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Editor-in-Chief

Dr. Subhash Kumar

Assistant Professor & HOD, Department: Botany,
CCR (PG) College, Muzaffarnagar,
Email: editor.agriphytologica@gmail.com
Contact No. +91-9837991612

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Agri Phytologica Insights is an international, peer-reviewed, open-access, multidisciplinary scholarly journal published by Learning Media Publication, Meerut, Uttar Pradesh, India. Established in 2026, the journal aims to provide an academic platform for the dissemination of original research, scientific findings, innovative methodologies, and critical reviews in agricultural sciences, plant sciences, biological sciences, environmental sciences, biotechnology, and related interdisciplinary fields.

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- Support research addressing food security, biodiversity conservation, environmental protection, and sustainable rural development.
- Provide an accessible platform for researchers, academicians, and practitioners to share scientific findings.
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- Agricultural Applications of Emerging Technologies

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- **First issue:** June
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Editorial Contact

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Patron
Dr. Devendra Kumar
(Principal Scientist(Rtd.))
Department of Crop Physiology, Biochemistry and Post Harvest Technology
ICAR-CPRI Regional Station, Modipuram, Meerut, Uttar Pradesh, India
Research Areas: Plant Stress Physiology, Ecophysiology, Biodiversity
Email: devkmalik@gmail.com

Editor-in-Chief
Dr. Subhash Kumar
Assistant Professor & HOD
Department of Botany, CCR (PG) College, Muzaffarnaga
Research Areas: Plant Stress Physiology, Ecophysiology, Biodiversity
Email: editor.agriphytologica@gmail.com
Contact No. : +91-9837991612

Managing Editor
Mr. Devansh Vikal
Email: agriphytologica@gmail.com
Mobile: +91 8449001390

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Prof. Dr. Ni Luh Suriani, S.Si., M.Si.
Professor
Department: Natural Sciences & Mathematics
Institution: Udayana University, Bali, Indonesia
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Dr. Yuwalee Unpaprom
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Department: Biotechnology
Institution: Maejo University, Chiang Mai, Thailand
Email: yuwalee@mju.ac.th
yuwaleeun@gmail.com
Research Areas: Functional Food, Bioactive Compound Extraction, Anti-aging Serum, Algae, Biomass, Biofuels

Editors

Dr. Dinesh Kumar (Editor)
Designation: Principal Scientist (Biochemistry)
Department: Quality & Basic Sciences
ICAR–Indian Institute of Wheat & Barley Research, Karnal, Haryana, India
Email: dinesh.kumar3@icar.org.in

Dr. Bandana
Designation: Senior Scientist
Department: Crop Physiology, Biochemistry & Post Harvest Technology
Institution: ICAR–Central Potato Research Institute, Regional Station, Modipuram, Meerut, Uttar Pradesh, India
Email: Bandana.biochem@gmail.com

Dr. Sandeep Kumar
Designation: Associate Professor
Department: Botany
Institution: Meerut College, Meerut, Uttar Pradesh, India
Email: sandeep.kumar@mcm.ac.in

Dr. Sushil Kumar
Designation: Associate Professor
Department: Botany
Institution: Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India
Email: sushil.kumar.bot@ccsuniversity.ac.in

Dr. Rohit Kundu
Designation: Scientist
Department: Division of Sample Surveys
Institution: ICAR–Indian Agricultural Statistics Research Institute, Pusa, New Delhi, India
Email: rohit.kundu1@icar.org.in

Dr. Tarun Verma
Designation: Assistant Professor
Department: Dairy Science & Food Technology
Institution: Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India
Email: tarunverma@bhu.ac.in

Prof. Awanish
Designation: Professor
Department: Botany
Institution: Mahatma Gandhi (P.G.) College, Gorakhpur, Uttar Pradesh, India
Email: awanish.bot@mgpgc.ac.in

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Designation: Scientist
Department: Social Science Research & Capacity Development Unit
Institution: ICAR–Central Institute for Research on Buffaloes (CIRB), Hisar, Haryana, India
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Dr. Sudhir Kumar Rajpoot
Designation: Assistant Professor
Department: Agronomy
Institution: Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India
Email: sudhir.k.rajpoot@bhu.ac.in

Dr. Sandeep Kumar
Designation: Assistant Professor
Department: Botany & Microbiology
Institution: Gurukula Kangri (Deemed to be University), Haridwar, Uttarakhand, India
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SPATIAL MULTI-SCALE FRACTAL ANALYSIS OF TOMATO LEAF STRUCTURAL DEGRADATION UNDER DISEASE PROGRESSION

Vanshul Malik*[1] and Dr. Rachna Tyagi[2]

[1]Department of Botany, D.A.V. (PG) College, Muzaffarnagar, Uttar Pradesh, India
[2]Assistant Professor, Department of Botany, D.A.V. (PG) College, Muzaffarnagar, Uttar Pradesh, India

***Corresponding Author Email**-vanshulmalik95@gmail.com

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ABSTRACT

Tomato (*Solanum lycopersicum*) is an important horticultural crop worldwide, and its productivity is significantly limited by the widespread occurrence of foliar diseases that gradually modify leaf morphology and physiological function. Disease progression in the leaves entails intricate spatial transformations like lesion development, vein proliferation and disruption in venation, tissue necrosis and disruption of normal laminar architecture. Often, these structural alterations may be challenging to express in a conventional visual or categorical scoring system which is often subjective and lacks granularity. In the present study, a spatial multi-scale fractal analysis framework has been proposed for the quantitative capture of structural degeneration in tomato leaves during disease progression. Leaf images from sources with known disease severity levels were analyzed through fractal geometry-based metrics, focusing on two key descriptors.fractal dimension (FD) and lacunarity.to express structural complexity and heterogeneity. Fractal dimension serves as a description of overall morphological complexity in this context, whereas lacunarity is widely used to quantify spatial irregularity and gap distributions within leaf tissues. Multi-scale analysis was employed to examine structural changes at different spatial resolutions. This helped us better understand both global shape changes and localized tissue damage. The findings demonstrate a steady reduction in fractal dimension as disease severity escalates, indicating a gradual deterioration of structural complexity. On the other hand, lacunarity values increase as the disease gets worse, which means that the leaf architecture becomes more varied and broken up. The results show that fractal-based spatial analysis is a strong, non-destructive, and objective way to measure how disease damages the structure of plant leaves. This technique holds considerable promise for utilization in automated plant disease diagnostic systems, precision agriculture, and digital plant phenotyping platforms.

Keywords: Tomato (*Solanum lycopersicum*), plant pathology, fractal dimension, lacunarity, image analysis, leaf morphology, disease progression, multi-scale spatial analysis, structural degradation, digital phenotyping.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most widely grown vegetables in the world. It is very important for global food security, nutrition, and the agricultural economy. But a lot of foliar diseases caused by fungi, bacteria, and viruses keep threatening tomato production. Diseases such as early blight, late blight, bacterial spot, and mosaic virus infection have a big impact on the structure of leaves, which makes them less able to photosynthesize, causes them to die too soon, and results in big losses in yield (Agrios, 2005).

The leaf is a very complicated biological structure that has a lot of different levels of organization, such as venation networks, epidermal patterns, and mesophyll arrangement. As the disease gets worse, this organized structure progressively deteriorates, which is shown by the formation of lesions, loss of chlorophyll, tissue necrosis, and damage to the vascular system. These structural changes aren't the same everywhere, and they often change depending on the size of the area being looked at. This makes it hard to measure them accurately using standard visual scoring methods (Barbedo, 2013).

Conventional methods for evaluating plant diseases rely primarily on subjective visual assessments or categorical indices of disease severity. Even though these methods are easy to use, they aren't very accurate, reliable, or sensitive to changes in structure that happen early on. Consequently, there is an increasing demand for sophisticated quantitative methodologies capable of precisely characterizing the progressive morphological changes occurring in infected plant tissues (Mohanty *et al*., 2016).

Benoit Mandelbrot came up with fractal geometry, which is a powerful way to use math to study natural structures that are complex and not perfectly regular. Biological systems, including leaves, roots, and vascular networks, frequently display self-similar and scale-invariant characteristics, rendering them appropriate for fractal-based analysis. Fractal dimension (FD) quantitatively assesses structural complexity, whereas lacunarity quantifies the heterogeneity and spatial distribution of gaps within a structure (Mandelbrot, 1982 and Cross, 1997).

In recent years, image-based fractal analysis has become a useful tool in plant phenotyping and pathology for finding small structural differences that people can't easily see (Backes *et al*., 2009). Nonetheless, the majority of studies concentrate on single-scale analysis, which may inadequately reflect the hierarchical characteristics of biological degradation processes.

To overcome this limitation, the present study applies a spatial multi-scale fractal analysis approach to investigate structural degradation in tomato leaves during disease progression. By integrating fractal dimension across multiple spatial scales, this study aims to provide a more comprehensive and quantitative understanding of how disease affects leaf architecture at both macro and micro structural levels.

The objective of this research is to establish a robust, non-destructive, and scalable framework for early detection and quantification of disease-induced morphological changes in tomato leaves, which can further contribute to the development of automated plant disease diagnostic systems.

MATERIALS AND METHODS

Dataset Source and Image Acquisition

The publicly available PlantVillage dataset has high-quality labeled images of healthy and diseased plant leaves taken under controlled conditions (Hughes and Salathé, 2015). We got tomato leaf images (Solanum lycopersicum) from this dataset.

For this study, images were categorized into three disease progression stages:

- Healthy (Control)
- Early infection stage
- Late infection stage

These categories represent progressive structural degradation in leaf morphology due to disease.

Image Preprocessing

Initial image preprocessing was performed using Fiji (ImageJ) to extract relevant structural features from the leaf images (Schindelin *et al*., 2012).

The preprocessing workflow included:

1. Conversion of RGB images into grayscale
2. Noise reduction using filtering techniques
3. Adjustment of brightness and contrast
4. Background removal to isolate the leaf region
5. Binary thresholding for segmentation of leaf structure

These steps ensured that only morphological features of the leaf were retained for subsequent analysis (Gonzalez and Woods, 2008).

Disease Progression Categorization

Leaf images were categorized into Healthy, Early, and Late infection stages based on visible symptoms such as lesion formation, discoloration, and tissue degradation (Barbedo, 2013). This classification enabled comparative analysis of structural changes associated with disease progression.

Multi-Scale Fractal Analysis

Fractal analysis was performed using a computational approach implemented in Python (Anaconda environment).

The box-counting method was used to compute fractal dimension (FD) from the binary leaf images (Mandelbrot, 1982). In this method, the image is overlaid with grids of varying box sizes, and the number of boxes containing part of the leaf structure is counted.

$$FD = \log N(\ ) / \log (1/\ )$$

Where: N( ) = number of occupied boxes, = box size

A multi-scale approach was applied by varying box sizes, allowing analysis of structural complexity at different spatial resolutions (Backes *et al*., 2009).

Spatial Heatmap Generation

Spatial heatmaps were generated using Python-based image processing techniques to visualize the distribution of structural intensity across the leaf surface.

Heatmaps enabled:

- Identification of regions with high structural degradation
- Visualization of lesion-dense areas
- Comparative assessment of spatial heterogeneity across disease stages

Graphical Representation

Graphical analysis was performed to illustrate variations in fractal dimension across disease stages.

Plots were generated using Python libraries (e.g., Matplotlib) to represent:

- Fractal dimension vs disease stage
- Comparative structural trends across categories
- These visualizations helped in clearly identifying trends in structural degradation.

Statistical Analysis

Statistical analysis was conducted using Microsoft Excel to evaluate the significance of differences in fractal dimension across disease stages.

The following statistical methods were applied:

- Calculation of mean and standard deviation for each group
- One-way Analysis of Variance (ANOVA) to test overall significance
- Post-hoc comparison using Tukey fs Honestly Significant Difference (HSD) test
- The Tukey HSD test was used to identify statistically significant differences between Healthy, Early, and Late stages of disease progression.ms and precision agriculture technologies.

RESULTS AND DISCUSSION

Fractal Dimension Analysis Across Disease Stages

Fractal dimension (FD) was used as a quantitative measure to evaluate the structural complexity of tomato leaves across different disease progression stages (Healthy, Early, and Late). The mean fractal dimension values obtained for each category are presented below:

Class	Mean FD
HEALTHY	1.923712
EARLY	1.67748
LATE	1.331023

Table 1. Multi scale fractal dimensions values

The results clearly indicate a progressive decline in fractal dimension with increasing disease severity. Healthy leaves exhibited the highest FD, reflecting a highly complex and well-organized structural architecture. In contrast, Early-stage leaves showed a moderate reduction, indicating initial disruption due to lesion formation. Late-stage leaves exhibited a substantial decrease in FD, suggesting severe structural degradation, fragmentation, and loss of venation integrity.

These findings are consistent with previous studies demonstrating that fractal dimension effectively captures structural complexity and its degradation in biological systems (Mandelbrot,1982 and Cross, 1997).

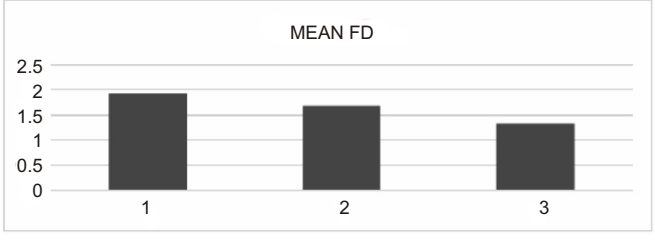

Figure 1. Mean fractal dimensions values across Healthy, Early, Late Stages

Multi-Scale Fractal Analysis

To better understand structural changes at different spatial resolutions, fractal dimension was analyzed at multiple scales (256, 128, and 64).

Scale	Healthy	Early	Late
256	1.923712	1.901043	1.901981
128	1.67748	1.610499	1.631949
64	1.331023	1.39625	1.471515

Table 2. Multi-scale fractal dimension values

At the macro-scale (256), minimal differences were observed among the three stages, indicating that overall leaf shape remains relatively unaffected during early disease progression.

At the meso-scale (128), a clear decline in FD from Healthy to Early stage was observed, suggesting initial structural disruption at intermediate levels such as lesion formation and tissue disorganization.

At the micro-scale (64), Late-stage leaves showed a higher FD compared to Healthy and Early stages, indicating increased structural irregularity and fragmentation at finer spatial levels.

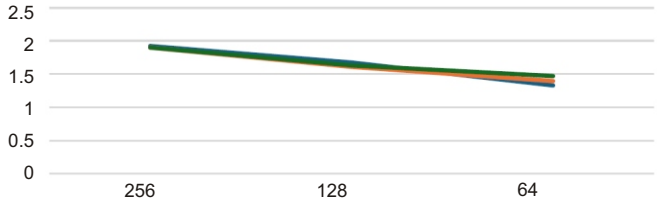

Figure 2. Variation of fractal dimensions across multiple spatial scales (256,128,64) for different disease stages.

These observations confirm that disease progression is scale-dependent, with more pronounced effects observed at smaller spatial scales (Backes *et al*., 2009).

Statistical Analysis (ANOVA) scale

To determine whether the differences in fractal dimension across disease stages were statistically significant, a one-way ANOVA was performed.

- F-value: 564.7652665834562
- p-value: 7.76697746435071 × 10.6¹

The extremely high F-value indicates substantial variation among the groups, while the very low p-value ($p \ll 0.05$) confirms that the differences are highly statistically significant. This demonstrates that disease progression has a strong and measurable impact on leaf structural complexity (Montgomery, 2017).

Post-hoc Analysis (Tukey HSD Test)

To further examine pairwise differences between disease stages, Tukey's Honestly Significant Difference (HSD) test was performed (Tukey, 1949).

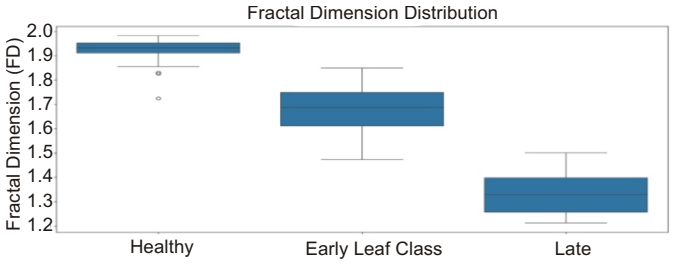

Figure 3. Post-hoc Tukey HSD test showing pairwise comparison of fractal dimension between Healthy, Early, and Late stages

Spatial Heatmap Analysis

Spatial heatmaps were used to visualize the distribution of structural complexity across leaf surfaces.

