PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.4910

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RESEARCH ARTICLE

Unravelling the biochemical and phytochemical responses of sapota (*Manilkara zapota* L.) P. Royen cv. DHS-1 on foliar feeding of plant growth regulators

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OPEN ACCESS

ARTICLE HISTORY
Received: 01 September 2024
Accepted: 16 December 2024
Available online
Version 1.0 : 20 February 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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CITE THIS ARTICLE
Gautam D, Acharya GC, Swain SC, Samal S, Samant D, Bhat NV, Sandeep, Mohapatra S. Unravelling the biochemical and phytochemical responses of sapota (*Manilkara zapota* L.) P. Royen cv. DHS-1 on foliar feeding of plant growth regulators. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.4910

Abstract

Foliar application of plant growth regulators (PGRs) is one of tactic for plant nourishment which directly enhances nutrient uptake, leading to a more balanced nutrient supply and improved accumulation in plant ultimately improving quality and yield. The current research covers to see the response of biochemical and phytochemical parameters of sapota cv. DHS-1; to the pre-harvest spray of the PGR i.e. naphthalene acetic acid (NAA) (50 and 100 ppm), 2-chloro-4-pyridyl) N′-phenyl urea (5 and 7.5 ppm), salicylic acid (SA) (150 and 300 ppm) and homo brassinolide (10 and 15 ppm); sprayed at flowering, pea stage and marble stage of plant growth. Treatments were imposed on 24-years-old, uniformly grown, sapota trees, planted at 10m x 10m spacing, at Central Horticultural Experiment Station, (ICAR-IIHR), Bhubaneswar during 2022 and 2023. In general, application of PGRs were found affecting the fruit quality significantly over control. However, application of NAA and CPPU performed better at higher dose. The treatment NAA at 100 ppm resulted in highest total soluble solids (°Brix), TSS: acid, ascorbic acid content, sugar content and minimum titratable acidity (%). Further, application of NAA at 100 ppm found to be most effective in boosting total flavonoids content, total phenol content and antioxidant activity. The possible correlations among these parameters were discussed which showed that the bio-active compounds such as vitamin C, phenol and flavonoid contributed towards the anti-oxidant capacity of fruit. Therefore, application of PGRs showed to effectively augment fruit quality by improving both biochemical and phytochemical properties when compared to control.

Keywords

biochemical; foliar application; phytochemical; plant growth regulators

Introduction

Sapota (*Manilkara zapota* L.) P. Royen is one of a significant tropical fruit from Sapotaceae family. It can be grown in tropical regions across the globe. Sapota is cherished in many countries for its sweet, soft, luscious and delightful fruits. In countries like Central and South America, the bark and fruits are utilized to produce milky latex for making gutta parcha, which is also a key component in chewing gum (chickle). The fruit pulp contains sapotin, used medicinally to reduce fever, along with saponin, fixed oils and bitter alkaloids (1).

In India, chiku ranks as the sixth most vital commercial fruit crop, after mango, banana, citrus, apple and guava. Commercial growing sapota considered as an important fruit crop due to its high nutritional value, economic benefits and adaptability to various climatic conditions. Sapota cultivation provides income opportunities for farmers and supports rural economies. Its adaptability to diverse soil types and climates also makes it a viable crop in different regions of India. Sapota is predominantly cultivated in several major states of India, known for their favorable climatic conditions. Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu, Gujarat, West Bengal, Odisha and Haryana contribute to the production, reflecting the fruit's adaptability across various regions of the country. These states collectively support the robust sapota industry, vital for both local consumption and commercial distribution. Odisha, located on the eastern coast of India, cultivates sapota in both coastal and interior districts, including Jagatsinghpur, Balasore, Khurda, Kendrapara, Puri, Cuttack and Ganjam.

From a nutritional perspective, sapota is rich in minerals, vitamins and phytonutrients, including phenols, flavonoids, fatty acids, ascorbic acid and antioxidants, which are beneficial for disease prevention (2). Therefore, investigating the effect of foliar feeding of PGRs on nutrient content and biochemical properties of sapota can be a crucial aspect in the field of sapota fruit research.

