

Exploring Comparative Surface Morphology of Two Varieties of *Piper betle* Linn. (Ayurvedic Medicinal Plant) Through SEM

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Abstract

Piper betle Linn. enjoys important place in Ayurveda and Hindu culture. It is important cash crop of India. Many varieties of this plant are available which are mainly based on colour, aroma, taste, size of leaves. For authentication and differentiation of these varieties modern tool SEM can be used. Two varieties of *Piper betle* investigated for the present study are Kapoori and Meetha cum bangla. SEM study revealed that stomata are present only on lower surface of leaves. SEM characteristics of lower epidermis of leaf are of taxonomic importance for differentiation of both varieties. Characteristics of upper epidermis of leaf and petiole of both varieties are similar except negligible differences. Hydathodes are present on upper as well as lower surfaces of leaves of both plants. Pearl glands are observed on all investigated parts of both plants. They are longer in Meetha cum Bangla and shorter in Kapoori. Stem showed presence of ridges, wax granules, non glandular trichomes on the epidermal surface which are distinct enough in both varieties; hence these characteristics can be used in delimitation of both the varieties. More elements are found in EDAX analysis on epidermal cell of stem as compared to that of leaf and petiole. Most of the elements found in EDAX analysis on leaf, petiole and stem of both varieties are same except a few.

Key words

P. betle var.Kapoori, *P. betle* var.Meetha cum bangla, SEM and EDAX Analysis

Introduction

Piper betle belongs to family Piperaceae. *Piper betle* is important cash crop of India and also known as 'green gold of India' (Guha, 2006; Biswas et al, 2022) Millions of people depend on this plant for source of income. It is versatile medicinal

plant used in Ayurveda. It is used in treatment of various diseases. Betle leaves are aphrodisiac, diuretic. Leaves are used in treatment of pulmonary infection, arthritis. It promotes secretion of milk during lactation. Juice of betle leaves is tonic to liver, heart, brain and other internal organs (Roy and Vijayalaxmi, 2013). It promotes healthy teeth and skin. Juice of betle leaves with honey is used to treat cough, dyspnoea, indigestion in children (Chauhan et al, 2016).

The following *Sanskrit* verse in *Mahaushadhanighantu* explains medicinal properties of betle leaves.

ताम्बूलं विशदं रूच्यं तीक्ष्णोष्णं तुवरं सरम् ।
बल्यं तिक्तं कटु क्षारं रक्तपित्तकरं लघु ।
वश्यं श्लेष्मास्यदौर्गन्ध्य-मलवातश्रमापहम् ॥५२॥

Piper betle plant is important source of essential oil. Betle leaves have an antioxidant action. When they are heated with fats and oil, they check development of rancidity in them. Hence effective in preserving oils and ghee (Pandey, 2004). *Piper betle* is important medicinal plant which has religious importance in Hindu culture.

About 100 varieties of *Piper betle* are found across the world (Biswas, 2022). Different varieties of *Piper betle* are due to variations in size, shape, colour, aroma and taste of leaves (Anonymous, 1989). This Paper reports epidermal micromorphological characteristics and EDAX analysis of leaf, petiole and stem of two varieties of *Piper betle* observed by Scanning Electron Microscope. The objectives of the present study are 1. To discover the extent to which micromorphological features of leaf, petiole and stem could aid in identification and differentiation of two varieties of *Piper betle*. 2. To study elemental analysis which is important for determining nutritional value.

Materials and Methods

The varieties of *Piper betle* investigated in the present work were Kapoori and Meetha cum bangla. Both the varieties were collected from Oilseed Research Station, Jalgaon. Scanning Electron Microscopy of leaf (upper and lower surface), petiole, stem of both plants was carried out at ICON Labs Pvt. Ltd., Navi Mumbai. Plant parts were

directly mounted on metallic stub using double sided carbon tape. Dirt on the material was removed with the help of blower. Then the sample is viewed with FEI Quanta 200 Environmental Scanning Electron Microscope with EDAX System and photomicrographs were taken at different magnifications.

EDAX analysis on epidermal cell and pearl glands on lower surface of leaf, petiole and stem was carried out with EDAX system of FEI Quanta 200 Environmental Scanning Electron Microscope.

Measurements were taken by the same Scanning Electron Microscope. Mean values of 15 observations with standard deviation were taken for length of pearl glands and non glandular trichomes. Mean values of 10 observations with standard deviation were taken for size of stomatal aperture.

Observations

All the surface micromorphological features of leaf, petiole and stem are as follows

Leaf Epidermis:

Leaves of *Piper betle* Linn. are hypostomatic.

A. Lower Epidermis:

Piper betle var. **Kapoori** (Fig.1 A)-

Stomata

1. Position-Stomata are at the same level of epidermis
2. Distribution-Distributed unevenly
3. Orientation -Oriented irregularly in any direction
4. Striae- Absence of striae associated with stomata
5. Shape – Elliptic
6. Cuticular rim – Stomata are with cuticular rim of uniform width or occasionally cuticular rim is present at both ends only

7. Types- Cyclocytic stomata surrounded by 4 or more subsidiary cells. Two contiguous stomata which are right angle or parallel to each other are also observed.
8. Dimensions- Maximum length of stomatal aperture on lower surface of leaf is 22.86 μm and minimum length of stomatal aperture is 14.86 μm . The mean length of stomatal aperture is $17.84 \pm 2.56 \mu\text{m}$. The maximum breadth of stomatal aperture is 3.57 μm and the minimum breadth is 1.68 μm . The mean breadth of stomatal aperture is $2.73 \pm 0.70 \mu\text{m}$.

Epidermal Cells

1. Arrangement – No definite pattern of arrangement or sometime arranged in linear fashion
2. Anticlinal Wall – Anticlinal wall position is indicated by narrow shallow groove
3. Periclinal wall – Flat or slightly convex
4. Cuticular ornamentation on periclinal wall- Smooth surfaced
5. Shape – Polygonal or elongated in any direction

Trichomes- Presence of pearl glands only. Non-glandular trichomes absent. Maximum length of pearl gland on lower surface of leaf is 37.14 μm and minimum length of pearl gland is 26.63 μm . The mean length of pearl gland is $30.87 \pm 3.37 \mu\text{m}$.

Hydathodes- Present which are surrounded by rosette of epidermal cells

Piper betle* var. *Meetha cum bangla (Fig.1 B)-

Stomata

1. Position-Stomata are slightly raised above epidermal surface
2. Distribution-Distributed unevenly
3. Orientation -Oriented irregularly in any direction
4. Striae- Absence of striae associated with stomata
5. Shape – Elliptic
6. Cuticular rim – Stomata are with cuticular rim at both ends only but rarely stomata with cuticular rim of even width present.