Healthy samples exhibited dense and continuous high-intensity regions, indicating uniform and intact structural organization.

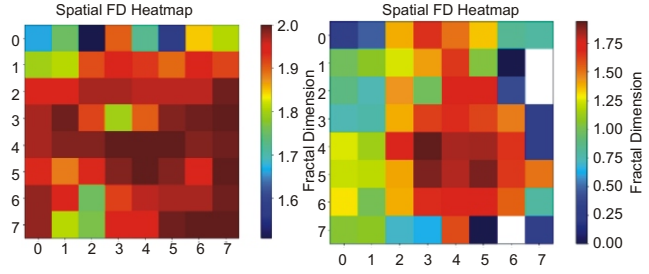

Figure 4. Spatial Heatmaps Showing Distribution Of Structural Complexity In Tomato Leaves 1 And 2 in Healthy

Early-stage samples showed mixed intensity patterns, reflecting localized disruption and partial structural degradation.

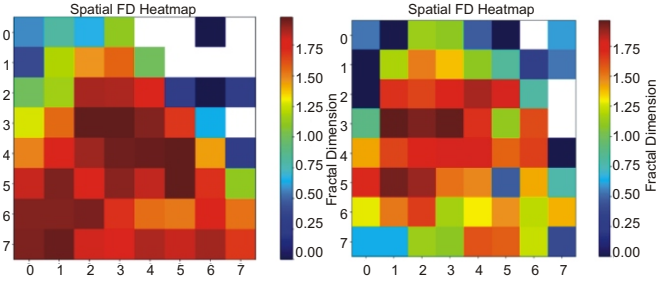

Figure 5. Spatial Heatmaps Showing Distribution Of Structural Complexity In Tomato Leaves 1 And 2 in Early.

Late-stage samples displayed highly heterogeneous and fragmented intensity distributions, indicating severe structural breakdown and loss of connectivity.

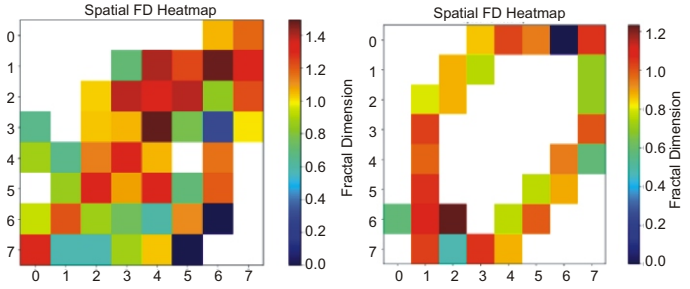

Figure 6. Spatial Heatmaps Showing Distribution Of Structural Complexity In Tomato Leaves 1 And 2 in Late

These spatial patterns clearly demonstrate that structural degradation becomes more localized and irregular as disease progresses, consistent with image-based plant disease analysis approaches (Barbedo, 2013).

Integrated Discussion

The results of this study clearly demonstrate that fractal dimension is a robust and sensitive parameter for quantifying structural degradation in tomato leaves during disease progression.

The observed decline in FD from Healthy to Late stages reflects a systematic loss of morphological complexity associated with tissue damage, lesion expansion, and venation disruption.

Multi-scale analysis further reveals that disease impact is not uniform across spatial levels. While macro-scale structures remain relatively stable during early stages, significant alterations occur at meso- and micro-scales, particularly in advanced infection stages. The increase in FD at micro-scale in Late-stage leaves suggests increased fragmentation and irregularity, which is characteristic of severe tissue breakdown.

The statistical analysis strongly supports these findings, confirming that the observed differences are highly significant and not due to random variation. The boxplot distribution further highlights the increasing variability and heterogeneity associated with disease progression.

Heatmap analysis provides complementary visual evidence, illustrating the transition from uniform structural organization in Healthy leaves to highly fragmented and irregular patterns in Late-stage leaves. This confirms that disease progression is both quantitatively measurable and spatially characterized.

Overall, the integration of fractal analysis, multi-scale evaluation, statistical validation, and spatial visualization provides a comprehensive framework for understanding disease-induced structural degradation. This approach offers a significant improvement over traditional qualitative methods and has strong potential for application in automated plant disease detection and precision agriculture systems.

CONCLUSION

The present study demonstrates that spatial multi-scale fractal analysis is an effective and reliable approach for quantifying structural degradation in tomato (*Solanum lycopersicum*) leaves under disease progression. The results clearly indicate a progressive decline in fractal dimension from Healthy to Late infection stages, reflecting a systematic loss of structural complexity associated with tissue damage, lesion expansion, and disruption of venation networks.

Multi-scale analysis revealed that the impact of disease is not uniform across spatial levels. While minimal variation was observed at the macro-scale, significant structural changes were evident at meso- and micro-scales, particularly in advanced stages of infection. This highlights the importance of scale-dependent analysis in capturing subtle morphological variations (Backes *et al*., 2009).

Spatial heatmap analysis further supported these findings by demonstrating a transition from uniform and continuous structural patterns in Healthy leaves to highly heterogeneous and fragmented distributions in Late-stage infected leaves. This confirms that disease progression leads to localized structural disruption and increased irregularity, consistent with image-based plant disease assessment approaches (Barbedo, 2013).

Statistical validation using ANOVA and Tukey HSD test confirmed that the differences in fractal dimension across disease stages are highly significant, establishing fractal dimension as a robust quantitative indicator of disease severity (Montgomery, 2017).

Overall, the integration of fractal analysis, multi-scale evaluation, spatial visualization, and statistical validation provides a comprehensive and objective framework for plant disease assessment. This approach has strong potential for application in digital plant pathology, automated disease detection systems, and precision agriculture for real-time crop health monitoring.

FUTURE SCOPE

The present study highlights the potential of spatial multi-scale fractal analysis as a quantitative tool for assessing structural degradation in plant leaves. Future research can integrate this approach with machine learning and deep learning techniques to enable automated and real-time disease detection systems.

Further expansion of this methodology to different crop species and disease types would improve its applicability in agricultural diagnostics. Additionally, combining fractal parameters with other image-based features, such as color and texture, may enhance the accuracy of disease severity estimation.

The use of advanced imaging technologies, including hyperspectral imaging and drone-based platforms, may facilitate large-scale field monitoring. Such developments can contribute significantly to precision agriculture by enabling early disease detection and improving crop management strategies.

CONFLICT OF INTREST

The authors declare that there is no conflict of interest.

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UNRAVELLING THE ETHNOMEDICINAL SIGNIFICANCE, PHYTOCHEMISTRY AND PHARMACOLOGICAL POTENTIAL OF *COPTIS TEETA* WALL

Everest Shiwach[1], Harveer Singh Cheema[2], Mitra Pal Singh[3] and Sandeep Kumar[4*]

[1]Department of Botany, Deva Nagri College, Meerut, Uttar Pradesh, India
[2,4]Department of Botany, Meerut College, Meerut, Uttar Pradesh, India
[3]Department of Management Studies, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
***Corresponding Author Email**-sandeep.kumar@mcm.ac.in

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ABSTRACT

The plant kingdom, one of the main life-supporting systems, was a gift from nature to the planet. The distribution of medicinal plants varies depending on the geographical zones around the world. Certain medicinal plants have huge potential for distribution, while others such as *Coptis teeta*, are gradually losing their natural habitat and facing extinction. The non-timber forest plant *Coptis teeta* is primarily found in northwest Yunnan (China), Bhutan, and northeast India, particularly in Arunachal Pradesh's temperate zones. The rhizome of this plant is well-known in Chinese and North East Indian traditional medicine for preventing and treating a variety of human illnesses. According to numerous reports and studies, *Coptis teeta* includes a number of alkaloids, such as berberine, palmatine, jatrorrhizine, coptisine, columbamine, and epiberberine, as well as secondary metabolites, lignans, phenylpropanoids, flavonoids, phenolic acids, saccharides, and steroids. In order to improve drug quality assessment and management, numerous high-content components in *Coptis* extracts will be studied qualitatively and quantitatively. Due to its diverse nature and extensive range of pharmacological actions, *Coptis teeta* cultivation and multiplication should be done with care and patience. This review's goals are to summarise the present level of knowledge regarding the *Coptis teeta* plant, outline its pharmacological functions, and identify the causes of its extinction.

Key Words: *Coptis teeta,* secondary metabolites, traditional medicine, Berberine

INTRODUCTION

Medicinal plants represent one of the oldest and most important sources of therapeutic agents used for the prevention and treatment of human diseases. Since ancient times, humans have relied on plants to meet

their basic healthcare needs, owing to their wide availability and diverse bioactive compounds. Traditional medical systems across different civilizations, including Ayurveda, Traditional Chinese Medicine, and Unani, have extensively utilized medicinal plants for the management of various ailments. Even today, plant-derived medicines continue to play a significant role in modern healthcare, serving as sources of numerous pharmaceutical drugs and lead compounds for new drug discovery (Cheema and Singh, 2021). Fruits, leaves, and tubers were first used by humans as food and medicine. As a result of these experiences, a knowledge system was created and assimilation into man culture, which was then passed down from generation to generation, culminating in the traditional knowledge system (De Pasquale, 1984). Similarly, there are a number of historical facts that suggest the ancient use of natural remedies to treat primary health problems in various parts of the world. With a growing global interest in adopting and researching traditional systems, as well as investigating their potential based on various health-care systems, an assessment of traditional medicine's rich legacy is critical. Furthermore, in the recent past, the use of plants as medicines has included the isolation of active chemicals, starting with the isolation of morphine from opium in the early nineteenth century (Cheema et al., 2014), *Rosa damascene* (Khare et al., 2018)*), Lawsonia inermis* (Singh et al., 2017), *Alnus nepalensis* (Saxena et al., 2016), *Pluchea lanceolata* (Mohanty et al., 2013), *Christia vespertilionis* (Upadhyay et al., 2013) and *Conyza sumatrensis (*Boniface et al., 2015*)*, as well as Phyto molecule derivates including ursolic acid derivatives (Kalani et al., 2014), all have medicinal properties.

The indigenous communities of Arunachal Pradesh possess extensive traditional knowledge of medicinal plants, which has been developed and preserved over generations. This ethnomedicinal knowledge includes the identification, preparation, and therapeutic use of a wide range of medicinal herbs for the treatment of various human ailments. While formal records are unavailable for this region, traditional knowledge remains an important repository of indigenous information. Its timely documentation is critical to safeguard this knowledge from permanent loss. R. Wilcox and Captain Bedford first described this herb in 1825, followed by Grifth in 1836 from the Mishmi Hills of Indian flora (Payum, 2017). *C. teeta* was utilised by the Mishmis and other tribes of Arunachal Pradesh to cure malaria, stomach discomfort, and diarrhoea etc (Shankar and Rawat, 2013). *Coptis teeta* is currently priced at around Rs.2000/kg (Bajpay *et al*., 2019). Nearly the last decade, the combined effects of unregulated collection and forest degradation have resulted in a population drop of over 60%. As a result, the species is listed in Red List Category as Endangered. India has around 90% of the *C. teeta* population, hence it is considered representative of the global population (Mukherjee, 2019).

Coptis teeta Wall. is a perennial herbaceous plant endemic to Arunachal Pradesh (Mishmi Hills) in India and widely dispersed in temperate regions such as Arunachal Pradesh, Sikkim, and Bhutan. Plants are small (30-50 cm), stemless, perennial, blooming, evergreen. Leaves are 5–20 cm in length, pinnatifid, with a 3-lobed lamina and glabrous petioles. Flowers are small, few flowered, white or yellowish in colour, and inflorescence is panicle. Rhizomes are horizontal to oblique (5–15 cm in length) with fibrous roots that have a bitter taste, external skin that is yellowish brown, and pith that is yellow–orange, with multiple nodes and rootlets. It has a brownish yellow rhizome and is widely used as a medication in the Ayurvedic, Siddha, and Unani medicinal systems. Inflammation, eye ailments, skin diseases, stomach issues, constipation, jaundice, and urine disorders, cancer and inflammation, clearing heat, eliminating dampness, purging fire, and detoxification are all treated with it. It is often used to treat bacillary dysentery, typhoid, tuberculosis, epidemic

cerebrospinal meningitis, empyrosis, pertussis, and other disorders. Rhizomes have a bitter taste due to many alkaloids (Berberine, Coptisine, and Palmatine, among others) that are helpful in inhibiting certain microorganisms and can be used to cure a variety of diseases (Bhattee and Beniwal, 1988, Payum, 2017, Latif *et al*., 2008). The main active element in this plant is berberine (6–8.5%) that possess antimicrobial and antibacterial activity, anti-diarrheal activity, anti-hypertensive activity, anti-arrhythmic activity, anti-hyperlipidemic activity, anti-inflammatory activity, anti-depressant activity, antioxidant activity, anti-trachoma, cholesterol reduction activity, and anti-diabetic activities (Payum, 2017, Sun *et al*., 2009).

PHYTOCONSTITUENTS

Coptis teeta rhizomes are a pungent and sour cooling herb that includes multiple active ingredients that are helpful in controlling various microorganisms and are also used to treat a variety of diseases while being safe and efficient. Berberine (13,13a-didehydro-9,10-dimethoxy-2,3-(methylenedioxy), the plant's major active element, is found in 6–8.5 percent of the rhizomes (Bajpay *et al*., 2019). Other compounds like 1,2,3, Propanetriol, Benzenepropanoic acid, methyl ester., 2-methoxy-4-venylphenol, Phenol,2,6-dimethoxy, Cysteamine Sulfonic Acid, Pentadecanoic Acid, n-Nonadecanol-1, 9,12-Octadecadienoic acid (Z,Z)-, Methyl Ester., 9,12,15-Octadecatrienoic acid, Methyl Ester(Z,Z,Z)-, Methyl Streate, Indeno[1,2-b] quinoxalin-11-one, 2-methyl, Stigmast-5-EN-3 -OL,(3.BETA.)-, Phenol,2,6-dimethoxy, Dichloroacetic Acid, undec-2-enyl ester, Benzoic acid, 4 Hydroxy-3-methoxy-, methyl ester, etc are also present (Payum, 2017, Mukherjee, 2019). Organic acids e.g. Quinic acid (27.85 mg/g), Acetic acid (0.18 mg/g), Malic acid (15.25 mg/g), Tartaric acid (0.46 mg/g), Oxalic acid (0.34 mg/g) and Citric acid (1.53 mg/g) are also present in *Coptis teeta* plant (Bhattee and Beniwal, 1988).

BIOLOGICAL ACTIVITIES OF *COPTIS TEETA*

Fresh and dried *Coptis teeta* rhizomes are commonly used as a remedy. The bitter flavour of the leaf and rhizome is caused by berberine and coptine. Due to a lack of resources, research on this endangered medicinal plant's various biological activities are limited, but it has potential and should be further examined for its therapeutic properties.

A. Antimicrobial activity:

Coptis teeta wall. is a medication preferred by the Unani system of medicine for treating eye conditions (Ates and Turgay, 2003). A number of the plant's compounds have been demonstrated to be both safe and effective in the treatment of bacterial diseases, including diarrhoea. (Preethi *et al*., 2010). Whooping cough, diphtheria, typhoid fever, tropical diarrhoea, eye irritation, and colds can all be treated with *Coptis teeta's* pharmacological properties (Hsieh *et al*., 2001). The growth inhibitory activity of extracts produced by organic solvents from the root of *Coptis teeta* in Myanmar (Burma) was investigated in axenic culture against *Giardia lamblia*, *Trichomonas vaginalis*, and *Entamoeba histolytica* (Lone *et al*., 2014, Kaneda *et al*., 1990). The rhizome of *Coptis teeta* is used by a tribe in north-east India to treat diarrhoea. Berberine inhibits *Shigella dysenteriae*, *Salmonella paratyphi*, and several *Klebsiella* species, which cause a variety of intestinal illnesses (Wagner, 2005). Berberine shows the anti-diarrheal properties by inhibiting the transit of the small intestine (Eaker and Sninsky, 1989). Berberine was found to have antibacterial action against all MRSA strains tested. Berberine's minimal inhibitory concentrations (MICs) against MRSA ranged from 32 to 128 mg/mL. With 64 mg/mL or less of berberine, MRSA was inhibited 95% of the time (Yu *et al*., 2005).