PGRs are essential for plant growth and development; typically function effectively at very low concentrations. PGRs operate within plant cells to either stimulate or hinder specific enzymes or their synthesis, thereby regulating plant metabolism. They trigger changes in physiological and biochemical traits, which ultimately affects the quantitative and qualitative performance of the crop. Foliar application of PGRs is an effective strategy for direct plant feeding through leaves, enabling quick uptake regardless of soil conditions (3). This method enhances nutrient balance and accumulation in crops, leading to improvement in fruits crops in terms of both quantity and quality. However, the effectiveness of foliar application can be influenced by several factors such as the type of nutrient or PGR, the concentration and formulation of the spray solution, environmental conditions (e.g., temperature, humidity and light) and the plant species and growth stage.

The findings from this study could significantly enhance farmers' proficiency in utilizing PGRs at optimal dosages and timings, thereby amplifying the benefits. Furthermore, it could help in boosting the individual and household nutrition and contribute to overall health benefits through sapota consumption. Therefore, the current study was designed to evaluate the response of phytonutrients like, total phenol, total flavonoids, free radical scavenging activity and different fruit quality parameters to pre harvest foliar spray of PGRs applied (each year) at flowering, fruit setting (pea stage) and one month after fruit setting (marble stage) stage of sapota tree growth cultivated on the eastern coast of India.

Materials and Methods

The current study was carried out at the research farm of the Central Horticultural Experiment Station (ICAR-IIHR) in Bhubaneswar, India, situated at a latitude of 20°15' N, longitude of 85°15' E and an altitude of approximately 42 meters above sea level. The investigation spanned two growing seasons in 2022 and 2023. The region experiences a tropical wet and dry climate, with an average annual temperature of 27.2°C, relative humidity of 78.2% and an annual rainfall of around 1500 mm. The soil in the experimental site was characterized by sandy loam in nature, consisting of 17.25% silt, 73.40% sand and 9.35% clay. It was highly acidic with a pH of 4.49 with available nitrogen (186.74 kg/ha) comprising low level of organic carbon (0.24%) and phosphorus (12.15 kg/ha), while potassium levels were moderate (181.51 kg/ha).

The research field was maintained according to established agricultural practices and protocols, ensuring proper care and management throughout the study. This included regular monitoring, application of necessary treatments and adherence to best practices for optimal field conditions. Healthy, uniformly growing sapota plants were randomly selected for the experiment and various doses of plant growth regulators (PGRs) were applied. The nine treatments of the following concentrations: NAA at 50 (T_1), 100 (T_2), CPPU at 5 ppm (T_3), 7.5 ppm (T_4), SA at 150 ppm (T_5), 300 ppm (T_6) and Homobrassinolide at 10 ppm (T_7), 15 ppm (T_8) keeping one treatment control (water spray) i.e., T_9 were incorporated through foliar spraying at the at flowering, fruit setting (pea stage) and one month after fruit setting (marble stage) each year. The experiment was carried out in an RCBD design where each treatment was repeated three times and each replication containing four plants. The fruits were picked at full mature stage. Fruit quality attributes were analysed at central laboratory in Central Horticultural Experiment Station, (ICAR-IIHR), Bhubaneswar as per the brief procedure given below:

1. Determination of biochemical attributes of sapota

a. Total soluble solids (˚B) Acidity (% citric acid), TSS: Acid ratio and sugars (reducing, non-reducing and total)

Total soluble solids (TSS) of beverage were determined with the help of digital refractometer (ATAGO, Japan) and the values were presented in °Brix after making corrections for difference in temperature. The acidity was determined using phenolphthalein indicator by titrating a known volume of sample against 0.1 N NaOH (4). Appearance of light pink colour was taken as an end point. The acidity was represented as per cent citric acid. TSS: acid ratio of fruit pulp was determined by dividing TSS with acidity of fruit pulp. Total sugars, reducing sugars and non-reducing sugars of sapota fruit samples were estimated copper titration method (5). Determination of non-reducing sugar was carried out by deducting the value of reducing sugar value from the total sugar value.

2. *Determination of phytochemical attributes of sapota*

b. *Vitamin C, phenol, flavonoids and antioxidant activities* : Estimation of ascorbic acid is based on the principle that the dye showing blue colour in alkaline solution and red colour in acid solution is reduced by ascorbic acid to a colourless form. Assay method was followed to calculate the ascorbic acid (vitamin C) (4). FRAP (ferric reducing antioxidant potential) method was used for the estimation of total antioxidants (6). Total flavonoid in the methanol extract and total phenols were estimated as per referred scientist (7, 8).