7. Types- Cyclocytic stomata surrounded by 4 or more subsidiary cells. Two contiguous stomata which are parallel to each other are also observed.
8. Dimensions- Maximum length of stomatal aperture on lower surface of leaf is 27.14 μm and minimum length of stomatal aperture is 17.20 μm . The mean length of stomatal aperture is $20.42 \pm 2.86 \mu\text{m}$. The maximum breadth of stomatal aperture is 3.01 μm and the minimum breadth is 1.43 μm . The mean breadth of stomatal aperture is $1.92 \pm 0.50 \mu\text{m}$

Epidermal Cells

1. Arrangement – No definite pattern of arrangement or sometime arranged in linear fashion
2. Anticlinal Wall – Anticlinal wall position is indicated by broad shallow groove
3. Periclinal wall – Slightly convex
4. Cuticular ornamentation on periclinal wall- Smooth surfaced
5. Shape – Polygonal or elongated in any direction

Trichomes- Presence of pearl glands only. Non-glandular trichomes absent. Maximum length of pearl gland on lower surface of leaf is 57.14 μm and minimum length of pearl gland is 27.14 μm . The mean length of pearl gland is $41.52 \pm 6.62 \mu\text{m}$.

Hydathodes- Present which are surrounded by rosette of epidermal cells

B. Upper Epidermis: (Fig.2)

Characteristics of upper epidermis of both varieties are similar except periclinal wall of epidermal cell and length of trichomes.

Stomata- Not observed

Epidermal Cells

1. Arrangement – No definite pattern of arrangement or sometime arranged in linear fashion
2. Anticlinal Wall – Anticlinal wall position is indicated by broad shallow groove

3. Periclinal wall – Flat or slightly convex in Kapoori and slightly convex in Meetha cum bangla
4. Cuticular ornamentation on periclinal wall- Smooth surfaced
5. Shape – Polygonal or elongated in any direction

Trichomes- Presence of pearl glands. Pearl glands are less abundant as compared to lower leaf surface. Non-glandular trichomes absent. Lengths of pearl glands on upper surface of leaf are

Name of variety	Min. (μm)	Max. (μm)	Mean \pm S.D. (μm)
Kapoori	29.15	42.86	37.14 \pm 3.83
Meetha cum bangala	28.57	51.43	42.76 \pm 7.80

Hydathodes- Present which are surrounded by rosette of epidermal cells

Petiole: (Fig.3)

Characteristics of petiole of both varieties are similar. No differences have been observed except length of trichomes.

Stomata- Not observed

Epidermal Cells

1. Arrangement –Cells are arranged in linear fashion in vertical rows
2. Anticlinal Wall – Anticlinal wall position is indicated by groove. Anticlinal wall in between cells of two adjacent vertical rows is indicated by deep groove while anticlinal wall in between cells of same vertical row is indicated by shallow groove
3. Periclinal wall –Convex
4. Cuticular ornamentation on periclinal wall- Smooth surfaced
5. Shape – Rectangular, elongated in vertical direction or rarely squarish

Trichomes- Presence of pearl glands and non-glandular trichomes. Lengths of pearl glands on petiole are

Name of variety	Min. (µm)	Max. (µm)	Mean ± S.D. (µm)
Kapoori	32.56	45.71	39.61 ± 3.47
Meetha cum bangala	28.52	51.43	41.43 ± 8.26

Lengths of non-glandular trichomes on petiole are

Name of variety	Min. (µm)	Max. (µm)	Mean ± S.D. (µm)
Kapoori	20.62	104.4	52.89 ± 29.05
Meetha cum bangala	24.63	75.67	43.31 ± 16.31

Stem: (Fig.4)

Stomata- Not observed

Epidermal Cells- Detailed study of epidermal cell is not possible because of presence of ridges which obscure surface. Wax is deposited in the form of granules on the epidermis. Ridges are denser and closely placed, wax granules are denser on stem of Kapoori variety while ridges are comparatively less dense and distantly placed, wax granules scattered and comparatively less dense on stem of Meetha cum bangala variety

Trichomes- Presence of pearl glands and non-glandular trichomes. Non glandular trichomes are abundant on stem of Kapoori variety while they are scanty on stem of Meetha cum bangala variety. Lengths of pearl glands on stem are

Name of variety	Min. (µm)	Max. (µm)	Mean ± S.D. (µm)
Kapoori	27.28	52.86	38.65 ± 6.47
Meetha cum bangala	28.78	57.24	45.37 ± 9.95

Lengths of non-glandular trichomes on stem are

Name of variety	Min. (µm)	Max. (µm)	Mean ± S.D. (µm)
Kapoori	14.29	72.85	26.27± 15.63
Meetha cum bangala	28.57	85.71	51.24± 16.71

Keys to varieties of *Piper betle*

1. Key based on SEM characteristics of Lower epidermis of leaf

- Stomata at the same level of epidermis and with cuticular rim of uniform width or occasionally cuticular rim is present at both ends only, position of anticlinal wall of epidermal cell is indicated by narrow shallow groove, periclinal wall of epidermal cell is flat or slightly convex, pearl glands are comparatively shorter ($30.87 \pm 3.37 \mu\text{m}$)

---Kapoori

- Stomata are slightly raised above epidermal surface with cuticular rim at both ends only but rarely stomata with cuticular rim of even width is present, position of anticlinal wall of epidermal cell is indicated by broad shallow groove, periclinal wall of epidermal cell is slightly convex, pearl glands are comparatively longer ($41.52 \pm 6.62 \mu\text{m}$)

---Meetha Cum Bangla

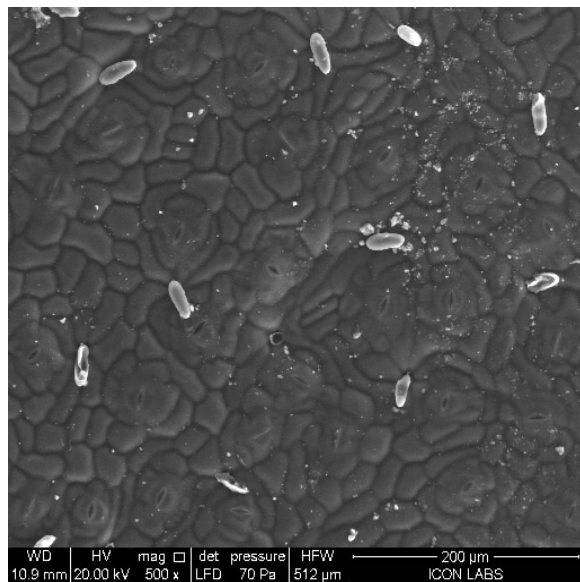
2. Key based on SEM characteristics of stem

- Ridges on the epidermal surface are more dense and closely placed, wax granules are dense, non-glandular trichomes denser and shorter ($26.27 \pm 15.63 \mu\text{m}$), pearl glands are comparatively shorter ($38.65 \pm 6.47 \mu\text{m}$)

