acid treatments, which were added in each application at the same concentration. Nitrogen and potassium were added in multiple applications according to the recommended fertilizer dose (600 kg ha⁻¹ of nitrogen N and 300 kg ha⁻¹ of potassium K₂O) (5). The field application of the research was completed on September 1, 2022.

Experimental design: The research was conducted as a factorial experiment within a randomized complete block design (RCBD) using 3 replications with three factors: phosphorus, silicon, and citric acid (P, S, C), each with three levels (3x3x4), resulting in 36 experimental units in each replication. The experiment was replicated three times, resulting in a total of 108 experimental units. The data were analyzed using the Genstat software, and the means were compared using the least significant difference (L.S.D.) test at a 5% level of significance (15).

Study indicators: Included the: lateral roots diameter (mm root^{-1}), root epidermis thickness (micrometers μm), roots vascular bundles thickness (μm), stem cuticle layer thickness (μm), stem vascular bundles thickness (μm), leaf cuticle layer thickness (μm), leaf mesophyll layer thickness (μm), leaf area ($\text{dcm}^2 \text{ plant}^{-1}$), and plant yield (kg plant^{-1}).

RESULTS AND DISCUSSION

Lateral roots diameter (mm root^{-1})

The lateral roots arise internally from the cells of pericycle. The research factors have a role in increasing the diameter of those roots (Table 2), P3 exceeded the increases in diameter ($7.26 \text{ mm root}^{-1}$), with a significant differences from the treatment P2 and the control treatment P1 (6.70 and $6.28 \text{ mm root}^{-1}$). S3 was superior in increasing the diameter of the lateral root ($7.68 \text{ mm root}^{-1}$) while S1 treatment gave lowest value ($5.40 \text{ mm root}^{-1}$). When adding citric acid, treatment C3 excelled in increasing the diameter to 6.96 mm

root^{-1} , but non-significant differences from treatments C1 and C4. The dual interaction between phosphorus and silicon led to the superiority of the P3S2 treatment ($8.08 \text{ mm root}^{-1}$) and P1S1 treatment gave $4.70 \text{ mm root}^{-1}$. At the interaction between phosphorus and citric acid, the treatment P3C3 excelled and reached $8.17 \text{ mm root}^{-1}$ with a non-significant difference from the treatment P3C4 ($7.74 \text{ mm root}^{-1}$). As for the two-way interaction between silicon and citric acid, the S3C1 treatment was significantly superior ($7.90 \text{ mm root}^{-1}$) compare with S1C3 which gave $5.37 \text{ mm root}^{-1}$. The interaction of the three factors of the study led to the superiority of treatment P3S2C3 ($10.10 \text{ mm root}^{-1}$) compared to all the treatments, but non significant differences from treatment P3S2C4 ($9.20 \text{ mm root}^{-1}$), while the control treatment P1S1C1 recorded the least diameter of lateral roots ($4.33 \text{ mm root}^{-1}$).

Table 2. Effect of phosphorus, silicon, citric acid on diameter of lateral roots (mm root^{-1})

Phosphorus (P)	Silicon (S) kg Ha^{-1}	Citric acid (C) kg Ha^{-1}				P * S
		C1 (0)	C2 (2)	C3 (4)	C4 (6)	
P 1 kg Ha^{-1} (0)	S1 (0)	4.33	4.73	4.93	4.80	4.70
	S2 (75)	6.83	5.80	6.90	6.50	6.50
	S3 (100)	8.85	6.70	7.75	7.30	7.65
P 2 kg Ha^{-1} (160)	S1 (0)	5.36	5.13	5.30	5.40	5.30
	S2 (75)	7.40	8.00	5.90	6.26	6.89
	S3 (100)	8.00	8.80	7.33	7.56	7.92
P 3 kg Ha^{-1} (320)	S1 (0)	6.56	6.33	5.90	6.10	6.22
	S2 (75)	6.93	6.10	10.10	9.20	8.08
	S3 (100)	6.85	6.63	8.53	7.93	7.48
L.S.D. (Interaction)		1.16				0.58
Phosphorus (P) * Citric acid (C)		C1	C2	C3	C4	Means (P)
P 1		6.67	5.74	6.52	6.20	6.28
P 2		6.92	7.31	6.17	6.41	6.70
P 3		6.78	6.35	8.17	7.74	7.26
L.S.D. (C * P)		0.67				0.33
Silicon (S) * Citric acid (C)		C1	C2	C3	C4	Means (S)
(S)						
S1		5.42	5.40	5.37	5.43	5.40
S2		7.05	6.63	7.63	7.32	7.16
S3		7.90	7.37	7.87	7.60	7.68
L.S.D. (C * S)		0.67				0.33
Means (C)		6.79	6.47	6.96	6.78	
L.S.D. (C)		0.39				

