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Identification of pollen grains of some species of *Eugenia* and *Syzygium* (Myrtaceae) using Fourier transform infrared spectroscopy

Heba Elazab Mohamed Elazab

Department of Botany, Faculty of Science, Ain Shams University P.O. Box 11566, Abbassia, Cairo, Egypt. hebazm20@yahoo.com.

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Four plant species have been collected from the parks and botanic gardens in Egypt; namely: *Eugenia supraxillaris*, *Eugenia uniflora*, *Syzygium cumini*, *Syzigium jambos*. These species were tentatively selected from the two genera *Eugenia and Syzygium* for studying their pollen morphology. Farther, their pollens have been undertaken as modules representing preliminary trials to test the efficiency of the Fourier transform infrared (FT-IR) spectroscopy as a tool for pollen grains identification. This methodology is applied as a novel tool to screen the chemical compositions of pollen grains. In case of successful application of this method, a larger number of Myrtle taxa can be subject to future studies for unraveling the knots of controversy and taxonomic arguments about the stability or segregation of some species of the two genera mentioned above. Pollen samples, belonging to the four species under investigation, were examined with light photomicroscope as regards to some morphological features, principally: pollen shape; size; aperture characters; and pollen class. The results obtained showed that the pollens of the examined species were oblatespheroidal toprolate spheroidal in shape. Tricolporate and tetracolporate types were detected in all the studied taxa.

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FT- IR identification revealed that all the studied species were closely similar with regard to their chemical compositions. Thus, the results obtained at this stage might assume that the pollen grains of the *Eugenia* and *Syzygium* species under investigation exhibit close similarity.

Key words**:** *Eguenia,* Myrtaceae, Pollen Morphology, *Syzygium*.

Introduction

Since Linnaeus' treatment of *Eugenia* in *Species Plantarum* (1753: 470), referred to the occurrence of more than 800 species that have been described or transferred to this genus. Many botanists have been dissatisfied with the wide range of difference inform shown by the members of the genus. One of the most comprehensive reclassifications of *Eugenia* was proposed by Niedenzu (1893). He established several segregate genera which were based mainly on the characters of the flower. His system was adopted by many workers, but, because of the lack of distinct generic limits, many other botanists continued to consider *Eugenia* in a broader sense. Later studies of the members of the group led more and more botanists to reject Niedenzu's classification and to return the segregated genera to *Eugenia.* Because of the great number of species described in *Eugenia* the group has become rather unwieldy and Merrill and Perry (1938a, b, 1939) proposed a new systematic treatment of the group. They redefined some of the earlier proposed segregates of the genus. The new systematic treatment which they proposed is based on the structure of the seed. According to them, species of *Eugenia* are diagnosed as having a pericarp which is easily crushed and "the seed is free, the testa is smooth, chartaceous to cartilaginous and mostly lustrous, and the cotyledons are mechanically inseparable, i.e., they have grown together in such a way that often the line of their opposing faces is scarcely distinguishable. *Syzygium,* one of the segregate genera, is described as having "fruits that when dried are not too easily broken, and when opened, the embryo (not the entire seed) falls out leaving the roughish seed coat more or less loosely adhering to the pericarp; the embryo has two distinct cotyledons usually attached near the middle of the opposing faces which conceal the hypocotyl within (Wilson,1957).Rather recently, Biffin *et al*. (2009) stated that Syzygieae and Myrteae were traditionally included in Myrtaceae subfamily Myrtoideae, due to the shared possession of a succulent pericarp. The relationships in Myrtaceae have been the focus of

several relatively recent studies (e.g. Sytsma *et al*., 2004; Wilson *et al*., 2005). Evidence from morphology (Johnson and Briggs, 1984) and molecular phylogenetic studies (Sytsma et al., 2004; Van der Merwe *et al*., 2005, Wilson *et al.*, 2005) suggested that the fleshy fruit of Myrtaceae has multiple origins, arising separately within Syzygieae (e.g. *Syzygium*), Myrteae (e.g. *Eugenia*) and other groups in the family. However, Biffin *et al*. (2009) assumed that similar phenotypes in Syzygieae and Myrteae might conflict shifts from dry to fleshy fruits according to different states among arborescent rainforest lineages. Most recently, transferability of microsatellites with microsatellite molecular markers (simple sequence repeats, SSR) between myrtle species revealed two groups, one composed by *Eugenia* and *Campomanesia* and another by *Syzygium* and *Myrciaria*, which indicates conserved microsatellite regions within and between genera (Nogueira *et al*., 2015). However, the use of such recent molecular methodologies did not also efficiently unravel the knots of the *Eugenia-Syzygium* controversy. Moreover, these methods are extremely costing and show limitation of the existing data as generally poor resolution of relationships among the tribes (Byng *et al*., 2015). Thus, in this respect, further methods, characterized by being rather easily applied and less costing, are required.