B. Antioxidant activity:

Plant parts such as fruits, vegetables, and medicinal herbs contain numerous antioxidant substances in the form of phenolic compounds

(phenolic acids, flavonoids, quinones, coumarins, lignans, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (carotenoids), and other endogenous metabolites, which act as free radical scavengers. The dried ethanol root extract of *Coptis teeta* contained alkaloids, flavonoids, and glycosides, according to preliminary phytochemical investigation. The ethanolic root extract from *Coptis teeta* has strong potential anti-oxidant activity (Lone *et al*., 2014, Tan *et al*., 2007). The extract had a significant IC_{50} against the *in vitro* results, indicating that it had significant free radical scavenging action (Pourmorad *et al*., 2006). Berberine, which is abundant in *Coptis teeta* rhizome, has been studied for its antioxidant properties. Both in cells cultivated with high glucose-containing medium (Liu *et al*., 2008) and in a range of diabetic animal models, berberine has been shown to suppress oxidative stress (Lao-ong *et al*., 2012).

C. Anti-inflammatory and Analgesic activity-

The anti-inflammatory effects of *Coptis* rhizome ethanol extract demonstrated the greatest reduction in inflammation. The pre-treatment with Methanolic extract of *C. teeta* revealed that the extract is helpful in the early phase of inflammation, which is predominantly caused by the release of histamine and serotonin. Up until the third hour of the experiment, the extract's anti-inflammatory impact continues to remain noticeable. The results of the study strongly suggest that Methanolic extract of *C. teeta* has peripheral analgesic efficacy, and that their mechanisms of action may be mediated by inhibition of local peritoneal receptors, possibly including COX inhibition. The study's findings indicate the analgesic and anti-inflammatory action of standardised methanolic extracts of *C. teeta* rhizome, as well as validating the pharmacological backdrop of *C. teeta* rhizome's traditional use (Chelleng *et al*., 2024). The initial immune response to red blood cells and antibody production were altered differently depending on the route of application of berberine *in vitro* treatment of splenocytes (Ivanovska and Philipov, 1996, Li *et al*., 2015).

D. Antidiabetic activity

Coptis teeta Wall. was the sole traditional use for diabetes treatment that had no mention of antidiabetic action, accounting for 10% of the research status. Several studies have been conducted to assess the efficacy of berberine in the treatment of diabetes. *C. teeta* rhizomes stimulated the increase in glucose uptake (Zhang *et al*., 2021). Berberine has the ability to lower and sustain blood sugar levels through two mechanisms: blocking sugar absorption from the intestine and increasing and boosting insulin production (Wang *et al*., 2011, Yin *et al*., 2008). Berberine has been demonstrated to increase cyclic AMP levels by inhibiting the phosphodiesterase enzyme. A rise in cyclic AMP levels has also been linked to an increase in insulin secretion. The hypoglycemic/antidiabetic efficacy of berberine could be due to these actions (Chander Semwal *et al*., 2009). To understand the specific mechanism of the hypoglycaemic action, further detailed chemical and pharmacological research is required.

E. Anticancer Activity

Berberine, its salts, and derivatives have been found as the active chemical for suppressing lung cancer selectively and potentially without hazardous side effects (X. Xu *et al*., 2025, Maung Tin-Wa, 2010). Berberine has been shown to inhibit a variety of cancers, including esophageal cancer (Z. Xu *et al*., 2017). Berberine has been shown in numerous studies to alter tumour cell development by inhibiting tumour cell growth and inducing apoptosis and cell cycle arrest (Da Liu *et al*., 2019). Berberine appears to impede tumour cell migration and invasion, according to a significant body of experimental research. Berberine boosted E-cadherin protein expression in human lung cancer A549 cells in a concentration- and time-dependent manner, while drastically downregulating N-cadherin expression; these alterations hindered invasion and metastasis. Furthermore, discovered that the effect of berberine

on cancer cell metastatic potential is mediated by activation of the AMPK signalling pathway, which inhibits tumour cell adhesion, migration, and invasion by reducing the activity of ERK and the expression of COX-2 (Jin et al., 2017).

F. Antiviral Activity

Berberine is a good candidate for use in new antiviral medicines and therapies since it targets distinct stages of the viral life cycle. Berberine has been found to inhibit virus replication by focusing on specific interactions between the virus and its host. Berberine binds to DNA, preventing DNA synthesis and reverse transcriptase activity. Herpes simplex virus (HSV), human cytomegalovirus (HCMV), human papillomavirus (HPV), and human immunodeficiency virus (HIV) replication are all inhibited by berberine (Warowicka *et al*., 2020). Berberine inhibits six HIV-1 clade B isolates entirely and shows antiviral activity in a concentration-dependent manner, with IC_{50} values ranging from 5.5 to 10.25 g/ml (Shao *et al*., 2020).

G. Antihypertensive Activity

Berberine has been shown in several studies to have the ability to lower blood pressure (Hui *et al*., 1991). Berberine delayed the onset and attenuated the severity of hypertension in SHR rats. In spontaneously hypertensive rats, the antihypertensive effects of the berberine derivative 6-protoberberine (PTB-6) were investigated. PTB-6 reduced systolic blood pressure in conscious SHRs in a dose-dependent manner (6-PTB: 5 mg/kg, –31.1 1.6 mm Hg; 10 mg/kg, –42.4 3.1 mm Hg) (J.-C. Liu *et al*., 1999).

H. Hepatoprotective Activity

Several investigations have been conducted to determine the effectiveness of berberine as a hepatoprotective agent. Berberine pre-treatment significantly reduced doxorubicin induced structural damage in the liver, including vascular congestion, inflammatory cell infiltration, hepatocellular degeneration and necrosis, and liver fibrosis, according to histopathological examinations (Zhao *et al*., 2012). A separate study examined the protective effects of alkaloid isoquinoline berberine on CCl (4)-induced hepatotoxicity in mice. Berberine's hepatoprotective actions may be linked to free radical scavenging and oxidative/nitrosative stress reduction, as well as the regulation of the inflammatory response in the liver (Domitroviæ *et al*., 2011). Berberine may also be effective for preventing hepatotoxicity caused by methotrexate by improving biochemical and oxidative stress indices, according to a recent study (Mehrzadi *et al*., 2018).

I. Antimalarial activity

Coptis teeta, a plant of immense significance to the tribes of northeastern India, holds great application in treating a range of ailments, including parasitic diseases such as malaria (Latif *et al*., 2008). The *Coptis* rhizome exhibited exceptional anti-malarial potency, with an IC50 of 1.9 µg/ml against 3D7 and 4.85 µg/ml against Dd2, along with an impressive selectivity index (SI) surpassing 263 (3D7) and 103 (Dd2). Coptis rhizome exhibits anti-malarial properties through three significant chlorinated compounds: coptisine, berberine, and palmatine. These compounds displayed respective IC50 values of 1.1, 2.6, and 6.0 µM against 3D7 strain and 3.1, 6.3, and 11.8 µM against Dd2 strain. Coptisine chloride demonstrated the most potent anti-malarial effects among the tested compounds, showing an IC50 of 1.1 µM against 3D7 and 3.1 µM against Dd2, along with corresponding selectivity indices (SI) of 37.8 and 13.2, respectively. Significant anti-malarial activity was observed in mice infected with the Plasmodium yoelii 17X strain when administered the herbal extract of Coptis rhizome and its primary active compound, coptisine chloride. The anti-malarial effects remained consistent from day 3 to day 7 after infection, with a range of 50.38% to 72.13% ($P < 0.05$) for Coptis rhizome and 81% to 89% ($P < 0.01$) for coptisine chloride (Teklemichael *et al*., 2020). The antimalarial efficacy of methanol extracts from *Coptis teeta* was examined *in vitro* in a

separate investigation, while the identification of potential leads was conducted through an *in-silico* study. In the 3D7 strain of *P. falciparum*, the IC50 of the methanol extract from *Coptis teeta* was documented as 0.08 ìg/ml, while in the Dd2 strain, it was found to be 0.7 ìg/ml. In comparison to chloroquine, noroxyhydrastatine displayed superior binding affinity as observed in the docking study (Goswami *et al*., 2022).

Table 1. Major compounds found in Coptis teeta and their biological activities.

S. No.	Compound Name	Biological Activities	Ref.
1.	Hexadecanoic Acid, Methyl Ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, 5-Alpha reductase inhibitor	Payum, 2017.
2	Pentadecanoic Acid	Antioxidant	Payum, 2017.
3	2-methoxy-4-venylphenol	Anti-tumour, Antimicrobial Anti-in?ammatory	Jeong *et al*., 2011, Jeong and Jeong, 2010.
4	9,12-Octadecadienoic acid (Z, Z)-, Methyl Ester.	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic	Payum, 2017
6	Berberine	Antitumour, analgesic, Anesthetic, antibacterial, Antimalarial	Sun *et al*., 2009. Jeong *et al*., 2011.
7	Indeno [1,2-b] quinoxalin-11-one, 2-methyl	Anti-cell proliferation of tumour cell	Gazit *et al*., 1996.
8	Stigmast-5-EN-3-OL, (3. BETA.)-	Potent anti-diabetic agent in regulating glucose transport	Sujatha *et al*., 2010.
9	n-Nonadecanol-1	Anti-microbial	Dalli *et al*., 2007.
10	Coptisine, Palmatine	Antimalarial	Jeong and Jeong, 2010.
11	Noroxyhydrastatine	Antimalarial	Jeong *et al*., 2011

REASON FOR DEPLETION OF *COPTIS TEETA*

The *Coptis teeta* herb is enlisted in Red List Category as endangered (A2cd ver 3.1) (IUCN Red List of threatened species). The combined e?ects of unregulated collection and degradation of forest over the last decade years has resulted in decline of the population of over 60%. Therefore, the species has been assessed as Endangered. About 90% *C. teeta* population is in India, therefore considered representative of that of the global population (Payum, 2017). As previously stated, there are various reasons for the extinction of *Coptis teeta* in the wild. The rising need for herbal items, such as medicine and cosmetics, has resulted in a very high demand for raw plant parts, putting significant strain on their natural environment. Due to population pressure and other construction activities in the highlands, the natural habitat of this *unique* species is shrinking. Natural resources are being exploited indiscriminately and excessively. Due to a lack of agro-technology for the highly desirable *Coptis teeta* plants, farmers are hesitant to adopt them in the field. There have been no major attempts to cultivate on a commercial basis. The devastation of these flora is exacerbated by forest fires set by local hill tribes to clear land. Illegal trade in high-value medicinal plants that have been prohibited. Animals, both tame and wild, graze excessively. Cutting medicinal trees for fuel, wood, and other purposes, as well as lopping leaves for fodder and animal bedding. Climate and weather patterns are changing. There is a lack of understanding about this important legacy.

CONCLUSION

Extensive reports paint a sobering picture of *Coptis teeta*, a critically endangered jewel of the Eastern Himalayas. This valuable medicinal plant faces imminent peril, demanding urgent action for its protection. Evidence from multiple studies indicates that this plant harbors an arsenal of beneficial chemicals, effectively targeting a range of health issues such as cancer, malaria, diabetes, and even diarrhoea. Despite some success with various propagation techniques, protecting and conserving this herb in its natural habitat is essential for ensuring its long-term survival. Investigating its medicinal properties holds immense promise for tackling various health issues, making further research in this area a critical priority. By nurturing this precious plant, we not only secure the balance of nature but also unlock a treasure trove of possibilities for ourselves. Protecting it shields our environment, propagating it ensures abundance, and researching it unlocks a wealth of knowledge for generations to come.

CONFLICT OF INTEREST

None to declare

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MORPHOLOGICAL RESPONSES OF SALICYLIC ACID AND JASMONIC ACID ON POTTED POTATO PLANTS (*SOLANUM TUBEROSUM* L.) UNDER DROUGHT STRESS CONDITION

Alisha Malik and Subhash Kumar*

Department of Botany CCR(PG) College, Muzaffarnagar, Uttar Pradesh, India

***Corresponding Author Email**-subhashvikal2012@gmail.com

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ABSTRACT

Agriculture faces growing threats from climatic variability and severe water scarcity. As a drought-sensitive horticultural crop, potato (*Solanum tuberosum* L.) experiences marked declines in growth and tuber yield under moisture deficit conditions. Phytohormones serve as essential signalling molecules that modulate plant physiological responses under abiotic stress. The present study evaluated the protective efficacy of exogenous Salicylic Acid (SA) and Jasmonic Acid (JA) on potato cultivar (cv. Kufri Chipsona-1) subjected to water stress. Eighteen potted plants were arranged in a Completely Randomized Design (CRD), comprising control and water-stressed regimes combined with foliar applications of SA and JA across three replications. Exogenous SA application significantly enhanced plant height, chlorophyll retention, and overall tuber yield, thereby effectively mitigating drought-induced damage. Conversely, while JA mediated defense-related responses, it demonstrated limited capacity for vegetative growth promotion under water-limited conditions.

Keywords: Water stress, Phytohormones, Salicylic acid, Jasmonic acid, Kufri Chipsona-1

INTRODUCTION

Agriculture faces growing threats from climatic variability and severe water scarcity (Aggarwal, P. K., 2008; Pathak, H., Aggarwal, P. K., and Singh, S. D., 2012)). As a major non-cereal food crop belonging to the family Solanaceae, potato (*Solanum tuberosum L.*) plays a vital role in national food and nutritional security in India—currently the world's second-largest producer (FAOSTAT, 2023; Singh *et al*., 2020). Predominantly cultivated in the Indo-Gangetic plains during the winter season, potato production is heavily concentrated across Uttar Pradesh, West Bengal, and Bihar (Ezekiel *et al*., 2012; Rana *et al*., 2017). However, because of its shallow root system, the potato crop is exceptionally sensitive to soil moisture deficits, making drought and climate-induced

water scarcity major limiting factors for tuber development, physiological activity, and final yield (Iwama, 2008; Obidiegwu *et al*., 2015).

Mitigating water stress in horticultural crops increasingly relies on understanding and exploiting endogenous signaling pathways (Ahmad *et al*., 2018). Phytohormones such as salicylic acid (SA) and jasmonic acid (JA) function as critical regulators of plant responses to environmental stressors (Bari and Jones, 2009; Wasternack and Hause, 2013). While SA is primarily associated with systemic acquired resistance, osmotic adjustment, and antioxidant defense under abiotic stress (Hayat *et al*., 2010; Khan *et al*., 2015), JA plays pivotal roles in mediating defense signaling and stomatal regulation (Raza *et al*., 2021; Sharma *et al*., 2020). This investigation evaluates the comparative efficacy of exogenous SA and JA applications in modulating growth metrics, physiological processes, and stress mitigation mechanisms in *Solanum tuberosum* subjected to moisture stress.

MATERIALS AND METHOD

Experimental Site and Climate

Pot trials were carried out in the greenhouse at CCR (PG) College, Muzaffarnagar, Uttar Pradesh (29° 28’ 12" N latitude, 77° 40’ 48" E longitude; elevation 272 m above mean sea level). The region experiences a monsoon-influenced humid subtropical climate with hot summers (June average: 30.2 °C) and cooler winters (January average: 12.5 °C). The mean annual temperature stands at 24.2 °C, with an annual rainfall of 929 mm, peaking in July (261.4 mm) and reaching its lowest in November (8 mm).

Soil Properties and Plant Material

The pot soil was characterized as a sandy loam with moderate organic carbon, low available nitrogen, medium available phosphorus and potassium, neutral-to-slightly alkaline pH (7.7), and normal soluble salt concentrations (EC). Tubers of Solanum tuberosum L. cv. ‘Kufri Chipsona-1’—a high-yielding (~40 t/ha), processing-grade variety maturing in 90–110 days—were acquired from the ICAR-Central Potato Research Institute Campus (CPRIC), Regional Station, Modipuram, Meerut (UP).

Experimental Design

Using a Completely Randomized Design (CRD), 18 potted plants were divided into control (normal) and water-stress conditions. Treatments evaluated two phytohormones (salicylic acid and jasmonic acid) across three replications.

Condition Factor (Control vs. Water Stress)

- Control (Well-Watered)
- Water Stress (Reduced Water Supply at tuber initiation stage)

Treatment Factor

- No Phytohormone (Control)
- Salicylic Acid
- Jasmonic Acid

Experimental Units

- **Pots:** Used uniform-sized plastic pots (5-liter pots) for growing potato plants.

Randomization

- Randomly assign each pot to one of the 6 treatment combinations (2 conditions × 3 treatments).