Statistical analysis

The data for both the years was pooled to evaluate comparative effect of PGRs on fruit quality parameters and phytonutrients. Recorded data of various parameters were statistically analysed using variance technique (9). Tukeys' HSD test ($p \leq 0.05$) was used for comparing the differences in means. Pearson correlation coefficients among different variables of sapota as regulated by use of growth regulators was worked out using KAUGRAPES software.

Results and Discussion

The significant influence of foliar feeding of PGRs on biochemical properties of sapota fruit is presented in Table 1 which revealed that maximum score of quality parameters such as TSS (22.23 °B), ascorbic acid (12.66%), reducing sugar (11.67%), total sugar (16.32%), non reducing sugar (4.98%) and lowest acidity (0.17 %), was observed under application of T_4 (NAA at 100 ppm), which was significantly higher than other treatments, followed by T_2 (CPPU 7.5 ppm). Whereas the minimum was recorded under control. It shows that foliar application of NAA at 100 ppm gave the best results among all the treatments.

The application of NAA during the fruit development stage led to increased total soluble solids (TSS), sugar content and reduced acidity. This may be due to the fact that once a fruit is set and begins to grow, it becomes an active metabolic sink, diverting the flow of food materials from vegetative organs to the fruit, even at the expense of vegetative growth (10). These findings are in close agreement with the results obtained in sapota (11) and in plum (12).

The rise in TSS as influenced by NAA treatment might be attributed to the enhanced gathering of metabolites and the rapid change of starch into soluble sugars throughout fruit development phase. The same outcomes are in line with other studies conducted by various researchers (12-16).

The application of NAA may lead to a reduction in fruit acidity, possibly because it hastens the ripening process, allowing for the earlier consumption of acids through post-harvest respiratory processes in sapota fruits (17). As the fruit matures earlier, the acids that would normally be present in higher concentrations in unripe fruit are metabolized more quickly, leading to a reduction in overall acidity. However, no significant influence of PGR spray on acidity of fruit was recorded in the investigation. The results are supported by the other researchers as well (14, 18, 19). A consistent acid content and upsurge in TSS resulted into a rise in TSS:acid ratio. The results are in line with result obtained from a study conducted in the year 2018 (12) in sapota.

The potential increase in reducing sugars observed with the use of NAA in sapota fruit could be attributed to the accelerated break down of starch into simpler sugars and their subsequent utilisation throughout the fruit development process. This mechanism suggests that NAA treatment may enhance the enzymatic activity responsible for starch breakdown, leading to a higher concentration of reducing sugars in the fruit. These results are closely supported by the results obtained by other researchers in sapota (12) and in guava (20).

The rise in total sugar content observed with the application of NAA in sapota fruit may be attributed to the accelerated ripening process and the enhanced activity of hydrolytic enzymes. These enzymes perform a key role in breaking down complex and composite carbohydrates into simpler sugars molecules, leading to an increase in the overall sugar content of the fruit. As the fruit ripens more quickly, the conversion of starches and other polysaccharides into sugars is expedited, resulting in a higher sugar content. These findings are in close agreement with the results reported in sapota (12) and in pomegranate (21).

Table I. Pooled data on impact of foliar feeding of plant growth regulators on biochemical parameters of sapota cv. DHS-1