FT-IR spectroscopy has been shown to be a very useful technique in several research fields and in particular for the analysis of organic material (Nabet and Pezolet 1997; Petibois *et al*. 2006). It provides information on the chemical bonding or molecular structure of materials. The basis of the technique is the property of chemical bonds and groups of bonds to vibrate at characteristic frequencies: a molecule that is exposed to infrared rays absorbs infrared energy at frequencies specifically characteristic of that molecule. During FT-IR analysis, a spot on the specimen is subjected to a modulated IR beam. The specimen's transmittance and reflectance of the infrared rays at different frequencies are translated into an IR absorption plot consisting of reverse peaks (Gottardini, *et al.*, 2007).An IR spectrum represents a fingerprint of a sample, with absorption peaks corresponding to the frequencies of vibrations between the bonds of the atoms making up the material. Because each material is made up of a unique combination of atoms, it is impossible to find two different compounds ever producing exactly the same IR spectrum. Therefore, IR spectroscopy can positively identify (qualitative analysis) each unique kind of material with the extra advantage of not destroying the sample (nondestructive method). In

addition, the size of the peaks in the spectrum is a direct indication of the amount of material analyzed (quantitative analysis), and it can provide a precise measurement without external calibration. Finally, FT-IR measurements do not require prior complex sample preparation. FT-IR spectroscopy has been successfully used in the past for the characterization and identification of microorganisms (Orsini *et al*. 2000; Mariey *et al*. 2001; Miguel Gomez *et al*. 2003; Pastuszka et al. 2005; Al-Holy et al. 2006). The first scientists to apply FT-IR spectroscopy for pollen identification were Pappas *et al.* (2003). They created a library consisting of the spectra of 20 pollen types, both in diffuse reflectance and KBr pellet. For each FT-IR spectrum, they identified a fingerprint region, between 1500–800 cm_1, which then enabled them to discriminate the pollen of different species. Their high match values were verified by comparing spectra of unknown pollen with the corresponding library.

This work was a pilot study to investigate the potential of using Fourier transforms infrared (FT-IR) spectroscopy for the rapid identification of pollen grains to evaluate the similarities between the studies species of *Eugenia* and *Syzygium*. The chemical composition variations and morphological characters of pollen grains of the studied taxa by using Light Microscopy and FT-IR spectroscopy were detected. The objective of the study reported here was to evaluate an alternative method to identified pollen grains associated with taxonomical significance. FT-IR spectroscopy seems to be easier for the sample analysis, requiring no specific palynological skills. Therefore, FT-IR spectroscopy could be a very helpful method for rapid pollen identification in different disciplines and in a large number of studies. FT-IR spectroscopy is excellent tool for biochemical analysis of pollen.

Material and methods

Flower buds of *Eugenia supraxillaris, Eugenia uniflora, Syzygium cumini, Syzigium jambos*were collected the parks and the gardens of Egypt, Cairo, having first been identified using the approach described by Baily (1976), and matched against authentic material at The Royal Botanic Gardens, Kew. Non-acetolysed materials were studied. Glycerine jelly was used as the mounting medium for Light Microscopy (LM) investigations. The measurements were carried out by taking the mean value of 20 counts of pollen grains following the work of Brookes and Thomas (1967). The dimensions of the polar and equatorial axis of pollen grains were measured.

A standard ×40 objective was used with a ×10 photo eyepiece. The terms used by Faegri and Iversen (1975) and Punt et al. (1994) for describing the pollen grains pattern have been adopted here .Pollen spectra were obtained in by means of Fourier transform infrared (FTIR) spectra at room temperature on a Nicolet 6700 FTIR spectrometer. The absorbance spectra were measured between 400 and 3900cm-1. The function groups are identified according to Silverstein *et al.*, (2005) (Table 1).

Table 1. The most important chemical bonds observed of using Fourier transform infrared spectroscopy (Silverstein *et al.*, 2005).

Chemical bond	Peak frequency (cm^{-1})	Function group
O-H stretch, H-bonded	3500-3200	alcohols, phenols
N-H stretch $1^{o,}$ 2^{o}	3400-3250	amines, amides
O-H stretch	3300-2500	carboxylic acids
-C≡C-H: C-H stretch	3330-3270	alkynes (terminal)
C-H stretch	3100-3000	aromatics
C-H stretch	3000-2850	alkenes
-C=C- stretch	1680-1640	alkenes
N-H bend	1650-1580	1^{o} amines
N-O asymmetric stretch	1550-1475	nitro compounds
C-C stretch (in-ring)	1500-1400	aromatic
N-O symmetric stretch	1360-1290	nitro compounds
C-N stretch	1335-1250	aromatic amine
C-O stretch	1320-1000	alcohols, carboxylic acids, esters, ethers
C-H wag (-CH2 X)	1300-1150	alkyl halides
C-N stretch	1250-1020	aliphatic amines
=C-H	1000-650	bend alkenes
C-H “oop”	900-675	aromatics
N-H wag	910-665	$1^{o,}$ 2^{o} amines
C-Cl stretch	850-550	alkyl halides
-C≡C-H: C-H bend	700-610	alkynes
C-Br stretch	690-515	alkyl halides

