ISSN: 2229-7359 Vol. 11 No. 3s, 2025

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FTIR Spectroscopy In Fire Forensics: A Novel Approach To Identify Pollen Signatures At Extreme Temperatures

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Abstract

Pollen grains from ten floral species were analysed using Fourier Transform Infrared (FTIR) spectroscopy to analyse their molecular stability under extreme temperatures, as encountered in fire-related forensic cases. Samples were subjected to temperatures ranging from 25°C to 1000°C for different duration. FTIR spectra revealed that up to 500°C, the molecular structure and composition of pollen grains remained largely unchanged up to 24 hours. At 1000°C spectral changes were observed, including the disappearance of some peaks; however, sufficient spectral features persisted to allow identification of pollen type. This study demonstrates the robustness of FTIR spectroscopy in identifying pollen grains exposed to high temperatures, offering a non-destructive, cost-effective and efficient alternative to electron microscopy. The findings highlight the potential of FTIR spectroscopy to expand forensic palynology applications by enabling the creation of a comprehensive spectral profile for high-temperature pollen analysis. The integration of FTIR spectroscopy in high-temperature pollen analysis provides a novel approach for solving crimes, particularly in cases such as fire accidents, vehicular fires and arson investigations. By combining botanical expertise with advanced microscopy and chemometric techniques, forensic palynology efficiently expands its role in legal investigations worldwide.

Keywords: Palynology, Non-destructive, FTIR Spectra, Fire Forensics, Forensic Investigation, High-Temperature Analysis

INTRODUCTION

Forensic palynology is a specialized field that examines pollen grains, spores, and other palynomorphs to establish connections between objects, individuals, and locations, providing valuable evidence in both criminal and civil legal proceedings [1]. Pollen grain analysis has become a crucial tool in forensic science due to its ability to provide trace evidence Pollen evidence can disclose the geographical history of a person or object, as different regions, countries, and even specific areas within a garden possess unique pollen profiles[2] Additionally, pollen analysis can determine the time of year when an object came into contact with the pollen, providing temporal context to the evidence[1]. The outermost layer of pollen grains, known as the exine, is primarily composed of sporopollenin, a remarkably resilient compound that can withstand extreme environmental conditions. This durability makes pollen grains invaluable in forensic investigations, particularly in fire forensics, where their resistance to high temperatures is crucial. The versatility of pollen analysis extends across multiple disciplines, including plant biology and forensic science, as it enables the rapid and precise identification and classification of biological samples.

FT-IR spectroscopy has proven to be an exceptionally valuable tool across various research disciplines, particularly excelling in the analysis of organic materials. Its versatility and precision make it an indispensable method for characterizing complex biological and chemical samples. [3][4][5] FT-IR spectroscopy provides critical insights into the chemical bonding and molecular structure of materials. This technique is based on the principle that chemical bonds and groups of bonds vibrate at specific characteristic frequencies. When a molecule is exposed to infrared radiation, it absorbs energy at frequencies unique to its structure. During FT-IR analysis, a modulated infrared beam is directed onto a specific area of the sample. The sample's ability to transmit and reflect infrared light at various frequencies is recorded and translated into an infrared absorption spectrum, which appears as a series of inverted

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peaks. These peaks serve as a molecular "fingerprint," enabling precise identification and characterization of the material [5]. FT-IR spectroscopy produces a molecular fingerprint unique to each sample, where absorption peaks correspond to specific vibrational frequencies of atomic bonds within the material. Since no two distinct compounds share identical atomic arrangements, their IR spectra are inherently unique, enabling unambiguous qualitative identification of substances while preserving the sample (nondestructive analysis). The technique also offers quantitative insights, as peak intensities directly reflect material concentrations, eliminating the need for external calibration, Requiring minimal sample preparation, FT-IR combines efficiency with precision, making it ideal for rapid analysis. Its proven utility extends to microbiology, where it reliably characterizes and identifies microorganisms through their distinct biochemical profiles, demonstrating its versatility across scientific disciplines.[6][7][8][9]. The first scientists to apply FT-IR spectroscopy for pollen identification were Pappas et al. (2003)[10]. The researchers developed a spectral reference library for 20 pollen types using both diffuse reflectance and potassium bromide (KBr) pellet FT-IR techniques. A diagnostic spectral region (1500–800 cm⁻¹) served as a unique biochemical fingerprint, enabling precise discrimination between species through comparative analysis with unknown samples. FT-IR spectroscopy detects pollen's diverse biochemical components—including proteins, lipids, carbohydrates, carotenoids, and sporopollenins—by analyzing their distinct vibrational signatures. This capability extends to monitoring chemical alterations in pollen exposed to environmental stressors, such as heat, by tracking shifts in molecular bonds. The method excels in probing intracellular components like lipids and carbohydrates due to its penetration depth, offering deeper insights into pollen's nutritional and structural chemistry compared to surface-focused techniques. By combining spectral libraries with chemometric validation, FT-IR provides a rapid, reliable alternative to traditional microscopy for species identification and environmental impact studies[7][11].