Treatments	TSS	Acidity	TSS: Acid	total sugar	Reducing sugar	Non reducing sugar
	(°B)	(%)		(%)	(%)	(%)
T_1	19.81±0.25^{d}	0.189±0.001^{e}	104.53±2.4^{d}	15.53±0.12^{b}	11.02±0.67^{bc}	4.51±0.54^{c}
T_2	21.77±0.21^{b}	0.180±0.00^{f}	119.96±2.5^{b}	16.08±0.21^{a}	11.24±0.75^{ab}	4.84±0.54^{b}
T_3	20.45±0.39^{c}	0.180±0.001^{f}	113.29±3.4^{c}	15.73±0.12^{b}	11.13±0.66^{ab}	4.60±0.54^{c}
T_4	22.23±0.18^{a}	0.169±0.002^{g}	131.95±2.7^{a}	16.32±0.30^{a}	11.35±0.77^{a}	4.98±0.47^{a}
T_5	17.24±0.17^{h}	0.230±0.001^{b}	74.80±1.43^{h}	14.43±0.30^{de}	10.63±0.84^{de}	3.81±0.54^{e}
T_6	17.61±0.11^{g}	0.219±0.001^{c}	80.23±1.29^{g}	14.26±0.22^{ef}	10.50±0.76^{e}	3.77±0.54^{e}
T_7	18.50±0.18^{f}	0.211±0.001^{d}	87.04±1.70^{f}	14.66±0.26^{cd}	10.81±0.80^{cd}	3.85±0.54^{de}
T_8	19.22±0.17^{e}	0.209±0.001^{d}	91.75±1.74^{e}	14.76±0.28^{c}	10.85±0.82^{cd}	3.91±0.54^{d}
T_9	16.46±0.22^{i}	0.241±0.001^{a}	67.86±1.50^{i}	14.02±0.15^{f}	10.43±0.69^{e}	3.60±0.54^{f}
Tukey's HSD at (p<0.05)	0.32	0.004	3.05	0.30	0.27	0.20

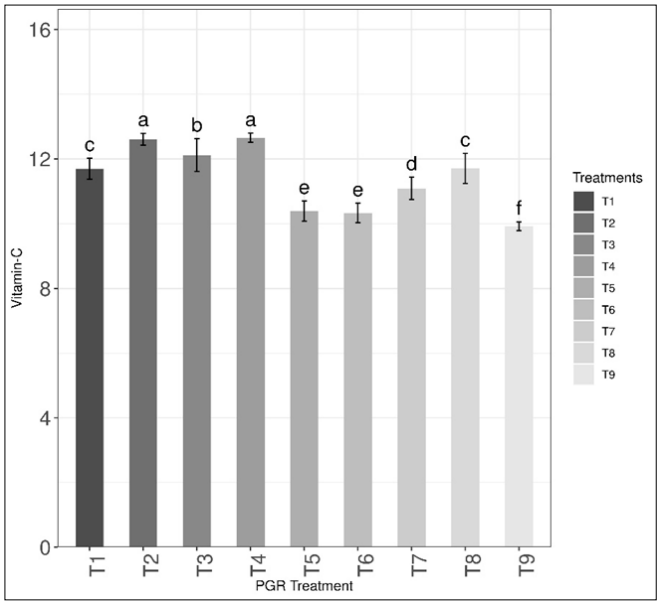
T_1: CPPU 5 ppm; T_2: CPPU 7.5 ppm; T_3: NAA 50 ppm; T_4: NAA 100 ppm; T_5: SA 150 ppm; T_6: SA 300 ppm; T_7: Homobrassinolide 10 ppm; T_8: Homobrassinolide 15 ppm; T_9: Control (Water spray)

The values marked with the same alphabets are non-significant at p>0.05

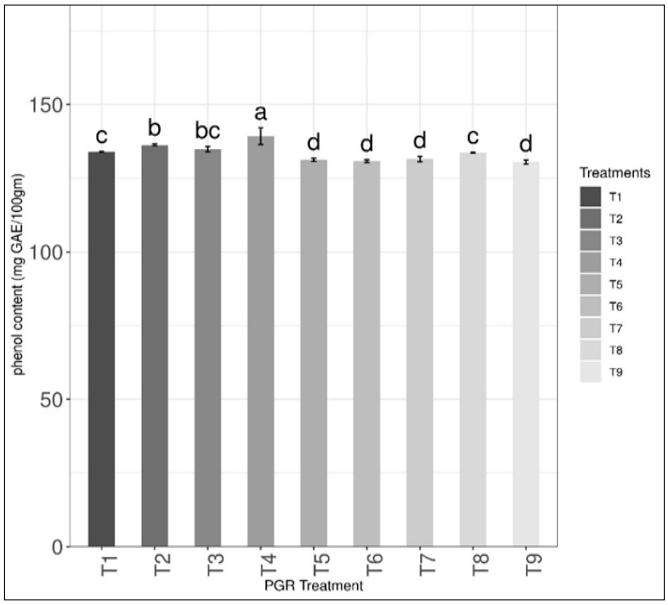
The significant influence of foliar feeding of PGRs on phytochemical properties of sapota fruit is presented in Fig. 1 (A-D) which revealed that maximum score of quality parameters such as vitamin C (12.66%), total phenol content (139.26 mg GAE/100gm FW), total flavonoid content (29.59 mg QE /100g FW) and antioxidant activity (22.39 mM Fe II/100g FW) was observed under application of T_4 (NAA at 100 ppm), which was significantly higher than other treatments, followed by T_2 (CPPU 7.5 ppm). Whereas the minimum was recorded under control.