Results

Pollen grain characteristics of *Eugenia supraxillaris* Spring (Plate 1, Figs 1-8)

Polarity: Isopolar.**Pollen shape class:** oblate to suboblate, P/E = 0.75. **Shape; Polar view:** Triangularand square, **Equatorial view:** Elliptical. **Pollen dimension:** P= (8.75-14) µm, E= (14-21) µm. **Symmetry:** radially symmetric. **Pollen class:** Tricolporateand tetracolporate.**Pollen size class:** Small. **Aperture:** Composite; **Ectoaperature:** Colpus; **Endoaperture:** pore. **Apocolpium:** Absent **(**syncolpate). **Sculpturing:** No prominent sculpturing elements are detected by the Light Microscopy. **Pollen shape class:** oblate to suboblate, P/E = 0.75. **Exine:** It is thicker around the colpi. **Intine:** Thin. **Tectum:** Tectate. **IR function group and chemical composition:**The typical spectra, chemical bonds, and function groups of *Eugenia supraxillaris* pollens are shown in Table 2 and Plate 1; Fig.2.

Table 2. The most important chemical bonds observed in pollen of *Eugenia supraxillaris* using Fourier transform infrared spectroscopy.

Chemical bond	Peak frequency (cm^{-1})	Function group
O-H stretch, H-bonded.	3358.0	alcohols, phenols.
O-H stretch, C-H stretch, -C≡C-H: C-H stretch.	2923.9, 2853.4	carboxylic acids, alkenes, alkynes (terminal).
N-H bend	1628.4	1^{o} amines
N-O asymmetric stretch.	1539.3	nitro compounds
C-C stretch (in-ring).	1447.6	aromatic
N-O symmetric stretch, C-N stretch.	1319.6	nitro compounds, aromatic amine
C-H wag (-CH2 X), C-N stretch.	1236.2	alkyl halides, aromatic.
C-O stretch	1319.6, 1236.2, 1099.5, 1038.5	alcohols, carboxylic acids, esters, ethers, aliphatic amines.
=C-H, C-H “oop”, C-Cl stretch, N-H wag	840.7, 776.7, 699.8	bend alkenes, $1^{o,}$ 2^{o} amines, alkynes, aromatics.
-C≡C-H: C-H bend, C-Cl stretch.	632.4	alkynes.
C-Br stretch	517.2	alkyl halides.

Fig.1. Morphology of *Eugenia supraxillaris*

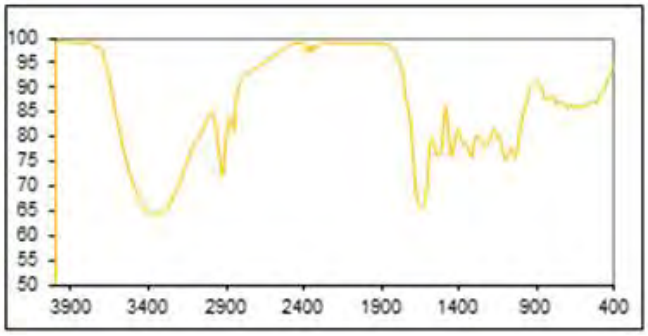

Fig. 2. Vibrational spectra of pollen of *Eugenia supraxillaris* showing spectra in the wavenumber range 400 cm^{-1} - 3900 cm^{-1}

Fig.3. Polar view of tricolporate pollen grain (optical section). (LM x 3720).

Fig. 5. Polar view of tri- and tetra-colporate pollen grains (optical section). (LM x 3720).

Fig. 7. Equatorial view showing exine thickness around colpus (optical section). (LM x 3720).

Fig.4. Polar view showing syncolpium pollen (high focus). (LM x 3720).

Fig.6. Polar view Polar view of tri- and tetracolporate pollen grains (high focus). (LM x 3720).

Fig.8. Equatorial view showing colporate pollen (high focus). (LM x 3720).

Plate 1

Pollen grain characteristics of *Eugenia supraxillaris*

Pollen grain characteristics of *Eugenia uniflora* L.

(Plate 2, Figs 1-8).

Polarity: Isopolar. **Pollen shape class:** Suboblate, P/E = 0.83. **Shape: Polar view:** Triangular and square**, Equatorial view:** Irregular elliptic. **Pollen dimension:** P = (12.25- 15.40) µm, E = (17.5- 21) µm. **Symmetry:** radially symmetric. **Pollen class:** Tricolporate and tetracolporate. **Pollen size class:** Small. **Aperture:** Composite; **Ectoaperature:** Colpus; **Endoaperture:** pore. **Apocolpium:** Relatively wide apocolpium. **Sculpturing:** Noprominent sculpturing elements are detected by the Light Microscopy. **Exine:** It is thicker around the colpi. **Intine:** Thin. **Tectum:** Tectate. **IR function group and chemical composition:** The typical spectra, chemical bonds, and function groups of *Eugenia uniflora* pollens are shown in Table 3 and Plate 2; Fig.2.

