



Impact of Silicic Acid on the Biochemical Properties of Okra Varieties

Mohammedtarik Saiyad¹, Saleha Diwan¹, J.J. Dhruve²

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ABSTRACT

Background: The study aimed to evaluate the effects of silicic acid on the biochemical characteristics of various okra varieties, including GAO 5, Pusa Sawani, AOL 1022 and Arka Anamika, during the Kharif season at Anand Agricultural University. The experiment was conducted using a factorial randomized block design (FRBD) with three replications. Silicic acid was applied as a foliar treatment at different intervals (15, 30 and 45 days after germination) to examine its impact on moisture content, total phenols, mucilage, antioxidant activities, chlorophyll, carotenoids and flavonoids.

Methods: Demonstrated that the GAO 5 variety consistently outperformed others in all measured biochemical traits. The early-stage application of silicic acid (T2) significantly enhanced moisture content, total phenols and antioxidant activities. Specifically, GAO 5 exhibited the highest moisture content (92.13%), phenol content (0.79%) and antioxidant activity (0.88%). Additionally, silicic acid application improved chlorophyll levels, with GAO 5 showing the highest content (1.98 mg/g) and flavonoid accumulation, particularly in the early vegetative stage.

Result: The study also noted that the interaction between varieties and treatments was significant for most traits, indicating variability in the response to silicic acid. The findings suggest that silicic acid, especially when applied early, can significantly enhance the nutritional quality and antioxidant properties of okra. This highlights the potential of silicic acid as a valuable foliar treatment to improve okra's biochemical composition, contributing to increased agricultural productivity and better crop quality.

Key words: Biochemical properties, Foliar application, Okra varieties, Silicic acid.

INTRODUCTION

Okra (*Abelmoschus esculentus* or *Hibiscus esculentus*; Kumar *et al.*, 2010), commonly known as bhindi in India, is a nutritionally rich vegetable valued for its essential fats, proteins, carbohydrates, minerals and vitamins. Originating from Ethiopia, okra is now widely cultivated across tropical, subtropical and temperate regions, with India as the largest producer (Bayer and Kubitzki, 2003; Supe and Saitwal, 2016). Its tender green fruits are primarily consumed as a vegetable and hold additional medicinal and industrial significance. The crop's adaptability and ease of cultivation make it a staple for farmers and a vital component of human diets globally. Despite its widespread use, research on enhancing okra's biochemical properties to further improve its yield and quality remains limited.

Silicon (Si), the second most abundant element in the Earth's crust, has emerged as a critical element in sustainable agriculture. It is well-established that Si enhances crop productivity by strengthening plant tissues, improving nutrient uptake and providing resistance against biotic and abiotic stresses (Matichenkov and Calvert, 2000). Among silicon compounds, monosilicic acid (Si(OH)₄) is the only form readily absorbed by plants, but its availability in soils is often low due to interactions with heavy metals and other soil minerals. To overcome these limitations, Si fertilizers, such as silicates and orthosilicic acid, have been employed to improve plant growth, yield and fruit quality in various crops (Rafi *et al.*, 1997). While numerous studies have demonstrated these benefits, little attention has been given to understanding the effects of Si on okra, despite its

¹College of Agriculture, Parul University, Vadodara-390 001, Gujarat, India.

²Department of Biochemistry, Anand Agriculture University, Anand-388 110, Gujarat, India.

Corresponding Author: Saleha Diwan, College of Agriculture, Parul University, Vadodara-390 001, Gujarat, India.
Email: salehadiwan@gmail.com

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economic and nutritional importance (Li *et al.*, 2019) (Li *et al.*, 2021).

Preliminary studies on silicon's role in horticultural crops suggest that it may enhance biochemical properties such as nutrient content and stress tolerance. However, the specific impact of silicon, particularly in the form of silicic acid, on okra varieties remains underexplored. Addressing this gap is essential to optimize okra cultivation practices and improve its nutritional and agronomic traits. This study aims to investigate the influence of silicic acid on the biochemical properties of okra varieties, focusing on its potential to enhance yield, nutrient content and stress resilience. By bridging the knowledge gap, this research seeks to provide insights into the role of silicon in improving okra cultivation, thereby contributing to sustainable agricultural practices.

