EFFICACY OF DIATOMACEOUS EARTH AND SILICIC ACID ON LEAF QUALITY AND BIOCHEMICAL PROPERTIES IN MULBERRY

ABSTRACT

A study on 'Diatomaceous earth (DE) and silicic acid as a source of silicon on mulberry leaf quality and biochemical attributes' was conducted during 2022-24. The experiment was laid out in Randomized Complete Block Design (RCBD) with thirteen treatment combinations and three replications. Diatomaceous earth (DE) was applied to soil in five split doses at 18 days after pruning (DAP) along with RDF and foliar silicic acid (FSA) spray was given at 28 DAP for five crops at varied levels. Observations were recorded at 30th, 45th and 60th DAP and pooled data of five crops were analysed. Among different treatments, combined application of 450 kg ha^{-1} DE + 4ml L^{-1} FSA (T_{10}) recorded highest moisture content (73.45 ± 3.01 %), highest moisture retention capacity (77.62 ± 5.28 %), crude protein (22.50 ± 1.28 %), crude fibre (12.59 ± 0.42 %) and crude fat (4.76 ± 0.17 %) content whereas, T_{11} (600 kg ha^{-1} DE + 4ml L^{-1} FSA) exhibited maximum carbohydrate (19.87 ± 0.19 %), total phenols (15.46 ± 0.51 mg GAE g^{-1} dw), chlorophyll 'a' (1.58 ± 0.05 mg g^{-1}), chlorophyll 'b' (0.98 ± 0.03 mg g^{-1}), total chlorophyll (2.56 ± 0.02 mg g^{-1}) and ash content (9.57 ± 0.21 %) in mulberry leaves. Further, T_{11} (600 kg ha^{-1} DE + 4ml L^{-1} FSA) and T_8 (600 kg ha^{-1} DE + 2ml L^{-1} FSA) exhibited on par results with T_{10} for all the leaf biochemical attributes of mulberry.

***Key words*:** V_1 Mulberry, Silicon, Diatomaceous earth, Silicic acid and Biochemical parameters.

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L. is an economically important insect, its significance is attributed to its silk secreting ability. The successful harvest of quality cocoons depends exclusively on the nutrition of the silkworm. Mulberry leaves serve as sole food source and provide various nutrients to carry out its physiological activities. Nearly 70 per cent of silk proteins produced by silkworm are absorbed directly from proteins of mulberry leaves. Therefore, leaves quality plays a vital role in larval growth and cocoon crop productivity (Kamala and Karthikeyan, 2019)

Soil fertility and nutrient management affect the sustained productivity and quality of foliage in mulberry. Despite, periodical application of 20 tons $ha^{-1}yr^{-1}$ of FYM and 350:140:140 kg NPK per hectare per year under irrigated conditions, mulberry production faces manifold challenges. In order to overcome these drawbacks, application of other secondary and micronutrients apart from primary nutrients is imperative to improve crop productivity. Since, mulberry responds well to foliar nutrition, which play a good role in supplying supplemental doses of major and minor nutrients, hormones, stimulants and other beneficial substances that can quickly improve plant metabolism inturn enhances the growth, yield and biochemical parameters (Banuprakash *et al*., 2024). However, the beneficial effects of Si on stimulating plant growth particularly in plants subjected to both abiotic (*e.g.*, aluminium, salt and heavy metal toxicity) and biotic (*e.g*., plant diseases and pests) stresses in other crops recently received increasing attention.

Silicon (Si) is the second most abundant element on the earth crust, is taken up by crops as monosilicic acid (H_4SiO_4) from the soil (Epstein, 1999). Silicon is the only element known that does not damage plants upon excess accumulation and its concentration in plants widely varies from 0.10 to 10% per dry mass depending on plant species. Characterization and categorization of Si in South Indian soils where sericulture is predominant revealed that these soils under intensive cultivation are deficit in plant available Si and responded well to the external application of Si. The most important characteristics of Si source to be considered useful as a Si fertilizer are high soluble Si content, suitable physical properties, ready availability for plants, cost effectiveness, easy and local availability, balanced ratios and amounts of calcium (Ca), magnesium (Mg) and silicon and absence of heavy metals (Prakash *et al*., 2017). Studies using different sources of silicon (calcium silicate, diatomite, foliar silicic acid, crop residues and biochar) gave promising results with different crops *viz*., rice, finger millet, maize, soybean, groundnut, tomato, potato and many other horticultural crops.