This pilot study explored the potential of Fourier Transform Infrared (FT-IR) spectroscopy as a rapid method for identifying pollen grains following exposure to various temperature conditions of 30°C, 200 °C, 500 °C, 800 °C and 1000 °C to evaluate the changes between the pre and post temperature studies with ten different flower species of Tecom stans, Clitoria ternatea, Onosma bracteatum, Ipomoea pandurata, Pseuderanthemum, Cordia angiocarpe, Leucophyllum frutescens, Datura stramonium, Bougainvillea glabra, Cape honeysuckle. This study examined the morphological features and chemical composition variations of pollen grains from the specified taxa using compound microscopy and FT-IR spectroscopy. The primary goal was to assess the potential of FT-IR spectroscopy as an alternative method for identifying pollen grains recovered as trace evidence from fire-related crime scenes. Unlike traditional palynological techniques, FT-IR spectroscopy offers a more accessible approach, requiring no specialized skills in pollen analysis. Its ability to rapidly and reliably identify pollen makes it particularly valuable for arson investigations, where time-sensitive and accurate analysis is crucial. This study evaluates FT-IR's applicability for pollen exposed to 200–1000°C addressing gaps in fire-forensic methodologies[7].

METHODOLOGY

Flower buds of *Tecom stans, Clitoria Ternatea*, *Onosma bracteatum, Ipomoea pandurata*, *Pseuderanthemum, Cordia angiocarpe*, *Leucophyllum frutescens*, *Datura stramonium, Bougainvilleaglabra, Cape honeysuckle* were collected from streets and gardens of Gujarat, India. The study focused on non-acetolysed materials, which were mounted using glycerine as the medium. Compound Microscopy (40x, Leica) was used to assess morphological features. Measurements were taken of the polar and equatorial axes of the pollen grains to determine their dimensions. Pollen samples were subjected to incrementally increasing temperatures (ranging from 30°C to 1000°C) to simulate fire conditions for 6-8 hours. A muffle furnace was used to maintain controlled heating conditions. Pollen spectra were acquired at room temperature using a Bruker Alpha II FTIR spectrometer equipped with an attenuated total reflectance (ATR) module. Spectral data were collected over the range of 4000–400 cm⁻¹ with a resolution of 4 cm⁻¹, averaging 26 scans per sample for precise analysis.Pre- and post-heating pollen samples were analysed using FTIR Spectroscopy to capture changes in molecular vibrations.

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RESULT

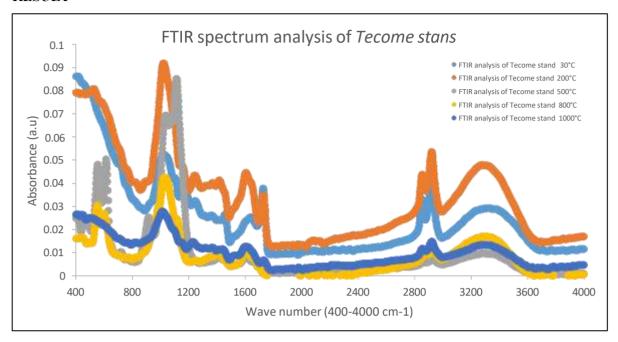


Figure 1A: Comparison of FTIR Absorbance vs. Wave number of *Tecom stans* Pollen grain at 30°C, 200 °C, 500 °C, 800 °C, and 1000 °C

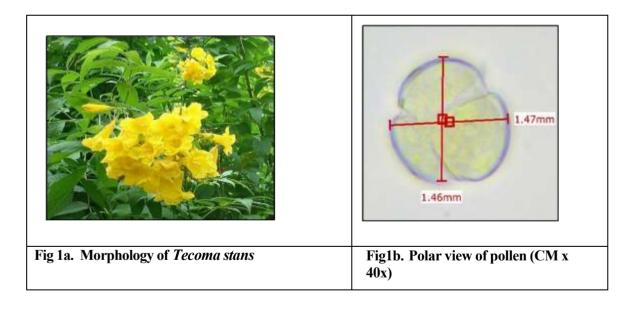
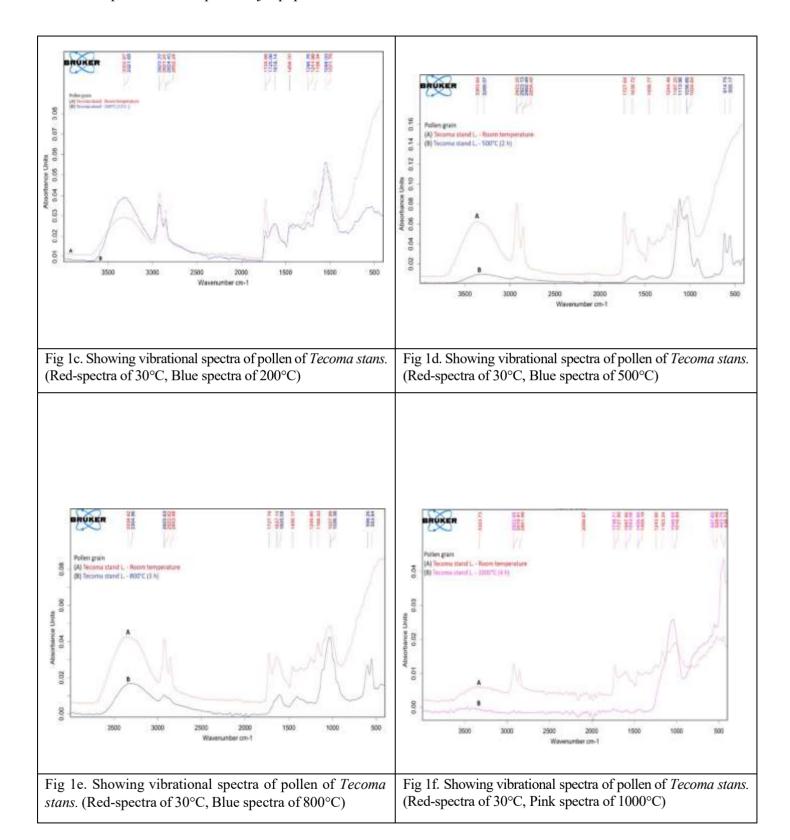