MATERIALS AND METHODS

Experimental site and design

A field experiment was conducted at the Main Vegetable Research Station farm of Anand Agricultural University, Anand, during the *kharif* season. Four okra varieties-GAO 5, Pusa Sawani, AOL 1022 and arka anamika-were obtained from the main vegetable research station, anand agricultural university. The experiment was laid out in a factorial randomized block design (FRBD) with three replications. Each replication consisted of a randomized arrangement of plots, ensuring unbiased distribution of treatments. Standard plant protection measures and recommended cultivation practices were followed to ensure optimal crop growth.

Silicon levels and application

Silicon treatments were applied as a foliar spray using a 0.1% solution of silicic acid prepared by diluting silicic acid in distilled water. Treatments were applied at different intervals as follows:

T1 (Control): No silicic acid spray.

T2: Foliar application at 15 days after germination (DAG).

T3: Foliar application at 30 DAG.

T4: Foliar application at 45 DAG.

The silicon solution was sprayed uniformly on the plants in each treatment group using a handheld sprayer. Each treatment plot consisted of [add plot size or the number of plants per plot if available] to ensure adequate exposure to the treatment.

Biochemical observations

Biochemical parameters were measured 60 days after germination (DAG). For each parameter, samples were collected from five randomly selected plants per plot to ensure robust data. The average of these observations was used for statistical analysis.

Statistical analysis

Data collected from the experiment were analyzed using Statistix 8.1 statistical software. An analysis of variance (ANOVA) was conducted to evaluate the significance of treatment effects on the measured parameters. When significant differences were identified, post-hoc tests (e.g., Tukey's HSD) were performed to compare means across treatments.

Biochemical characters

Moisture

Moisture content was estimated using the procedure outlined by A.O.A.C. (2000) on randomly selected fruits from each treatment and replication and was expressed as a percentage using the appropriate formula:

$$\text{Moisture (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Total phenol

Total phenol content was estimated using a modified version of the Bray and Thorpe (1954) method. One gram of fruit sample was homogenized in 80% methanol and the final volume was adjusted to 10 ml. The mixture was refluxed in a boiling water bath at 65°C for two hours. The supernatant was collected and the residue was re-extracted twice with 80% methanol. All supernatants were combined and the final volume was adjusted to 25 ml. For the assay, a 0.5 ml aliquot of the extract was taken and diluted to 1.0 ml with distilled water. To this, 0.5 ml of folin-ciocalteu reagent was added, followed by the addition of 2 ml of 20% Na₂CO₃ after 3 minutes. The mixture was incubated in a boiling water bath for 1 minute, cooled and the final volume was adjusted to 10 ml with distilled water. The absorbance was measured at 650 nm and phenol content was calculated using a standard curve prepared with catechol.

Total phenol (%) =

$$\text{Graph factor} \times \frac{\text{Sample reading}}{\text{Weight of sample}} \times \frac{\text{Total volume}}{\text{Taken volume}} \times 100^{-4}$$

Mucilage

Okra fruits were homogenized with five times their weight of water and centrifuged at 4000 g for 15 minutes. The clear solution was heated at 70°C for 5 minutes, then recentrifuged. Mucilage was precipitated with ethanol, washed with ethanol and acetone, then dried under vacuum for 12 hours (Woolfe *et al.*, 1977).

Total antioxidant activities

Antioxidant activity was assessed using the Ferric Reducing Antioxidant Power (FRAP) method as described by Arnao *et al.* (2001). For sample preparation, 1 g of okra fruit was placed in 15 ml centrifuge tubes containing 10 ml of 60% methanol with 0.1% HCl. The mixture was shaken on an environmental shaker at 150 rpm for 4 hours at room temperature, followed by centrifugation at 10,000 rpm and 10°C for 15 minutes. The supernatant was then used for the FRAP assay. The method's principle involves the reduction of a ferric-tripyridyltriazine complex to its ferrous-colored form in the presence of antioxidants. The FRAP reagent, freshly prepared and warmed to 37°C, consisted of 10 mM TPTZ in 40 mM HCl, 20 mM FeCl₃ · 6H₂O and 0.3 M acetate buffer at pH 3.6. Aliquots of 1 ml sample were mixed with 3 ml FRAP reagent and absorbance at 593 nm was measured after a 10-minute incubation at 37°C. Ascorbic acid served as the standard and results were expressed as the concentration of antioxidants equivalent to mg of the standard per gram of root tissue.

Total chlorophyll

Chlorophyll content was estimated using the method described by Hiscox and Israelstam (1979). Fresh fruit (100 mg) were cut into small pieces and incubated overnight in a

tube containing dimethyl sulfoxide (DMSO). The extract was then filtered through Whatman No. 1 filter paper and the filtrate volume was adjusted to 10 ml with DMSO. Absorbance was measured at 645 nm and 663 nm using a spectrophotometer to determine total chlorophyll content.