Table 3. The most important chemical bonds observed in pollen of *Eugenia uniflora* using Fourier transform infrared spectroscopy.

Chemical bond	Peak frequency (cm^{-1})	Function group
O-H stretch, H-bonded	3401.1	alcohols, phenols.
O-H stretch, C-H stretch	2925.2, 2853.7	carboxylic acids, alkenes,
-C≡C-H: C-H stretch.		alkynes (terminal).
-C=C- stretch	1654.6	alkenes.
N-O asymmetric stretch	1539.2	nitro compounds.
C-C stretch (in-ring)	1447.9	aromatics.
N-O symmetric stretch	1339.5	nitro compounds.
C-H wag (-CH2X)	1234.2	alkyl halides.
C-O stretch, C-N stretch.	1234.2, 1081.4, 1042.9	alcohols, carboxylic acids, esters, ethers, aliphatic amines.
=C-H, N-H wag,C-H “oop”, C-Cl stretch.	835.4	bend alkenes, $1^{o,}$ 2^{o} amines, aromatics, alkyl halides.
C-Cl stretch, -C≡C-H: C-H bend, C-Br stretch.	622.0	alkynes, alkyl halides.

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Fig.1. Morphology of *Eugenia uniflora*

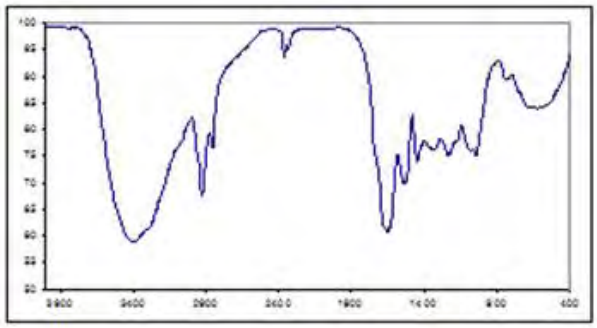

Fig 2. Vibrational spectra of pollen of *Eugenia uniflora* showing spectra in the wavenumber range 400 cm^{-1} - 3900 cm^{-1}.

Fig.3. Polar view of tricolporate pollen (optical section). (LM x 3720).

Fig. 5. Polar view of tetra-colporate pollen (optical section). (LM x 3720).

Fig. 7. Equatorial view showing exine thickness (optical section). (LM x 3720).

Fig.4. Polar view showing apocolpium pollen (high focus). (LM x 3720).

Fig. 6. Polar view showing apocolpium pollen (high focus). (LM x 3720).

Fig.8. Equatorial view showing colporate pollen (high focus). (LM x 3720).

Plate 2

Pollen grain characteristics of *Eugenia uniflora*

Pollen grain characteristics of *Syzygium cumini* (L.) Skeels (*Eugenia cumini* (L.) Druce) (Plate 3, Figs 1-8).

Polarity: Isopolar .**Pollen shape class:** Oblate spheroidal to prolate spheroidal, P/E = 1. **Shape: Polar view:** Triangular and rhomboidal**, Equatorial view:** Irregular elliptic. **Pollen dimension:** P = (8.75-10.5) µm, E = (14- 15.75) µm. **Symmetry:** radially symmetric. **Pollen Class:** Tricolporate and tetracolporate. **Pollen size class:** Small. **Aperture:** Composite; **Ectoaperature:** Colpus; **Endoaperture:** pore. **Apocolpium:** Relativelywide. **Sculpturing:** No prominent sculpturing elements are detectedby the light microscopy. **Exine:** It is thicker around the colpi. **Intine:** Thin. **Tectum:** Tectate. **IR function group and chemical composition:** The typical spectra, chemical bonds, and function groups of *Syzygiumcumini* pollens areshown in Table 4 and Plate 3; Fig. 2.

Table 4. The most important chemical bonds observed in pollen of *Syzygium cumini* using Fourier transform infrared spectroscopy.

Chemical bond	Peak frequency (cm^{-1})	Function group
O-H stretch, H-bonded	3294.3	alcohols, phenols, carboxylic acid
-C≡C-H: C-H stretch, N-H stretch	$1^{o,}$ $2^{o,}$	alkynes (terminal), amines, amides.
C-H stretch, O-H stretch	2925.0, 2853.7	alkenes, carboxylic acids.
-C=C- stretch.	1653.5	alkenes.
N-O asymmetric stretch	1539.0	nitro compounds.
C-C stretch (in-ring).	1448.3	aromatics.
N-O symmetric stretch, C-N stretch	1318.5	nitro compounds, aromatic amine.
C-H wag (-CH2 X).	1234.9, 1165.0	alkyl halides.
C-O stretch.	1318.5, 1234.9, 1165.0 1094.1, 1036.7	alcohols, carboxylic acids, esters, ethers.
C-N stretch	1234.9, 1165.0, 1094.1, 1036.7	aliphatic amines.
N-H wag, -C-H “oop”	779.5, 839.6	aromatics, $1^{o,}$ 2^{o} amines,
C-Cl stretch		alkyl halides.
=C-H, -C≡C-H: C-H bend	663.3	bend alkenes, alkynes.
C-Cl stretch, C-Br stretch		alkyl halides.
C-Br stretch	518.8	alkyl halides