Diatomaceous earth (DE) or diatomite are the sedimentary rocks that result from the deposition of Si-rich unicellular life forms known as diatoms. Amorphous silica is the main

component of DE and has high plant available Si, highly porous in structure with low density, high surface area and CEC for retaining nutrients in soil proving to be useful in improving the physical properties of soils (Anitha and Prakash, 2015). Applying silicic acid (SA) as a source of Si through foliar spray can enhance the use efficiency and bioavailability of Si to plants. Mulberry leaf quality is mainly dependent on its biochemical composition. Nitrogen is a major component of amino acids, the building blocks of proteins, its deficiency reduces the protein and water content of the leaves, thereby reducing the nutritive value of the leaves which is essential in mulberry sericulture. Fertilizers provide essential nutrients for plant growth, including proteins, fats, ash, fibre and carbohydrates. Si being beneficial nutrient when applied externally, combined with other fertilizers, helps in improving the uptake and use efficiency, controlled release of these nutrients ensures availability to the plants over an extended period, reducing the risk of nutrient leaching and waste. This steady nutrient supply supports the synthesis of proteins, fats and carbohydrates in plant tissues (Dhiraj and Kumar, 2012).

MATERIAL AND METHODS

The experiment was conducted during 2022-24 in well-established V1 mulberry garden with a spacing of 90×90 cm at Department of Sericulture, GKVK, Bengaluru. The field is located at a latitude of 12°58′ N and longitude of 77°35′ E and at an altitude of 930 m above mean sea level in the Eastern Dry Zone (Zone-5) of Karnataka and receives an average rainfall is 915.8 mm.

Field experiments were laid out to study the effect of diatomaceous earth and silicic acid as sources of silicon on growth and yield of mulberry under field conditions by pruning the crop for five times. The experiment was laid out in a randomized complete block design (RCBD) with thirteen treatments and three replications. Si sources tested were T_1- Foliar application of silicic acid (FSA) @ 2ml L^{-1}; T_2 -Foliar application of silicic acid (FSA) @ 4ml L^{-1}; T_3- Soil application of diatomaceous earth (DE) @ 300 kg ha^{-1}; T_4-Soil application of diatomaceous earth (DE) @ 450kg ha^{-1}; T_5- Soil application of diatomaceous earth (DE) @ 600 kg ha^{-1}; T_6 – Combination of DE @ 300 kg ha^{-1} and 2ml L^{-1} FSA; T_7- Combination of DE @ 450 kg ha^{-1} and 2ml L^{-1} FSA; T_8- Combination of DE @ 600 kg ha^{-1} and 2ml L^{-1} FSA; T_9 – Combination of DE @ 300 kg ha^{-1} and 4ml L^{-1} FSA; T_{10}- Combination of DE @ 450 kg ha^{-1} and 4ml L^{-1} FSA; T_{11}- Combination of DE @ 600 kg ha^{-1} and 4ml L^{-1} FSA. Apart from these, T_{12} and T_{13} were included as RDF (Control) and absolute control (without RDF), respectively. All other practices of mulberry cultivation were followed as per standard package of practices (Dandin and Giridhar, 2014). The data on growth

parameters of mulberry plant were recorded at 30th, 45th and 60th DAP in each treatment on randomly selected five plants from each replication and mean value of 5 crops were calculated. Mulberry leaves collected from the field on 45th day after pruning (DAP) were subjected to analyse the biochemical composition.

Sample Preparation: Fresh leaves were collected from mulberry garden on 45th DAP and immediately dried in an oven drier at 50-55 °C for 8-10 h. Thereafter, these were ground in an electric grinder to pass through a 40-mesh sieve. The samples were stored in clean double-sealed polythene bags in the refrigerator during the investigation (A.O.A.C., 2005).

Estimation of Biochemical Parameters in Mulberry Leaf: Quality parameters of mulberry leaf such as moisture content, moisture retention capacity, chlorophyll content, total carbohydrates, proteins, crude fiber, phenols, ash and crude fat contents of the leaf were estimated according to standard procedures given below.

Leaf moisture content (%): Moisture content in the mulberry leaves was expressed in percentage on wet basis and it was determined by drying mulberry leaves in hot air oven (60 ± 1°C for 24 hours) and the same procedure continued till constant weight was obtained and then moisture content was determined by following formula (A.O.A.C., 1970).

$$\text{Moisture content (\%)} = \frac{(\text{Initial wt. of sample}) - (\text{Final wt. of sample})}{(\text{Initial weight of the sample})}$$

Leaf moisture retention after 6 hr of harvest (%): To determine the leaf moisture after six hours of harvest, a composite sample of tenleaves was collected and fresh weight was taken. The leaves were kept open under laboratory condition and the weight was recorded at 6 hours after harvest. The leaves weredried thoroughly at 80^0 C in the oven. Dry weight was taken; the moisture retention capacity was calculated by using the formula below (A.O.A.C., 1970).

$$\text{Moisture retention (\%)} = \frac{(\text{Weight after 6 hrs.}) - (\text{Dry weight})}{(\text{Fresh weight}) - (\text{Dry weight})}$$

Estimation of crude protein: Protein content of the leaf was assessed after determining the total nitrogen content in the leaf (0.5 g leaf sample) using Micro-Kjeldhal method. The protein content of

the leaf was computed by multiplying the per cent nitrogen of the sample with the factor 6.25 (A.O.A.C., 1980).

$$\text{Crude protein (\%)} = \text{N (\%)} \times 6.25$$

Estimation of total carbohydrate: Total carbohydrate of mulberry leaf was estimated by following the method of (Dubois *et al*., 1956) using glucose as standard. The total anthrone positive substances were expressed as mg of carbohydrate g^{-1} dry weight of leaf sample.