Figure 1B: Pollen grain characteristics of Tecoma stans.

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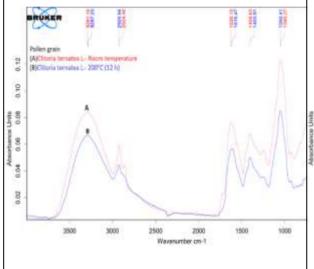
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0.80 m 0.85 mm

Fig 2a. Morphology of *Clitorea* ternatea.

Fig 2b. Polar view of pollen (CM x 40x)



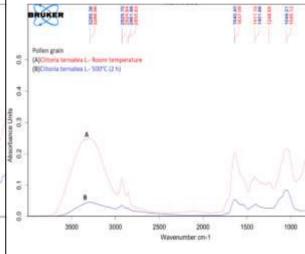
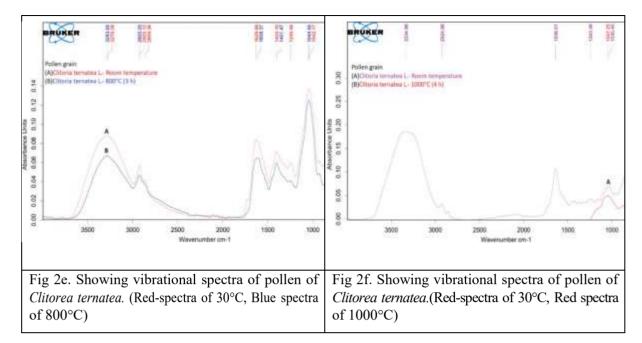


Fig 2c. Showing vibrational spectra of pollen of *Clitorea ternatea*.(Red-spectra of 30°C, Blue spectra of 200°C)

Fig 2d. Showing vibrational spectra of pollen of *Clitorea ternatea*.(Red-spectra of 30°C, Blue spectra of 500°C)

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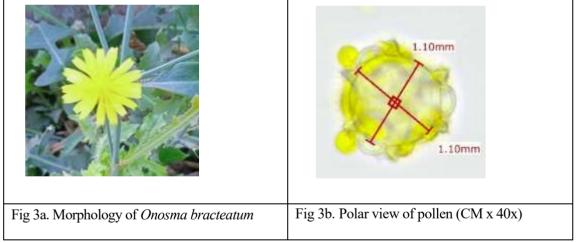


Figure 2: Pollen grain characteristics of Clitorea ternatea

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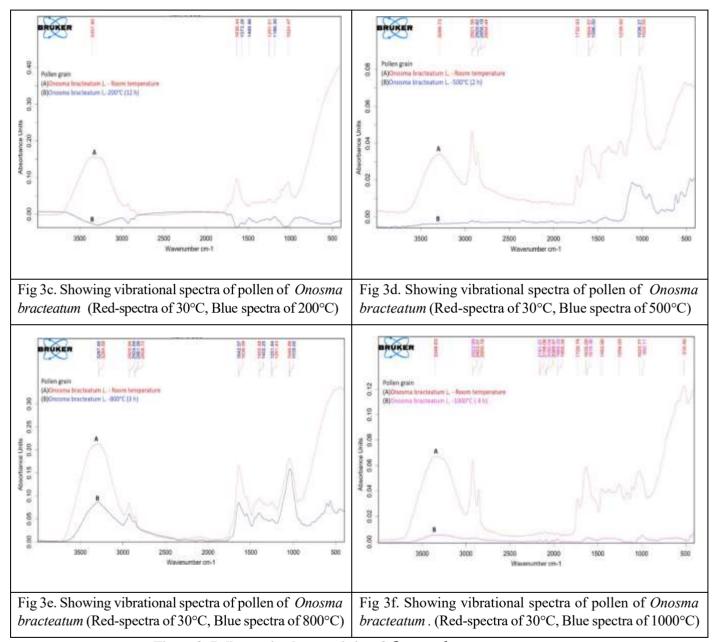
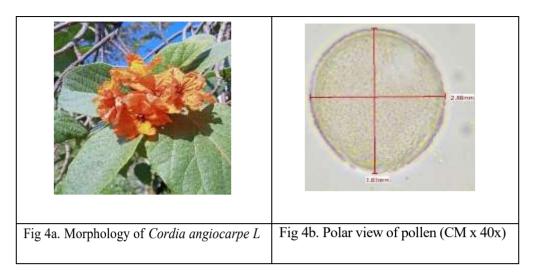