Root epidermis thickness (μm): Root epidermis is the outer layer surrounding the root, It is very important in the absorption. The treatment P3 was significantly superior in increasing the thickness to $18.93 \mu\text{m}$ in compare with the lowest value in P1 gave

$17.42 \mu\text{m}$ (Table 3). Treatment S3, excelled in increasing the thickness to $19.65 \mu\text{m}$ in compare with the lowest value in S2 ($17.37 \mu\text{m}$). The treatment C4 excelled in increasing the thickness to $20.19 \mu\text{m}$ and the lowest value in C1 ($17.41 \mu\text{m}$) and the

bilateral interaction between phosphorous and silicon led to the superiority of the treatment P2S3 (23.25 μm) in compare with the lowest value in P2S2 (15.07 μm). Interaction of phosphorus with citric acid, the significant superiority of the treatment P1C4 (24.92 μm). and the lowest value in P1C2 (14.64 μm). As

for the interaction of silicon with citric acid, the treatment S3C4 excelled and reached 23.51 μm and the lowest value in S2C2 (14.88 μm). Treatment P1S3C4 was significantly superior to all treatments, and the thickness of the root cuticle reached (30.93 μm) and the lowest value in P2S2C1 (9.77 μm).

Table 3. Effect of phosphorous, silicon and citric acid on root epidermis thickness

Phosphorus (P)	Silicon (S) kg Ha ⁻¹	Citric acid (C) kg Ha ⁻¹				P * S
		C1 (0)	C2 (2)	C3 (4)	C4 (6)	
P 1 kg Ha ⁻¹ (0)	S1 (0)	15.63	15.93	18.7	22	18.07
	S2 (75)	18.53	14.53	15.47	21.83	17.59
	S3 (100)	12.2	13.47	9.83	30.93	16.61
P 2 kg Ha ⁻¹ (160)	S1 (0)	14.43	23.7	13.77	14.9	16.7
	S2 (75)	9.77	16.43	20.57	13.53	15.07
	S3 (100)	21.87	25.87	23.17	22.1	23.25
P 3 kg Ha ⁻¹ (320)	S1 (0)	25.87	11.57	14.43	21.13	18.25
	S2 (75)	22	13.67	24.33	17.8	19.45
	S3 (100)	16.37	23.9	18.57	17.48	19.08
L.S.D. (Interaction)		2.25				1.12
Phosphorus (P) * Citric acid (C)						
Phosphorus (P)		C1	C2	C3	C4	Means (P)
P 1		15.46	14.64	14.67	24.92	17.42
P 2		15.36	22	19.17	16.84	18.34
P 3		21.41	16.38	19.11	18.81	18.93
L.S.D. (C * P)		1.30				0.65
Silicon (S) * Citric acid (C)						
(S)		C1	C2	C3	C4	Means (S)
S1		18.64	17.07	15.63	19.34	17.67
S2		16.77	14.88	20.12	17.72	17.37
S3		16.81	21.08	17.19	23.51	19.65
L.S.D. (C * S)		1.30				0.65
Means (C)		17.41	17.67	17.65	20.19	
L.S.D. (C)		0.75				