Replication

Taken 3 replications per treatment combination to ensure statistical robustness.

Treatments

T_1: Control + No Phytohormone

T_2: Control + Salicylic Acid

T_3: Control + Jasmonic Acid

T_4: Water Stress + No Phytohormone

T_5: Water Stress + Salicylic Acid

T_6: Water Stress + Jasmonic Acid

Repeated above process for 3 replications to ensure each treatment combination is adequately represented.

Pots were irrigated at 6 DAP (days after planting), 27 DAP, 42 DAP, 63 DAP and 80 DAP during the year 2023-24.

The water stress was imposed by withholding water in T4, T5 and T6 treatments at tuber initiation stage according to Table 1.

The growth stage were identified and confirmed by uprooting the plants and by examining the stage of tuber development. Experimental pots were dehaulmed at 90 DAP and harvesting was done 10-15 days after of dehaulming so that tuber skin is matured.

Table 1. Details of treatments in pot experiments during year 2023-24

Treatment	Growth stage	Duration of water stress (days)
		2023-24
T_1, T_2, T_3	All growth stages	Nil
T_4	Tuber initiation	7(27 to 34DAP*)
T_5	Tuber initiation	7(27 to 34 DAP*)
T_6	Tuber initiation	7(27 to 34 DAP*)

* DAP= Days after planting

Phytohormone Treatments

Foliar applications were administered manually at 35 days after planting (DAP) following plant establishment. Salicylic acid was applied at a concentration of 0.5 mM, while jasmonic acid was sprayed at 150 ìg mL ^{1}.

Pot Setup and Sowing

Eighteen 5-liter plastic pots were filled with sandy loam soil collected from the research farm of CCR (PG) College, Muzaffarnagar (U.P.). Well-chitted, disease-free seed tubers of *Solanum tuberosum* L. cv. 'Kufri Chipsona-1' were planted manually at a depth of 3–4 cm in moist soil, using one whole tuber per pot.

Fertilizer Application and Irrigation

Recommended fertilizer rates were 180 kg N, 80 kg P_2O_5, and 100 kg K_2O ha ^{1}, recalculated proportionally per pot volume. Full doses of phosphorus and potassium alongside half of the nitrogen were applied at planting. The remaining 50% nitrogen was top-dressed during hoeing and weeding. All pots received initial irrigation immediately following plant emergence, after which irrigation schedules were adjusted based on water-stress treatment protocols.

Sampling and Morphological Measurements

Observations under well-watered (control) and water-stressed treatments were recorded at peak stress conditions, immediately prior to re-watering:

Shoot Height: Measured in centimeters from the ground line to the youngest apical leaf on randomly selected shoots.

Root Depth: Measured in centimeters using washed root samples, taking length from the hypocotyl transition zone to the distal root tip.

Leaf Area: Quantified per plant using a portable leaf area meter (LI-3000, LI-COR Inc., USA).

Stolon and Tuber Counts: Evaluated by manually counting uprooted, washed root systems (n=3 per replication).

Dehaulming, Harvest, and Grading

Haulms were severed at 90 DAP to facilitate skin maturation and minimize tuber infection. Harvesting occurred 15 days after dehaulming using manual hand hoes. Tubers were sorted into marketable (>30g) and non-marketable (<30g) categories, noting structural defects (e.g., pointed or dumbbell shapes, surface cracking). Total tuber yield was recorded using a digital scale.

Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA) for a Completely Randomized Design (CRD) following the standard methods outlined by Gomez and Gomez (1984). Treatment means were compared using the critical difference (CD) test at a 5% level of significance ($P \leq 0.05$) when the F-test indicated significant variance.

RESULTS AND DISCUSSION

Table 2. Plant height, leaf area, number of tubers and total tuber yield of cultivar Kufri Chipsona-1

Pot Number	Condition	Treatment	Plant Height (cm)	Number of Leaves	Root Length (cm)	Tuber Yield (g)
1	Control	No Phytohormone	25	10	15	200
2	Control	Salicylic Acid	30	12	18	250
3	Control	Jasmonic Acid	28	11	17	230
4	Water Stress	No Phytohormone	18	8	10	150
5	Water Stress	Salicylic Acid	22	9	12	180
6	Water Stress	Jasmonic Acid	20	9	11	160

Well-Watered (Control) Conditions

In the absence of exogenous phytohormones, control plants exhibited the lowest growth and productivity, recording a mean plant height of 25 cm and a tuber yield of 200 g (Table 2). Exogenous treatment with Salicylic Acid (SA) significantly improved morpho-physiological traits, achieving the highest plant height (30 g) and tuber yield (250 g). These observations align with previous findings demonstrating that SA application stimulates cell division, expands leaf area, and enhances photosynthetic capacity under non-stress conditions (Hayat *et al*., 2010).

Conversely, Jasmonic Acid (JA) application resulted in moderate growth (28 cm) and yield (230 g). While JA plays a beneficial physiological role, its principal signaling pathways govern defense responses and secondary metabolite biosynthesis, which can induce growth trade-offs under non-stressed, optimal conditions (Wasternack and Hause, 2013).

Water-Stress Conditions

Drought stress severely impaired vegetative growth and yield in untreated plants, reducing height to 18 cm and tuber yield to 150 g (Table 2). However, foliar application of SA effectively alleviated drought-induced inhibition, maintaining higher plant height (22 cm) and tuber yield (180 g). The stress-mitigating capacity of SA can be attributed to its role in regulating stomatal conductance, maintaining cell turgor, improving water use efficiency (WUE), and enhancing antioxidant defense mechanisms (Miura and Tada, 2014).

Foliar application of JA under water stress provided moderate recovery (20 cm height, 160 g tuber yield) compared to untreated stressed plants. Although JA induces protective stress-response and osmoprotectant accumulation, its efficacy in promoting net structural growth under drought remains lower than that of SA (Sharma *et al*., 2018).

Overall, exogenous Salicylic Acid proved to be the most effective phytohormone for improving growth parameters, chlorophyll retention, and tuber yield in ‘Kufri Chipsona-1’ under both irrigated and drought-stressed environments.

CONCLUSION

This study highlights the physiological efficacy of exogenous salicylic acid in enhancing growth performance and drought tolerance in *Solanum tuberosum* cv. ‘Kufri Chipsona-1’. SA application significantly counteracted water-stress penalties by preserving vegetative vigor, chlorophyll content, and final tuber yield. While jasmonic acid conferred moderate stress resilience, its growth-promoting effect was less pronounced. Consequently, foliar treatment with SA represents a promising agronomic strategy to mitigate drought stress and stabilize potato productivity under increasingly variable climatic conditions.

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Research Article

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EFFECT OF INTEGRATED NUTRIENT MANAGEMENT ON STEM GROWTH PARAMETERS OF OKRA (*ABELMOSCHUS ESCULENTUS* L.MOENCH) C.V. ARKA ANAMIKA

Ankul Kumar* and Giriraj Kishore

Department of Horticulture, CCR(PG) College, Muzaffarnagar, Uttar Pradesh, India

***Corresponding Author Email**-ankul2017@gmail.com

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ABSTRACT

Okra is an essential vegetable / food crop cultivated worldwide including India. This crop, contributes to the livelihoods of millions of small farmers (FAO, 2023b). India is the largest producer of okra, accounting for ~70% of the world's production (FAO, 2021). Integrated Nutrient Management (INM) has emerged as an effective approach for improving crop productivity while maintaining soil health. The present study, conducted during the Zaid season of 2022-23, 2023–24 at the Horticulture Research Farm, CCR(PG) College, Muzaffarnagar (U.P.), evaluated the effects of Integrated Nutrient Management (INM) on growth and yield of okra (*Abelmoschus esculentus* L. Moench) Cv. Arka Anamika. The experiment included eight treatments combining organic manures (FYM, poultry manure, vermicompost) and inorganic fertilizers, laid out in a Randomized Block Design with three replications. Among the treatments, T6 (FYM @ 10 t/ha + VC @ 5 t/ha) and F9 (NPK @ 50% RDF + VC @ 5 t/ha) significantly enhanced plant height, number of leaves, branches, nodal development, fruit yield, and biomass accumulation. Maximum yield (48.32 q/ha) was recorded with T6. The study highlights the potential of INM, particularly the synergistic use of FYM and vermicompost, in improving okra productivity and soil health. Adoption of such sustainable nutrient strategies can reduce dependency on chemical fertilizers while enhancing crop performance.

Keywords: Organic Manure, vermicompost, , NPK, Morphology, Growth, Okra

INTRODUCTION

Okra, commonly referred as bhendi (*Abelmoschus esculentus* (L.) Moench), is an annual vegetable belonging to the family Malvaceae (Puneeth *et al*., 2022). Okra is a warm-season vegetable well adapted to tropical and subtropical climates. Okra is an essential food crop cultivated worldwide including India. This crop, contributes to the livelihoods of millions of small farmers (FAO, 2023b). India is the largest producer of

okra, accounting for ~70% of the world's production (FAO, 2021). It is a highly versatile crop with significant applications in culinary, medicinal, and industrial fields. The tender green pods are widely used in cooking, adding flavor and texture to dishes such as curries, stews, and gumbo. Due to its mucilaginous nature, okra also serves as a natural thickening agent in soups and sauces. In many cultures, it is pickled or served as a side dish. Nutritionally, okra is an excellent source of dietary fiber, vitamins A, C, and K, folate, and antioxidants, all of which contribute to improved digestion, cardiovascular health, and blood sugar regulation.

Organic manures, including farmyard manure, compost, and vermicompost, are derived from natural sources and play a vital role in enhancing soil health. These inputs improve soil structure, increase water-holding capacity, and stimulate beneficial microbial activity, thereby contributing to overall soil fertility. (Gayathri, K. and Krishnaveni, K., 2015)They supply essential macronutrients such as nitrogen, phosphorus, and potassium in a slow-release form, promoting sustained nutrient availability and balanced plant growth over time.

Application of inorganic fertilizers can lead to negative consequences, including soil acidification, nutrient leaching, and environmental pollution. Prolonged dependence on chemical fertilizers may also reduce soil organic matter content, ultimately compromising long-term soil fertility and sustainability (Bodamwal *et al*., 2006). In okra cultivation, the application of organic manures has been shown to improve plant vigor, enhance root development, and boost the plant's resistance to biotic stresses such as pests and diseases (Kachari and Gogoi, 2020). Moreover, these amendments strengthen the soil's resilience against erosion and contribute to sustainable farming practices by minimizing environmental degradation.

Although organic manures generally contain lower nutrient concentrations compared to synthetic fertilizers—necessitating the application of larger quantities to meet crop nutrient requirements—they remain indispensable for restoring soil organic matter and maintaining long-term ecological balance (Kaur *et al*., 2022).

Arka Anamika is a prominent okra variety developed by the ICAR–Indian Institute of Horticultural Research. It is widely recognized for its high yield potential, resistance to pests, and adaptability to diverse agro-climatic conditions. Despite these advantages, maximizing the performance of *Arka Anamika* remains a challenge due to various biotic and abiotic stresses, particularly those arising from suboptimal soil fertility and improper nutrient management practices (Narwariya *et al*., 2023). This study, therefore, seeks to develop an optimized INM approach tailored to the specific needs of this cultivar, thereby contributing to both scientific knowledge and practical agricultural advancement.

To address these challenges, Integrated Nutrient Management (INM) has emerged as an effective approach for improving crop productivity while maintaining soil health. INM promotes the balanced use of chemical fertilizers, organic manures, and bio-fertilizers to optimize nutrient availability, enhance soil biological activity, and improve overall soil structure (Panta and Parajulee, 2021). This integrated approach not only rectifies nutrient imbalances but also reduces reliance on synthetic inputs and contributes to environmentally sustainable agriculture (Choudhary *et al*., 2015).

In light of these considerations, the present investigation has been conducted with aiming To investigate the individual and combined effects of organic manures and inorganic fertilizers on the stem growth parameters of okra.

MATERIALS AND METHOD

The present field experiments were conducted during the Zaid season of 2023–24 at the Horticulture Research Farm, Department of Horticulture, CCR (PG) College, Muzaffarnagar, Uttar Pradesh (India) situated at 29.01°N latitude and 77.43°E longitude, with an elevation of 222 meters above mean sea level. The site is geographically located in the subtropical belt of Northern India. The meteorological data recorded during the experimental period are presented in Table 1. The maximum temperature ranged up to 29.2°C, while the minimum dropped to 4.9°C. Relative humidity varied between 88.2% and 35.31%, and a total of 64.32 cm rainfall was received during the month of May.

Table- 1 Mean monthly meteorological data recorded during the experimental period (2023-24)

SMW	Week Period	2023	2024	2024	2024	2023	2023	2024	2024
		Temp. Max (°C)	Temp. Min (°C)	Temp. Max (°C)	Temp. Min (°C)	RH Max (%)	RH Min (%)	RH Max (%)	RH Min (%)	Rainfall (cm)	Rainfall (cm)
10	Mar 5 – Mar 11	29.2	14.1	30.2	13.1	88.2	40.23	80.2	38.5	0	0
11	Mar 12 – Mar 18	29.2	14.1	30.1	15.1	88.2	40.23	85.2	38.8	0	0
12	Mar 19 – Mar 25	29.2	14.1	32.2	14.1	88.2	40.23	86.2	38.5	0	0
13	Mar 26 – Apr 1	29.2	14.1	31.1	14.1	88.2	40.23	87.2	38.9	0	0
14	Apr 2 – Apr 8	25.1	10.8	26.5	11.9	84.67	35.31	85.87	35.89	0	0
15	Apr 9 – Apr 15	25.1	10.8	26.5	12	84.67	35.31	85.87	35.78	0	0
16	Apr 16 – Apr 22	25.1	10.8	26.5	12.5	84.67	35.31	84.67	35.31	0	0
17	Apr 23 – Apr 29	25.1	10.8	27	12.5	84.67	35.31	86.5	35.68	0	0
18	Apr 30 – May 6	21	4.9	22	6	91.2	41.21	91.2	42.21	16.08	0
19	May 7 – May 13	21	4.9	23	6	91.2	41.21	93	42.2	16.08	0
20	May 14 – May 20	21	4.9	22	6.5	91.2	41.21	92.5	42.3	16.08	0
21	May 21 – May 27	21	4.9	24	6	91.2	41.21	92	44	16.08	0
22	May 28 – Jun 3	21	4.9	22	5	91.2	41.21	92	43.3	0	0
23	Jun 4 – Jun 10	21.3	8	23.3	8.5	86.53	45.41	85.5	45.2	0	11
24	Jun 11 – Jun 17	21.3	8	22	8.9	86.53	45.41	85.7	44.5	0	10
25	Jun 18 – Jun 24	21.3	8	22.2	8.8	86.53	45.41	87.5	44.8	0	15
26	Jun 25 – Jul 1	21.3	8	22.9	8.9	86.53	45.41	85.5	49.2	0	16

The topography of the field experiment was fairly uniformed. The soil samples were collected before the sowing of okra from the depth 0-15 cm from the soil surface was air dried, sieved through 2 mm stainless steel sieved and then stored in the plastic bag for physical and chemical analysis. The soil sample was analyzed from the soil testing lab Meerut. On the bases of analysis, the physical and chemical properties of experimental field soil are present in Table 2 showed that the soil was loamy sand and texture.