Fig.1. Morphology of *Syzygium cumini*

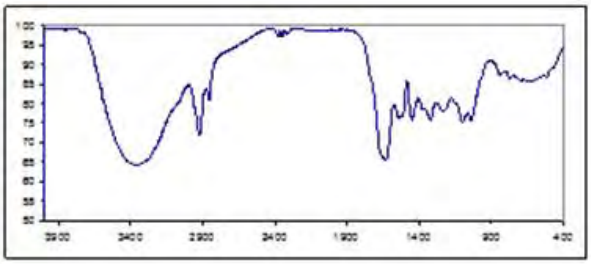

Fig 2. Vibrational spectra of pollen of *Syzygium cumini* showing spectra in the wavenumber range 400 cm^{-1} - 3900 cm^{-1}.

Fig.3. Polar view of tricolporate pollen (optical section). (LM x 3720).

Fig. 5. Polar view of tetracolporate pollen (optical section). (LM x 3720).

Fig. 7. Equatorial view showing colporate pollen (optical section). (LM x 3720).

Fig.4. Polar view showing apocolpium pollen (high focus). (LM x 3720).

Fig.6. Polar view of tetracolporate pollen (high focus). (LM x 3720).

Fig.8. Equatorial view showing thickness of exine around the colpus (high focus). (LM x 3720).

Plate 3

Pollen grain characteristics of *Syzygium cumini*

Pollen grain characteristics of *Syzigium jambos* (L.) Alston (*Eugenia jambos* L.) (Plate 4, Figs 1-8).

Polarity: Isopolar. **Pollen shape class:** Oblate spheroidal to prolate spheroidal, P/E = 1. **Shape: Polar view:** semicircular and rhomboid**, Equatorial view:** Elliptic. **Pollen dimension:** P = (10.5-14) µm, E = (17.5-21) µm. **Symmetry:** radially symmetric.**Pollen class:** Tricolporate and tetracolporate. **Pollen size class:** Small. **Aperture:** Composite; **Ectoaperature:** Colpus; **Endoaperture:** pore. **Apocolpium:** Relatively wide. **Sculpturing:** No prominent sculpturing elements are detected by the Light Microscopy. **Exine:** It is thicker around the colpi. **Intine:** Thin. **Tectum:** Tectate. **IR function group and chemical composition:** The typical spectra, chemical bonds, and function groups of *Syzygium jambos* pollens are shown in Table 5 and Plate 4; Fig. 2.

Table 5. The most important chemical bonds observed in pollen of *Syzygium jambos* using Fourier transform infrared spectroscopy.

Chemical bond	Peak frequency (cm^{-1})	Function group
O-H stretch, H-bonded	3343.7	alcohols, phenols.
C-H stretch, O-H stretch,	2925.1, 2853.5	alkenes, carboxylic acids,
-C≡C-H: C-H stretch.		alkynes (terminal).
-C=C- stretch	1655.1	alkenes.
N-H bend	1626.3	1^{o} amines.
N-O asymmetric stretch	1540.0	nitro compounds.
C-C stretch (in-ring)	1448.9	aromatic.
N-O symmetric stretch, C-N stretch	1318.5	nitro compounds, aromatic amine.
C-H wag (-CH2 X).	1234.3, 1168.8	alkyl halides.
C-O stretch	1318.5, 1234.3, 1168.8, 1079.4	alcohols, carboxylic acids, esters, esters, ethers.
C-N stretch	1234.3, 1168.8, 1079.4	aliphatic amines.
=C-H, N-H wag,	829.2,779.1, 671.0	bend alkenes, $1^{o,}$ 2^{o} amines,
-C≡C-H: C-H bend, C-Cl stretch.		alkyl halides, alkynes.
C-H “oop”	829.2, 779.1	aromatics.
C-Br stretch	519.7	alkyl halides.

Fig.1. Morphology of *Syzigium jambos*

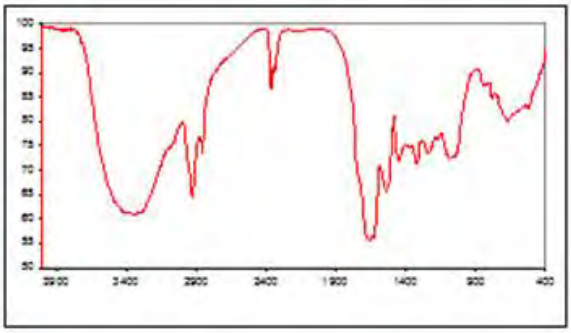

Fig 2. Vibrational spectra of pollen of *Syzigium jambos* showing spectra in the wave number range 400 cm^{-1} - 3900 cm^{-1}.