Total Phenols (mg gallic acid equivalents g^{-1} dw): Phenol content from selected mulberry crosses was estimated by spectrophotometric method by using Folin Ciocalteu Reagent (FCR). Five grams of dried mulberry powder pre-incubated at room temp with 80 per cent Methanol (20ml) for 72 hrs, was homogenized with 20 ml of methanol (80%) in pestle and mortar for 2-3 times. Pooled the extracts and made up the volume to 50 ml. Then 0.5 ml of extract was taken in test tube, 0.2 ml of Folin-Ciocalteau's Phenol Reagent was added followed by 3.3 ml of distilled water and mixed well. After 2 min, 1 ml of sodium carbonate solution was added and thoroughly mixed. The mixture was incubated at room temperature for 30 min and then intensity of blue color was measured in spectrophotometer at 700 nm. Preparation of standard curve for phenols was done using gallic acid (GA) as standard (Singleton *et al*., 1999).

Total phenol content (mg gallic acid equivalents g^{-1} dw) =

$$\frac{\text{OD700 nm} \times \text{Standard value (µg/OD)} \times \text{Total volume of extract} \times 100}{\text{Assay volume} \times \text{Weight of sample (g)} \times 1000}$$

Chlorophyll estimation: The content of chlorophyll in mulberry leaf was calculated by the following procedure defined by Hiscox and Isrealstam (1979). The leaf chlorophyll content was determined using the formula proposed by Arnon (1949).

$$\text{Chlorophyll 'a'} = \frac{[12.7\ (\text{O. D. } 663) - 2.69\ (\text{O. D. } 645)]\ V}{1000 \times W}$$

$$\text{Chlorophyll 'b'} = \frac{[22.9\ (\text{O.D. } 645) - 4.68\ (\text{O.D. } 663)]\ V}{}$$

$$1000 \times W$$

$$\text{Total chlorophyll} \frac{\text{mg}}{\text{g}} \text{fresh weight} = \frac{20.2(O.D.645) + 8.02(O.D.663) \times \text{Volume}}{1000 \times W}$$

W- Weight of leaves

Estimation of crude fibre in plant sample: The crude fibre of the sample was estimated by taking 2g sample with ether or petroleum ether and boiled (initial boiling temperature of 35-38°C and final temperature of 52 °C). Then 200 ml of sulphuric acid was added and boiled for 30 min. Filtered through muslin cloth and washed with boiling water until washings were free of acid. Again, boiled the residue with 200 ml of sodium hydroxide for 30 min. filtered thorough muslin cloth, again washed with 25 ml of boiling sulphuric acid, three 50 ml portions of water and 25 ml of alcohol. The residue was removed and transferred to pre-weighed ashing dish (W1, g). The residue was dried for 2hrs at 130°C, cooled in a desiccator and weighed (W2, g), Ignited for 30 min at 600 °C and then cooled in a desiccator and reweighed (W3, g) (A.O.A.C., 2005). The fiber content of sample was calculated by:

$$\text{Crude Fibre (\%)} = \frac{(W2 - W1) - (W3 - W1)}{\text{weight of the sample (g)}} \times 100$$

Estimation of Ash (%): The ash content in selected leaves of mulberry crosses were estimated by adopting by using A.O.A. C. (2012) method and expressed in percentage.

Crude Fat content (%): The fat content of mulberry leaf was estimated by Soxhlet method. 5g of powdered leaf sample was taken in thimble and placed in previously weighed flask with stones (W_1). Later 100ml of petroleum ether (boiling range of 40-60 ^{0}C) was added to these flasks and kept in soxtherm. Then, run the instrument for one and half hour. After complete extraction, flasks were kept in hot air oven @ 105 ^{0}C for 30 min and weight (W_2) of flask was recorded after cooling (A.O.A.C., 2005).

$$\text{Crude fat content (\%)} = \frac{W_2 \text{ g (flask + stones + oil)} - W_1 \text{ g (flask + stones)}}{\text{Weight of leaf sample (g)}} \times 100$$

Statistical analysis: All assays were conducted in triplicate. The experimental data collected on various components of plant were subjected to Fisher's method of Analysis of Variance (ANOVA) as outlined by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Application of diatomite and silicic acid revealed notable variation with respect to the quality parameters of mulberry *viz*., moisture content, moisture retention capacity after 6 hr, chlorophyll, crude protein, crude fibre, total carbohydrate, fat, ash and phenol contents in mulberry leaves estimated at 45th day after pruning. The mean data of five crops are subjected for analysis of variance and the results are furnished in Table 1 to 4.