Table-2: Physical and Chemical value of experimental field soil (2024)

S. No.	Soil characteristics	Value obtained	Method used
1	Fined sand	55.1 %	Hydrometer Method
2	Silt	24.3 %	Hydrometer Method
3	Clay	21%	Hydrometer Method
4	Texture	Sandy loam	International pipette Method
5	Organic carbon	0.44%	Black's Method
6	Total Nitrogen	0.051%	Subbiah Method
7	Available Phosphorus	0.078 %	Olsen' s Method

8	Available Potash	0.0201%	Flame Photometer Method
9	Zink	0.009%	Flame Photometer Method
10	Boron	0.0022%	Flame Photometer Method
11	Molybdenum	0.0012%	Flame Photometer Method
12	pH	7.4	Beckman's pH meter
13	Electric Conductivity (EC) m. mhos./cm	3.1	Conductance Method

Table -3: Details of experimental treatment

Sr. No	Notation	Treatment
1	T	Control
2	T_2	Farm yard manures (FYM)@ 20 t/ha
3	T_3	Poultry manure (PM)@10t/ha
4	T_4	Vermin-compost (VC) @ 10t/ha
5	T_5	Farm yard manures (FYM)@ 10 t/ha+ PM@5t/ha
6	T_6	Farm yard manures (FYM)@ 10 t/ha+ VC@5t/ha
7	T_7	Poultry manure (PM)@ 5t/ha+VC@5t/ha
8	T_8	NPK@50%RDF+FYM@10t/ha
9	T_9	NPK@50%RDF+VC@5t/ha
10	T_{10}	NPK@50%RDF+PM@5t/ha

Design and Layout of the Experiment

Variety	Arka Anaika
Design of layout	Randomized block design (RBD)
Number of treatments	10
Number of replications	3
Number of plants per plot	15
Plant distance	50x40 cm2 (Row x Plant)
Net plot size	1.215x27= 32.805 m2
Width of path	1 meter
Width of main irrigation channel	1 meter
Width of experimental border	30 cm
Width of experimental field	13.85 m
Length of experimental field	24.30 m
Size of experimental field	13.5x7.2= 97.2 m2

The land was brought to a fine tilt by ploughing with mould board plough and harrowing twice after the harvest of the previous crops. The plots were laid out as per the plan of the layout. Organic manures were applied according to the treatment specifications (Table 3) after the layout of the experimental field was completed.

Arka Anamika is a prominent okra variety which was used in experiments. This okra variety was developed through interspecific hybridization between Abelmoschus esculentus and A. manihot ssp. tetraphyllus var. tetraphyllus, followed by backcross breeding. Released by ICAR-IIHR, Bengaluru. Hybrid variety of okra (Arka Anamika) was used in the study which was sowed on 1st of March, 2023 by hand dibbling of one and two seeds per holl at 2-3cm depth with a spacing of 50x40 cm between plant to plant and row to row respectively. Gap filling was done two weeks after sowing of the seeds in order to maintain proper

plant population.

Irrigation was given once in 8-10 days during the crop growth till harvesting period depending upon the weather condition. In order to check weed growth and to maintain the field weed free field, manual weeding with the help of khurpi was done regularly during the early crop growing days. 18 Hoeing and thinning at the plant quality or proper distance in plants a know control of disease and air circulation plants interval of 15 days starting from 30 days after sowing. A mixture of Chlorpyriphos 20% E.C and Thiamethoxam 25% WG power was sprayed to prevent caterpillars and green aphids from feeding on the foliage.The fruit was harvest manually by hand picking at an interval of 2-3 days and. A total number of six pickings were done,

In order to assess the effect of different foliar nitrogen spray on the growth and yield of the crop, periodical observations were recorded from each net plot, five plants were randomly selected and observations on growth was recorded periodically 30,45, 60, Days After Sowing (DAS) and at harvest.

RESULTS

Plant height (cm)

Plant height of okra increased progressively with the advancement of crop age and was significantly influenced by the foliar application of various nitrogen sources. At 30 DAS, the tallest plants (16.63 cm) were observed in treatments involving integrated nutrient application (Table 5, Figure 1). The final plant height at the time of first picking ranged significantly among treatments, with the maximum height (79.69 cm) recorded in treatment T6 (FYM @ 10 t/ha + VC @ 5 t/ha), followed closely by T9 (NPK @ 50% RDF + VC @ 5 t/ha) which recorded 76.74 cm. These treatments showed the highest percentage increase in plant height over the control (60.3 cm), with T6 showing a 32.15% increase and T9 a 27.26% increase. In contrast, the lowest growth was observed in the untreated control (T1), which also recorded the minimum per cent increase. These results clearly indicate that the combined application of organic manures and reduced levels of inorganic fertilizers significantly enhances vegetative growth in okra.

Stem base diameter (cm)

Integrated nutrient management practices significantly influenced the stem base diameter of okra plants (Table 2, Figure 2). The thickest stems were observed in T6 (FYM @ 10 t/ha + VC @ 5 t/ha), which recorded a stem base diameter of 2.25 cm, corresponding to a 33.14% increase over the control (T1: 1.69 cm). Among the other treatments, T9 (NPK @ 50% RDF + VC @ 5 t/ha) and T7 (PM @ 5 t/ha + VC @ 5 t/ha) showed notable improvements, with stem diameters of 2.07 cm (22.49% increase) and 1.95 cm (15.38% increase) over the control, respectively. The significant increase in stem base diameter under integrated nutrient treatments highlights the positive impact of combining organic and inorganic nutrient sources on okra plant vigor.

Number of branch per plants (cm)

At 30 DAS, the highest number of branches (3.44) was recorded in treatment T9 (NPK @ 50% RDF + VC @ 5 t/ha), while the lowest (1.23) was recorded in the control (T1) (Table 7, Figure 3). Regardless of the treatment, the number of branches increased progressively with plant age. At the time of harvest, the maximum number of branches (3.98) per plant was observed in treatment T7 (PM @ 5 t/ha + VC @ 5 t/ha), whereas the control consistently showed the lowest number of branches. These results emphasize the positive impact of integrated nutrient management on branching in okra, particularly when poultry manure and vermicompost are used in combination.

Fresh weight of plants (g)

The highest mean fresh weight at harvest (14.43 g) was recorded in treatment T9 (NPK @ 50% RDF + VC @ 5 t/ha), which was significantly superior to the control (8.90 g). However, the maximum fresh weight (15.48 g) and per cent increase over control (89.01%) were recorded in treatment T6 (FYM @ 10 t/ha + VC @ 5 t/ha) (Table 7, Figure 4). These results suggest that integrated nutrient management involving both organic and inorganic sources significantly enhances the biomass accumulation in okra plants.

Dry Weight of Plant (g/plant)

The dry weight of okra plants was significantly influenced by different nutrient management treatments. The highest dry plant weight (4.11 g) was recorded in treatment T9 (NPK @ 50% RDF + VC @ 5 t/ha), which was significantly greater than that of all other treatments (Table 8, Figure 5).. However, the maximum dry weight (5.01 g) and corresponding percent increase were achieved with T6 (FYM @ 10 t/ha + VC @ 5 t/ha), emphasizing the synergistic effect of combined organic manures in enhancing total dry matter accumulation in okra.

Table 5. Effect of Integrated Nutrient Management on plant height (cm) of Okra

Treatments	Notation	Plant height (cm)	Per cent increase over control
Control	T_1	60.3	----------
Farm yard manures (FYM)@ 20 t/ha	T_2	74.83	24.09
Poultry manure (PM)@10t/ha	T_3	74.47	23.49
Vermin-compost (VC) @ 10t/ha	T_4	74.88	24.17
Farm yard manures (FYM)@ 10 t/ha+ PM@5t/ha	T_5	74.4	23.38
Farm yard manures (FYM)@ 10 t/ha+ VC@5t/ha	T_6	79.69	32.15
PM@ 5t/ha+VC@5t/ha	T_7	75.57	25.32
NPK@50%RDF+FYM@10t/ha	T_8	75.49	25.09
NPK@50%RDF+VC@5t/ha	T_9	76.74	27.26
NPK@50%RDF+PM@5t/ha	T_{10}	75.29	24.85
CD at 5%		0.538	
SEm (±)		0.180	

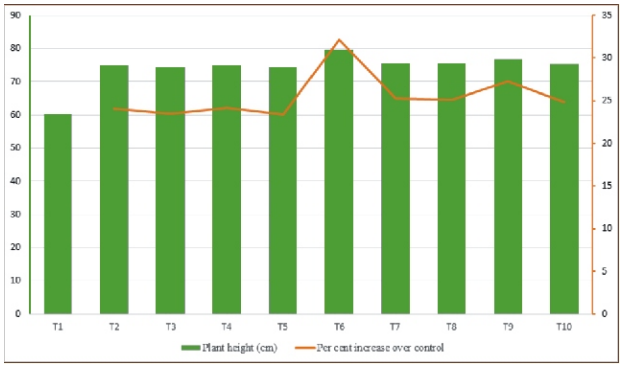

Fig 1. Effect of Integrated Nutrient Management on plant height (cm) of Okra

Table 6. Effect of Integrated Nutrient Management on Stem base diameter (cm) on Orka

Treatments	Notation	Stem base diameter(cm)	Per cent increase over control
Control	T_1	1.69	----------
Farm yard manures (FYM)@ 20 t/ha	T_2	1.81	7.10%
Poultry manure (PM)@10t/ha	T_3	1.8	6.51%
Vermin-compost (VC) @ 10t/ha	T_4	1.84	8.88%
Farm yard manures (FYM)@ 10 t/ha+ PM@5t/ha	T_5	1.79	5.92%
Farm yard manures (FYM)@ 10 t/ha+ VC@5t/ha	T_6	2.25	33.14%

PM@ 5t/ha+VC@5t/ha	T_7	1.95	15.38%
NPK@50%RDF+FYM@10t/ha	T_8	1.89	11.83%
NPK@50%RDF+ VC@5t/ha	T_9	2.07	22.49%
NPK@50%RDF+PM@5t/ha	T_{10}	1.85	9.47%
CD at 5%		0.200	
SEm (±)		0.067	

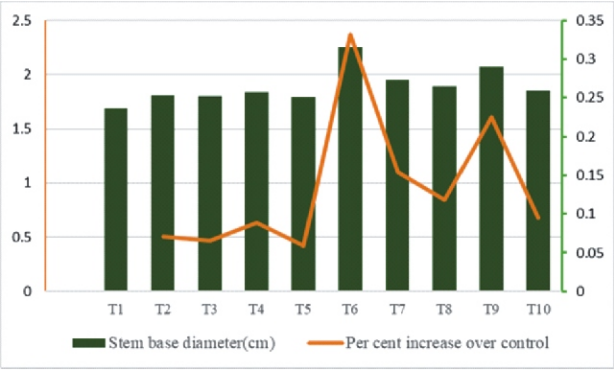

Figure 2. Effect of Integrated Nutrient Management on Stem base diameter (cm) of Okra

Table 4. Effect of Integrated Nutrient Management on Number of branch/plants of Okra

Treatments	Notation	Number of branch/ plants	Per cent increase over control
Control	T_1	1.23	----------
Farm yard manures (FYM)@ 20 t/ha	T_2	3.13	154.47
Poultry manure (PM)@10t/ha	T_3	3.12	153.65
Vermin-compost (VC) @ 10t/ha	T_4	3.04	147.15
Farm yard manures (FYM)@ 10 t/ha+ PM@5t/ha	T_5	3.12	153.65
Farm yard manures (FYM)@ 10 t/ha+ VC@5t/ha	T_6	3.98	223.57
PM@ 5t/ha+VC@5t/ha	T_7	3.1	152.03
NPK@50%RDF+FYM@10t/ha	T_8	3.07	149.59
NPK@50%RDF+ VC@5t/ha	T_9	3.44	179.67
NPK@50%RDF+PM@5t/ha	T_{10}	3.22	161.78
CD at 5%		0.087	
SEm (±)		0.029	

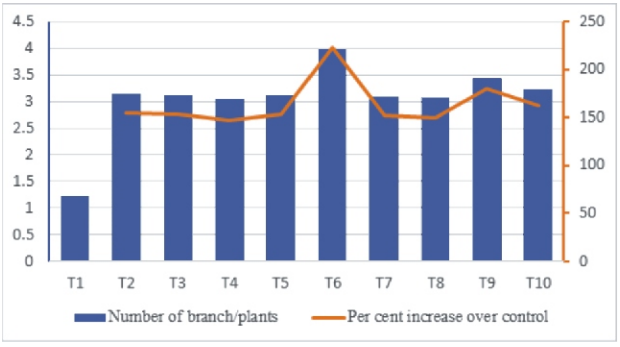

Fig 3. Effect of Integrated Nutrient Management on number of branch/plants of Okra

Table 8. Effect of Integrated Nutrient Management on Dry weight of plant of Okra

Treatments	Notation	Dry weight of plant	Per cent increase over control
Control	T_1	2.18	----------
Farm yard manures (FYM)@ 20 t/ha	T_2	4.04	85.32
Poultry manure (PM)@10t/ha	T_3	4.06	86.23
Vermin-compost (VC) @ 10t/ha	T_4	4.42	102.75
Farm yard manures (FYM)@ 10 t/ha+ PM@5t/ha	T_5	4.08	87.15
Farm yard manures (FYM)@ 10 t/ha+ VC@5t/ha	T_6	5.11	134.4
PM@ 5t/ha+VC @ 5t/ha	T_7	4.15	90.36
NPK@50%RDF+FYM @10t/ha	T_8	4.24	94.49
NPK@50%RDF+VC@5t/ha	T_9	4.88	123.85
NPK@50%RDF+PM@5t/ha	T_{10}	4.36	100
CD at 5%		0.091	
SEm (±)		0.030	

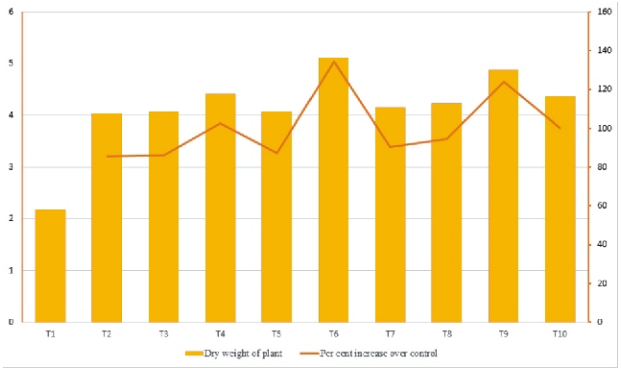

Fig 5. Effect of Integrated Nutrient Management on Dry weight of plant (g) of Okra

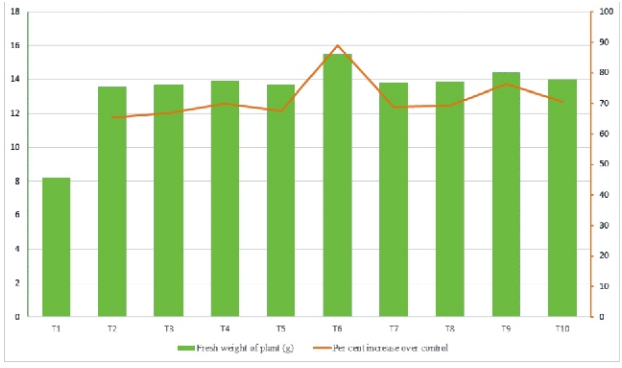

Fig 4. Effect of Integrated Nutrient Management on Fresh weight of plant (g) of Okra

Table 7. Effect of Integrated Nutrient Management on Fresh weight of plant (g) of Okra

Treatments	Notation	Fresh weight of plant (g)	Per cent increase over control
Control	T_1	8.19	----------
Farm yard manures (FYM)@ 20 t/ha	T_2	13.54	65.32
Poultry manure (PM)@10t/ha	T_3	13.67	66.91
Vermin-compost (VC) @ 10t/ha	T_4	13.92	69.96
Farm yard manures (FYM)@ 10 t/ha+ PM@5t/ha	T_5	13.72	67.52
Farm yard manures (FYM)@ 10 t/ha+ VC@5t/ha	T_6	15.48	89.01
PM@ 5t/ha+VC@5t/ha	T_7	13.82	68.74
NPK@50%RDF+ FYM@10t/ha	T_8	13.86	69.23
NPK@50%RDF+ VC@5t/ha	T_9	14.43	76.19
NPK@50%RDF+ PM@5t/ha	T_{10}	13.97	70.57
CD at 5%		0.112	
SEm (±)		0.037	

DISCUSSION

Plant height (cm)

Plant height in okra increased progressively with crop age and was significantly influenced by the foliar application of various nitrogen sources. At 30 DAS, the tallest plants (16.63 cm) were observed in treatments receiving integrated nutrient application.

At the time of first picking, the maximum plant height (79.69 cm) was recorded in T6 (FYM @ 10 t/ha + VC @ 5 t/ha), followed by T9 (NPK @ 50% RDF + VC @ 5 t/ha) with 76.74 cm. Both treatments showed notable increases over the control (60.3 cm), with T6 and T9 reflecting 32.15% and 27.26% increases, respectively.

The improved plant height under integrated treatments may be attributed to the continuous nutrient release from organic sources, enhancing nitrogen availability and uptake. Nitrogen plays a crucial role in cell elongation and division, thereby promoting vegetative growth. These results align with findings by Chaudhary *et al*. (2018) and Miaha *et al*. (2020), who also reported enhanced growth with integrated nutrient management in okra. Kumar *et al*. (2019) reported that the combined application of organic and chemical fertilizers enhances the plant height of okra. Integrated nutrient management (INM) helps maintain soil organic matter while

improving the nutrient content and structural condition of the soil, thereby positively influencing the vegetative growth and reproductive traits of okra.