Fig.3. Polar view of tricolporate pollen (optical section). (LM x 3720).

Fig. 5. Polar view of tetracolporate pollen (optical section). (LM x 3720).

Fig. 7. Equatorial view showing thickness of exine around the colpus (optical section). (LM x 3720).

Fig. 4. Polar view showing abocolpium pollen (high focus). (LM x 3720).

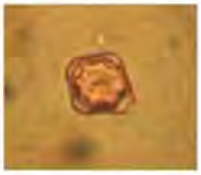

Fig 6. Polar view of tetracolporate pollen (high focus). (LM x 3720).

Fig.8. Equatorial view showing colporate pollen (high focus). (LM x 3720).

Plate 4

Pollen grain characteristics of *Syzigium jambos*

Assessment of pollen FTIR spectra of the studiedtaxa

The typical spectra of *Eugenia supraxillaris, Eugenia uniflora, Syzygium cumini* and *Syzigium jambos* of pollen grains are shown in Fig. 1, along with vibrational band assignment of major biochemical components.The identification of the of species of *Eugenia* and *Syzygium*s hows minute spectral differences among the *E. supraxillaris, E. uniflora, S. cumini,* and *S. jambos.* The typical wave number range between 400 and 3900 cm^{-1} are reported and the most important peaks are indicated on the spectra. According to Silverstein *et al.,* (2005), the peaks identify to typical chemical bonds present in the pollen matter. The most important chemical bonds of the studied taxa by FT-IR spectroscopy and the typical frequencies are listed in Tables (2-5).In addition, the comparison among the most important chemical bonds in pollen of four the studied taxa are listed in Table 6.

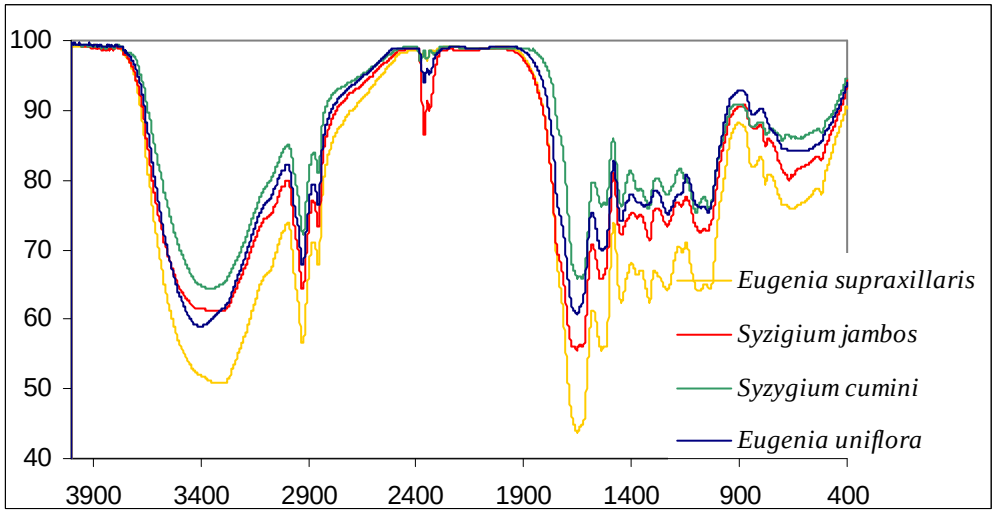

Fig. 1. Vibrational spectra of the four study species showing spectra in the wave number range 400 cm^{-1} - 3900 cm^{-1}. Colour description; *Eugenia supraxillaris*: orange, *Eugenia uniflora*: blue, *Syzygium cumini*: green, *Syzigium jambos*: red.

Table 6. Comparison of the most important chemical bonds in pollen grains of *Eugenia supraxillaris, Eugenia uniflora, Syzygium cumini* and *Syzygium jambos* using Fourier transform infrared spectroscopy.

Chemical bond	*E. supraxillaris*	*E. uniflora*	*S. cumini*	*S. jambos*
-OH stretch, H-bonded	+	+	+	+
O-H stretch	+	+	+	+
-C≡C-H: C-H stretch	+	+	+	+
C-H stretch	+	+	+	+
-C=C- stretch	-	+	+	+
N-H bend	+	-	-	+
N-O asymmetric stretch	+	+	+	+
C-C stretch (in-ring)	+	+	+	+
N-O symmetric stretch	+	+	+	+
C-N stretch	+	+	+	+
C-O stretch	+	+	+	+
C-H wag (-CH2 X)	+	+	+	+
C-N stretch	+	+	+	+
=C-H	+	+	+	+
C-H “oop”	+	+	+	+
N-H wag	+	+	+	+
C-Cl stretch	+	+	+	+
-C≡C-H: C-H bend	+	+	+	+
C-Br stretch	+	+	+	+