Figure 3: Pollen grain characteristics of *Onosma bracteatum*

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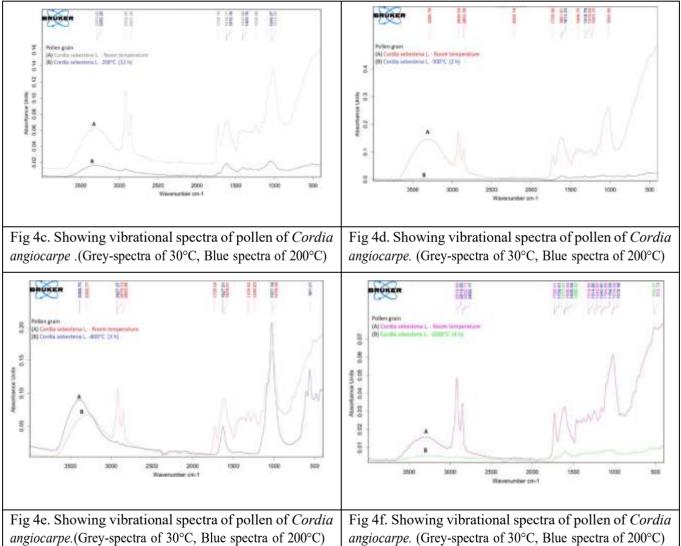


Figure 4: Pollen grain characteristics of Cordia angiocarpe

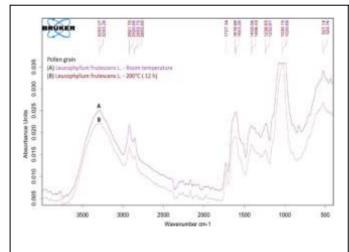
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1.82mm 0.97mm

Fig 5a. Morphology of *Leucophyllum* frutescens.

Fig 5b. Polar view of pollen (CM x 40x)



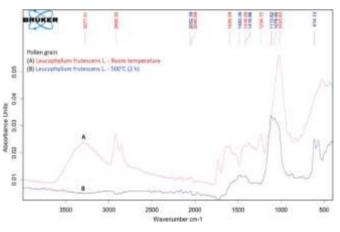
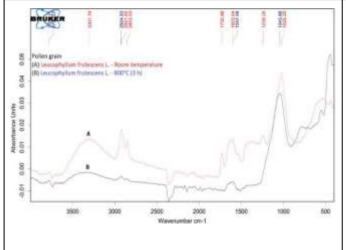


Fig 5c. Showing vibrational spectra of pollen of *Leucophyllum frutescens*. (Purple-spectra of 30°C, Red spectra of 200°C)

Fig 5d. Showing vibrational spectra of pollen of *Leucophyllum frutescens*. (Red-spectra of 30°C, Blue spectra of 500°C)

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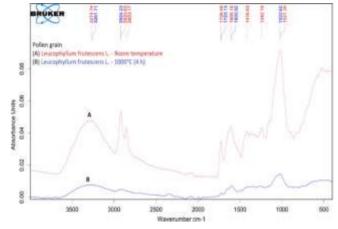


Fig 5e. Showing vibrational spectra of pollen of *Leucophyllum frutescens*. (Red-spectra of 30°C, Blue spectra of 800°C)

Fig 5f. Showing vibrational spectra of pollen of *Leucophyllum frutescens*. (Red-spectra of 30°C, Blue spectra of 1000°C)

Figure 5: Pollen grain characteristics of Leucophyllum frutescens.