Stem base diameter (cm)

The stem base diameter of okra plants was also significantly increased under INM treatments. The maximum stem diameter (2.25 cm) was observed in T6, indicating a 33.14% improvement over control (T1: 1.69 cm). Other treatments, such as T9 (2.07 cm; 22.49% increase) and T7 (1.95 cm; 15.38% increase), also showed marked improvements.

The thickening of the stem base may be due to better root proliferation and vegetative vigor promoted by integrated nutrient application. Organic amendments not only improve nutrient uptake but also enhance soil structure and water-holding capacity. Similar findings were reported by Chaudhary *et al*. (2018), Miaha *et al*. (2020), and Kumar *et al*. (2019), who demonstrated that the integration of organic and inorganic sources improves overall plant robustness and health.

Number of branch per plants (cm)

At 30 DAS, the highest number of branches (3.44) was observed in treatment T9 (NPK @ 50% RDF + VC @ 5 t/ha), while the control (T1) recorded the lowest (1.23). A consistent increase in the number of branches was noted as the plants matured. At harvest, the maximum branching (3.98 branches per plant) was recorded in treatment T7 (PM @ 5 t/ha + VC @ 5 t/ha), whereas the control remained lowest throughout the growth period.

These results clearly indicate the beneficial effect of integrated nutrient management in promoting lateral growth and branching in okra. The combination of poultry manure and vermicompost in T7 appears particularly effective, likely due to enhanced nutrient availability and improved soil health. Anand and Kadam (2016) found that, maximum number of branches was observed in the treatment of 60% N through neem cake with 40% N through urea. As the nitrogen is essential for the synthesis of proteins and it might have helped in production of more number of branches. Patil *et al*. (2004) found the same result in tomato plant. Thus it can be inferred from this result that application of INM (organic and inorganic) significantly affected the number of branches per plant.

Fresh weight of plants (g)

The highest mean fresh weight at harvest (14.43 g) was recorded in treatment T9 (NPK @ 50% RDF + VC @ 5 t/ha), which was significantly higher than the control (8.90 g). However, the maximum fresh weight (15.48 g) and the highest percentage increase over control (89.01%) were observed in treatment T6 (FYM @ 10 t/ha + VC @ 5 t/ha).

These results underscore the positive influence of integrated nutrient management in enhancing biomass accumulation in okra. The superior performance of T6 and T9 may be attributed to the combined effects of organic and inorganic nutrient sources, which improve nutrient availability, soil structure, and microbial activity. While poultry manure and vermicompost increased fresh weight significantly, the highest gain was achieved when FYM was combined with vermicompost, likely due to the sustained nutrient release and improved root zone environment.

These findings are consistent with the observations reported by Bhushan *et al*. (2013) and Amran *et al*. (2014) who also noted increased fresh biomass in okra under integrated nutrient regimes.

Dry Weight of Plant (g/plant)

The dry weight of okra plants was significantly affected by the various nutrient management

treatments. Treatment T9 (NPK @ 50% RDF + VC @ 5 t/ha) recorded the highest dry plant weight among the inorganic-integrated treatments (4.11 g), while the maximum dry weight overall (5.01 g) and the greatest percent increase over control were observed in T6 (FYM @ 10 t/ha + VC @ 5 t/ha).

These results underscore the synergistic effect of combining organic manures in enhancing total dry matter accumulation. The improved biomass may be attributed to the nutrient-solubilizing capacity of vermicompost, which enhances nutrient uptake, particularly NPK. Organic manure also plays a critical role in supporting plant growth by releasing essential macro- and micro-nutrients through mineralization, while simultaneously improving soil structure and microbial activity.

Similar trends have been reported by Abduli *et al*. (2012) who found that organic nutrient sources significantly boost dry matter production in okra.

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Research Article

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PHYTOCHEMICAL-DRIVEN SYNTHESIS OF SILVER NANOPARTICLES USING *OCIMUM SANCTUM* (TULSI) LEAF EXTRACT

Subhash Kumar*, Shailly Chaudhary, Akshita Dixit, Anjul, Anshika, Himani, Himani Saini, Juvariya, Jyoti and Km Saloni

Department of Botany CCR(PG) College, Muzaffarnagar, Uttar Pradesh, India

***Corresponding Author Email**-subhashvikal2012@gmail.com

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ABSTRACT

Green synthesis offers an environmentally sustainable alternative to conventional chemical methods for the production of silver nanoparticles. In the present study, an aqueous leaf extract of Ocimum sanctum was employed as a biological reducing and stabilizing agent for the synthesis of silver nanoparticles. The study was conducted in the Department of Botany, CCR College, Muzaffarnagar, in collaboration with BIO-COSMOS Lab, Meerut, during 2025–2026. The synthesized nanoparticles were characterized using UV–visible spectroscopy and agarose gel electrophoresis. The appearance of a characteristic surface plasmon resonance peak near 420 nm was used to confirm nanoparticle formation, while agarose gel electrophoresis was used to investigate nanoparticle–DNA interactions and their potential effects on DNA integrity. The findings are expected to contribute to a better understanding of the role of *O. sanctum* phytochemicals in the bioreduction and stabilization of silver nanoparticles and to support the development of safer, plant-mediated nanomaterials.

Keywords: Green synthesis, silver nanoparticles, Ocimum sanctum, plant-mediated synthesis, nanoparticle characterization

INTRODUCTION

The development of green nanotechnology has emerged as a cost-effective and environment friendly alternative to conventional chemical synthesis methods (Mishra *et al*., 2024; Sood and Chopra, 2017). Plant-mediated synthesis (i.e. utilizing biological materials such as Tulsi leaves) offers a sustainable strategy to mitigate the environmental hazards associated with toxic chemical reducing agents (Rout, 2012; Tangod and M, 2024). In this context, *Ocimum sanctum* serves as a potent biological platform, with its rich profile of bioactive phytochemicals—including eugenols and terpenes—acting as essential reducing and stabilizing agents during nanoparticle formation (Ramteke *et al*., 2012). The present study was conducted in department of Botany, CCR(PG) College,

Muzaffarnagar (U.P.) during 2025-26. In this study, we aim to synthesize silver nanoparticles using an aqueous extract of *Ocimum sanctum* leaves and investigate their synthesis through UV-Vis spectroscopy and agarose gel electrophoresis. (A. and R., 2021; Goyal, 2020; P.R. *et al*., 2025). The UV-V is spectrum of AgNPs synthesized from *Ocimum sanctum* typically falls within the range of 420 to 450 nm. These analytical techniques will elucidate the crystalline nature and size distribution of the particles, facilitating a deeper understanding of the bio-reduction mechanisms involved (Mishra *et al*., 2023; Sulaiman *et al*., 2024).

The specific phytochemical profile of Ocimum sanctum—particularly its abundance of methoxy and allyl groups—facilitates the efficient bioreduction of Ag+ ions into highly stable nanoparticles (Bindhani and Panigrahi, 2015; Mallikarjuna *et al*., 2011). Complementary to these reductive mechanisms, established characterization protocols—such as UV-visible spectroscopy—are frequently employed to confirm the success of the synthesis through the observation of Surface Plasmon Resonance peaks, typically centered near 420 nm (Sulaiman *et al*., 2024).

Agarose gel electrophoresis has been established as a powerful tool for investigating interactions between nanoparticles and DNA. Several studies have explored the interaction of DNA with nanoparticles, indicating that these nanoparticles can cause DNA strand breaks, which can be monitored using agarose gel electrophoresis.

Ocimum sanctum is a sacred plant of South Asia belonging to family Lamiaceae. It is well-known for its use in Ayurvedic and homeopathic medicine, particularly in treating respiratory problems, digestive issues, skin diseases, and skin inflammation. This plant is a rich source of various phytochemicals, including eugenol, rosmarinic acid, flavonoids, and terpenes, which contribute to its medicinal properties. Additionally, these compounds serve as natural reducers and stabilizers in the synthesis of nanosilver, facilitating the reduction of silver ions (Ag) into silver nanoparticles (AgNPs). These components also coat and stabilize the synthesized nanosilver by forming a thin layer around them.

MATERIALS AND METHODS

The present study was conducted in department of Botany, CCR(PG) College, Muzaffarnagar (U.P.) during 2025-26 in colaboration with BIO-COSMOS Lab, Meerut.

Collection and Preparation of Plant Material

Fresh and healthy leaves of *Ocimum sanctum* were collected from Herbal cum Medicinal graden of CCR(PG) College campus, Muzaffarnagar. Fresh *Ocimum sanctum* leaves were thoroughly washed with distilled water, crushed into a fine power. An aqueous leaf extract (5% w/v) was prepared by boiling 5 grams of leaf powder in 100 ml of sterile distilled water at 60°C for 40 minute to extract bioactive metabolites (Alex *et al*., 2024).

The resulting light brown aqueous extract was filtered by Whatsman filter paper No. 1, to remove particulate matter and subsequently refrigerated at temperatures below 4 °C to maintain the chemical integrity of the active constituents (Das *et al*., 2024).

Synthesis of Silver Nanoparticles

For the synthesis of silver nanoparticles, the prepared leaf extract was combined with a 1 mM silver nitrate solution, typically in a specific ratio (1:9) to initiate the reduction process (Banerjee *et al*., 2014; Melkamu *et al*., 2022). The reaction is maintained at 30 °C, during which a distinct color shift from light green to brown indicates the successful transformation of silver ions into nanoparticles (Anandalakshmi, 2021; Ansari *et al*., 2023). Following synthesis, the resulting suspension is subjected to rigorous characterization, starting with UV-Vis spectrophotometry to identify the characteristic surface plasmon resonance peak, typically observed near 420 nm (Manimaran *et al*., 2016).

After formation, the synthesized silver nanoparticles (AgNPs) were collected and purified by centrifugation at 12,000 rpm for 30 minutes, followed by repeated washing three times with Milli-Q water, and then lyophilized at -20°C before further experiments.

Characterization of Silver Nanoparticles

The formation of the synthesized silver nanoparticles was analyzed using UV-visible spectroscopy, where the absorption spectra were measured by scanning the wavelength range from 300 nm to 700 nm. The presence of sharp peaks in the range of 400 nm to 450 nm indicated the successful formation of silver nanoparticles due to surface plasmon excitation. (A. V. Narasimha Swamy and R. Srinu Venkat Rao., 2021)

Table 1: Optimization Parameters for AgNP Synthesis

Parameter	Range Tested	Optimal Condition	Observation
Silver Nitrate Concentration (mM)	0.5 - 5.0	1.0 mM	Maximum SPR intensity at 1.0 mM; higher concentrations led to aggregation
Extract-to-$AgNO_3$ Ratio (v/v)	1:10 - 1:30	1:20	Optimal reduction and stability; lower ratios gave incomplete reduction
pH of Reaction Mixture	4.0 - 10.0	7.0 (Neutral)	Maximum nanoparticle formation at neutral pH; acidic pH inhibited synthesis
Temperature (°C)	25 - 90	60 - 75	Faster synthesis at elevated temperature; room temperature required 24-48 hours
Incubation Time (hours)	1 - 72	24 hours	Complete reduction achieved within 24 hours; prolonged incubation caused aggregation
Extract Concentration (% w/v)	2 - 10	5%	Optimal capping and stabilization at 5%

Agarose Gel Electrophoresis

The potential interaction of AgNPs with plasmid DNA was monitored using agarose gel electrophoresis. A plasmid (pBR322) with a DNA concentration of 1 µg per ml in sterile distilled water was mixed with different concentrations (0.1, 0.5, and 1.0 µg/mL) of AgNPs. The mixture was incubated for 30 minutes at 37°C, followed by electrophoresis of the mixed samples in 1% agarose gels using Tris–borate–EDTA (TBE) buffer at pH 7.2 and a current of 300 mA for 30 minutes. DNA bands were visualized using a UV transilluminator, and the rate of transformation of supercoiled DNA into an open circular DNA form was analyzed.

RESULTS AND DISCUSSION

Visual Observation and UV-Vis Spectroscopic Analysis

The successful synthesis of silver nanoparticles using Ocimum sanctum leaf extract was observed through visual analysis. A colloidal dark brown suspension of AgNPs was detected after 24 hours, with the color change in the solution shifting from light brown to dark brown(A. and R., 2021). This color change can be attributed to Surface Plasmon Resonance, which occurs when incident light excites the natural oscillation and movement of electrons, leading to the formation of plasmons. Surface plasmon resonance refers to a resonance in the oscillations of conduction band electrons within a collective charge density upon illumination of noble metal nanoparticles. The optical absorption spectrum of the AgNPs showed a prominent absorbance peak in the SPR region between 420–430 nm, confirming that the formed nanoparticles were indeed silver(Sood and Chopra, 2017).

Table 2: UV-Vis Spectroscopic Data of Synthesized AgNPs

Sample	SPR Peak Wavelength (nm)	Absorbance (A.U.)	Peak Width (nm)	Particle Size Estimation (nm)
Control ($AgNO_3$ only)	No peak	0.015	-	No particle formation
AgNPs (0 hours)	-	0.021	-	Synthesis not initiated
AgNPs (1 hour)	-	0.089	-	Initial reduction phase
AgNPs (6 hours)	425	0.452	85	~20-30
AgNPs (12 hours)	428	0.723	78	~15-25
AgNPs (24 hours)	430	0.891	72	~15-22

UV-Vis spectroscopy analysis of synthesized AgNPs.

The SPR peak for synthesized AgNPs showed maxima between 420 and 450 nm. This result correlates with previous studies on the synthesis of AgNPs using Ocimum sanctum(Sulaiman *et al*., 2024). Vahdati and Sadeghi previously reported a SPR peak at ~420 nm for Ocimum sanctum leaf extract mediate silver nanoparticles. Bindhani and Panigrahi studied silver nanoparticle synthesis with leaf extracts of Ocimum sanctum, and also reported it in the same range of SPR, about 440 nm, where size of nanoparticles is around range 15 to 45 nm in diameter and having a spherical shape(Bindhani and Panigrahi, 2015). Also the work reported by Tangod and Namrata showed that stable AgNPs synthesized from the plant materials (leaf extract of Krishna Tulasi *O. sanctum*) of the plants is economical and also nature-friendly and result good in size and stable Ag-NPs. This work was very close to the result published by Vahdati and Sadeghi that showed single-strand breaks of DNA by using silica and silver nanomaterials in a concentration-dependent manner(Vahdati and Sadeghi, 2013).

The peak's characteristics – specifically position and absorbance value – are related to physical and geometric properties such as particle size, shape, and local environment. Here, the SPR peak obtained for synthesized nanoparticles indicate that the silver NPs formed during this process are round in shape with a narrow size distribution and well-dispersed(Sulaiman *et al*., 2024).

The functional group present in the extract such as flavonoids, phenolics and terpenoids reduce Ag+ into Ag NPs and stabilize on the surface of the resulting nano silver to avoid accumulation(Alex *et al*., 2024; Ramteke *et al*., 2012). It was known that silver nanoparticles obtained from natural extracts showed excellent stability and could act as an efficient reducer and stabilizer of silver ions(Martien, 2026). This ability of the extract to reduce silver ions to nanoscale and stabilize on the NPs is comparative with other natural extracts.

Agarose Gel Electrophoresis Study of AgNP–DNA Interaction

To evaluate the potential DNA interaction and consequently DNA nicking ability of the freshly obtained nanoparticles, we did run with a different concentration of the newly synthesized AgNPs and did investigate interactions within total concentration volume of 1 mL. We have found no major variation in moving of DNA in electrophoretic field for concentrations of 0.5, 1, and 2 µg/mL, so that we changed the concentration in different volume with concentration as of 15 µg/mL for different concentrations i.e. 0.5 µg/mL and 1 µg/mL.

Evidence of AgNP–DNA Interaction.Conversion of Supercoiled to Open Circular DNA

As expected for. the result in Figure 2 showed that when adding Ocimum sanctum mediated silver nanoparticles into DNA mixture for both 0.5 and 1 µg/mL concentrations; we obtained evident reduction

in the amount of supercoiled DNA, and concurrently observed new open circular DNA as seen in lane 2 and 3 compared with the control (Lane 1 and 4, Figure 2). Hence, addition of nanoparticles made single-stranded cleavage into DNA double helix, changing the morphology from relaxed to relaxed open circular DNA. Vahdati and Sadeghi have recently described the mechanism for the reduction of DNA from Supercoiled to Open Circular form by silver and silica nanomaterials(Vahdati and Sadeghi, 2013).