Moisture content (%)

Moisture content of mulberry leaves varied among different treatments with the values ranging from 64.04 % to 75.10 %. (Table 1). Combined application of DE @ 450 kg per ha through soil application and 4ml L^{-1} FSA (T_{10}) has recorded a higher moisture percentage (73.45 ± 3.01 %) when compared to RDF - control (70.68 ± 2.52 %) and notably least in the leaves harvested from $T_{13,}$ absolute control (67.52 ± 1.48 %).

Moisture retention after 6 hr (%)

Moisture retention capacity after 6 hr of leaf harvest in mulberry ranged between 65.82% to 79.36 % among different treatment combinations (Table 1). Similar to the moisture content results, T_{10} (77.62 ± 5.28 %) exhibited the highest moisture retention capacity after 6 hr of leaf harvest and that were statistically on par to T_8 which received 600 kg ha^{-1} DE + 2ml L^{-1} FSA (77.04 ± 5.24 %), suggesting that the combination of diatomite and silicic acid positively affected the ability of mulberry leaves to retain moisture over time. RDF control (T_{12}) has moderate moisture retention capacity (74.11 ± 5.04 %) and minimum was noticed in T_{13} (69.40 ± 4.72 %) leaves from non-treated plot**.**

The increase in moisture content in the leaves may be attributed due to the enhancement of hydrogen ion concentration in plant due to the accumulation of chlorides and less moisture loss by evapotranspiration and thereby enhanced the moisture content in the leaves and fresh leaf weight. Usually moisture content in mulberry leaves fluctuate from 64-83 per cent. Nitrogen has the highest influence on leaf quality and moisture content of leaves (Harshitha Mala *et al*., 2024).

Table 1: Effect of different levels of of diatomaceous earth and silicic acid on moisture content and moisture retention capacity of V1 mulberry leaves at 45th day after pruning

Treatments	Moisture content (%)		Moisture retention after 6hrs (%)	
	Range	Mean	Range	Mean
T_1	68.58-71.81	70.24 ± 2.46	71.36-74.72	73.08 ± 4.97
T_2	69.08-72.33	70.75 ± 1.82	72.03-75.42	73.77 ± 5.02
T_3	69.75-73.04	71.44 ± 2.79	72.77-76.19	74.52 ± 5.07
T_4	70.07-73.37	71.76 ± 3.29	73.21-76.66	74.98 ± 5.10
T_5	70.80-74.13	72.51 ± 2.56	74.36-77.86	76.16 ± 5.18
T_6	70.19-73.50	71.89 ± 5.87	73.67-77.14	75.45 ± 5.14
T_7	70.94-74.28	72.65 ± 3.35	75.01-78.54	76.82 ± 5.23
T_8	71.63-75.00	73.36 ± 8.50	75.22-78.77	77.04 ± 5.24
T_9	70.76-74.09	72.47 ± 9.60	73.84-77.32	75.63 ± 5.15
T_{10}	71.72-75.10	73.45 ± 3.01	75.79-79.36	77.62 ± 5.28
T_{11}	71.59-74.96	73.32 ± 2.76	74.89-78.42	76.70 ± 5.22
T_{12}	69.01-72.26	70.68 ± 2.52	72.36-75.77	74.11 ± 5.04
T_{13}	64.04-71.13	67.52 ± 1.48	65.82-73.12	69.40 ± 4.72
F test		NS		NS
S.Em ±		2.68		3.06
CD @5%		-		-
C.V(%)		-		-

NS- Non Significant; Values in columns represent mean ± SD (Deviation between pooled replication data); Range of five crops; DAP- Days After Pruning;

Crude Protein (%)

The results revealed that significant variations with respect to crude protein content of V1 mulberry leaf (Table 2). T_{10} (450 kg ha^{-1} DE + 4ml L^{-1} FSA) had the highest crude protein content of 22.50 ± 1.28 per cent, which is 1.93 % higher than the RDF control (20.57 ± 1.11 %)**.** Whereas, T_{11} (600 kg ha^{-1} DE + 4ml L^{-1} FSA) also showed a significant increase with 22.23 ± 0.40 per cent and the lowest protein content was registered in leaves harvested from T_{13} *i.e.,* without RDF application

plot in T_{13} (18.86 ± 1.05 %).

Application of silicic acid at higher concentration might have involved in the biosynthesis of cell wall components which could have enhanced the protein content in mulberry leaves. Higher protein yield was observed in treatment with the 450 kg ha^{-1} DE along with 4ml L^{-1} FSA (T_{10}) over control, which can be attributed to higher nitrogen content recorded in the same treatment. This may be due to Si influences cell wall components, such as pectic acid and protein. Similarly, increase in protein content was also noticed in wheat (Gong *et al*., 2003), with the application of sodium silicate by foliar application of silicon aqueous solution in paddy (Ahmad *et al*., 2013) and in soybean through silicic acid foliar spray (Shwethakumari and Prakash, 2018). Improved protein content in mulberry leaf samples may be due to beneficial effect of Si released from soil application of diatomaceous earth (DE), further created favourable soil physical and chemical properties which lead to accumulation of nutrients and higher N uptake and increased photosynthetic area which leads to enhanced photosynthetic rate and ultimately accumulation of nutrient in mulberry leaves. These results are in line with findings of Kadalli *et al*. (2017) who noticed higher protein and starch content in potato tubers with soil application of DE.