Discussion

The pollen morphological characters by light microscopy indicate the small size of the pollen grain and the occurrence of the little variation of all the studied taxa. The pollen grains of the four the studied showed great similarities in their pollen attributes of polarity, symmetry, pollen shape, pollen class, aperture, and pollen size. According to Erdtman (1952), the pollen shape class found to be oblate spheroidal to prolate spheroidal in *Syzygium cumini* and *Syzigium jambos*, while it was oblate to suboblate in *Eugenia supraxillaris* and suboblate in *Eugenia uniflora.* Relatively wide area of apocolpium is observed in all studied taxa except in *Eugenia supraxillaris*, the syncolpium was detected (Plate 1, Fig.4). So no clear significant variation was observed in pollen morphology by Light Microscopy.

The characterization of pollen grains by FT-IR has several favorable qualities. The desiccated nature of grains provides relatively stable biochemical composition, and thus enables simple manipulation and measurement of pollen grains (Gottardini, 2007). Moreover, our results identify biochemical variability of pollen grains at taxonomical significance of two the related genera. The spectral measurement of the four studied taxa of *Eugenia* and *Syzigium*, indicates that the variation was limited among them (Fig.1 & Table 6). The results of our investigation show that FT-IR spectroscopy provides specific spectra of the chemical bonds in pollen grains of *Eugenia* and *Syzygium* species and show slightly differentiation between them. In the present study, The vibrational spectra of all studied species show that they have many common features in terms of chemical characteristics of their pollen except N-H bend bond is not recorded in *Eugenia uniflora* and *Syzygium cumini* and also -C=C- stretch bond is not recorded in *E. supraxillaris* (Fig. 1 & Table 6). Therefore, IR spectra can be applied on a larger number of the two genera of *Eugenia* and *Syzygium* subjected to future studies for unraveling the knots of controversy and taxonomic arguments about the stability or segregation of them by several authors (Wilson, 1957; Sytsma *et al*., 2004; Wilson *et al*., 2005; Biffin et al., 2009; Byng et al., 2015; Nogueira et al., 2015). Development of standardized database of pollen IR spectra would allow rapid expansion of worldwide plant data by the global scientific community, and would serve as a starting point for identification, classification, biochemical characterization and general data mining. Implementation of the global spectral database covering a range of plant samples, with different taxonomical, temporal and spatial origin, should lead to a novel understanding of the pollen compositions. In addition, The IR spectrum of pollen grains contains the signatures of lipids, proteins, carbohydrates and grain wall biopolymers called sporopollenins. Moreover, the features related to these compounds are responsible of phenotypic attributes (Zimmermann and Kohler, 2014).

Conclusions

The study of pollen characters by light microscope and IR identification are revealed that the four species of the studied taxa are relatively similar. The results of the preliminary investigation are not support to transfer of the studied taxa of *Eugenia* to *Syzygium*. This work consider as a pilot study to investigate the potential of Fourier transform infrared (FT-IR)

microspectroscopy for rapid detect the similarities between *Eugeni a*and *Syzygium*. The tool of use Fourier transforms infrared (FT-IR) spectroscopy for pollen identificationarerecommended to be applied in all species of *Euginia* and *Syzigium* to get more significant data. On the other hand, the identification of pollen grains via FT-IR microspectroscopy can give a significant contribution to other scientific fields, such as in forensic science or in paleopalinology, or in any other application in which an objective approach for resolving doubts in pollen taxa identification is necessary. In addition, FT-IR measurements do not require prior complex sample preparation and less costly than other tools of pollen identification.

References

Al-Holy, M.A., Lin, M., Al-Qadiri, H., Cavinato, A.G. and Rasco B.A. 2006. Classifications of foodborne pathogens by Fourier transform infrared spectroscopy and pattern recognition techniques. *J. Rapid Meth. Autom. Microbiol*., **14**:189–200.

Bailey, L.H. 1976. *Hortus third*: *A concise Dictionary of Plants cultivated in the United States and Canada* .Macmillan Publishing Co., Inc. New York. 1290 pp.

Biffin, E., Lucas E.J., Craven L.A., da Costa I.R., Harrington, M.G. and Crisp, M.D. 2009 Evolution of exceptional species richness among lineages of fleshy-fruited Myrtaceae. *Annal. Bot*.**106**: 79-93.

Brookes, D. and Thomas, K.W. **1967**. The distribution of pollen grains on microscope slides part I. The non-randomness of the distribution. *Pollen Spores***.9**: 921–629.

Byng, J.W., Phillipson, P.B. and Snow, N. 2015. Nomenclatural notes on Malagasy *Syzygium* Gaertn. (Myrtaceae). *Candollea* **70**: 151–155.

Erdtman, G, 1952. *Pollen Morphology and Plant Taxonomy (Angiosperms).* Almqvist and Wiksell, Stockholm, 593pp.

Fægri, K. and Iversen J. 1975. *Textbook of pollen analysis*: 3rd ed. Copenhagen: Scandinavian University Books.294pp.