The potential increase in ascorbic acid (vitamin C) with the use of NAA might be due to the continuous synthesis of glucose-6-phosphate during the course of the growth and development of the fruit, which is considered a precursor of vitamin C (22). Additionally, PGRs spray may enhance cell size and intercellular spaces, along with the accumulation of sugars, water and other soluble solids in greater amounts due to the increased translocation of metabolites towards the fruits. This accumulation can raise the osmotic pressure, leading to the increased synthesis and accumulation of organic acids, including ascorbic acid (23). The similar results have been observed in a recent study conducted in daisy mandarin (23) and pomegranate (24).

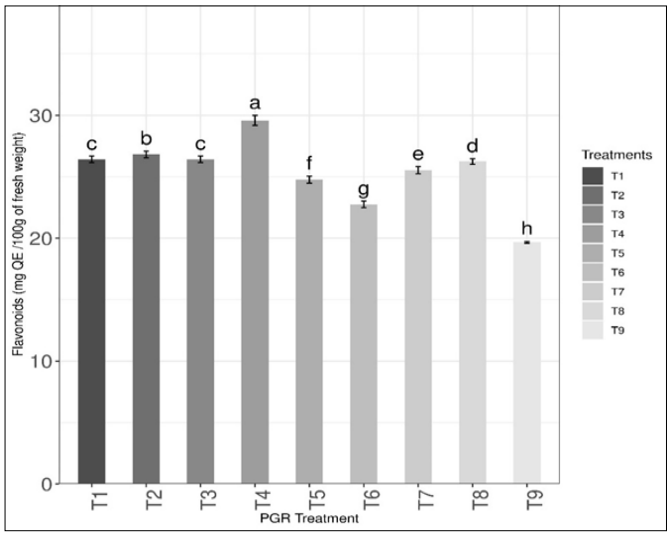
All PGR treatments significantly increased total flavonoids content and phenols content compared to control. Auxins are known to regulate the metabolism of phenolics and flavonoids by increasing the enzymatic activities such as chalcone synthase (CHS) and phenylalanine ammonia-lyase (PAL). These enzymes are key player in the biosynthesis pathway of phenolics and flavonoids compounds (25). The findings revealed that fruits which were treated with PGRs possessed a more antioxidant capacity compared to those in the untreated control fruits. NAA, may influenced the plants by activating abiotic stress mechanism and boosting the production of antioxidant compounds (26). These findings are consistent with another study which indicated that flavonoid and phenolic substances may be the primary factors prevailing antioxidant activity in the samples of wax apple fruit (25).



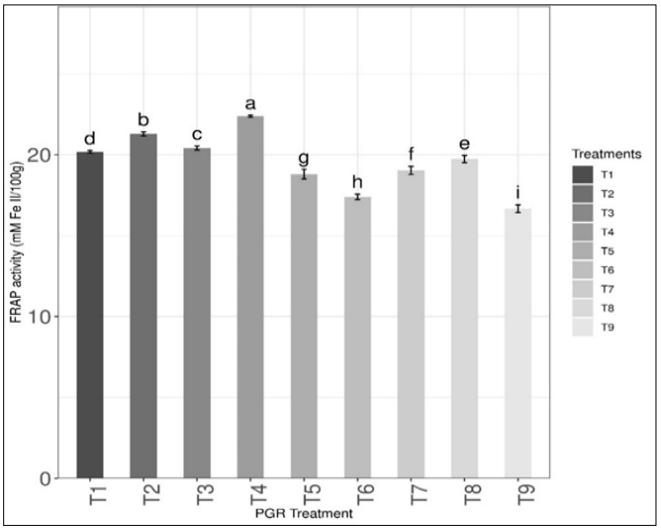
A. Vitamin C content (% citric acid).