Fig 6a. Morphology of Pseuderanthemum

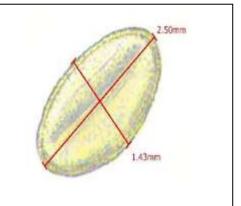


Fig 6b. Polar view of pollen (CM x 40x)

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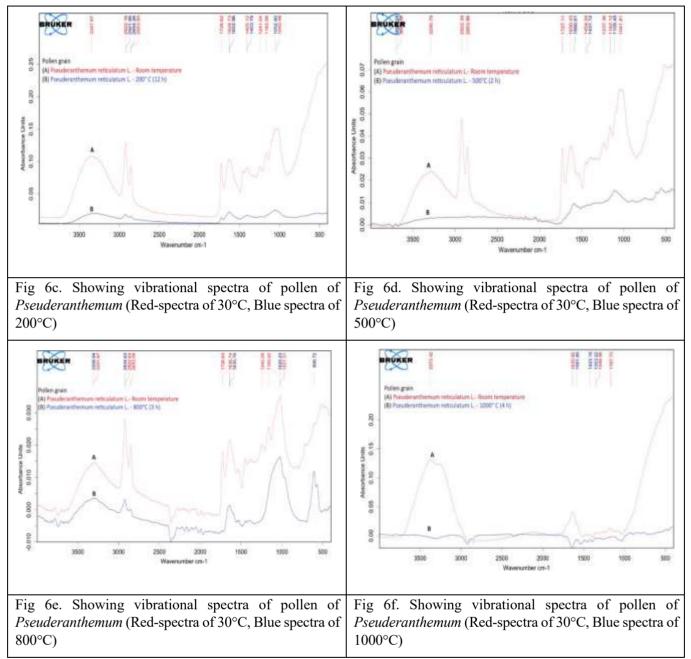


Figure 6: Pollen grain characteristics of *Pseuderanthemum*

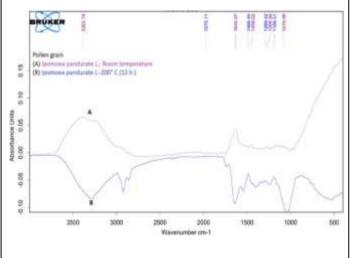
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Fig 7a. Morphology of *Ipomoea* pandurata

Fig 7b. Polar view of pollen (CM x 40x)



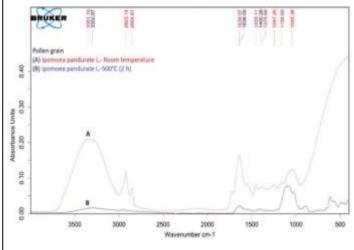


Fig 7c. Showing vibrational spectra of pollen of *Ipomoea pandurata*. (Purple-spectra of 30°C, Blue spectra of 200°C)

Fig 7d. Showing vibrational spectra of pollen of *Ipomoea* pandurata (Red-spectra of 30°C, Blue spectra of 500°C)

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pandurata (Red-spectra of 30°C, Blue spectra of 800°C)

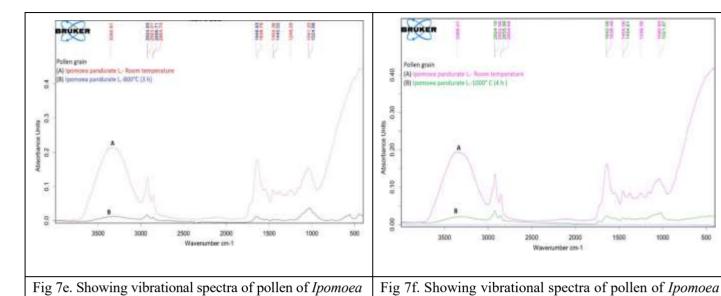
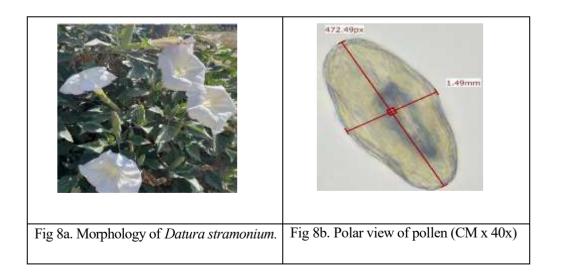


Figure7: Pollen grain characteristics of Ipomoea pandurata

pandurata (Pink-spectra of 30°C, Green spectra of 1000°C)



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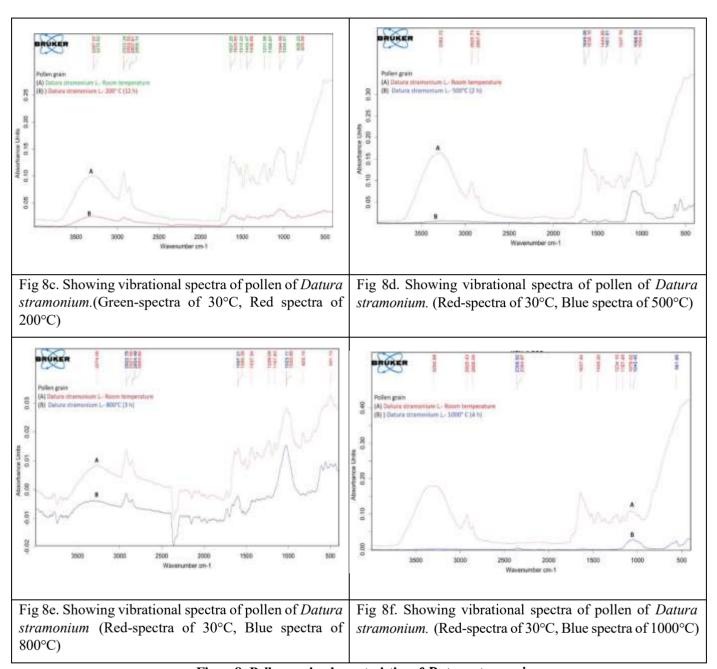