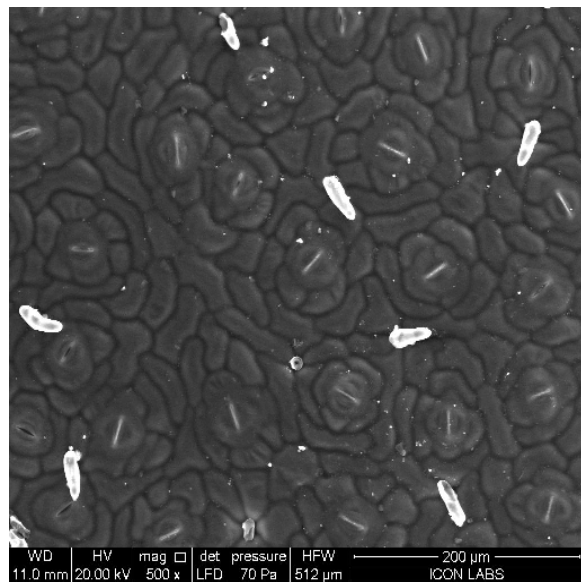
---Kapoori

- Ridges on the epidermal surface are comparatively less dense and distantly placed, wax granules scattered and less dense, non-glandular trichomes less dense and longer ($51.24 \pm 16.71 \mu\text{m}$), pearl glands are comparatively longer ($45.37 \pm 9.95 \mu\text{m}$)

---Meetha Cum Bangla

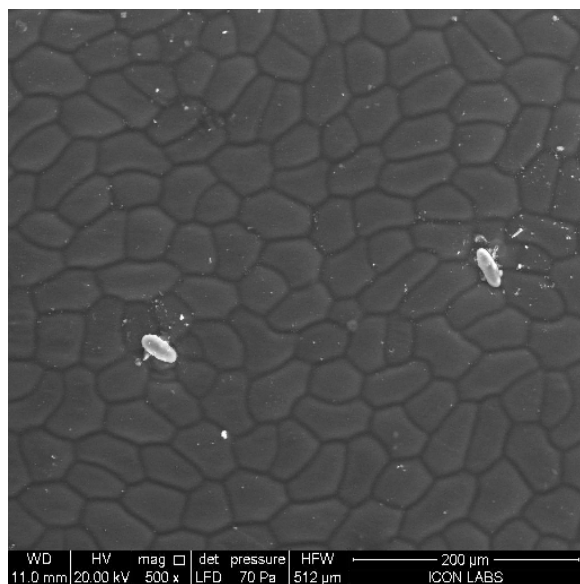


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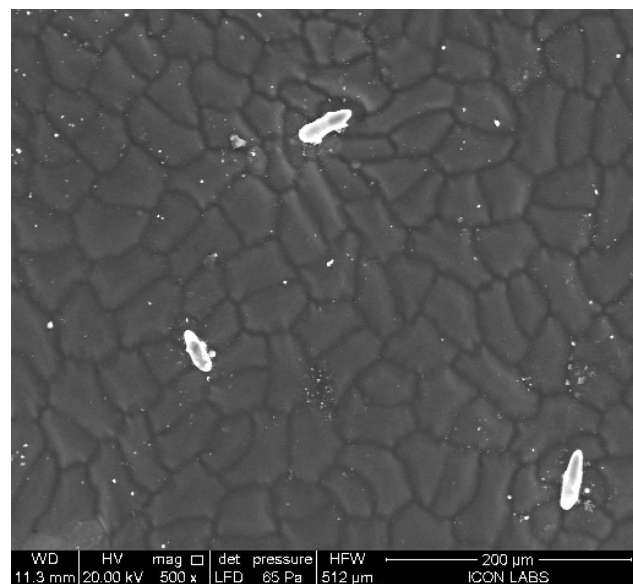


B

Fig.1.SEM Photomicrographs of lower surface of leaf of variety **A.** Kapoori **B.** Meetha cum Bangala

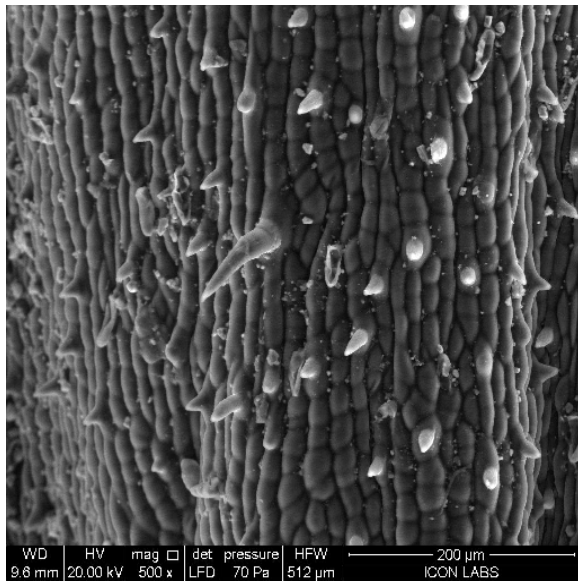


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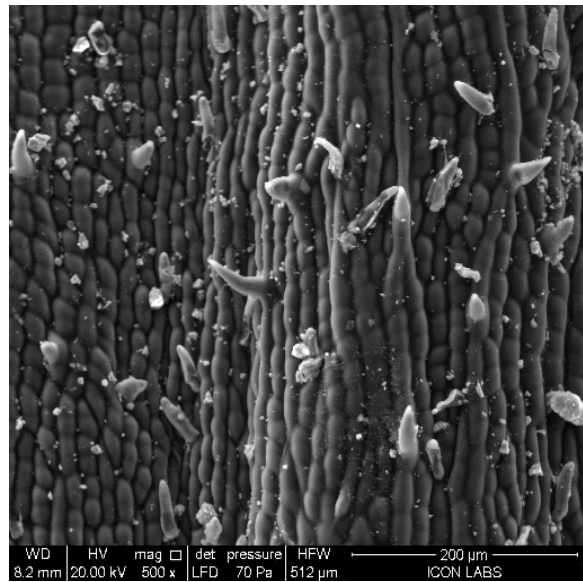


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Fig.2.SEM Photomicrographs of upper surface of leaf of variety **A.** Kapoori **B.** Meetha cum Bangala

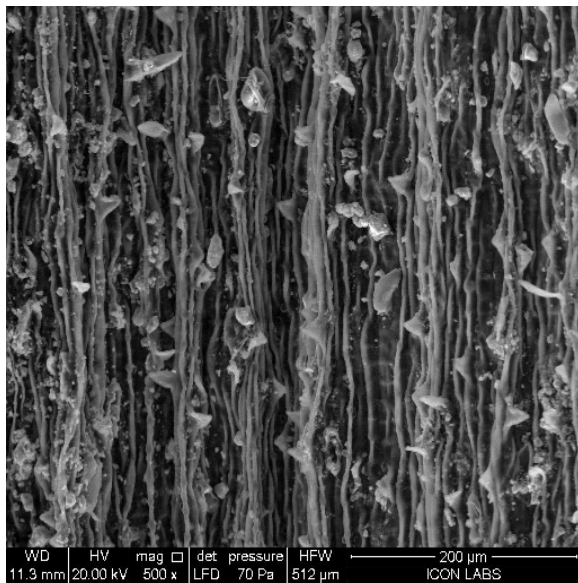


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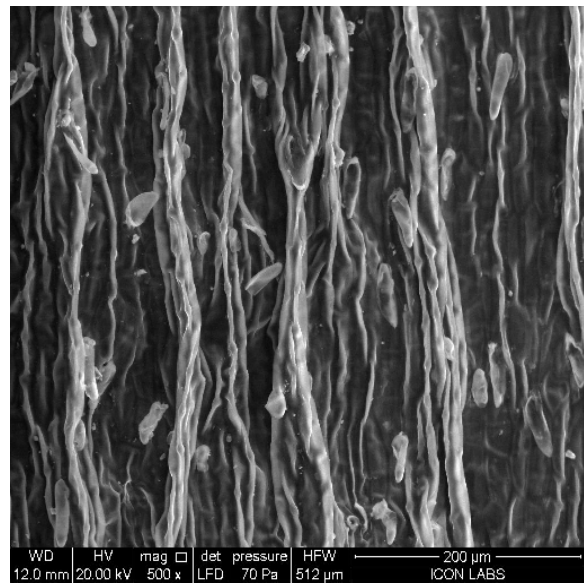


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Fig.3. SEM Photomicrographs of petiole of variety **A. Kapoori** **B. Meetha cum Bangala**



A



B

Fig.4. SEM Photomicrographs of stem of variety **A. Kapoori** **B. Meetha Cum Bangla**

EDAX Analysis:

Epidermal cell and pearl glands on lower leaf surface, petiole and stem were quantitatively analyzed by EDAX microanalysis. The results were summarized in the following tables (Table 1, 2, 3, 4).