Carbohydrate (%)

The total carbohydrate content in mulberry leaves has varied significantly due to various levels of silicon application (Table 2). T_{11} (600 kg ha^{-1} DE + 4ml L^{-1} FSA) recorded highest carbohydrate content at 19.87 ± 0.19 per cent, which is 2.29 per cent higher than the RDF control (17.58 ± 0.63 %) followed by T_{10} (450 kg ha^{-1} DE + 4ml L^{-1} FSA) with 19.32 ± 0.68 per cent and lowest carbohydrate was noticed in T_{13} (13.55 ± 0.80 %) in non-treated plots. The enhanced carbohydrate status in the present study was attributed due to higher photosynthetic efficiency because of presence of higher chlorophyll levels. Similar observations were made by Ramachandra *et al*. (2008). The synergistic effect of Si on photosynthesis improved markedly the carbohydrate biosynthesis, supply of cell wall material and eventually the dry matter production, total sugars and glucose, corroborating the present study (Jeer *et al*., 2022).

Rajanna *et al.* (2000) reported that the mulberry raised with recommended NPK had significantly higher total soluble carbohydrates (17.61 %) and crude protein content (17.89 %). Similarly, Dhiraj and Kumar (2012) opined that the treatment of nitrogen and potassium recorded higher biochemical constituents in mulberry leaf which indicated a significant difference in total soluble protein, total reducing sugars and total chlorophyll.

Table 2: Effect of different levels of diatomaceous earth and silicic acid on crude protein, carbohydrate and total phenols of V1 mulberry leaves at 45th day after pruning

Treatments	Crude protein (%)		Carbohydrate (%)		Total phenols (mg GAE g dw $^{-1}$)	
	Range	Mean	Range	Mean	Range	Mean
T_1	20.09-21.15	20.59 ± 0.37	17.34-18.15	17.76 ± 0.86	13.47-14.11	13.80 ± 1.47
T_2	20.16-21.28	20.71 ± 0.77	17.49-18.31	17.91 ± 0.29	13.62-14.26	13.95 ± 1.05
T_3	20.34-21.41	20.84 ± 0.09	17.68-18.52	18.11 ± 0.45	13.84-14.49	14.17 ± 0.48
T_4	20.45-21.52	20.95 ± 1.30	17.94-18.78	18.37 ± 0.62	14.28-14.96	14.63 ± 0.83
T_5	20.79-21.88	21.30 ± 0.76	18.31-19.17	18.75 ± 2.41	14.67-15.36	15.02 ± 0.46
T_6	20.55-21.63	21.05 ± 0.77	17.89-18.74	18.33 ± 1.58	14.22-14.89	14.56 ± 0.52
T_7	20.91-22.01	21.42 ± 1.06	18.45-19.32	18.90 ± 0.51	14.49-15.17	14.84 ± 0.74
T_8	21.40-22.52	21.92 ± 0.58	18.61-19.48	19.06 ± 0.83	14.73-15.43	15.09 ± 0.35
T_9	20.68-21.77	21.19 ± 0.70	18.13-18.98	18.57 ± 0.55	14.42-15.10	14.77 ± 0.62
T_{10}	21.96-23.27	22.50 ± 1.28	18.86-19.75	19.32 ± 0.68	14.94-15.64	15.30 ± 0.34
T_{11}	21.79-23.09	22.23 ± 0.40	19.41-20.32	19.87 ± 0.19	15.10-15.81	15.46 ± 0.51
T_{12}	20.07-21.14	20.57 ± 1.11	17.17-17.98	17.58 ± 0.63	13.48-14.12	13.81 ± 0.86
T_{13}	18.17-19.74	18.86 ± 1.05	13.11-14.05	13.55 ± 0.80	12.03-13.36	12.68 ± 0.59
F test		*		*		*
S.Em ±		0.50		0.58		0.44
CD @5%		1.47		1.69		1.27
C.V(%)		4.14		5.53		5.23

*- Significant @ 0.05; Values in columns represent mean ± SD (Deviation between pooled replication data); Range of five crops; DAP- Days After Pruning;

Total phenols (mg GAE g^{-1} *dw*):

Phenolic compounds in mulberry leaves are important because of antioxidant, anti-inflammatory, and anti-proliferative properties which is essential for silkworm growth and development. Highest total phenols was noticed in T_{11} (600 kg ha^{-1} DE + 4ml L^{-1} FSA) (15.46 ± 0.51 mg GAE g^{-1} *dw*) which was significantly on par to T_{10} (450 kg ha^{-1} DE + 4ml L^{-1} FSA) (15.30 ± 0.34 mg GAE g^{-1} *dw*)

when compared to RDF control (T_{12}) (13.81 ± 0.86 mg GAE g^{-1} *dw*). The lowest phenol content was recorded in non-treated T_{13} (12.68 ± 0.59 mg GAE g^{-1} *dw*) plot.