Figure8: Pollen grain characteristics of Datura stramonium

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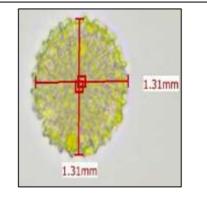
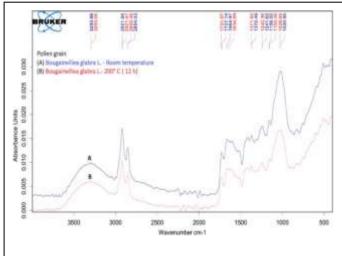


Fig 9a. Morphology of *Bougainvillea glabra*.

Fig 9b. Polar view of pollen (CM x 40x)



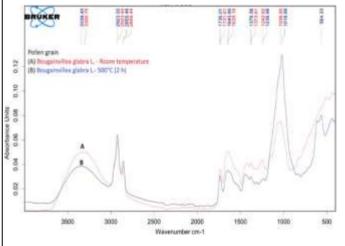


Fig 9c. Showing vibrational spectra of pollen of *Bougainvillea labra*. (Blue-spectra of 30°C, Red spectra of 200°C)

Fig 9d. Showing vibrational spectra of pollen of *Bougainvillea labra*. (Red-spectra of 30°C, Blue spectra of 500°C)

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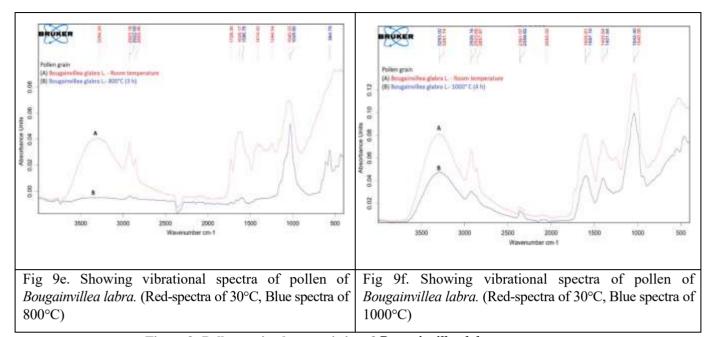
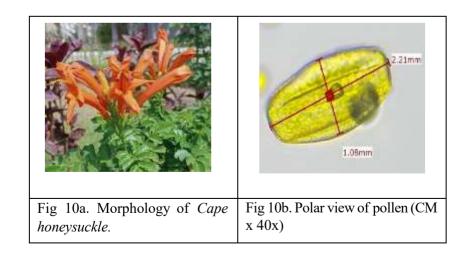


Figure 9: Pollen grain characteristics of *Bougainvillea labra*



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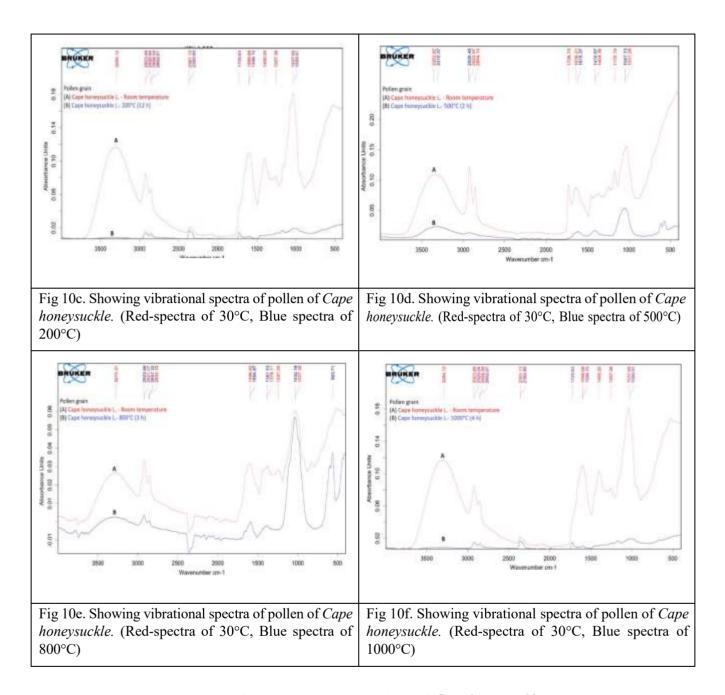


Figure 10: Pollen grain characteristics of Cape honeysuckle

Name of plant	Family	FTIR Spectrum Analysis						
(Scientific name)								
		30°C	200°C	500°C	800°C	1000° C		
Tecom stans	Bignoniaceae	√	√	✓	✓	✓		