Roots vascular bundles thickness

The vascular bundle consists of xylem and phloem. In Table 4, we notice that the treatment P1 was superior in increasing the thickness of the vascular bundle to 323.3 μm in compare with the lowest value in P3 (218.2 μm). The treatment S2 was significantly superior (232.3 μm) in compare with the lowest value in S1 (218.1 μm). With regard to the single effect of citric acid, it had no role, as the superiority was attributed to the control treatment C1 (238.2 μm) and the lowest value in C3 (208.2 μm). As for the bilateral interaction between phosphorus and silicon, it led to the superiority of the treatment P1S2

(255.4 μm) and the lowest value in P3S2 (206.0 μm). The treatment P2C1 was significantly superior to the rest of the treatments (249.4 μm) and the lowest value in P3C3 (177.3 μm). As for the interference of silicon with citric acid, the significant superiority of treatment S2C4 was observed, reaching 257.6 μm and the lowest value in S3C3 (188.9 μm). As for the triple interaction between the factors of the study, the treatment P3S3C2 was significantly superior in increasing root vascular bundle thickness compared to all treatments (284.9 μm) and the lowest value in P3S2C3 (174.1 μm).

Table 4. Effect of phosphorous,silicon,citric acid on root vascular bundles thickness

Phosphorus (P)	Silicon (S) kg Ha ⁻¹	Citric acid (C) kg Ha ⁻¹				P * S
		C1 (0)	C2 (2)	C3 (4)	C4 (6)	
P 1 kg Ha ⁻¹ (0)	S1 (0)	228.4	203.1	219.1	210.6	215.3
	S2 (75)	274.4	241.4	222.8	283.1	255.4
	S3 (100)	240.2	233.1	212.6	219.1	226.3
P 2 kg Ha ⁻¹ (160)	S1 (0)	255.2	213.8	258	189.2	229
	S2 (75)	235.9	224.6	252.6	228.8	235.5
	S3 (100)	257.1	207.6	176.7	233.2	218.6
P 3 kg Ha ⁻¹ (320)	S1 (0)	224.6	208.8	180.3	225.7	209.9
	S2 (75)	185.6	203.5	174.1	261	206
	S3 (100)	242.4	284.9	177.4	250.5	238.8
L.S.D. (Interaction)		19.2				9.6
Phosphorus (P) *		Citric acid (C)				
Phosphorus (P)		C1	C2	C3	C4	Means (P)
P 1		247.7	225.9	218.2	237.6	232.3
P 2		249.4	215.3	229.1	217.1	227.7
P 3		217.5	232.4	177.3	245.7	218.2
L.S.D. (C * P)		11.1				5.5
(S)		Silicon (S) *				
		Citric acid (C)				
		C1	C2	C3	C4	Means (S)
S1		236	208.6	219.1	208.5	218.1
S2		232	223.2	216.5	257.6	232.3
S3		246.6	241.9	188.9	234.3	227.9
L.S.D. (C * S)		11.1				5.56
Means (C)		238.2	224.5	208.2	233.5	
L.S.D. (C)		6.4				

Cuticle layer thickness: The cuticle is a layer consisting of cutin and esters that surrounds the plant organs except the roots. This study factors had a role in increasing its thickness (Table 5), as the treatment P3 excelled in increasing the thickness to 4.81 μm and the lowest value in P1 (4.33 μm). When adding silicates, the thickness also increased, and the treatment S3 excelled at (4.81 μm) and the lowest value in S2 (4.24 μm). As for citric acid, it did not had a role on its own, as control treatment C1 excelled in increasing thickness (4.83 μm) and the lowest value in C3 (4.21 μm). The interaction between phosphorous and silicon led to significantly superiority of the treatment P2S3 over all treatments (5.99 μm) and the lowest value in P2S2 (2.95 μm). When phosphorus interacted with citric acid, treatment P3C1 was significantly superior to other treatments (5.55 μm), but with a non-significant differences from treatment P1C4 (5.50 μm) and the lowest value in P1C2 (3.65 μm). When the silicon overlapped with citric acid, the significant superiority was given to treatment S2C2 (6.67 μm) compared to all treatments and the lowest value in S3C3 (4.03 μm). The interaction between the factors of the study resulted in a significant superiority of the P3S3C2 treatment over all treatments (6.63 μm) treatments and the lowest value in P1S3C3 (2.53 μm).