Concordant to the present investigation, several studies have revealed the enhanced performance of crop in response to Si nutrition. Wasti *et al*. (2017) reported that fertilization with Si positively enhanced on yield and fruits quality traits in tomato. This enhancement effects of Si could be the sum of increasing the activity of many antioxidant enzymes, inhibiting H_2O_2 activity in addition to enhancement of chlorophyll content and photochemical efficiency and governing uptake and balance of K and Na due to enhanced water relations, membrane stabilization and altering the plant hormones such as auxin, cytokinin and abscisic acid (ABA). Photosynthesis is the physiological basis of biomass formation, which provides raw material and energy for the growth and development of plants. The Si nutrition proved to help in expression of photosynthesis-related genes and regulation of the photochemical process, thus promoting photosynthesis of tomato seedlings in turn contributing to crop yields The beneficial effect of Si in mulberry, a non-accumulator of Si suggests a possible involvement of Si in the physiological and biochemical parameters.

Bhavya *et al*. (2011) showed that the foliar application of Si along with boron significantly enhanced the quality parameters *viz.*, total soluble solids, acidity, total sugar, reducing sugar and non-reducing sugars of Bangalore blue grapes; Pallavi and Prakash (2021) studied the effect of soil drenching of silicic acid on yield, quality and nutrient content of tomato and revealed that, soil drenching of silicic acid @ 4 mL L^{-1} at 15, 30 and 45 days after planting (DAP) significantly increased the yield attributes quality parameters such as total soluble solids (TSS) and lycopene content of tomato, thus implying that soil drenching of silicic acid (@ 4 mL L^{-1}), as a novel way to enhance yield, quality and nutrient content of tomato which are in accordance with current study.

Chlorophyll content (mg/g)

Application of different levels of diatomite and silicic acid on V1 mulberry leaves exhibited significant influence in the chlorophyll content of leaf estimated on 45th day after pruning. Significantly highest chlorophyll ‘a’ content was observed in T_{11} (1.58 ± 0.05 mg g^{-1}) which was treated with combined application of DE @ 600 kg ha^{-1} DE + 4ml L^{-1} FSA followed by T_8 (1.57 ± 0.04 mg g^{-1}). The minimum chlorophyll ‘a’ content was recorded in T_{13} (absolute control) (1.17 ± 0.05 mg g^{-1}). Maximum chlorophyll ‘b’ content was shown in T_{11} **(**0.98 ± 0.03 mg g^{-1}) on 45th DAP which was found on par with T_8 (600 kg ha^{-1} DE soil application along with 2ml L^{-1} FSA) (0.95 ± 0.02 mg g^{-1}) and least was recorded in T_{13} (absolute control) (0.65 ± 0.03 mg g^{-1}). Total chlorophyll content among

different treatment combinations varied between 1.73 mg g^{-1} to 2.61 mg g^{-1}**.** Significantly highest total chlorophyll on 45th DAP was observed in T_{11} (2.56 ± 0.02 mg g^{-1}) followed by T_8 (2.53 ± 0.11 mg g^{-1}) and T_{10} (2.49 ± 0.09 mg g^{-1}) when compared to RDF Control (2.27 ± 0.08 mg g^{-1}) (Table 3).

Table 3: Effect of different levels of of diatomaceous earth and silicic acid on chlorophyll content of V1 mulberry leaves at 45th day after pruning

Treatments	Chlorophyll 'a' (mg g^{-1})		Chlorophyll 'b' (mg g^{-1})		Total chlorophyll (mg g^{-1})	
	Range	Mean	Range	Mean	Range	Mean
T_1	1.44-1.50	1.47 ± 0.16	0.79-0.83	0.81 ± 0.09	2.23-2.34	2.29 ± 0.11
T_2	1.45-1.52	1.48 ± 0.11	0.79-0.83	0.81 ± 0.06	2.24-2.35	2.30 ± 0.04
T_3	1.46-1.53	1.50 ± 0.05	0.81-0.84	0.83 ± 0.03	2.27-2.38	2.32 ± 0.06
T_4	1.48-1.55	1.51 ± 0.09	0.82-0.86	0.84 ± 0.05	2.30-2.41	2.35 ± 0.08
T_5	1.50-1.57	1.53 ± 0.05	0.83-0.87	0.85 ± 0.03	2.33-2.44	2.39 ± 0.31
T_6	1.49-1.56	1.52 ± 0.05	0.82-0.86	0.84 ± 0.03	2.31-2.42	2.36 ± 0.20
T_7	1.51-1.58	1.54 ± 0.08	0.86-0.90	0.88 ± 0.04	2.37-2.48	2.42 ± 0.07
T_8	1.54-1.61	1.57 ± 0.04	0.93-0.97	0.95 ± 0.02	2.47-2.58	2.53 ± 0.11
T_9	1.50-1.57	1.53 ± 0.06	0.84-0.88	0.86 ± 0.04	2.34-2.45	2.40 ± 0.07
T_{10}	1.52-1.59	1.55 ± 0.03	0.92-0.98	0.94 ± 0.02	2.43-2.55	2.49 ± 0.09
T_{11}	1.54-1.61	1.58 ± 0.05	0.94-1.02	0.98 ± 0.03	2.50-2.61	2.56 ± 0.02
T_{12}	1.44-1.50	1.47 ± 0.09	0.78-0.82	0.80 ± 0.05	2.22-2.32	2.27 ± 0.08
T_{13}	1.11-1.23	1.17 ± 0.05	0.62-0.68	0.65 ± 0.03	1.73-1.92	1.82 ± 0.11
F test		*		*		*
S.Em ±		0.05		0.03		0.07
CD @5%		0.13		0.07		0.22
C.V(%)		5.30		5.19		5.50