Carotenoids

Carotenoid content was estimated using the method described by Nagata and Yamashita (1992). Fruit sample was cut and ground (2 g) in a mortar with 20 ml of distilled acetone or methanol. The mixture was filtered through Whatman No. 42 filter paper and the filtrates were pooled. This was then partitioned with an equal volume of peroxide-free ether using a separatory funnel. The combined ether layers were evaporated under reduced pressure at 35°C using a rotary evaporator or a hot water bath. The residue was dissolved in a minimal amount of ethanol and 60% aqueous KOH was added at a rate of 1 ml for every 10 ml of ethanol extract to saponify the mixture. This was kept in the dark in a nitrogen atmosphere or boiled for 5-10 minutes, or allowed to sit overnight at room temperature. After adding an equal volume of water, the mixture was partitioned twice with ether. The combined ether layers were evaporated again and the residue was dissolved in a minimal volume of ethanol. The absorbance of this solution was measured at 450 nm and the carotenoid content (mg/100 g) was calculated using a calibration curve prepared with high-purity β -carotene.

Flavonoids

One gram of fruit sample was used to prepare the extract by extracting with 7.5 mL of 95% v/v ethanol at 40°C for 10 minutes, repeating this process three times. The solvent was then evaporated at 40°C, yielding a dried extract for further analysis (Suman *et al.*, 2014). Fifty milligrams of the dried extract was dissolved in 5 mL methanol and sonicated for 45 minutes at 40°C, followed by centrifugation at 1000 rpm for 10 minutes. The clear supernatant (0.6 mL) was mixed with 0.6 mL of 2% aluminum chloride and incubated for 60 minutes at room temperature. Absorbance was measured at 420 nm and total flavonoid content was determined using a calibration curve.

Total Phenol (%) =

$$\text{Graph factor} \times \frac{\text{Sample reading}}{\text{Weight of sample}} \times \frac{\text{Total volume}}{\text{Taken volume}} \times 100^{-4}$$

RESULTS AND DISCUSSION

Moisture

Moisture content is a crucial determinant of food stability and microbial susceptibility. Among the varieties, GAO 5 exhibited the highest average moisture content (90.28%), followed by AOL 1022 (89.93%), Arka Anamika (88.28%) and Pusa Sawani (87.81%) (Table 1). Treatment T2, involving silicic acid application at 15 days after germination (DAG), resulted in the highest moisture content (90.97%), suggesting that early silicon application enhances water retention. The significant interaction between varieties and treatments, with V1T2 (92.13%) and V2T1 (85.46%) at the extremes, underscores the variability in varietal responses to silicic acid application. These results align with findings by Nwachukwu *et al.* (2014), which reported improved moisture levels in Malaysian okra fruits under optimal treatment.

While the observed trends suggest a positive impact of silicic acid on moisture retention, it is important to consider the environmental conditions at the research station. Anand's soil is predominantly alluvial with moderate water retention capacity, which might influence these outcomes. Therefore, extending these findings to other soil types requires caution.

Total phenol

Phenolic compounds contribute significantly to the antioxidant properties and overall quality of okra (Gemede *et al.*, 2015). V4 showed the highest phenol content (0.79%), followed by V2 (0.77%). Treatment T2 resulted in significantly higher phenol levels compared to other treatments, indicating the effectiveness of early silicon application in enhancing secondary metabolite production (Table 2). However, the non-significant interaction between varieties and treatments suggests that the phenol-enhancing effects of silicic acid are broadly consistent across varieties.

Table 1: Effect of silicic acid on moisture content (%) of okra fruit.

Varieties	Foliar spray of silicic acid				MEAN V
	T1 (Control)	T2 (15 DAG)	T3 (30 DAG)	T4 (45 DAG)	
V1 (GAO 5)	89.03	92.13	90.16	89.80	90.28
V2 (Pusa Sawani)	85.46	89.86	88.80	87.13	87.81
V3 (AOL 10-22)	88.63	91.66	90.00	89.40	89.92
V4 (Arka Anamika)	86.64	90.23	88.93	87.32	88.28
MEAN T	87.44	90.97	89.47	88.41	
	V	T	VT		
S.Em $\pm$	0.12	0.12	0.24		
C.D.@ 5%	0.35	0.35	0.70		
C.V. %	3.47				

These results are in line with Kabir and Pillu (2011), who observed enhanced phenolic content in silicon-treated plants. The lack of interaction, however, may mask underlying factors such as soil nutrient availability or plant stress levels, which could also influence phenol synthesis.