B. Total phenol content (mg GAE 100gm FW).



C. Total flavonoid content (mg QE /100g FW).



D. Antioxidant activity (mM Fe II/100g FW).

Fig. 1: Phytochemical parameters as affected by foliar feeding of plant growth regulators in sapota cv. DHS-1. (A-D).

Bars show standard errors (±). Within a bar, different lowercase shows a significant difference P≤0.05 between treatments.

Correlation study between antioxidant activity and bioactive compounds such as phenols, flavonoids and vitamin C in sapota fruit

Pearson correlation coefficient analysis was conducted on pooled data to examine the relationships between antioxidant activity and bioactive compounds of sapota fruit as affected by use of growth regulators. It is clear from the data illustrated in the Fig. 2 that there is substantial positive correlation between antioxidant activity and bioactive compounds such as phenols, flavonoids and vitamin C of sapota fruit. Therefore, the correlation studies indicated that the bio-active compounds like vitamin C, phenol and flavonoid contribute towards the anti-oxidant capacity of fruit in sapota fruit. Many scientists reported positive correlation between antioxidant activity and bioactive compounds such as phenols, flavonoids and vitamins in fruits (27-29).

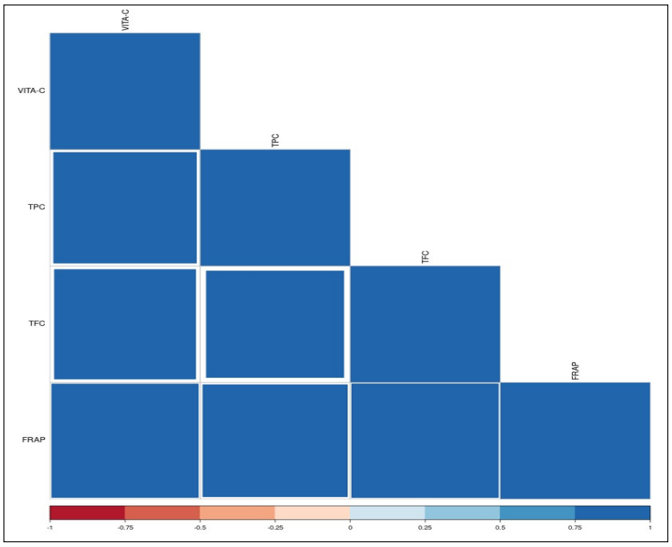


Fig. 2. Correlation study among vitamin C content, total phenol content, total flavonoid content and antioxidant activity of sapota fruit pulp.

TPC- total phenol content, TFC- total flavonoid content, FRAP antioxidant activity.

Conclusion

Therefore, from two year study it can be concluded that foliar feeding of PGRs, particularly NAA at 100 ppm, significantly enhances the biochemical and phytochemical properties of sapota fruit, leading to improved fruit quality. Key findings include increased total soluble solids, reduced acidity and boosted antioxidant activity and flavonoid content. These results underscore the potential of PGRs in enhancing sapota cultivation, offering a promising avenue for improving fruit quality and nutritional value.

Acknowledgements

The authors acknowledge the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore for granting permission for this study and offering support during the collection and analysis of insect samples. Authors extend sincere gratitude to the Director of ICAR-IIHR, Bengaluru and Vice-Chancellor, OUAT, Bhubaneswar, for their invaluable support and providing the essential facilities during the research period. Their assistance has greatly contributed to the success of this study.

Authors' Contributions

All the authors contributed significantly to the research work, including the design of the experiment, data collection, statistical analysis, interpretation of results and manuscript preparation. The conceptualization of the research was carried out by DG and GCA. The experimental design was formulated by DG, GCA and DS. The experimental materials were provided by GCA and DS. The execution of field and laboratory experiments, along with data collection, was conducted by DG, GCA, DS and NVB. The data analysis and technical guidance were provided by DG, SCS, SS, S and SM. The manuscript was prepared by DG, GCA, DS and NVB, while S and SM contributed to modifications and coordination.

Compliance with Ethical Standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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