*- Significant @ 0.05; Values in columns represent mean ± SD (Deviation between pooled replication data); Range of five crops; DAP- Days After Pruning;

Chlorophyll plays an important role in the photosynthesis. The nitrogen is an essential constituent of chlorophyll as it harvests solar energy and aids in photosynthesis (Sujathamma and Dandin, 2000). Photosynthetic efficiency is indicated by the increased amount of chlorophyll content in leaves; thus, it can be used as one of the criteria for photosynthetic rate quantification in mulberry.

Janardhan *et al*. (2008) reported that more amount of nitrogen (614 kg ha^{-1}) combined with recommended dose of FYM and fertilizers compared to other treatments (0 to 250 kg ha^{-1}) lead to more chlorophyll content and higher mulberry leaf yield.

Exogenously applied DE was also shown to consequentially improve photosynthesis and yield related parameters in wheat crop (Jeer *et al*., 2021), chlorophyll content of leaves in pomegranate (Kalatippi *et al*., 2017). Increase in chlorophyll content may be due to application of DE and application of foliar silicic acid which attributed to the availability of N, Fe and Mg necessary for the synthesis of chlorophyll, which improved net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and the contents of photosynthetic pigments. Addition of 0.5 mM of Si to the nutrient solution without iron, initially or continuously during the experiment, prevented the chlorophyll degradation of soybean (Gonzalo *et al*. 2013). Similar results were reported for chlorophyll content with the application of Si in different crops, such as cucumber (Feng *et al*., 2010), potato (Pilon *et al*., 2013), hydroponically grown soybean (Lee *et al*., 2010) and broad bean (Ghasemi *et al*., 2013) through soil Si application.

It was also suggested that the main role of Si in increasing the chlorophyll levels arises from its maintenance of the chloroplast ultrastructure concomitant with the enhancement of biosynthetic enzymes or depression of chlorophyll- degrading enzymes. The decrease in chlorophyll content in control plants may be due to formation of proteolytic enzymes such as chlorophyllase, which is responsible for chlorophyll degradation (Savvas *et al*., 2009). These results support with present findings by increase in chlorophyll content may be due to application of DE and application of foliar silicic acid.

Crude fibre (%)

Crude fibre determination is used to estimate the quality of contents in leaf which constitutes their least digestible fraction. Significant variation was noticed among different treatments with regard to crude fibre content of V1 mulberry leaf harvested on 45th DAP. Higher DE doses combined with 4ml L^{-1} FSA, T_{10} recorded significantly highest crude fibre content (12.59 ± 0.42 %), which is 2.69 % increment over T_{12} - RDF control (9.90 ± 0.62 %). The results were found on par to T_{11} (600 kg ha^{-1} DE + 4ml L^{-1} FSA) (12.07 ± 0.27 %) and T_8 (600 kg ha^{-1} DE + 2ml L^{-1} FSA) (11.79 ± 0.28 %). However, the lowest crude fiber content (8.32 ± 0.39 %) was registered in the leaves obtained from the treatment T_{13} (Absolute control) (Table 4).

Table 4: Effect of different levels of diatomaceous earth and silicic acid on crude fibre, ash and crude fat content of V1 mulberry leaves at 45th day after pruning