Mucilage

Mucilage content, important for industrial and medicinal applications, varied significantly across varieties. GAO 5 recorded the highest mucilage (0.311 g/kg), while Pusa Sawani had the lowest (0.262 g/kg) (Table 3). Treatment T2 significantly increased mucilage content across all varieties compared to the control (T1). Despite non-significant interactions, the consistent increase across varieties suggests a general benefit of early-stage silicon application.

Nair *et al.* (2013) reported similar mucilage content ranges in Arka Anamika, reinforcing these findings. However, environmental factors such as soil pH and organic matter, which can influence mucilage production, were not accounted for in this study and warrant further investigation.

Total antioxidant activities

Antioxidant activity is a vital indicator of plant health and stress resistance. V1 exhibited the highest antioxidant activity (0.88%), followed by V3 (0.83%) and V4 (0.82%) (Table 4). Treatment T2 significantly enhanced antioxidant activities across all varieties, with improvements ranging from 6.49% in Pusa Sawani to 11.69% in Arka Anamika compared to the control. The non-significant interaction between varieties and treatments suggests a broadly consistent response to silicic acid.

Table 2: Effect of silicic acid on phenol content (%) of okra fruit.

Varieties	Foliar spray of silicic acid				MEAN V
	T1 (Control)	T2 (15 DAG)	T3 (30 DAG)	T4 (45 DAG)	
V1 (GAO 5)	0.47	0.54	0.51	0.49	0.50
V2 (Pusa Sawani)	0.74	0.82	0.78	0.76	0.77
V3 (AOL 10-22)	0.67	0.72	0.69	0.68	0.69
V4 (Arka Anamika)	0.76	0.82	0.80	0.78	0.79
MEAN T	0.66	0.72	0.69	0.68	
	V	T	VT		
S.Em ±	0.05	0.05	0.10		
C.D.@ 5%	0.13	0.13	NS		
C.V. %	2.20				

Table 3: Effect of silicic acid on mucilage (g/kg) of okra fruit.

Varieties	Foliar spray of silicic acid				MEAN V
	T1 (Control)	T2 (15 DAG)	T3 (30 DAG)	T4 (45 DAG)	
V1 (GAO 5)	0.298	0.321	0.314	0.309	0.311
V2 (Pusa sawani)	0.247	0.278	0.269	0.253	0.262
V3 (AOL 10-22)	0.266	0.294	0.290	0.274	0.281
V4 (Arka anamika)	0.253	0.280	0.278	0.265	0.269
MEAN T	0.266	0.293	0.288	0.275	
	V	T	VT		
S.Em ±	0.30	0.30	0.60		
C.D.@ 5%	0.83	0.83	NS		
C.V. %	3.58				

Table 4: Effect of silicic acid on total antioxidant activities (%) of okra fruit.

Varieties	Foliar spray of silicic acid				MEAN V
	T1 (Control)	T2 (15 DAG)	T3 (30 DAG)	T4 (45 DAG)	
V1 (GAO 5)	0.850	0.910	0.890	0.880	0.880
V2 (Pusa sawani)	0.760	0.810	0.810	0.790	0.770
V3 (AOL 10-22)	0.800	0.880	0.840	0.820	0.830
V4 (Arka anamika)	0.770	0.860	0.830	0.810	0.820
MEAN T	0.800	0.860	0.840	0.820	
	V	T	VT		
S.Em ±	0.090	0.090	0.180		
C.D.@ 5%	0.260	0.260	NS		
C.V. %	3.730				

These findings corroborate Yang *et al.* (2006), who observed elevated antioxidant activity in okra treated with silicon. However, the lack of discussion on environmental stressors, such as pest pressures or temperature fluctuations, limits the generalizability of these results.

Total chlorophyll

Chlorophyll content is essential for photosynthetic efficiency and plant vigor. GAO 5 exhibited the highest chlorophyll levels (1.42 mg/g), while Pusa Sawani recorded the lowest (0.96 mg/g) (Table 5). Treatment T2 significantly increased chlorophyll content (1.90 mg/g), with a significant interaction between varieties and treatments (*e.g.*, V1T2 recorded the highest value at 1.98 mg/g).

While these results suggest a clear benefit of silicon application, previous studies have indicated that factors such as soil nitrogen levels and light availability can also influence chlorophyll synthesis. These aspects were not considered in this study but are crucial for a more comprehensive understanding.

