

Morphological and Micromorphological Characterization of Selected Leguminous Seeds from Chandrapur District, Maharashtra, India: A SEM and EDS Study

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ABSTRACT

The present study was carried out to investigate macromorphology, micromorphology and elemental composition of leguminous seeds from Chandrapur district (MS), India. This study critically examined seeds of *Cassia siamea*, *Cassia occidentalis*, *Tephrosia villosa*, *Tephrosia purpurea* and *Abrus precatorius*. The quantitative morphological characteristics of seeds, such as length, width, length/width ratio and mass were determined. The qualitative morphological characteristics of seeds such as shape, colour, apex, base and hilum feature were recorded. Micromorphological features (testa topography patterns) of seeds were recorded with the help of Scanning Electron Microscopy (SEM). The chemical characterization and elemental analysis of seeds were determined using the analytical technique, Energy Dispersive X-Ray Spectroscopy (EDS). Variations have been observed in quantitative (length, width, length/width ratio and mass) as well as qualitative (shape, colour, apex, base and hilum features) morphological characters of seeds. Micromorphological features revealed by SEM exhibit variation in testa topography patterns among the studied taxa; where three basic types of testa topography patterns i.e., levigate, rugulate and multi-reticulate were recorded. EDS study reported the variation in elemental concentration of the examined seeds. In all the seeds Carbon (C) and Oxygen (O) elements were detected in larger amounts. The features of the seeds reported in this investigation are distinctive and may be helpful in the identification of taxa.

Keywords: Leguminosae, seed, morphology, micromorphology, scanning electron microscopy, energy dispersive x-ray spectroscopy.

INTRODUCTION

The family Leguminosae is the third-largest family of flowering plants, behind only the Orchidaceae and Asteraceae. Hutchinson (1948) classified Leguminosae as "Leguminales" with three families: Caesalpiniaceae, Mimosaceae, and Fabaceae (Papilionaceae). However, according to Willis (1976), it comprises three subfamilies, namely, Caesalpinioideae, Mimosoideae and Papilionoideae. Recent trends consider these three sub-families under the broadly confined single Leguminosae or Fabaceae family.

Seeds possess great diversity in their quantitative morphological features, such as size and mass, as well as qualitative morphological features such as shape, colour, apex, base and hilum features. Seed micromorphological features, particularly revealed under SEM and elemental composition analysis by Energy Dispersive X-Ray Spectroscopy

(EDS/ EDX) are unique to the species but quite variable between different species. These features can form the basis of systematic importance (Chowdhury and Buth, 1970, Waffa, 2004 and Gohary and Mohammed, 2007). Morphological characteristics of seeds are used to establish taxonomic relationships in many plant families (Zhang et al., 2005 and Gohary and Mohammed, 2007). Many studies investigate Leguminosae seed morphology and structure, including Jones & Geneve (1995) and Hussein *et al.* (2002), Salimpour *et al.* (2007) etc.

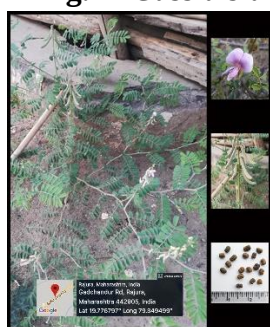
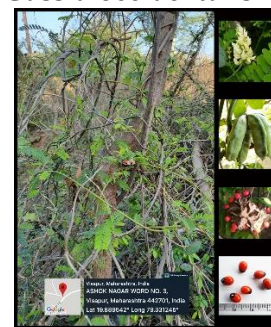
The present work has been undertaken to describe and investigate the morphology, micromorphology (seeds testa topography pattern) and elemental composition of leguminous seeds belonging to 5 legume species viz. *Cassia siamea* Lam., *Cassia occidentalis* L., *Tephrosia villosa* (L.) Pers., *Tephrosia purpurea* (L.) Pers and *Abrus precatorius* L. growing in Chandrapur district of Maharashtra, India. Detailed studies on the seeds of the investigated taxa here have not been reported in the literature.

MATERIALS AND METHODS

For the present study seeds of *Cassia siamea* Lam. (Fig.1), *Cassia occidentalis* L. (Fig.2), *Tephrosia villosa* (L.) Pers. (Fig.3), *Tephrosia purpurea* (L.) Pers (Fig. 4) and *Abrus precatorius* L. (Fig.5) were utilized. Fresh and mature fruits were collected from different localities (Table-1) of the Chandrapur district (North Latitude-18-4 to 20-5 (19.57'), East Longitude-78-5 to 80-6 (79.18') and Altitude-189) Maharashtra, India. Seeds were obtained by manual separation from fruit and allowed to shed dry. Dried seeds were washed with 70% ethanol to remove impurities and kept in airtight zipper bags. Morphological features of the seeds were studied with the aid of a stereoscopic microscope.