Elements	Leaf		Petiole		Stem	
	Kapoori Wt %	Meetha Cum Bangla Wt %	Kapoori Wt %	Meetha Cum Bangla Wt %	Kapoori Wt %	Meetha Cum Bangla Wt %
C	19.5	19.7	34.0	54.7	56.5	68.0
O	79.3	77.9	64.2	43.7	33.4	23.5
Si	0.1	-	0.1	0.5	2.3	0.5
S	0.1	-	-	-	-	0.1
K	1.0	0.3	1.1	0.5	4.8	6.3
N	-	2.1	-	-	-	-
Mg	-	0.1	-	-	0.2	-
Ca	-	-	0.5	0.3	1.2	0.6
Al	-	-	-	0.2	0.4	-
P	-	-	-	-	0.1	0.2
Cl	-	-	-	0.1	0.1	0.8
Fe	-	-	-	-	0.8	-

Table 1: Comparative EDAX Analysis on Epidermal Cell of two varieties of Piper betle.

Elements	Kapoori leaf			Meetha cum Bangala leaf		
	Pearl Gland 1 Wt %	Pearl Gland 2 Wt %	Pearl Gland 3 Wt %	Pearl Gland 1 Wt %	Pearl Gland 2 Wt %	Pearl Gland 3 Wt %
C	27.9	22.8	21.7	27.6	28.8	28.7
O	66.3	72.4	71.9	67.2	67.6	68.2
Mg	0.2	0.3	0.2	0.3	-	0.1
Al	0.2	-	0.1	0.1	-	-
Si	4.6	3.2	4.9	4.0	3.2	2.2
K	0.5	0.9	0.9	0.3	0.2	0.3
Ca	0.2	0.4	0.4	0.3	0.2	0.5
Mo	0.2	-	-	-	-	-
P	-	0.1	-	-	-	-
S	-	-	-	-	-	0.0

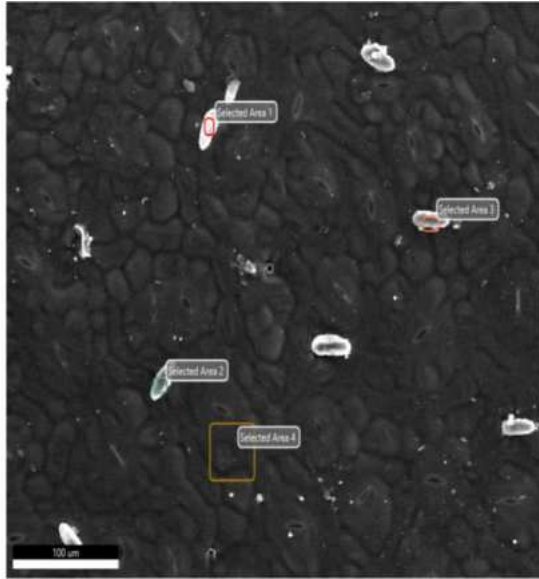
Table 2: EDAX Analysis on pearl glands on lower surface of leaf of *Piper betle* Linn.

Elements	Kapoori Petiole			Meetha cum Bangala Petiole		
	Pearl Gland 1 Wt %	Pearl Gland 2 Wt %	Pearl Gland 3 Wt %	Pearl Gland 1 Wt %	Pearl Gland 2 Wt %	Pearl Gland 3 Wt %
C	23.8	38.8	39.0	51.8	55.4	49.9
O	72.8	56.2	57.5	45.6	43.1	48.0
Mg	0.2	-	0.3	0.1	0.1	0.1
Al	0.1	0.3	0.1	0.1	0.1	0.1
Si	2.5	1.4	1.6	1.3	0.7	1.2
K	0.4	1.4	0.7	0.4	0.4	0.4
Ca	0.2	2.0	0.3	0.5	0.2	0.2
P	-	-	0.2	0.1	-	-
S	-	-	0.1	-	-	-
Cl	-	-	0.1	0.1	0.1	0.1

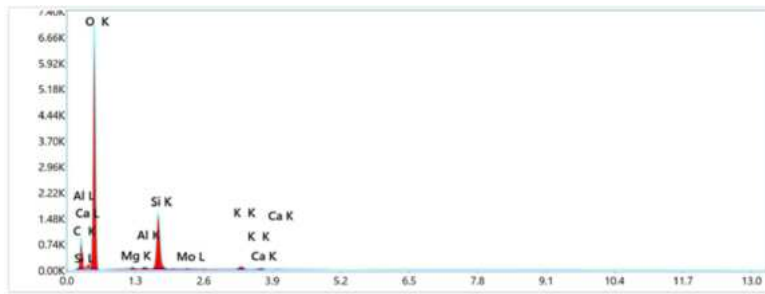
Table 3: EDAX Analysis on pearl glands on petiole of *Piper betle* Linn.

Elements	Kapoori Stem			Meetha cum Bangala Stem		
	Pearl Gland 1 Wt %	Pearl Gland 2 Wt %	Pearl Gland 3 Wt %	Pearl Gland 1 Wt %	Pearl Gland 2 Wt %	Pearl Gland 3 Wt %
C	59.6	49.0	51.1	70.7	52.0	58.4
O	29.0	37.1	39.2	17.6	37.8	34.3
Mg	0.1	0.5	0.4	-	-	-
Al	0.5	0.5	0.6	-	-	-
Si	2.1	3.3	4.7	6.8	6.8	4.5
K	4.4	6.3	1.7	3.9	2.4	2.3
Ca	1.4	1.1	1.0	0.3	0.3	-
P	-	-	-	-	0.1	0.1
S	-	-	-	-	0.1	0.1
Cl	0.2	0.1	0.1	0.6	0.4	0.3
Fe	2.7	2.3	0.9	-	-	-
Ti	-	-	0.1	-	-	-

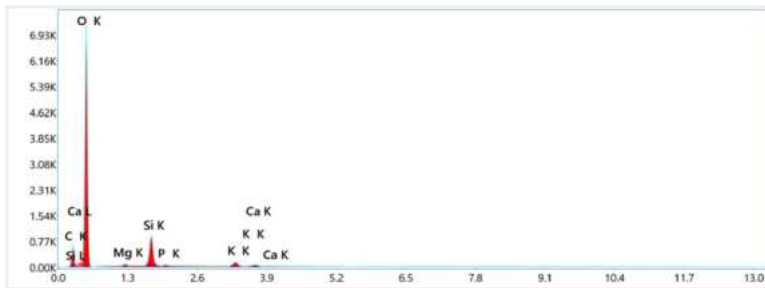
Table 4: EDAX Analysis on pearl glands on stem of *Piper betle* Linn.