Vascular bundles Thickness in the stem (μm): The results in Table 6 indicate that adding phosphorus different significantly effect when the compared treatment excelled as it achieved the largest thickness of the vascular bundle (186.4 μm) and the lowest value in P3 (137.5 μm).As for the silicon, it had a significant effect represented in treatment S2 (174.7) and the lowest value in S1 (140.0 μm). Citric acid had an effect, as the C4 treatment was superior in increasing the thickness of the vascular bundle to 167.20 μm and the lowest value in C2 (154.3 μm).The interaction between phosphorous and silicon, the treatment P1S2 showed a significant superiority over all treatments (238.5 μm) and the lowest value in P3S1 (120.6 μm). The treatment P1C3 was significantly superior in increasing the thickness of the vascular bundle to 198.0 μm and the lowest value in P3C2 (121.9 μm).When the interaction between silicon and citric acid, the S3C3 treatment was significantly superior to all treatments in increasing the thickness of the vascular bundle in the leg to 204.2 μm and the lowest value in S3C1 (135.6 μm). The interaction of the three factors of the study, P1S2C4 was superior to treatment (279.9 μm) and the lowest value in P3S1C4 (97.5 μm).

Table 5. Effect of phosphorous, silicon and citric acid on the cuticle layer in the stem

Phosphorus (P)	Silicon (S) kg Ha ⁻¹	Citric acid (C) kg Ha ⁻¹				P * S
		C1 (0)	C2 (2)	C3 (4)	C4 (6)	
P 1 kg Ha ⁻¹ (0)	S1 (0)	4.93	4.16	5.1	4.46	4.66
	S2 (75)	4.46	5.06	3.33	5.73	4.65
	S3 (100)	3.26	2.61	2.53	6.3	3.67
P 2 kg Ha ⁻¹ (160)	S1 (0)	5.46	5.9	3.86	4.06	4.82
	S2 (75)	2.66	3.06	3.26	2.83	2.95
	S3 (100)	6.06	6.53	5.83	5.53	5.99
P 3 kg Ha ⁻¹ (320)	S1 (0)	6.2	3.43	4.03	4.6	4.56
	S2 (75)	6.26	2.9	6.2	5.1	5.11
	S3 (100)	4.2	6.63	3.73	4.46	4.75
L.S.D. (Interaction)		0.44				0.22
Phosphorus (P) * Citric acid (C)		C1	C2	C3	C4	Means (P)
P 1		4.22	3.95	3.65	5.5	4.33
P 2		4.73	5.16	4.32	4.14	4.59
P 3		5.55	4.32	4.65	4.72	4.81
L.S.D. (C * P)		0.25				0.12
Silicon (S) * Citric acid (C)		C1	C2	C3	C4	Means (S)
(S)						
S1		5.53	4.5	4.33	4.37	4.68
S2		4.46	6.67	4.26	4.55	4.24
S3		4.51	5.26	4.03	5.43	4.81
L.S.D. (C * S)		0.25				0.12
Means (C)		4.83	4.48	4.21	4.78	
L.S.D. (C)		0.14				

Table 6. Effect of phosphorous, silicon, citric acid on vascular bundles thickness in the stem