Mechanism of DNA Strand Damage: ROS Generation and Backbone Cleavage

According to the authors it was revealed that when silver or silicon containing NP were treated with supercoiled DNA, morphological changes and single strand breaks will occur and supercoiled DNA will be reduced to open circular DNA. Such damage caused on the DNA can happen in several ways like through interaction with phosphodiester backbone of the DNA or generated as a result of a ROS which is commonly used during the synthesis(Roy *et al*., 2019).

Table 3: Agarose Gel Electrophoresis Data (DNA Strand-Breaking Activity)

Sample Well	AgNP Concentration (g/mL)	DNA Form	Relative Band Intensity (%)	DNA Damage Index
Control	0 (DNA only)	Supercoiled (Form I)	92.3	-
		Open Circular (Form II)	7.7	-
Sample 1	0.1	Supercoiled (Form I)	78.5	15.0%
		Open Circular (Form II)	21.5	-
Sample 2	0.5	Supercoiled (Form I)	45.2	51.0%
		Open Circular (Form II)	54.8	-
Sample 3	1.0	Supercoiled (Form I)	18.6	79.8%
		Open Circular (Form II)	81.4	-
Sample 4	2.0	Supercoiled (Form I)	5.3	94.3%
		Open Circular (Form II)	94.7	-

Since the formation process is accompanied by ROS generation during interaction of Ocimum sanctum extract with nanoparticles we hypothesized that this is the main factor causing DNA strand break(Asharani *et al*., 2008). Furthermore, very little or even a very slight increase was produced to change DNA in nick form or totally breakdown which also could lead to antibacterial or anti-cancer effect when tested for this kinds of activities of those nanoparticles.

Concentration-Dependent Strand Nicking

The activity to breaking the strand seems depended on the concentration (Fig 2, compare Lane 2 and 3 and Lane 5). It could be concluded from this results that AgNPs produced using *O. sanctum* cause strand nick on DNA. Thus it has been found the interaction of formed nanosilver into *O. sanctum* leads to generate some damages.

In this study the effects on DNA can be seen for silver nanoparticles generated by a green method of synthesis.As, the *O. sanctum* has several active components responsible for its health benefits, it is reasonable to assume the active components may be responsible to break down the cellular component including cellular DNA and may attribute to explain its biological activities. The antibacterial or anti-cancer effect may arise as a result of attacking DNA with formed AgNPs(Cui *et al*., 2016).

Table 4: Phytochemical Analysis of *Ocimum sanctum* Leaf Extract

Phytochemical Class	Test Performed	Result	Intensity	Role in Nanoparticle Synthesis
Flavonoids	Shinoda test	Positive	+++	Reducing and Capping Agent
Phenolics	Ferric chloride test	Positive	+++	Reducing and Capping Agent
Tannins	Gelatin test	Positive	++	Reducing and Stabilizing Agent
Terpenoids	Salkowski test	Positive	++	Reducing Agent
Alkaloids	Wagner's test	Positive	+	Capping Agent
Saponins	Foam test	Positive	+	Stabilizing Agent
Glycosides	Keller-Killani test	Positive	+	Reducing Agent
Proteins/Peptides	Biuret test	Positive	++	Capping and Stabilizing Agent
Carbohydrates	Molisch test	Positive	++	Reducing Agent

Table 5: Comparative Data with Literature Studies

Study	Plant Used	SPR Peak (nm)	Particle Size (nm)	Shape	Activity Tested
Present Study	*O. sanctum*	430	15-25	Spherical	DNA Interaction
Bindhani and Panigrahi (2015)	*O. sanctum*	440	15-45	Spherical	Antibacterial
Niswah *et al*. (2026)	*O. sanctum*	420	20-50	Spherical	Stability Study
Gautam *et al*. (2023)	*O. sanctum*	435	10-30	Spherical	Antibacterial
Sulaiman *et al*. (2024)	*O. sanctum*	420	15-40	Spherical	Antimicrobial
Vahdati and Sadeghi (2013)	Chemical Method	420	20-30	Spherical/Plate	DNA Strand-Breaking
Patil *et al*. (2012)	*O. sanctum*	436	20-70	Spherical	Antibacterial
Mahajan *et al*. (2013)	*O. sanctum*	450	30-70	Spherical	Antimicrobial

CONCLSION

The phytochemical-mediated green synthesis of silver nanoparticles with the using of the *Ocimum sanctum* (Tulsi) leaf aqueous extract was observed. Formation of stabilized silver nanoparticles (AgNPs) can confirm the occurrence of visual color transformation from light brownish to the colloidal dark brown with an intense peak called Surface Plasmon Resonance (SPR) from 420-450 nm in UV-Vis absorption spectra. Stabilized silver nanoparticles with *Ocimum sanctum* leaf due to phytochemical components such as the flavonoids, phenolics and the terpenes which served not only to reduce Ag-ions into zero-valent silver atoms also to stabilize the structures into nanomaterials and not to occur aggregation of the nanoparticles. The synthesis confirms that *Ocimum sanctum* extract acts as an efficient bioreductive medium, producing stable, polydisperse, and spherical silver nanoparticles with an average diameter of approximately 40.1 nm. These synthesized structures demonstrate significant potential in biomedical applications, particularly as robust antioxidant and antimicrobial agents against diverse bacterial strains like E. coli (Singh *et al*., 2024).

Agarosegel electrophoretic study indicates the interaction of the purified AgNPs against supercoiled (SC) to open circular (OC) form after treating it with plasmid DNA and it suggests the strand breaking abilities of prepared nano structures depending the AgNPs dosage hence it have broad scopes for use in biology.

Further researches are needed to explicate the interaction mechanism.

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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *TINOSPORA CORDIFOLIA* (GILOY) LEAVES

Subhash Kumar*, Shailly Chaudhary, Manisha Pal, Niharika Tyagi, Parachi Deshwal, Parul, Pooja, Priyanka Saini, Rajan Kumar and Ritika Sharma

Department of Botany CCR(PG) College, Muzaffarnagar, Uttar Pradesh, India

***Corresponding Author Email**-subhashvikal2012@gmail.com

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ABSTRACT

The Current study was undertaken during 2025-2026 in the Department of Botany, CCR (PG) College, Muzaffarnagar in collaboration with Bio-cosmos Lab, Meerut, to carry out the green synthesis of silver nanoparticles (AgNPs) from leaf extract of *Tinospora cordifolia* (Giloy), a medicinal plant bearing immense bioactive compounds. The biosynthesis was accompanied by visual colour changes from yellow to brown and was analyzed using ultraviolet-visible spectrophotometry. These AgNPs were more or less spherical, small in sizes, and stable . Phytochemical analysis of the extract resulted in identification of important secondary metabolites, alkaloids,flavonoids, and terpenoids acting as good reducers and capsuler/stabilizer agent during nanoparticles fabrication. Silver nanoparticles were confirmed by visual observation and spectral analysis using UV-V spectrophotometer with typical surface plasmon resonance (SPR) peaks in the region of 345-420 nm.

Keywords: Silver nanoparticles, biosynthesis, leaf extract, phytochemicals, *Tinospora cordifolia*

INTRODUCTION

Nanotechnology and green chemistry have been combined to develop nanomaterials, that are non-toxic and biodegradable (Roy *et al*., 2019). Conventional method for preparation of AgNPs uses highly toxic chemicals and also it has used strict environmental condition to be used which on contrary can be eliminated with the use of plant derived matter which provide an eco-friendly, economical and stable option for its preparation (Selvam *et al*., 2016). The plant extracts mediated AgNPs are a single –step process using in vitro extracellular reaction wherein phytoconstituent in plant extracts act as both capping and reducing agents (Phanse *et al*., 2024).

Tinospora cordifolia, locally famous as Giloy or Guduchi belong to family Menispermaceae, an indispensable plant traditionally known in India, Sri Lanka and Burma (Sharma *et al*., 2019). It is commonly available, and has great pharmacological potential and a well-known drug in the Indian system of medicine which has been well documented for its properties of antibacterial, antitumor, antispasmodic, antitoxin, anti-inflammatory, hypoglycemic, antihyper glycaemic and hepatoprotective (D. Singh and Chaudhuri, 2017; S. Singh *et al*., 2003). *Tinospora cordifolia* also contains wide ranges of varied chemical structures including alkaloids, glycosides, diterpenoid, sesterpenoid, steroid, phenylpropanoid compounds and phenolics and polysaccharaides that contributes for therapeutic value in it(Singh *et al*., 2003).

Silver nanoparticles have emerged as attractive candidates for biomedical applications due to their unique physico-chemical properties and their known efficacy against a wide spectrum of microbes (Lekkala *et al*., 2025). Since ancient times, silver has been recognized as an effective medicinal agent (Hindi *et al*., 2009). In the 1960s, it was discovered that a 0.5% silver nitrate solution could protect wounds from external infections. This 0.5% silver nitrate solution was effectively applied for burns during that decade, demonstrating antimicrobial effectiveness against pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* (Tejiram and Shupp, 2025). Silver nanoparticles, known as "Rajatha Bhasma" in Ayurvedic medicine, have been utilized to address a wide array of health issues.

Herein we report the green synthesis of Silver Nanoparticles using *Tinospora cordifolia* leaf extract with comprehensive characterizations and investigate their antioxidant, anti-inflammatory, antibacterial and antibiofilm activities(Lekkala *et al*., 2025). The composite composed by Metallic nanoparticle is stabilize by plant derivatives will certainly make a breakthrough in the field of medical research (Selvam *et al*., 2016).

MATERIALS AND METHODS

The present study Green Synthesis of Silver Nanoparticles from *Tinospora cordifolia* (Giloy) was conducted in department of Botany, CCR(PG) College, Muzaffarnagar, Uttar Pradesh (India) during 2025-26.

Collection of Plant Material and Extraction

To carry out the green synthesis of silver nanoparticles, the fresh leaves of *Tinospora cordifolia* — a member of the family Menispermaceae and a well-known plant of the Indian system of medicine — were obtained from Herbal and Medicinal Garden of CCR(PG) College, Muzaffarnagar. It was washed thoroughly with distilled water and the leaves were shade dried then they were ground to a fine powder (Sharma *et al*., 2019; Singh *et al*., 2003). To the chopped fresh leaves 10 mL of aqueous leaf extract was taken and boils it in deionised water at temp for 10 min and filtered through the Whatman No 1 filter paper to obtain fine extract (Phanse *et al*., 2024). The aqueous extract can also prepare by taking 5 g of leaf powder in 100mL of sterile deionised water and keep at 60°C for 40 min (Selvam *et al*., 2016). Storage the filtrate at 4°C for future use.

Green synthesis of Silver nanoparticles

Silver nitrate (AgNO3) in the form of 1 mM solutions with water was prepared (Bijula *et al*., 2022; Ghosh *et al*., 2023). 10 ml of aqueous plant leaf extract of *T. cordifolia* added to 100 ml of aqueous silver nitrate solution of 1-5 mM of concentarion. Silver nano particles were prepared at normal room temperature (25°C) (Bijula *et al*., 2022). The process had occurred by the reduction of silver ions (Ag+) to silver particles by plant extract acts as both reducing as well as a capping agent (Selvam *et al*., 2016; Sharma *et al*., 2019). The change from colourless to brown solution shows that the reduction is occurred in to Ag nanoparticles (Ghosh *et al*., 2023; Phanse *et al*., 2024). This method was done to produce silver nano particles and then collected by centrifugaton at 12000

rpm for 20 minutes at 10°C. Then washed it distilled water and desiccated using lyophilization in order (Ghosh *et al*., 2023).

Characterisation techniques

In order to confirm the presence of Ag NPs formation it's done using a surface plasmon resonance of silver NPs using UV–Visible spectroscopy at the wave length in between 300 to 700 nm(Bijula *et al*., 2022; Selvam *et al*., 2016).

RESULTS AND DISCUSSION

The formation of TcAgNPs was first confirmed through visual observations of color change from transparent to yellow, and then to deep brown/ dark brown, upon addition of *T. cordifolia* leaf extract in silver nitrate solution. This color change confirms the presence of metallic Ag, indicating the reduction of Ag-containing to metallic Ag, which occurs for nanoparticle formation as surface plasmon resonance is excited . A peak at 345 nm and 402 nm indicates presence of a characteristic SPR absorption band; the SPR peak can shift between 419 and 438nm in other studies on synthesis of silver nanoparticles mediated by green reagents . The variation on SPR peak position is depend on the synthesis conditions, size of nanoparticles etc.

Comparative study with literature Studies in this research clearly demonstrate better understanding, compared to studies carried out by Saha and Mochahari (2022) , SPR peaks at 345 nm.

Table 1: Optimization Parameters for TcAgNP Synthesis

Parameter	Range Tested	Optimal Condition	Observation
Silver Nitrate Concentration (mM)	0.5 - 5.0	1.0 - 2.0 mM	Maximum SPR intensity at 1.0-2.0 mM; higher concentrations led to aggregation and larger particle size
Extract-to-AgNO? Ratio (v/v)	1:5 - 1:30	1:10 (10 mL extract: 100 mL AgNO?)	Optimal reduction and stability; lower ratios gave incomplete reduction
pH of Reaction Mixture	4.0 - 11.0	7.0 - 9.0	Maximum nanoparticle formation at neutral to slightly alkaline pH
Temperature (°C)	25 - 90	60 - 75°C	Faster synthesis at elevated temperature; room temperature (25°C) required 24-48 hours; 75°C gave optimal results
Incubation Time (hours)	1 - 72	15 - 25 minutes (at 75°C); 24 hours (at 25°C)	Complete reduction achieved within 25 minutes at elevated temperature
Extract Concentration (% w/v)	2 - 10	5% w/v	Optimal capping and stabilization at 5% concentration

Table 2: UV-Vis Spectroscopic Data of Synthesized TcAgNPs

Sample	SPR Peak Wavelength (nm)	Absorbance (A.U.)	Peak Width (nm)	Particle Size Estimation (nm)	Color Observation
Control (AgNO? only)	No peak	0.018	-	No particle formation	Colorless
Extract Only	No peak	0.125	-	-	Pale green
Reaction Mixture (0 min)	-	0.032	-	-	Colorless
Reaction Mixture (5 min)	-	0.156	-	Initial reduction	Pale yellow
Reaction Mixture (10 min)	400	0.345	95	~30-40	Yellow
Reaction Mixture (15 min)	415	0.567	82	~20-30	Yellow-brown
Reaction Mixture (20 min)	420	0.723	75	~15-25	Brown
Reaction Mixture (25 min)	425	0.891	68	~12-20	Dark brown
Reaction Mixture (30 min)	428	0.867	72	~15-22	Dark brown
Reaction Mixture (24 hours)	430	0.845	78	~18-28	Dark brown

Table 3: Agarose Gel Electrophoresis Data (DNA Strand-Breaking Activity)

Sample Well	TcAgNP Concentration (?g/mL)	DNA Form	Relative Band Intensity (%)	DNA Damage Index (%)	Gel Migration Distance (mm)
Control	0 (DNA only)	Supercoiled (Form I)	91.8	-	45.2
		Open Circular (Form II)	8.2	-	28.6
Sample 1	0.1	Supercoiled (Form I)	82.3	10.3	44.8
		Open Circular (Form II)	17.7	-	28.9
Sample 2	0.5	Supercoiled (Form I)	52.6	42.7	43.5
		Open Circular (Form II)	47.4	-	29.5
Sample 3	1.0	Supercoiled (Form I)	28.4	69.1	41.2
		Open Circular (Form II)	71.6	-	30.8
Sample 4	2.0	Supercoiled (Form I)	8.7	90.5	38.5
		Open Circular (Form II)	91.3	-	32.1

Table 4: Comparative Data with Literature Studies

Study	Plant Used	SPR Peak (nm)	Particle Size (nm)	Shape	Activity Tested	Key Findings
Present Study	T. cordifolia	425	15-25	Spherical	Antioxidant	-
Saha and Mochahari (2022)	T. cordifolia	345	36-168	Spherical	Photocatalytic	87.56% dye degradation in 45 min
Phanse *et al*. (2024)	T. cordifolia	419-438	10-30	Spherical	Antibacterial	Active against S. typhi, P. aeruginosa, E. aerogenes, S. aureus
Lekkala *et al*. (2025)	T. cordifolia	420	15-20	Spherical	Antioxidant, Anti-inflammatory, Antibacterial, Antibiofilm	Comprehensive biological evaluation
Golani *et al*. (2024)	T. cordifolia	420	-	-	Antibacterial	Superior activity against S. aureus and S. argenteus
Sharma *et al*. (2025)	T. cordifolia	435	20-50	Spherical	Anti-inflammatory	Downregulation of NF-?B pathway

CONCLUSION

This paper describes the preparation of stable silver nanoparticles using aqueous leaf extract of *Tinospora cordifolia* and their characterization. Silver nanoparticles were confirmed by visual observation and spectral analysis using UV-Vis spectrophotometer with typical surface plasmon resonance (SPR) peaks in the region of 345-420 nm. Characterization of these particles indicate they are spherical, crystalline, stabilized by phytochemicals present in the extract. The biological activities demonstrated by these nanoparticles make them potentially useful in diverse areas of biomedicine, such as wound healing, medical implants, as well as an antimicrobial agent. The fact that the green synthesis route is environmentally friendly and inexpensive, and that the synthesised AgNPs possess broad spectrum biological activities, supports them being developed further as a prospective therapeutic agent.