Treatments	Crude fibre (%)		Ash content (%)		Crude fat (%)	
	Range	Mean	Range	Mean	Range	Mean
T_1	9.65-10.10	9.88 ± 1.05	8.31-8.70	8.51 ± 0.15	3.93-4.12	4.03 ± 0.20
T_2	9.72-10.18	9.96 ± 0.75	8.45-8.84	8.65 ± 0.32	4.01-4.20	4.11 ± 0.07
T_3	10.25-10.74	10.50 ± 0.36	8.53-8.94	8.74 ± 0.04	4.14-4.33	4.24 ± 0.11
T_4	10.57-11.07	10.83 ± 0.61	8.58-8.99	8.79 ± 0.31	4.23-4.43	4.33 ± 0.15
T_5	10.81-11.32	11.07 ± 0.34	8.71-9.12	8.92 ± 0.51	4.36-4.57	4.47 ± 0.58
T_6	10.68-11.19	10.94 ± 0.39	8.64-9.05	8.85 ± 0.44	4.25-4.45	4.35 ± 0.37
T_7	10.90-11.41	11.16 ± 0.56	9.08-9.51	9.07 ± 0.30	4.43-4.64	4.54 ± 0.12
T_8	11.51-12.05	11.79 ± 0.28	8.86-9.27	9.30 ± 0.25	4.51-4.72	4.62 ± 0.20
T_9	10.76-11.27	11.02 ± 0.46	8.90-9.32	9.12 ± 0.33	4.24-4.44	4.34 ± 0.13
T_{10}	12.29-12.87	12.59 ± 0.42	9.16-9.59	9.38 ± 0.17	4.65-4.87	4.76 ± 0.17
T_{11}	11.79-12.34	12.07 ± 0.27	9.34-9.78	9.57 ± 0.21	4.59-4.81	4.70 ± 0.05
T_{12}	9.67-10.12	9.90 ± 0.62	8.44-8.83	8.64 ± 0.47	4.05-4.24	4.15 ± 0.15
T_{13}	7.89-8.77	8.32 ± 0.39	7.39-8.21	7.79 ± 0.43	2.82-3.13	2.97 ± 0.18
F test		*		*		*
S.Em ±		**0.32**		**0.19**		**0.14**
CD @5%		**0.93**		**0.56**		**0.40**
C.V(%)		**5.11**		**3.78**		**5.56**

*- Significant @ 0.05; Values in columns represent mean ± SD (Deviation between pooled replication data); Range of five crops; DAP- Days After Pruning;

Crude Fat (%)

Crude fat is used to estimate the lipid fraction to assess the nutritional value of a sample. The results revealed that significant differences with respect to crude fat content among the different treatments. Higher fat content (4.76 ± 0.17 %) was found in the leaves obtained from the treatment T_{10} (450 kg ha^{-1} DE + 4ml L^{-1} FSA) which was on par with that of T_{11} (600 kg ha^{-1} DE + 4ml L^{-1} FSA) (4.70 ± 0.05 %), T_8 (600 kg ha^{-1} DE + 2ml L^{-1} FSA) (4.62 ± 0.20 %) and T_7 (450 kg ha^{-1} DE + 2ml L^{-1}

FSA) (4.54 ± 0.12 %). The lowest fat content (2.97 ± 0.18 %) was registered in leaves harvested from (T_{13}) without RDF application plots (Table 4).

Ash content (%)

Ash content in mulberry leaf is important to determine the inorganic matter remained after ashing organic matter. Ash value is useful in determining purity of sample. The ash content in leaves varied significantly due to varied levels of diatomite and silicic acid application. The highest ash content was found in T_{11} (600 kg ha^{-1} DE + 4ml L^{-1} FSA) with 9.57 ± 0.21 per cent, showing an increase of 0.93 % over RDF control (8.64 ± 0.47 %) which was on par with T_{10} (450 kg ha^{-1} DE + 4ml L^{-1} FSA) (9.38 ± 0.17%) and T_8 (600 kg ha^{-1} DE + 2ml L^{-1} FSA) (9.30 ± 0.25 %). The lowest ash content (7.79 ± 0.43 %) was observed in T_{13} (Table 4).

Foliar application of silicic acid in mulberry might have enhanced the nucleic acid metabolism in plants, which resulted in higher crude fat, crude fibre, ash and protein yield in mulberry leaf samples. These results are supported by Shwethakumari and Prakash (2018) which resulted in higher oil content in soybean seeds. Application of DE as Si source increased growth, yield and quality of several horticultural crops such as pomegranate (Swamy *et al*., 2017), tomato (Ashok *et al*., 2017) and potato (Kadalli *et al*., 2017).

CONCLUSION:

Enrichment of the mulberry leaves shall lead to the increased productivity of leaves which are essential for the silkworm crop productivity also. Among different treatments examined, combined application of 450 kg ha^{-1} DE + 0.4 % silicic acid foliar spray (T_{10}) recorded maximum moisture content, crude protein, carbohydrate, total phenols, total chlorophyll, crude fibre and other quality parameters. However, T_{11} (600 kg ha^{-1} DE + 0.4 % SA) and T_8 (600 kg ha^{-1} DE + 0.2 % SA) exhibited on par results with T_{10} for all the leaf biochemical attributes of mulberry. Present study provides sufficient evidence on beneficial effects of DE and silicic acid in improving leaf quality and biochemical parameters of mulberry through its high bioavailability of silicon and enhanced uptake of nutrients by plants.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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1.

2.

3.

Author's contribution:

[1]Conceptualization, Guidance, data analysis, draft writing, review and editing manuscript
[2,3]Supervision, designing of experiment, contribution of experimental materials and lab space;

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