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Clitoria ternatea	Fabaceae	V	✓	√	✓	✓
Onosma bracteatum	Boraginaceae	√	✓	√	✓	√
Ipomoea pandurata	Convolvulacea e	√	√	V	√	✓
Pseuderanthemum	Acanthaceae	V	✓	√	✓	✓
Cordia angiocarpe	Boraginaceae	V	✓	√	✓	✓
Leucophyllum frutescens	Cycadaceae	V	✓	✓	✓	✓
Datura stramonium	Solanaceae	V	✓	✓	✓	✓
Bougainvillea glabra	Nyctaginaceae	V	✓	✓	√	✓
Cape honeysuckle	Bignoniaceae	V	✓	✓	✓	✓

Table 1 showing the compilation of data of floral species and the results of temperature analysis

Morphological Analysis using compound microscope in all ten floral species exhibited minimal exine deformation from 30–200°C(Fig1b-10b) so pollen grains were recognizable but from 500°C temperature onwards exine thinning and pore structure is lost and from 800–1000°C exine is fragmented so pollen grain remained like a charred structure beyond identification.

FT-IR Spectral Findings

Assessment of FTIR spectra of *Tecom stans, Clitoria ternatea*, *Onosma bracteatum, Ipomoea pandurata*, *Pseuderanthemum, Cordia angiocarpe*, *Leucophyllum frutescens*, *Datura stramonium, Bougainvillea glabra*, and *Cape honeysuckle* pollen grains was done for temperature 30°C, 200 °C, 500 °C, 800 °C and 1000 °C were done(Figure1-10). The FT-IR spectra spanned a wavenumber range of 400 to 4000 cm⁻¹, highlighting key peaks that correspond to characteristic chemical bonds within the pollen material. Notably, when pollen is heated to 200–400°C, initial degradation of proteins occurs, marked by reduced absorption in the C−H stretching region (2850–2920 cm⁻¹) and diminished Amide I and II bands at 1650 cm⁻¹ and 1550 cm⁻¹, respectively[12]. **From 400–600°C there is** loss of carbohydrate absorption peaks (1000–1200 cm⁻¹) and increased intensity of aromatic carbon signals (~1600 cm⁻¹) whereas at ≥500°C lipid is degraded shown by reduced ester between C=O peak at 1745 cm⁻¹.From **600–800° there is c**omplete loss of organic functional groups, with the dominance of charred material signatures. From **800–1000°C there are**

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predominant inorganic residues (Si-O stretching at 1000–1100 cm⁻¹) indicating advanced carbonization and mineralization. Aromatic C=C (1600 cm⁻¹) and ether (1250 cm⁻¹) peaks persisted up to 1000°C suggestive of Sporopollenin Stability. Unique carbohydrate (1150–950 cm⁻¹) and carotenoid (2900 cm⁻¹) patterns enabled identification despite thermal stress. Table 1 shows the comparison of FTIR spectrum of all ten pollen grain from temperature range of 30°C-1000 °C [7][11][12][13].

The FT-IR spectra of pollen subjected to increasing temperatures revealed progressive chemical and structural changes. The changes in FT-IR spectra showed degradation of organic functional groups, including lipids and proteins, and eventual carbonization at high temperatures.

Figure 1A shows the graphical representation of comparison of FTIR Absorbance vs. Wave number of *Tecom stans* Pollen grain at 30°C, 200 °C, 500 °C, 800 °C, and 1000 °C strongly suggesting that we can identify the pollen grain despite its exposure to a high temperature. This study was performed to evaluate an alternative method for identifying pollen grains retrieved from fire-related crime scenes as trace evidence.

The above mentioned findings demonstrate that FT-IR spectroscopy can successfully track the thermal degradation of pollen grains, making it a powerful tool in fire forensics. The ability to identify pollen remnants at different stages of combustion allows forensic experts to infer fire conditions, including temperature exposure and potential fire origins.

DISCUSSION

Compound microscopy revealed the morphological characteristics of pollen grains, highlighting their small size and variability across all studied taxa. The pollen grains of the ten examined taxa exhibited diverse attributes, including differences in polarity, symmetry, shape, class, aperture, and size, underscoring the complexity and range of pollen morphology within these species as shown from Fig 1b-10b[7]. The compound microscopy results indicate that pollen grains remain largely identifiable up to 200°C, with minimal deformation of the exine. However, beyond 500°C, exine thinning and pore structure loss were observed, and at 800–1000°C, pollen grains were reduced to a charred, unrecognizable state

The characterization of pollen grains via FT-IR spectroscopy presents numerous benefits[7]. The desiccated state of pollen grains maintains a stable biochemical profile, allowing for easy manipulation and precise measurement of these samples[4][5]. Moreover, the morphological and FT-IR spectral analysis of pollen grains subjected to increasing thermal stress provides crucial insights into their structural and chemical stability.