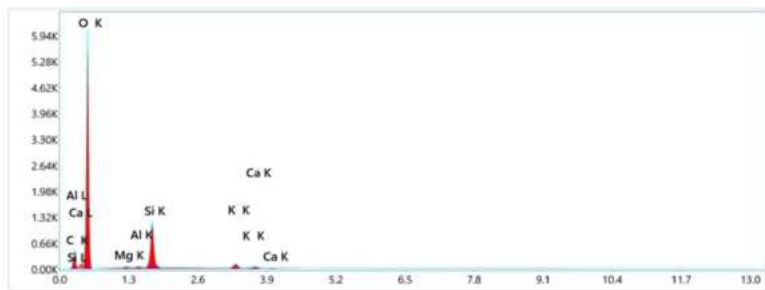
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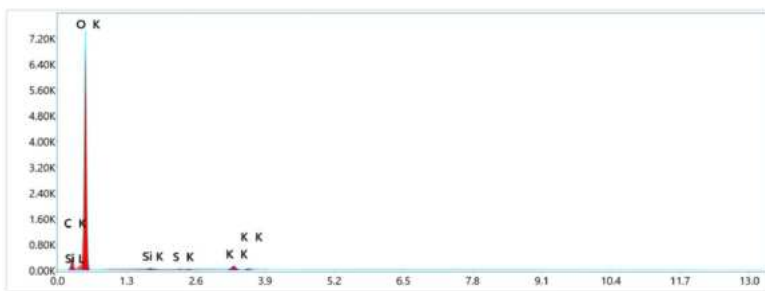
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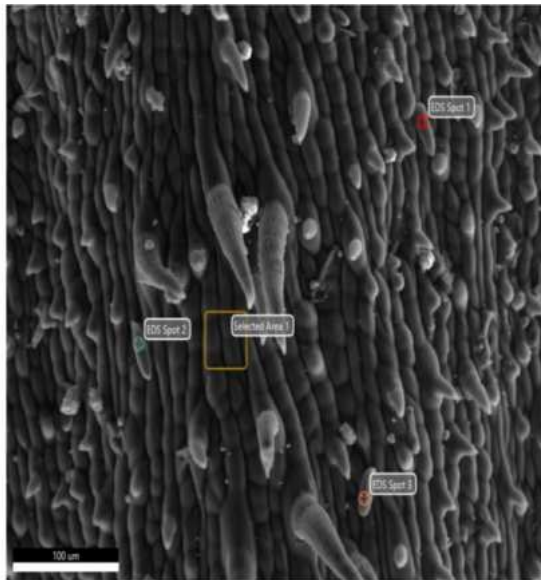


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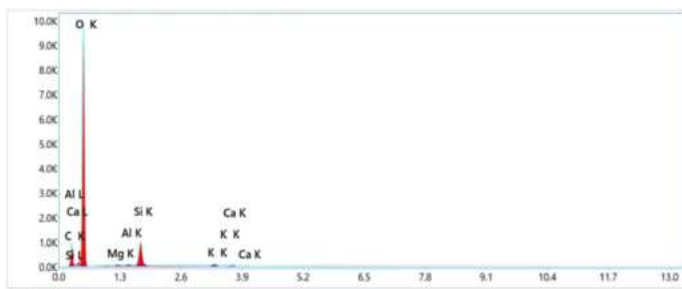


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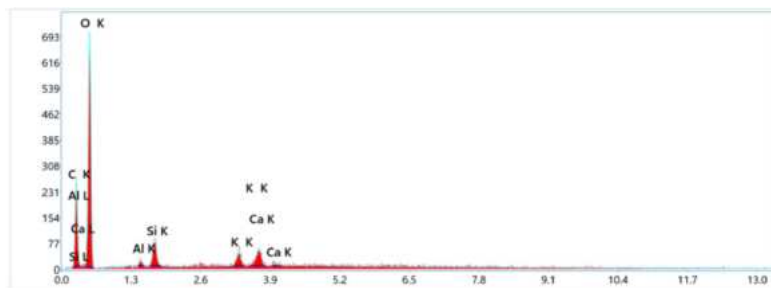
Fig.5 (A-E). EDAX analysis on pearl glands and Epidermal cell of lower surface of leaf of *Piper betle* Var. Kapoori **A.** SEM photomicrograph of lower surface of leaf of variety Kapoori marked with selected area for EDAX analysis, **B.** EDS spectrum showing elemental analysis of selected area 1(Pearl gland), **C.** EDS spectrum showing elemental analysis of selected area 2 (Pearl gland), **D.** EDS spectrum showing elemental analysis of selected area 3 (Pearl gland), **E.** EDS spectrum showing elemental analysis of selected area 4 (Epidermal cell).



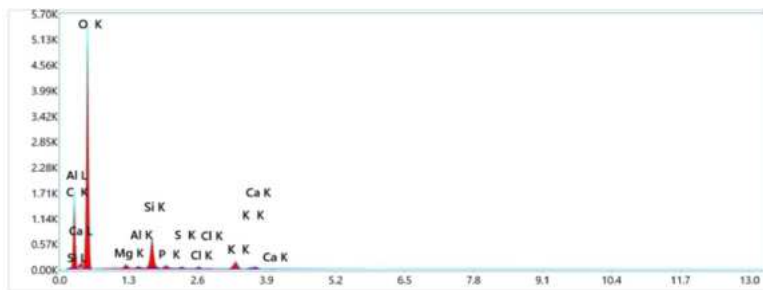
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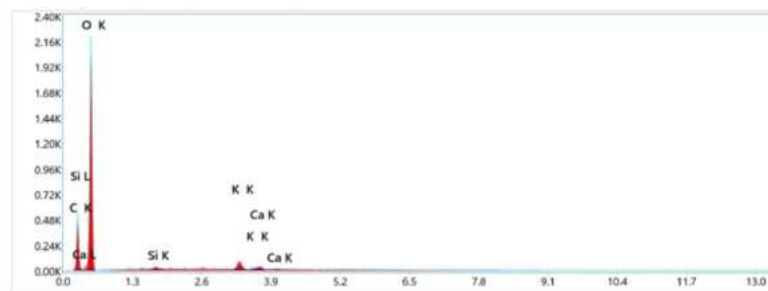
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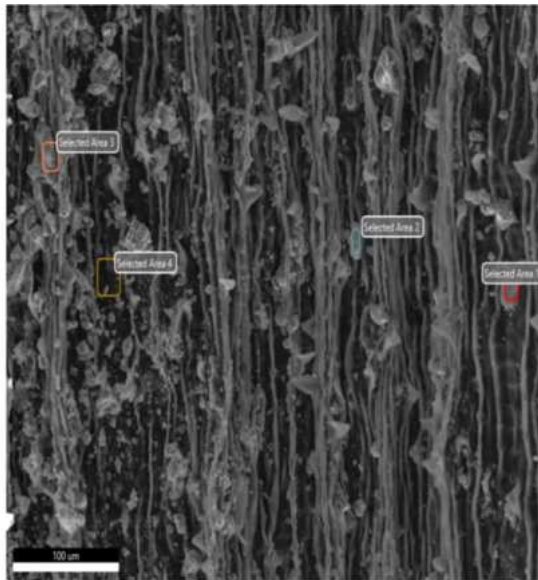