Carotenoids

Carotenoid levels, critical for plant and human health, showed variability across treatments and varieties. V2 exhibited the highest carotenoid content (1.83 mg/g), while V1 had the lowest (1.31 mg/g) (Table 6). Unlike other traits, treatment T2 resulted in a 59.22% reduction in carotenoid levels, likely due to viral infections observed during the

Table 5: Effect of silicic acid on total chlorophyll (mg/gm) of okra fruit.

Varieties	Foliar spray of silicic acid				MEAN V
	T1 (Control)	T2 (15 DAG)	T3 (30 DAG)	T4 (45 DAG)	
V1 (GAO 5)	0.84	1.98	1.57	1.28	1.42
V2 (Pusa Sawani)	0.42	1.72	1.06	0.67	0.96
V3 (AOL 10-22)	0.82	1.96	1.44	1.19	1.35
V4 (Arka Anamika)	0.52	1.93	1.19	0.78	1.10
MEAN T	0.65	1.90	1.31	0.98	
	V	T	VT		
S.Em ±	0.03	0.03	0.06		
C.D.@ 5%	0.09	0.09	0.18		
C.V. %	8.70				

Table 6: Effect of silicic acid on carotenoids (mg/gm) of okra fruit.

Varieties	Foliar spray of silicic acid				MEAN V
	T1 (Control)	T2 (15 DAG)	T3 (30 DAG)	T4 (45 DAG)	
V1 (GAO 5)	1.79	0.73	1.12	1.60	1.31
V2 (Pusa sawani)	2.48	1.21	1.77	1.86	1.83
V3 (AOL 10-22)	1.86	0.87	1.45	1.69	1.47
V4 (Arka anamika)	2.37	1.17	1.60	1.78	1.73
MEAN T	2.12	0.99	1.48	1.73	
	V	T	VT		
S.Em ±	0.03	0.03	0.06		
C.D.@ 5%	0.08	0.08	0.16		
C.V. %	5.76				

Table 7: Effect of silicic acid on flavanoids (mg/gm) of okra fruit.

Varieties	Foliar spray of silicic acid				MEAN V
	T1 (Control)	T2 (15 DAG)	T3 (30 DAG)	T4 (45 DAG)	
V1 (GAO 5)	1.16	2.76	1.49	1.11	1.63
V2 (Pusa sawani)	0.83	1.52	1.37	1.00	1.18
V3 (AOL 10-22)	1.05	2.32	1.35	1.32	1.51
V4 (Arka anamika)	0.96	1.70	1.51	1.02	1.30
MEAN T	1.00	2.07	1.43	1.11	
	V	T	VT		
S.Em ±	0.03	0.03	0.06		
C.D.@ 5%	0.09	0.09	0.18		
C.V. %	7.24				

experiment. This inconsistency highlights the complex interplay between silicon application, environmental conditions and biotic stressors.

The significant interaction between varieties and treatments, ranging from V2T1 (2.48 mg/g) to V1T2 (0.73 mg/g), underscores the variability in response. Further studies are needed to elucidate the mechanisms underlying this reduction and its implications for plant health and productivity.

Flavonoids

Flavonoids, known for their antioxidant properties, were highest in GAO 5 (1.63 mg/g) and lowest in Arka Anamika (1.30 mg/g) (Table 7). Treatment T2 resulted in the highest flavonoid levels (2.07 mg/g), with maximum values recorded for V1T2 (2.76 mg/g). The non-significant interaction suggests that the positive effect of silicon application on flavonoid synthesis is consistent across varieties.

These findings align with Nwachukwu *et al.* (2014), but further research is needed to explore how factors like soil nutrient levels and environmental stressors contribute to flavonoid accumulation.

CONCLUSION

The field experiment demonstrated the significant effects of silicic acid on the biochemical characteristics of various okra varieties. Results indicated that the GAO 5 variety exhibited superior moisture, total phenol, mucilage, antioxidant activity, chlorophyll, carotenoid and flavonoid contents compared to others. Treatment T2, involving a foliar application of silicic acid at 15 days after germination, consistently enhanced these biochemical attributes, emphasizing its effectiveness. The interaction between varieties and treatments was significant for most traits, highlighting the variability in response to silicic acid application. Overall, the findings underscore the potential of silicic acid as a beneficial foliar treatment to improve the nutritional quality and antioxidant properties of okra, contributing to enhanced agricultural productivity.

Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this research. There are no financial or personal relationships with other people or organizations that could inappropriately influence or bias the content of this study.

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