Gottardini, E., Rossi, S., Cristofolini, F. and Benedetti, L, **2007**. Use of Fourier transform infrared (FT-IR) spectroscopy as a tool for pollen identification. *Aerobiologia.* **23**:211-219.

Johnson, L.A.S., Briggs, B.G. **1984**. Myrtales and Myrtaceae: a phylogenetic analysis. *Ann. Missouri Bot. Gard***. 71**: 700–756.

Mariey, L., Signolle, J.P., Amiel, C., Travert, J. 2001. Discrimination, classification, identification of. microorganisms using FTIR

spectroscopy and chemometrics. *Vibrational Spectroscopy* **26**: 151–159.

Merrill, E.D., Perry, M. 1938a. On the Indo-Chinese Species of *Syzygium* Gaertner.*Arnold Arboretum, Jour.* **19(2**):99-116.

----------**1938b**. The Myrtaceae of China. *Arnold Arboretum, Jour.* **19(3):** 191-247.

----------.**1939**. The Myrtaceous Genus *Syzygium.*Gaertner in Borneo. *Amer. Acad. Arts. Sci., Memo.* **18(3):** 135-202.

Miguel Gomez, M.A., BratosPe´rez, M.A., Martin Gil, M.A., Duenas Diez, A., Martin Rodriguez, J.F., Gutie´rrez Rodriguez, P., Orduna Domingo, A., Rodriguez Torres, A. 2003. Identification of species of Brucella using Fourier transform infrared spectroscopy. *Jour. Microbiol. Meth.,* **55**:121–131.

Nabet, A, Pezolet, M. 1997. Two-Dimensional FT-IR Spectroscopy: A Powerful Method to Study the Secondary Structure of Proteins Using H-D Exchange. *Applied Spectroscopy,* **51**:466–469.

Niedenzu, F. 1893. Myrtaceae. In Engler, A. and K. Prantl. .*Die Naturlichen Pflanzenfamilien,* **3(7):**78-86. W. Engelmann, Leipzig.

Nogueira, A.M., Ferreira, A. Ferreira, M.F. **2015**. Transferability of Microsatellites from *Psidiumjuajava* to *Eugenia, Myrciaria, Campomanesia*, and *Syzygium* Species (Myrtaceae). *Plant Molecular Biology Reporte*r: 09 Jul 2015.

Orsini, F., Ami, D., Villa, A.M., Sala, G., Bellotti, M.G., Doglia, S.M. **2000**. FT-IR microspectroscopy for microbiological studies. *Jour. . Microbiol. Meth.*, **42**: 17–27.

Pappas, C.S., Tarantilis, P.A., Polissiou, M.G., Harizanis, P. C. **2003**. New method for pollen identification b y FTIR spectroscopy. *Applied Spectroscopy,* **57**: 23–27.

Pastuszka, J.S., Talik, E., Hacura, A., Sloka, J., Wlazlo, A. 2005. Chemical characterization of airborne bacteria using X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIRS). *Aerobiologia,* **21:**181–192.

Petibois, C., Gionnet, K., Goncalves, M., Perromat, A., Moenner, M. and De´le´ris, G. 2006. Analytical performances of FT-IR spectrometry and imaging for concentration measurements within biological fluids, cells, and tissues. *Analyst.,* **131**: 640–647.

Punt, W., Blackmore S., Nilsson, S. and Le Thomas, A. 1994. *Glossary of pollen and spore terminology: LPP Contributions series No. 1.*

Netherlands: Laboratory of Palaeobotany and Palynology, University of Utrecht.71pp.

Silverstein, R.M., Webster, F.X., Kiemle D.J., 2005. *Spectrometric Identification of Organic Compounds 7th Edition,* New York: John Wiley & Sons, Inc

Sytsma, K.J, Litt, A, Zjhra, M.L., 2004. Clades, clocks, and continents: historical and biogeographical analysis of Myrtaceae, Vochysiaceae, and relatives in the Southern Hemisphere. *Inter. Journ. Pl. Sci.,* **165**: 85–105.

Van der Merwe, M.M., Van W.Y.K, A.E., Botha and A.M. 2005. Molecular phylogenetic analysis of *Eugenia* L. (Myrtaceae), with emphasis on southern African taxa. *Pl. Syst. . Evol*., **251(1):** 21-34.

Wilson, K.A., 1957. A Taxonomic Study of the Genus *Eugenia* (Myrtaceae) in Hawii. *Pacific Science*, **11(2):** 161-180.

Wilson, P.G., O'Brien, M.M., Heslewood, M.M., Quinn and C.J. 2005. Relationships within Myrtaceae*sensulato* based on a matK phylogeny. *Pl. Syst*. *Evol*., **251**: 3–19.

Zimmermann, B. and Kohler, A. 2014. Infrared spectroscopy of pollen identifies plant species and genus as well as environmental conditions**.** *Plos One*, **9 (4):** 1-12.