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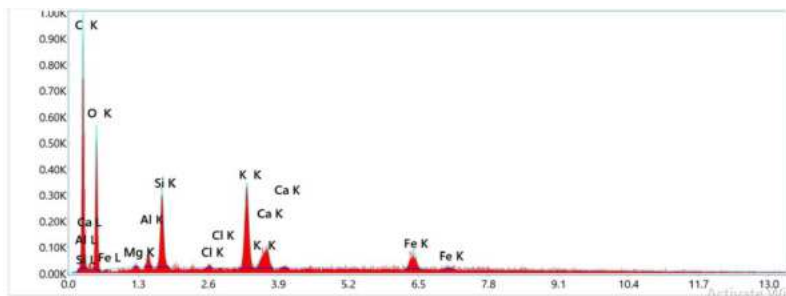


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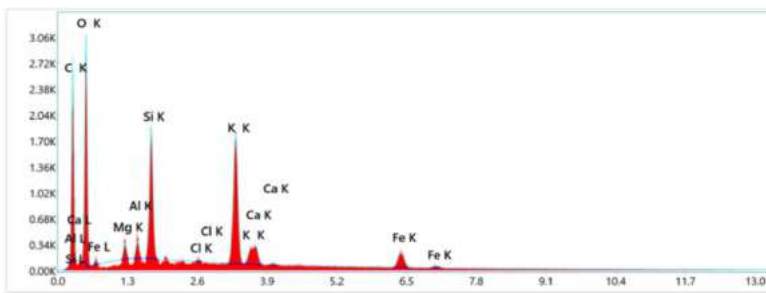
Fig. 6.(A-E). EDAX analysis on pearl glands and Epidermal cell of petiole of *Piper betle* Var. Kapoori **A.** SEM photomicrograph of petiole of variety Kapoori marked with selected area for EDAX analysis, **B.** EDS spectrum showing elemental analysis of selected area 1(Marked as EDS Spot 1, Pearl gland), **C.** EDS spectrum showing elemental analysis of selected area 2 (Marked as EDS Spot 2, Pearl gland), **D.** EDS spectrum showing elemental analysis of selected area 3 (Marked as EDS Spot 3, Pearl gland), **E.** EDS spectrum showing elemental analysis of selected area 4 (Marked as Selected area 1, Epidermal cell)



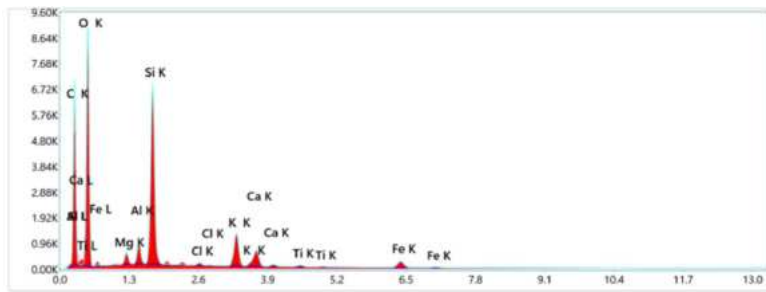
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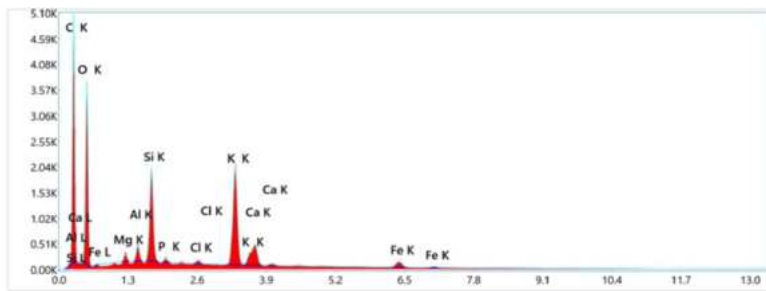
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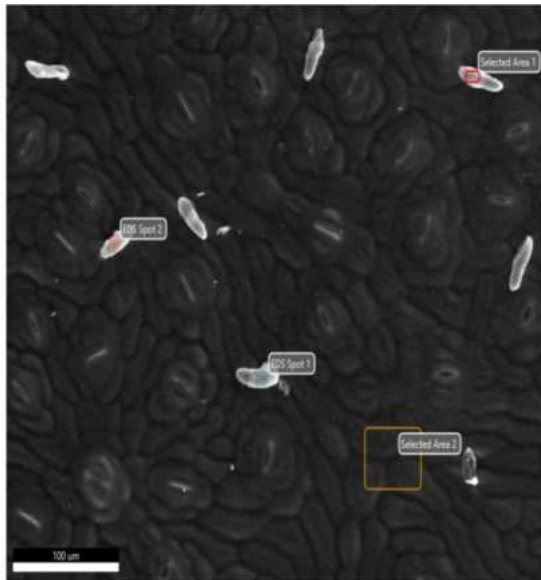


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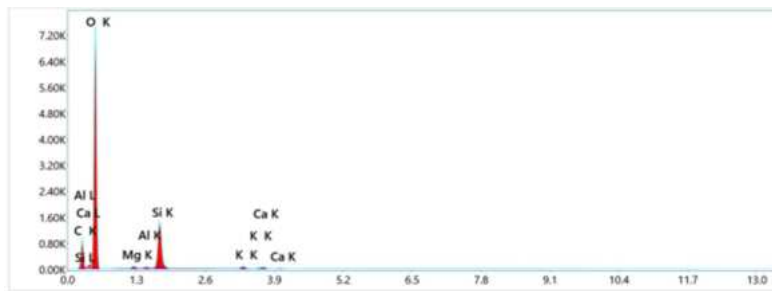


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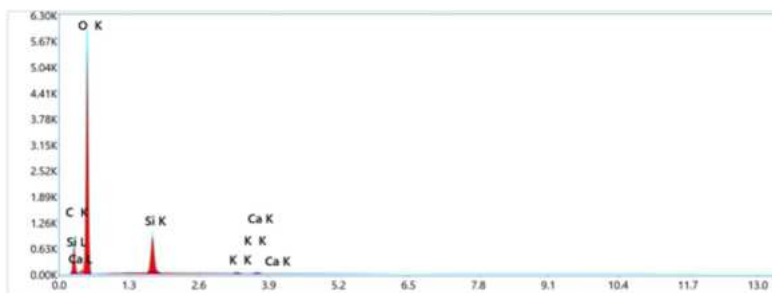
Fig.7. (A-E). EDAX analysis on pearl glands and Epidermal cell of stem of *Piper betle* Var. Kapoori **A.** SEM photomicrograph of stem of variety Kapoori marked with selected area for EDAX analysis, **B.** EDS spectrum showing elemental analysis of selected area 1 (Pearl gland), **C.** EDS spectrum showing elemental analysis of selected area 2 (Pearl gland), **D.** EDS spectrum showing elemental analysis of selected area 3 (Pearl gland), **E.** EDS spectrum showing elemental analysis of selected area 4 (Epidermal cell)