Phosphorus (P)	Silicon (S) kg Ha ⁻¹	Citric acid (C) kg Ha ⁻¹				P * S
		C1 (0)	C2 (2)	C3 (4)	C4 (6)	
P 1 kg Ha ⁻¹ (0)	S1 (0)	188.0	202.1	129.2	100.4	154.9
	S2 (75)	227.4	202.9	243.8	279.9	238.5
	S3 (100)	129.73	178.6	220.8	133.9	165.8
P 2 kg Ha ⁻¹ (160)	S1 (0)	93.5	102.4	159.7	222.9	144.6
	S2 (75)	218.3	155.8	102.2	174.7	162.8
	S3 (100)	122.6	181.3	182.9	190.8	169.4
P 3 kg Ha ⁻¹ (320)	S1 (0)	153.1	121.8	110	97.5	120.6
	S2 (75)	131.1	112.8	124.6	123.0	122.9
	S3 (100)	154.4	131.2	208.8	181.8	169.0
L.S.D. (Interaction)		18.7				9.3
Phosphorus (P) * Citric acid (C)		C1	C2	C3	C4	Means (P)
P 1		181.7	194.5	198.0	171.4	186.4
P 2		144.8	146.5	148.3	196.1	158.9
P 3		146.2	121.9	147.8	134.1	137.5
L.S.D. (C * P)		10.8				5.4
Silicon (S) * Citric acid (C)		C1	C2	C3	C4	Means (S)
(S)						
S1		144.8	142.1	133	140.3	140.0
S2		192.3	157.2	156.9	192.5	174.7
S3		135.6	163.7	204.2	168.8	168.1
L.S.D. (C * S)		10.8				5.4
Means (C)		157.6	154.3	164.7	167.2	
L.S.D. (C)		6.23				

Cuticle leaf thickness (µm)

Treatment P3 excelled in increasing the thickness to 3.81 µm (Table 7) and the lowest value in P1 (3.34µm). As for silicon, the S3 treatment was superior and led to an increase

in thickness (3.80 µm) and the lowest value in S2 (3.19 µm). While citric acid had no role in increasing the thickness, as the control treatment C1 excelled (3.83 µm) and the lowest value in C3 (3.20µm). As for the

interaction between phosphorus and silicon, the treatment P2S3 excelled significantly compared to the rest of the treatments (4.99 μm) and the lowest value in P2S2 (1.81 μm). and when phosphorus interacted with citric acid, the treatment P3C1 had a significant superiority compared to the rest of the treatments (4.57 μm), but with a non-significant difference from the treatment P1C4

(4.45 μm). As for the interaction between silicon and citric acid, the comparison treatment S1C1 excelled significantly compared to the rest of the treatments (4.58 μm). The treatment P3S3C2 was significantly superior to all treatments, as the thickness of the cuticle layer in its leaves was 5.63 μm and the lowest value in P2S2C4 (1.40 μm).

Table 7. Effect of phosphorous, silicon, citric acid on thickness of the cuticle layer in the Leaf

Phosphorus (P)	Silicon (S) kg Ha ⁻¹	Citric acid (C) kg Ha ⁻¹				P * S
		C1 (0)	C2 (2)	C3 (4)	C4 (6)	
P 1 kg Ha ⁻¹ (0)	S1 (0)	4.20	3.16	4.10	3.33	3.70
	S2 (75)	3.36	4.16	2.33	4.73	3.65
	S3 (100)	2.26	1.66	1.53	5.30	2.69
P 2 kg Ha ⁻¹ (160)	S1 (0)	4.33	4.90	2.83	3.06	3.78
	S2 (75)	1.53	2.06	2.26	1.40	1.81
	S3 (100)	5.06	5.53	4.76	4.60	4.99
P 3 kg Ha ⁻¹ (320)	S1 (0)	5.23	2.43	3.03	3.56	3.56
	S2 (75)	5.30	1.90	5.20	4.10	4.12
	S3 (100)	3.20	5.63	2.73	3.40	3.74
L.S.D. (Interaction)		0.34				0.17
Phosphorus (P) * Citric acid (C)		C1	C2	C3	C4	Means (P)
P 1		3.27	3.0	2.65	4.45	3.34
P 2		3.64	4.16	3.28	3.02	3.53
P 3		4.57	3.32	3.65	3.68	3.81
L.S.D. (C * P)		0.19				0.09
Silicon (S) * Citric acid (C)		C1	C2	C3	C4	Means (S)
(S)		C1	C2	C3	C4	Means (S)
S1		4.58	3.50	3.32	3.32	3.68
S2		3.4	2.71	3.26	3.41	3.19
S3		3.51	4.27	3.01	4.43	3.80
L.S.D. (C * S)		0.19				0.09
Means (C)		3.83	3.49	3.2	3.72	
L.S.D. (C)		0.11				