Table 1: Collection sites of examined taxa.

Sr. No.	Plant Species	Collection Site	Coordinates
1	<i>Cassia siamea</i>	G. N. College, Ballarpur	Lat 19.840143° Long 79.368669°
2	<i>Cassia occidentalis</i>	Sasti	Lat 19.805576° Long 79.341042°
3	<i>Tephrosia villosa</i>	Rajura, Gadchandur road	Lat 19.776797° Long 79.349499°
4	<i>Tephrosia purpurea</i>	Chandrapur, Ballarshah road	Lat 19.907256° Long 79.327588°
5	<i>Abrus precatorius</i>	Visapur	Lat 19.889542° Long 79.331245°

**Fig. 1- *Cassia siamea*****Fig. 2- *Cassia occidentalis*****Fig. 3- *Tephrosia villosa*****Fig. 4- *Tephrosia purpurea*****Fig. 5- *Abrus precatorius***

To evaluate quantitative morphological features of seeds, visually healthy and non-deformed seeds were selected to form a sample composed of 50 seeds. The data obtained were tabulated and subject to descriptive analysis. The charts and tables were prepared considering the mean value of the data obtained for each parameter using Microsoft-Excel 2019. Quantitative morphological features such as seed length, width, length/width ratio, thickness and mass were determined. The longitudinal length of seeds was determined from the base to the tip with the help of a ruler while the width and thickness were measured at the middle of the seed, using a digital caliper at an accuracy of 0.05 mm. The mass of the seeds was recorded with the help of an electronic digital balance. Qualitative morphological characters including seed shape, colour, apex, base and hilum features, were recorded with the help of a stereoscopic microscope (BLISCO make).

Micromorphology of seed i.e., testa topography pattern, was examined by using a scanning electron microscope (SEM)-JOEL-JSM-7610F. For the SEM study, complete, dried, mature and visually healthy seeds were selected for mounting on the stub. Platinum sputtering was carried out to inspect and photograph the seed under SEM. The terminology proposed by Lersten (1981) was adopted to describe the micromorphological characteristics of the seeds. For the determination of the chemical characterization and elemental analysis of seeds analytical technique, Energy Dispersive X-Ray Spectroscopy (EDS) was used.

RESULTS AND DISCUSSION

In this study, macromorphological characters (quantitative and qualitative), SEM-based micromorphological (testa topography patterns) and elemental composition of seeds using EDS technique were investigated. Studied taxa showed considerable variations in the parameter studied. Variations were observed in macromorphological quantitative characters among the examined taxa. Seeds of *Cassia siamea* were, on average 8.00, 6.00 and 1.00 mm in length, width and thickness, respectively. Mean length of 4.00 mm, width of 3.00 mm and thickness of 1.00 mm was recorded for *Cassia occidentalis* seeds. Mean length, width and thickness of 3.00 mm, 2.00 mm and 1.00 mm, respectively was reported in *Tephrosia villosa*. In *Tephrosia purpurea* average seed length, width and thickness was measured 4.00 mm, 1.50 mm and 1.00 mm, respectively. Seeds with 6.00 mm length, 3.00 mm width and 3.00 mm thickness on average was recorded in *Abrus precatorius*. The highest length/width (L/W) ratio was recorded in *Tephrosia purpurea* (2.66 mm), followed by 2.00 in *Abrus precatorius*. L/W ratio of seeds measured for *Cassia siamea*, *Cassia occidentalis* and *Tephrosia villosa* was 1.33 mm, 1.33 mm and 1.50 mm respectively. Seeds with the highest mass of 130 mg were observed in *Abrus precatorius* among the taxa examined. *Cassia occidentalis* and *Tephrosia purpurea* have on average, 7.1 mg mass per seed. In *Cassia siamea* and *Tephrosia villosa* seeds were found to have 8.3 mg and 4.1 mg mass respectively.

Table 2: Quantitative characters of examined taxa.