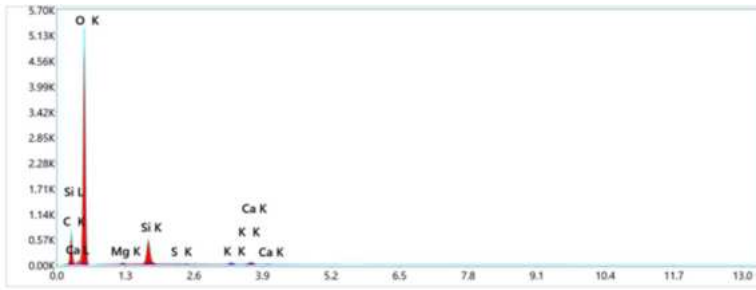
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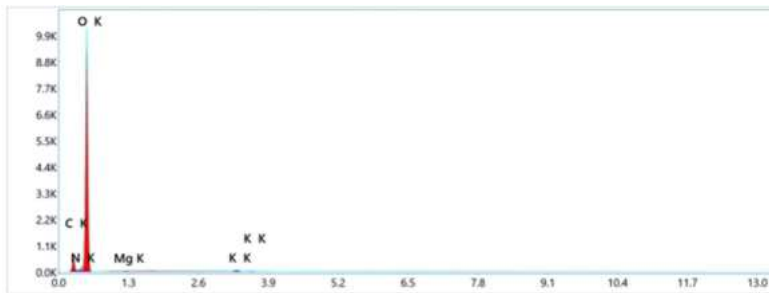
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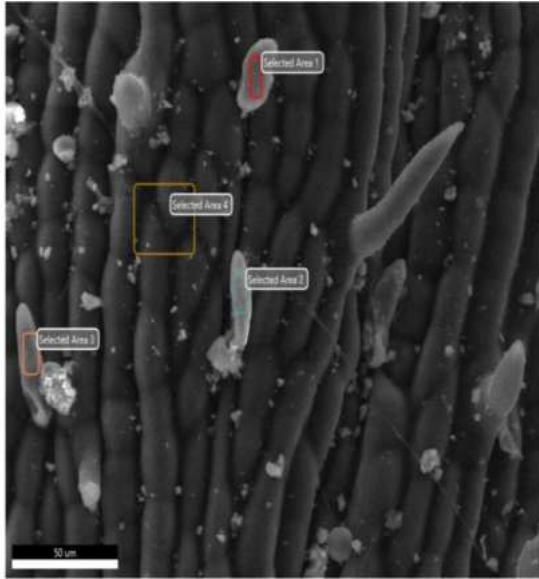


D

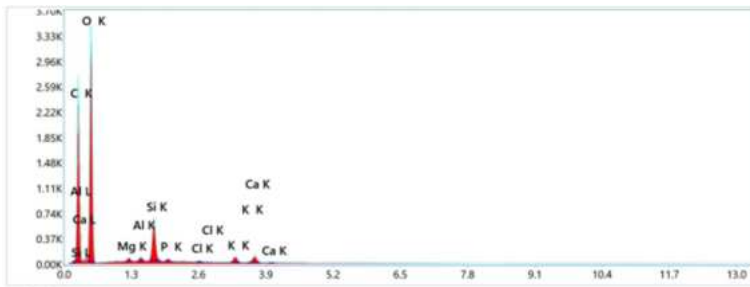


E

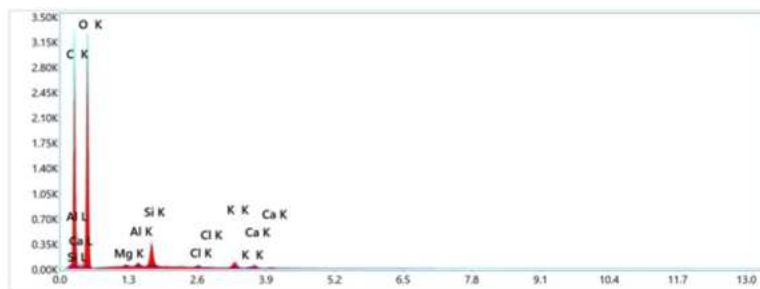
Fig.8. (A-E). EDAX analysis on pearl glands and Epidermal cell of lower surface of leaf of *Piper betle* Var. Meetha cum Bangla **A.** SEM photomicrograph of lower surface of leaf of *Piper betle* Var. Meetha cum Bangla marked with selected area for EDAX analysis, **B.** EDS spectrum showing elemental analysis of selected area 1(Marked as selected area 1, Pearl gland), **C.** EDS spectrum showing elemental analysis of selected area 2 (Marked as EDS Spot 1, Epidermal cell), **D.** EDS spectrum showing elemental analysis of selected area 3 (Marked as EDS Spot 2, Pearl gland), **E.** EDS spectrum showing elemental analysis of selected area 4 (Marked as selected area 2, Epidermal cell)