FT-IR spectral findings further corroborate these morphological changes, demonstrating a progressive breakdown of key biochemical components in pollen grains. The reduction in C–H stretching (2850–2920 cm⁻¹) and diminished Amide I/II bands (1650 cm⁻¹ and 1550 cm⁻¹) at 200–400°C indicate initial protein degradation. As temperature increases to 400–600°C, the loss of carbohydrate absorption peaks (1000–1200 cm⁻¹) and intensified aromatic carbon signals (~1600 cm⁻¹) suggest significant structural modifications. The degradation of lipids, evident from the reduction of ester C=O peaks at 1745 cm⁻¹ at ≥500°C, confirms the loss of essential organic compounds.

At temperatures between 600–800°C complete depletion of organic functional groups occurs, leaving behind a largely charred material. Notably at 800–1000°C the emergence of inorganic residues particularly Si-O stretching at 1000–1100 cm⁻¹, signifies advanced carbonization and mineralization. The persistence of aromatic C=C (1600 cm⁻¹) and ether (1250 cm⁻¹) peaks up to 1000°C suggests that sporopollenin is a key biopolymer in pollen and it retains some structural integrity even under extreme heat exposure. Moreover, unique carbohydrate (1150–950 cm⁻¹) and carotenoid (2900 cm⁻¹) patterns enable the identification of pollen grains

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In this study, the vibrational spectra of all examined species reveal significant similarities in the chemical characteristics of their pollen, highlighting shared biochemical traits[7] [12] [14][15][16][17]. The development of a standardized database of pollen IR spectra holds immense potential for the global scientific community, enabling the rapid expansion of plant data worldwide. Such a database would serve as a foundational resource for the identification, classification, biochemical characterization, and data mining of pollen samples. By incorporating plant samples with diverse taxonomic, temporal, and spatial origins, this global spectral repository could lead to groundbreaking insights into pollen composition and its environmental adaptations[7].

dditionally, the IR spectra of pollen grains capture biochemical signatures of key components such as lipids, proteins, carbohydrates, and sporopollenins—the biopolymers that form the robust grain wall. These chemical fingerprints provide valuable information for understanding plant-environment interactions and advancing research in fields like ecology, climate science, and forensic investigations.[16][17].

These findings highlight the resilience of sporopollenin and the potential of FT-IR spectroscopy in forensic applications. The ability to trace biochemical alterations in pollen grains at different temperature exposures provides a robust analytical framework for fire forensic investigations. Identifying pollen remnants at varying combustion stages allows forensic experts to infer the conditions and temperature intensity of fire events. Furthermore, as pollen grains are often found at crime scenes, this technique offers a non-destructive, reliable means of linking environmental evidence to criminal investigations[13][16].

Compared to microscopy, FT-IR eliminated the need for intact morphological features, crucial in fire-damaged samples. Limitations include a small reference library and unresolved interference from charred matrix components.

CONCLUSION

The examination of pollen characteristics using compound microscopy and IR identification revealed distinct patterns across all ten species of the studied taxa[7]. The FTIR spectrum of samples exposed from 200C to 1000C is in line with that of the 30C exposed sample. This study serves as a pilot investigation into the potential of Fourier Transform Infrared (FT-IR) microspectroscopy for the rapid detection of floral species that have been completely burned[9]. Additionally, the identification of pollen grains through FT-IR microspectroscopy offers significant contributions to various scientific fields, including forensic science, paleopalynology, and other applications where an objective and reliable method for resolving uncertainties in pollen taxa identification is essential. FT-IR spectroscopy proves indispensable for pollen analysis in fire-affected evidence, overcoming microscopy's limitations through sporopolleninspecific spectral resilience[7]. By tracking thermal degradation stages (protein denaturation → lipid oxidation \rightarrow carbonization), it provides forensic investigators with temperature-exposure timelines and taxonomic identification—even in severely charred remains[2]. Furthermore, FT-IR measurements offer the advantage of requiring minimal sample preparation, making them more efficient and cost-effective compared to other pollen identification techniques. The application of FTIR spectroscopy in analyzing pollen grains subjected to high temperatures offers a promising tool for fire forensics. The technique offers rapid, non-destructive identification of pollen traces at crime scenes, particularly in cases of arson and vehicular fires, where extreme temperatures is there from 800–1200°C[16][18]. .By understandingS how pollen chemistry changes under different thermal conditions, investigators can gain insights into the history and dynamics of fires, ultimately aiding in criminal investigations and environmental assessments. FT-IR spectroscopy emerges as a powerful tool for the biochemical analysis of pollen grains exposed to fire, offering a novel approach for forensic investigations. To fully harness its potential in forensic science, further research is warranted to expand the pollen species database and integrate FT-IR spectroscopy with complementary forensic techniques, thereby enhancing its applicability and effectiveness in fire-related forensic analyses.

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Its application could streamline evidence processing in arson cases, particularly where traditional palynology fails.

Conflict of Interest The authors declare that they have no conflicts of interest.

Funding: No Funding agency has funded the above studies.

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