Mesophyll layer thickness

Mesophyll is a tissue of parenchyma cells located between the upper and lower epidermal layers of the leaf responsible for photosynthesis. The results in Table 8 indicate that there is non-significant effect of the added phosphorus or silicon alone (table 8). As for citric acid, it had a significant effect on increasing the thickness of the mesophyll layer, as treatment C4 led to an increase in thickness (72.30 μm) and the lowest value in C1 (63.57 μm). The treatment P2S1 outperformed all treatments (78.70 μm) and

the lowest value in P3S3 (61.30 μm). When phosphorus interacted with citric acid, it was observed that the treatment P3C4 excelled in increasing the thickness to 76.72 μm . At the bilateral interaction between silicon and citric acid, the significant superiority of treatment S1C4 (81.98 μm). The interaction between phosphorus, silicon and citric acid led to an increase in the thickness of the mesophyll layer in the leaves of P1S2C2 treatment plants (106.03 μm) and the lowest value in P1S1C1 (53.33 μm).

Table 8. Effect of phosphorus, silicon, citric acid on leaf mesophyll layer thickness

Phosphorus (P)	Silicon (S) kg Ha ⁻¹	Citric acid (C) kg Ha ⁻¹				P * S
		C1 (0)	C2 (2)	C3 (4)	C4 (6)	
P 1 kg Ha ⁻¹ (0)	S1 (0)	53.33	54.93	62.30	94.43	66.25
	S2 (75)	54.4	106.03	63.20	53.43	69.27
	S3 (100)	72.17	61.77	95.30	80.90	77.53
P 2 kg Ha ⁻¹ (160)	S1 (0)	85.33	93.13	71.27	65.07	78.70
	S2 (75)	60.40	65.60	59.83	57.67	60.88
	S3 (100)	71.70	66.40	50.9	69.03	64.51
P 3 kg Ha ⁻¹ (320)	S1 (0)	57.73	66.67	76.83	86.43	71.92
	S2 (75)	58.37	63.60	60.73	68.23	62.73
	S3 (100)	58.70	53.97	57.03	75.48	61.30
L.S.D. (Interaction)		8.20				4.10
Phosphorus (P)		Citric acid (C)				Means (P)
		C1	C2	C3	C4	
P 1		59.97	74.24	73.60	76.26	71.02
P 2		72.48	75.04	60.67	63.92	68.03
P 3		58.27	61.41	64.87	76.72	65.32
L.S.D. (C * P)		4.74				2.37
(S)		Silicon (S) * Citric acid (C)				Means (S)
		C1	C2	C3	C4	
S1		65.47	71.58	70.13	81.98	72.29
S2		57.72	78.41	61.26	59.78	64.29
S3		67.52	60.71	67.74	75.14	67.78
L.S.D. (C * S)		4.74				2.37
Means (C)		63.57	70.23	66.38	72.30	
L.S.D. (C)		2.73				

Leaf area (dcm² plant⁻¹)

The results in Table 9 showed the significant superiority of treatment P2 (454.2 dcm² plant⁻¹) compared to the control treatment (374.5 dcm², plant⁻¹). Likewise, for silicon, as its third concentration was higher, represented by treatment S3 (462.6 dcm² plant⁻¹) and the lowest value in S1 (377.9 dcm² plant⁻¹). While citric acid did not had a significant effect, there were no significant differences between its four concentrations. The bilateral interaction between phosphorus and silicon led

to the P2S3 treatment significantly superior to the rest of the treatments (527.0 dcm² plant⁻¹). When phosphorus interacted with citric acid, the P2C1 treatment excelled as it reached 476.2 dcm² plant⁻¹. The interaction between silicon and citric acid shows superior of the treatment S3C3 (516.2 dcm² plant⁻¹). As for the triple interaction among the study factors, it was noted in the same table the significant superiority of the P2S3C3 treatment over all treatments (631.3 dcm² plant⁻¹) and the lowest value in P1S1C1 dcm² plant⁻¹.