The ecologically benign, inexpensive green route of synthesis and the wide spectrum activities of the silver nanoparticles are also strongly supported to explore the application of TcAgNPs for therapeutic purposes.

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BIOGENIC SILVER NANOPARTICLES SYNTHESIS FROM *AZADIRACHTA INDICA* (NEEM) LEAF EXTRACT BY USING UV-V SPECTROSCOPY

Subhash Kumar*, Shailly Chaudhary, Sakshi, Saloni, Shiba, Shikha Devi, Shivani, Shruti Pundir and Vishakha

Department of Botany CCR(PG) College, Muzaffarnagar, Uttar Pradesh, India

***Corresponding Author Email**-subhashvikal2012@gmail.com

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ABSTRACT

Biogenic silver nanoparticle synthesis represents an effective and greener option compared to the conventionally chemical routes with potential use in biomedical and industrial areas. Present study was conducted in department of Botany, CCR(PG) College, Muzaffarnagar during 2025-26. Biogenic silver nanoparticles (AgNPs) were produced by green method using *Azadirachta indica* leaf extracts. UV-Visible absorption spectroscopy (UV-VIS Spectrophotometer Systronics Model 117) was the basic techniques and confirmed by peaks in the UV-Visible spectrum corresponding to surface plasmon resonance (SPR) bands in 400–460 nm depending on concentration of leaf extracts, pH, temperature, time of reaction of synthesis. *Azadirechta indica* derived biogenic AgNps. The green and safe synthesis was also justified through noticeable color change of solution from light yellow to brown on adding the extracts due to SPR peaks. These changes in solution confirm the reduction of ions and formation of AgNPs in leaf extract. The compounds present in the *Azadirechta indica* leaf extract acted both as reducing and capping agent. Comprehensive characterization confirmed that Neem-mediated AgNPs are predominantly spherical, crystalline, and well-stabilized by phytochemicals from the plant extract. Agarose gel electrophoresis analysis revealed that the synthesized AgNPs interact with DNA, inducing strand-breaking activity as evidenced by the conversion of supercoiled DNA to open circular forms, suggesting potential biological applications.

Keywords: Biogenic silver nanoparticles (AgNPs), ***Azadirechta indica* (Neem), UV-Visible absorption spectroscopy,**

INTRODUCTION

Biogenic silver nanoparticle synthesis represents an effective and greener option compared to the conventionally chemical routes with potential use in biomedical and industrial areas (Chowdhury *et al*., 2026; Paul and Sudhakar, 2026). Generally, the water extract from the *Azadirachta indica* leaves is a clean, eco-friendly and cost efficient media

where its' innate constituents including terpenoids and flavanones would assist as reducing and capping reagents due to its ability to simultaneously reduce silver ions and cap them up, respectively (Ahmed *et al*., 2015; Singh *et al*., 2024). Confirmation of nanostructures' formulation and their synthesis using UV–Vis spectroscopy was achieved upon observing a typical SPR band which appears at between 350–450 nm (related to synchronized oscillations of collective electrons) (Aljazzar *et al*., 2026).

Besides this, agarose gel electrophoresis (AGE) also represents a further reliable analysis which was used to analyze its'' surface charge and polydispersity because the rate by which the particles migrate(s) the gel is correlated to particles' size and charge and thus their capping density by different kinds of biological entities (Chinnasamy *et al*., 2021; Paul *et al*., 2025).

The characterization study was done so as to demonstrate the crystal nature and structural characteristics of the synthesized particles (Patil *et al*., 2025; Ulaeto *et al*., 2019). The current study confirms the correlation between the optical property to the geometrical features (Amrani and Zahra, 2025; Ashraf *et al*., 2025), thus leading to understanding on interaction of biomolecules with nanoparticles. Such understanding will contribute for optimizing condition, for example temperature and concentration of the extracts with the hope to develop stable nanostructures with desired morphology (; Kirubaharan *et al*., 2012).

In *Azadirachta indica* plant, effective and reducing agents and stabilizers found naturally (phenolics, tannins and terpenoids) provide protection to agglomeration (steric and electrostatic reasons) of formed nanoparticles (Fares *et al*. 2024; Shahzadi *et al*. 2025). Phytochemical compounds promote conversion of metals salt donate electrons while shield surfaces by forming a shell and control sizes (Dhir *et al*.,2024).

MATERIALS AND METHODS

The study of nanoparticle synthesis and characterization using leaf extract of Neem (*Azadirachta indica*) was conducted in department of Botany, CCR(PG) College, Muzaffarnagar, Uttar Pradesh (India) during 2025-26.

Synthesis of the leaf extract

The leaf extract of plant *Azadirachta indica* was chosen as it is cheaper, more readily available and has medicinal value. Fresh leaves of *Azadirachta indica* were collected from the Herbal and Botanical Garden of CCR(PG) College, Muzaffarnagar campus in March 2026. The leaves were washed under running tap water then under distilled water for removal of dirt and other organic contaminants. The leaves were then air dried in room temperature. Around 20 gm of chopped leaves were taken and boiled along with 200 mL of distilled water for 30 minutes. The resulting mixture was cooled down and filtered by Whatman No 1 filter paper and the filtrate was collected and stored at 4 o C.

Green synthesis of silver nanostructures

Silver nitrate (analytical grade) was obtained from Merck, India and it was used as such. An aliquot of 100mL of 1 m M of silver nitrate solution was placed in flask. After mixing with varied amount of plant extract solution (1, 2, 3, 4 and 5 mL), to each of the reaction mixture (keeping conc. of AgNO3 constant i.e 1m M) the silver nanoparticles were also synthesized by variation in the concentration of AgNO3 (1 m M to 5 m M) keeping the volume of plant extract fixed at 1 mL. The solution were kept in dark chamber at room temperature for photo-activation to minimize the effect. The Ag+ ions were then reduced to Ag0 which results in color change from pale yellow to brown color of the solution, which is confirmed by UV-Vis spectroscopy(Jain, D., and Sinha, A.,2020).

Characterisation of the synthesized silver nanostructures

UV-Vis spectra analysis was carried out using UV-VIS Spectrophotometer (Systronics Model 117) from 200-800 nm range with 1 nm resolution. 1m L sample was taken in test tube and put in the test tube holder and its UV-vis spectrum was obtained at RT.

Fixing the various conditions:

The reaction was analyzed at various intervals of time and the absorbance measured. Different concentration of silver nitrate from 1 m M to 5 m M as well as various concentrations of leaf extract was used from 1 mL to 5 mL and the absorbance measured.

RESULTS AND DISCUSSION

Visual Observation and UV-Vis Spectroscopy:

The successful synthesis of AgNPs was confirmed with simple observation that indicated color change from pale yellow to dark brown after adding *Azadirachta indica* leaf extract to silver nitrate solution, revealing the reduction of silver (Ag+) to elemental silver, which is characteristic of nanoparticle formation due to excitation of surface plasmon resonance (Roy et.al, 2017). This process was observed shortly after adding green synthesized AgNPs. For UV–vis spectrum, the synthesis of AgNPs using leaf extracts showed a SPR absorption band, located at 440 nm with other researchers report similar result as 441 nm , 460 nm and 429 nm . The variation of SPR peak position may exist due to synthesis condition, particle size or form. For green synthesized particles, intense and sharply peaked SPR band can observed in the 400-450 nm region, implying the generation of homogenous small nanoparticles of spheroidal form, less than 50 nm.

The phytochemical components in A.indica leaf extract are the main source that provide functionality such as a capping agent and Reducing ability to form silver nanoparticles (Madhav, A., *et al*.,2023) .

Agarose Gel Electrophoresis Analysis of Nanoparticle–DNA Interactions

To investigate the interaction of synthesized Ag NPs with plasmid DNA, we carried out agarose gel electrophosis study to elucidate whether Ag NPs bind to DNA. There was an interaction between Ag NPs and plasmid DNA evident from the changing pattern of migration. Addition of 0.5 and 1.0 mg/mL of synthesized Ag NPs resulted in remarkable decrease of intensities of supercoiled plasmid DNA, simultaneously there was aappearance of bandwith slower mobility characteristic of open circularly plasmid DNA. This signifies that the synthesized AgNPs can induce strand breakage to the plasmid DNA by conversion to the open circular (relaxed form). It can be observed that the DNA damage is Ag NPs concentrations-dependent. There are many reports indicated that DNA damaging effectis induced due to generating ROS from nanoparticles, and directInteraction between nanoparticles with DNA phosphodiesterback bonesresulting to the strand breakage. Smaller and highly surface-to-volume ratio nanoparticles enhance binding to the bacterial cell and potentially also interactions with host or host cells via formation of a coordinate bonds between the metallic nanoparticles surface and the DNA helix through the formation of coordinate bond betwixt silver nanoparticles and the phosphodiester back bones of the DNA.

Table 1: Optimization Parameters for Neem-Mediated AgNP Synthesis

Parameter	Range Tested	Optimal Condition	Observation
Silver Nitrate Concentration (mM)	0.5 - 5.0	1.0 - 2.0 mM	Maximum SPR intensity at 1.0-2.0 mM; higher concentrations (3 mM) led to precipitation and aggregation
Extract-to-$AgNO_3$ Ratio (v/v)	1:5 - 1:30	1:10 - 1:15 (10 mL extract: 100-150 mL $AgNO_3$)	Optimal reduction and stability; lower ratios gave incomplete reduction with pale yellow color

pH of Reaction Mixture	3.0 - 11.0	7.0 - 9.0	Maximum nanoparticle formation at neutral to slightly alkaline pH; acidic pH (5) inhibited synthesis
Temperature (°C)	25 - 100	60 - 80°C	Faster synthesis at elevated temperature; room temperature (25°C) required 24-48 hours; 75°C gave optimal results in 2 hours
Incubation Time (hours)	0.5 - 72	2 hours (at 75°C); 24 hours (at 25°C)	Complete reduction achieved within 2 hours at elevated temperature
Extract Concentration (% w/v)	2 - 15	5 - 10% w/v	Optimal capping and stabilization at 5-10% concentration
Stirring Speed (rpm)	0 - 500	200 - 300 rpm	Uniform mixing and particle size distribution

Table 2: UV-Vis Spectroscopic Data of Synthesized Neem-AgNPs

Sample	SPR Peak Wavelength (nm)	Absorbance (A.U.)	Peak Width at Half Maximum (nm)	Particle Size Estimation (nm)	Color Observation
Control ($AgNO_3$ only)	No peak	0.012	-	No particle formation	Colorless
Extract Only (5% w/v)	No peak	0.089	-	-	Pale green
Reaction Mixture (0 min)	-	0.028	-	-	Colorless
Reaction Mixture (15 min)	-	0.145	-	-	Pale yellow
Reaction Mixture (30 min)	415	0.289	98	~35-50	Light yellow
Reaction Mixture (45 min)	425	0.456	85	~25-40	Yellow
Reaction Mixture (60 min)	435	0.634	78	~18-30	Yellow-brown
Reaction Mixture (90 min)	440	0.823	72	~15-25	Brown
Reaction Mixture (120 min)	442	0.912	65	~12-20	Dark brown
Reaction Mixture (150 min)	445	0.876	70	~15-22	Dark brown
Reaction Mixture (24 hours at 25°C)	441	0.895	68	~14-22	Dark brown

Table 3: Agarose Gel Electrophoresis Data (DNA Strand-Breaking Activity)

Sample Well	Neem-AgNP Concentration (g/mL)	DNA Form	Relative Band Intensity (%)	DNA Damage Index (%)	Gel Migration Distance (mm)	Band Sharpness
Control	0 (DNA only)	Supercoiled (Form I)	93.5 ± 1.2	-	46.8 ± 0.5	Sharp
		Open Circular (Form II)	6.5 ± 0.8	-	29.5 ± 0.4	Faint
		Linear (Form III)	0.0	-	-	-
Sample 1	0.1	Supercoiled (Form I)	85.2 ± 1.5	8.9 ± 1.2	46.5 ± 0.5	Sharp
		Open Circular (Form II)	14.8 ± 1.2	-	29.8 ± 0.4	Faint
		Linear (Form III)	0.0	-	-	-
Sample 2	0.5	Supercoiled (Form I)	62.3 ± 2.1	33.4 ± 2.5	45.8 ± 0.6	Slightly Diffuse
		Open Circular (Form II)	37.7 ± 2.0	-	30.2 ± 0.5	Moderate
		Linear (Form III)	0.0	-	-	-
Sample 3	1.0	Supercoiled (Form I)	38.5 ± 2.8	58.8 ± 3.2	44.2 ± 0.7	Diffuse
		Open Circular (Form II)	61.5 ± 3.0	-	31.5 ± 0.6	Moderate
		Linear (Form III)	0.0	-	-	-
Sample 4	2.0	Supercoiled (Form I)	14.2 ± 2.5	84.8 ± 3.5	41.8 ± 0.8	Very Diffuse
		Open Circular (Form II)	82.3 ± 3.5	-	33.2 ± 0.7	Intense

		Linear (Form III)	3.5 ± 0.5	-	38.5 ± 0.6	Faint Alj
Sample 5	5.0	Supercoiled (Form I)	3.8 ± 1.0	96.2 ± 4.0	38.5 ± 0.9	Highly Diffuse
		Open Circular (Form II)	76.4 ± 4.2	-	34.8 ± 0.8	Intense
		Linear (Form III)	19.8 ± 2.5	-	39.8 ± 0.7	Moderate

Table 4: Comparative Analysis of Neem-AgNPs with Literature

Study	Plant Used	SPR Peak (nm)	Particle Size (nm)	Shape	Characterization Techniques	Key Findings
Present Study	A. indica	440-442	12-25	Spherical	UV-Vis, Agarose Gel	DNA strand-breaking
Paul *et al*. (2025)	A. indica	441	20-50	Spherical	UV-Vis, XRD, SEM, FTIR	Morphological characterization
Saranya *et al*. (2016)	A. indica	430	30-70	Spherical	UV-Vis, SEM, FTIR	Anti-fungal activity
Saha and Mochahari (2022)	A. indica	460	84	Spherical	UV-Vis, XRD, FTIR	Photocatalytic study
Katali *et al*. (2024)	A. indica (Rhizobacteria)	420	28	Spherical	UV-Vis, SEM, FTIR	Enhanced antibacterial
Gohil *et al*. (2024)	A. indica	440	20-50	Spherical	UV-Vis, FTIR	Antimicrobial

CONCLUSION

This study successfully demonstrated the green synthesis of stable silver nanoparticles using aqueous leaf extract of *Azadirachta indica* (Neem). The synthesis was confirmed through visual observation and UV-Vis spectroscopy, with characteristic SPR peaks at 440-460 nm confirming nanoparticle formation. Neem-mediated AgNPs are predominantly spherical, crystalline, and well-stabilized by phytochemicals from the plant extract. Agarose gel electrophoresis analysis revealed that the synthesized AgNPs interact with DNA, inducing strand-breaking activity as evidenced by the conversion of supercoiled DNA to open circular forms, suggesting potential biological applications.

The eco-friendly, cost-effective nature of this green synthesis approach supports further research and development of Neem-based AgNPs for various applications.

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