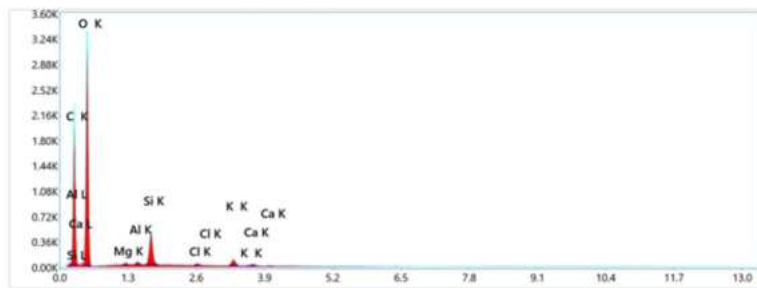
A



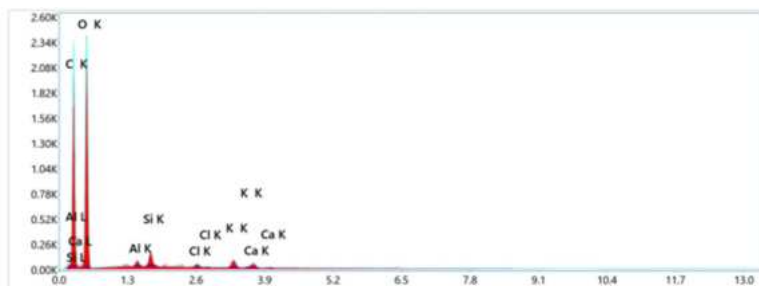
B



C

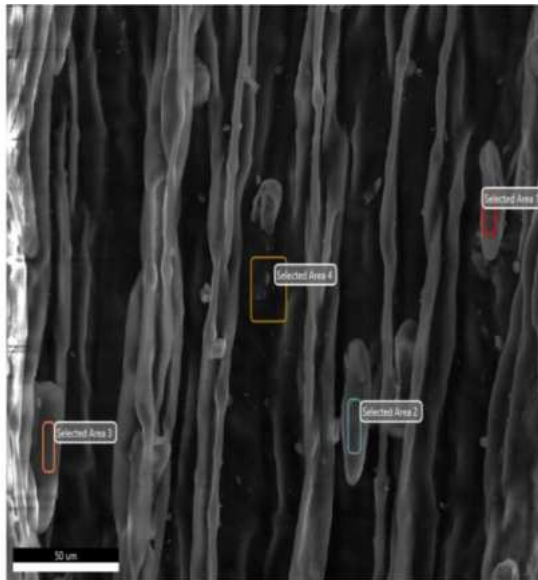


D

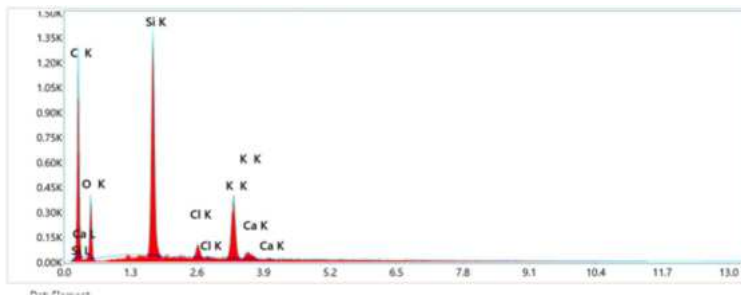


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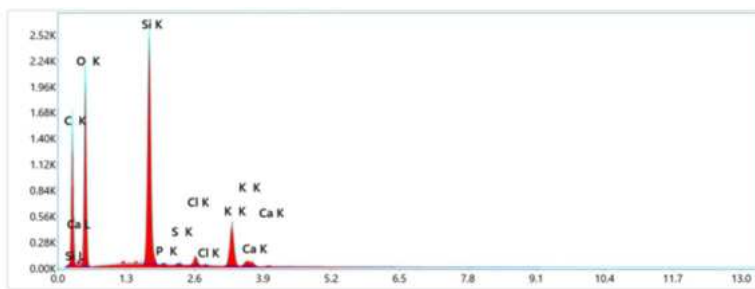
Fig. 9. (A-E). EDAX analysis on pearl glands and Epidermal cell of petiole of *Piper betle* Var. Meetha cum Bangla A. SEM photomicrograph of petiole of variety Meetha cum Bangla marked with selected area for EDAX analysis, **B.** EDS spectrum showing elemental analysis of selected area 1(Pearl gland), **C.** EDS spectrum showing elemental analysis of selected area 2 (Pearl gland), **D.** EDS spectrum showing elemental analysis of selected area 3 (Pearl gland), **E.** EDS spectrum showing elemental analysis of selected area 4 (Epidermal cell).



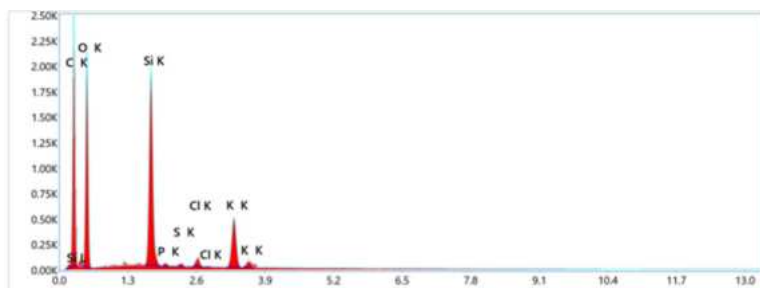
A



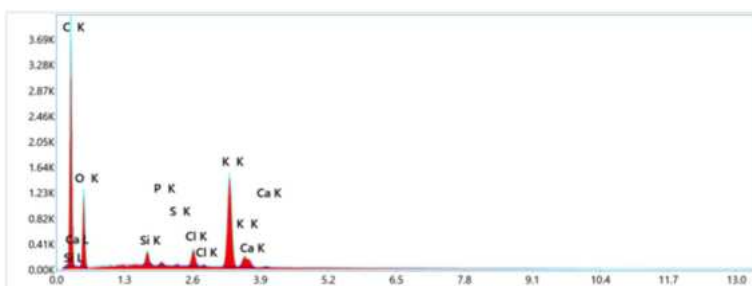
B



C



D



E

Fig. 10. (A-E). EDAX analysis on pearl glands and Epidermal cell of stem of *Piper betle* Var. Meetha cum Bangla **A.** SEM photomicrograph of stem of variety Meetha cum Bangla marked with selected area for EDAX analysis, **B.** EDS spectrum showing elemental analysis of selected area 1(Pearl gland), **C.** EDS spectrum showing elemental analysis of selected area 2 (Pearl gland), **D.** EDS spectrum showing elemental analysis of selected area 3 (Pearl gland), **E.** EDS spectrum showing elemental analysis of selected area 4 (Epidermal cell).

Pearl glands in both the varieties have oval body. Presence of pearl glands in family Piperaceae is recorded by Metcalfe and Chalk (1950). Pearl glands are more abundant on lower surface of leaf as compared to upper surface of leaf. Pearl glands in Meetha cum Bangla are longer than Pearl glands in Kapoori. Non-glandular trichomes on stem of Meetha cum Bangla are longer as compared to Non-glandular trichomes on stem of Kapoori while non-glandular trichomes on petiole of Meetha cum Bangla are shorter as compared to non-glandular trichomes on petiole of Kapoori.

As far as my knowledge goes, I found only one work of Mubeen et al (2014) in which they studied SEM characteristics of leaf and petiole and EDAX analysis of leaf of Sirugamani 1 (SGM1) variety of *Piper betle*. They reported presence of pearl gland and cyclocytic stomata on leaf epidermis and numerous covering trichomes on petiole. This is in conformity with my observations.

More elements are found in EDAX analysis on epidermal cell of stem as compared to that of leaf and petiole. EDAX analysis on pearl glands on lower surface of leaf showed presence of C, O, Mg, Al, Si, K, Ca. Element Mo and P are found additionally in pearl glands on leaf of Kapoori. EDAX analysis on pearl glands on petiole showed C, O, Mg, Al, Si, K, Ca, P, Cl. But pearl glands on petiole of Kapoori showed presence of additional element S. Common elements found in EDAX analysis on pearl glands on the stem of both taxa are C, O, Si, K, Ca, Cl. Additional elements Mg, Al, Fe, Ti are present in pearl glands on Kapoori stem while additional elements P, S are present in pearl glands on Meetha cum Bangla stem. Mubeen et al (2014) found Zn in EDAX analysis of dried leaf of variety Sirugamani 1 but I have not found Zn in EDAX analysis of both investigated varieties of *Piper betle*. Presence of minerals in investigated plant parts proves its nutritional value.

Conclusion

SEM characteristics of lower leaf surface and stem are taxonomically important and can be used as complementary tool in differentiating variety Kapoori from Meetha cum Bangla. Most of the elements found in EDAX analysis on leaf, petiole and stem of both varieties are same except a few.

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