Table 9. Effect of phosphorous, silicon and citric acid on leaf area (dcm² plant⁻¹)

Phosphorus (P)	Silicon (S) kg Ha ⁻¹	Citric acid (C) kg Ha ⁻¹				P * S
		C1 (0)	C2 (2)	C3 (4)	C4 (6)	
P 1 kg Ha ⁻¹ (0)	S1 (0)	210.7	274.4	295.5	341.9	280.6
	S2 (75)	381.8	459.4	428.4	395.8	416.4
	S3 (100)	452.5	387	443.2	422.8	426.4
P 2 kg Ha ⁻¹ (160)	S1 (0)	377.9	365.3	323.2	411.5	369.5
	S2 (75)	449.3	526.3	463.3	425.7	466.2
	S3 (100)	601.3	377.5	631.3	497.7	527.0
P 3 kg Ha ⁻¹ (320)	S1 (0)	499.2	502.7	473.6	459	483.6
	S2 (75)	417.4	449.5	408.4	448	430.8
	S3 (100)	411.2	430.2	474	422.3	434.4
L.S.D. (Interaction)		66.6				33.3
Phosphorus (P)		Citric acid (C)				Means (P)
		C1	C2	C3	C4	
P 1		348.3	373.6	389.0	386.8	374.5
P 2		476.2	423.1	472.6	445.0	454.2
P 3		442.6	460.8	452.0	443.1	449.6
L.S.D. (C * P)		38.4				19.2
(S)		Silicon (S) * Citric acid (C)				Means (S)
		C1	C2	C3	C4	
S1		362.6	380.8	364.1	404.1	377.9
S2		416.2	478.4	433.4	423.2	437.8
S3		488.3	398.2	516.2	447.6	462.6
L.S.D. (C * S)		38.4				19.2
Means (C)		422.4	419.2	437.9	425.0	
L.S.D. (C)		N.S				

Yield per plant (kg plant⁻¹)

The results in Table (10) show significant effect of phosphorus (P3) in increasing the plant yield to 3.09 kg Plant⁻¹ and the lowest value in P1 (2.59 kg Plant⁻¹). When studying the effect of silicon, the results showed that treatment S3 was significantly superior in increasing the yield up to 3.04 kg and the lowest value in S1 (2.56 kg Plant⁻¹). Treatment C4 was superior in increasing the plant yield to 2.95 kg plant⁻¹ and the lowest value in C1 (2.79 kg Plant⁻¹). As for the binary interaction between phosphorous and silicon, the

treatment P3S3 excelled in increasing the plant yield to 3.29 kg and the lowest value in P1S1 (2.01 kg Plant⁻¹). In the bilateral interaction between phosphorous and citric acid, the significant superiority was given to the treatment P3C4 (3.20 kg plant⁻¹). When silicon interacted with citric acid, the superiority of the two treatments S3C3 and S3C4 was observed (3.08 kg plant⁻¹). When the three factors of the study overlapped, P3S3C4 (3.48 kg plant⁻¹) was superior to all treatments and the lowest value in P1S1C1 (1.60 kg Plant⁻¹).

Table 10. Effect of phosphorus, silicon and citric acid on plant yield (kg plant⁻¹)

hosphorus (P)	Silicon (S) kg Ha ⁻¹	Citric acid (C) kg Ha ⁻¹				P * S
		C1 (0)	C2 (2)	C3 (4)	C4 (6)	
P 1 kg Ha ⁻¹ (0)	S1 (0)	1.60	1.90	2.19	2.36	2.01
	S2 (75)	2.72	3.02	3.10	2.92	2.94
	S3 (100)	2.79	2.7	2.90	2.63	2.75
P 2 kg Ha ⁻¹ (160)	S1 (0)	2.81	2.63	2.56	2.94	2.74
	S2 (75)	3.07	2.78	2.79	2.98	2.91
	S3 (100)	3.01	2.94	3.21	3.14	3.08
P 3 kg Ha ⁻¹ (320)	S1 (0)	2.96	2.92	2.83	2.93	2.91
	S2 (75)	2.86	3.21	3.02	3.20	3.07
	S3 (100)	3.24	3.32	3.12	3.48	3.29
L.S.D. (Interaction)		0.37				0.19
		Phosphorus (P) * Citric acid (C)				
Phosphorus (P)		C1	C2	C3	C4	Means (P)
P 1		2.37	2.54	2.73	2.64	2.59
P 2		2.97	2.78	2.85	3.02	2.91
P 3		3.02	3.15	2.99	3.20	3.09
L.S.D. (C * P)		0.21				0.11
		Silicon (S) * Citric acid (C)				
(S)		C1	C2	C3	C4	Means (S)
S1		2.46	2.48	2.53	2.75	2.56
S2		2.89	3.00	2.97	3.03	2.99
S3		3.01	2.99	3.08	3.08	3.04
L.S.D. (C * S)		0.21				0.11
Means (C)		2.79	2.82	2.86	2.95	
L.S.D. (C)		0.12				

Phosphorus fertilization led to an increases in most anatomical traits due to its role in increasing the division of protoderm, which is responsible for producing epidermis cells, leading to an increases in its thickness (Table 3). Additionally, phosphorus directly participates in the composition of nucleic acids RNA and DNA, as well as phospholipids in cell membranes. Phosphate, with its negative charge, acts as a link between the lipophilic cholesterol and hydrophilic choline, and it is involved in the synthesis of energy compounds ATP and ADP (9,11). All of these factors contribute to an increase in carbohydrate accumulation, which the plant utilizes for the growth of its organs, including the primary

root, lateral roots, and an increase in root hair density (12,26). Increasing the thickness of lateral roots leads to an increase in plant absorption rate and reduces the chances of damage, and the effect of phosphorus extends beyond the diameter of lateral roots. It also affects the thickness of the root epidermis (Table 3), indicating an increase in root hair activity. Root hairs are extensions of a single cell from the epidermal cells, and this enhances root function. Phosphate fertilization also increased the thickness of the cuticle layer in the stem and leaves of pepper plants (Tables 5 and 7). This layer, composed of cutin and wax, plays a significant role in reducing water loss, regulating transpiration, gas, and solute

transport, as well as protecting the plant from biotic stresses such as insect attack and abiotic stresses like drought (7). Furthermore, phosphate fertilization led to increases in leaf area (Table 9), indicating plant stimulation by increasing the number of leaves and single leaf area. Regarding silicon, the addition of potassium silicate has led to an increase in the diameter of lateral roots, root epidermis, and vascular bundle thickness in the pepper plant root (Table 2, 3, and 4) this is due to its role in activating the roots, as confirmed by (22), which leads to an increase in the efficiency of water and mineral absorption, thereby enhancing the efficiency of photosynthesis and the production of essential compounds in the plant's growth and development, subsequently activating all growth parameters, especially the roots (34). The results had emphasized the importance of citric acid, as it has led to an increase in the thickness of root epidermis, vascular bundle thickness in the pepper plant stem, and the thickness of the mesophyll layer in the leaf (Table 3, 6, and 8). Organic acids produced in plants, such as citric acid, gluconic acid, oxalic acid, lactic acid, tartaric acid, and aspartic acid, play a crucial role in lowering soil pH, releasing phosphorus ions, chelating iron and aluminum ions, and calcium ions (10). Citric acid also affects silicon availability indirectly, as its availability depends on various factors, including soil acidity. Plant roots can only absorb silicon in the form of silicic acid (H_4SiO_4). Therefore, the presence of citric acid in the plant root environment can lower the soil pH, even for a limited period, allowing the roots to absorb silicon.

CONCLUSION

Phosphorus, silicon and citric acid have an indirect role in increasing production by strengthening the anatomical structure starting from the roots to the other parts, and this is what the research results have proven. One of the most important problems of the pepper plant is the exposure of its branches to breakage or bending during the fruit harvesting process or even due to other service operations. This may be due to the weakness of the branch connection area or due to the weight of the fruits, leading to damage to the

branch bearing the fruit, leading to a decrease in the plant's productivity.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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