Species	Length (mm)	Width (mm)	L/W ratio (mm)	Thickness (mm)	Mass (mg)
<i>Cassia siamea</i>	8.00	6.00	1.33	1.00	8.3
<i>Cassia occidentalis</i>	4.00	3.00	1.33	1.00	7.1
<i>Tephrosia villosa</i>	3.00	2.00	1.50	1.00	4.1
<i>Tephrosia purpurea</i>	4.00	1.50	2.66	1.00	7.1
<i>Abrus precatorius</i>	6.00	3.00	2.00	3.00	130

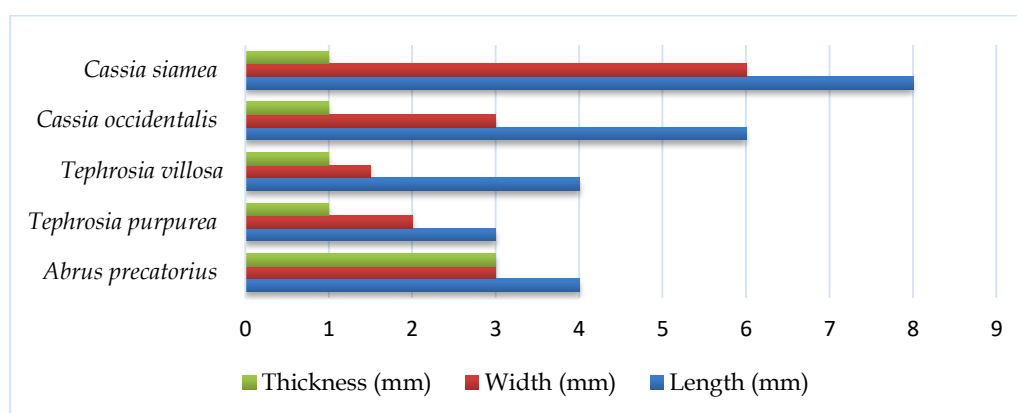


Fig.1 Comparative size of seeds and thickness in millimeters.

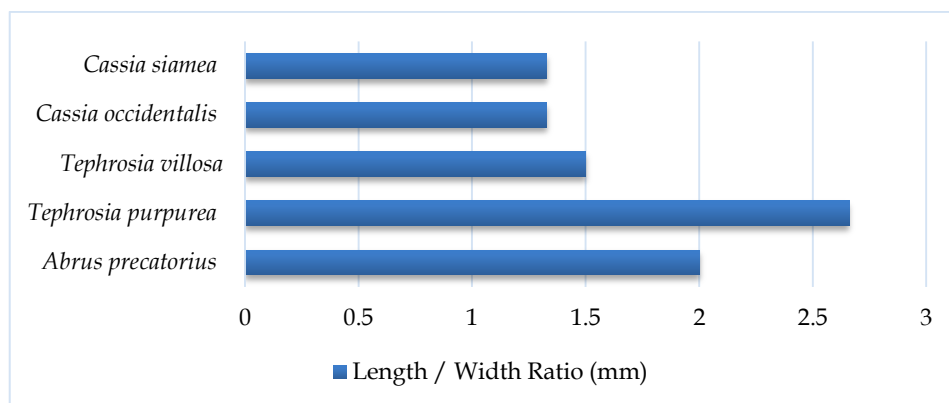


Fig. 2 Comparative length and width ratio of seeds in millimeters.

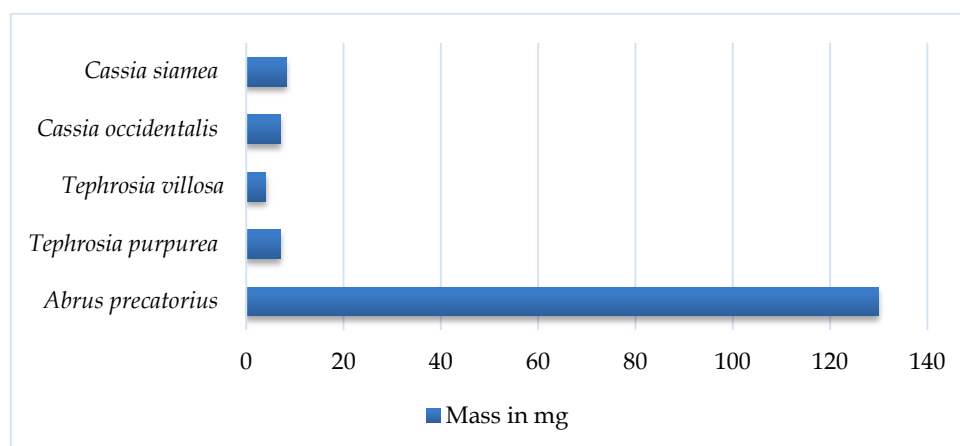


Fig. 3 Comparative mass of seeds in milligrams.

As can be seen variations were observed in the macromorphological qualitative characteristics of the seeds among examined taxa. Four different seed shapes were noted during the analysis. Elliptical, kidney and oblong-shaped seeds were found in *Cassia siamea*, *Tephrosia villosa* and *Tephrosia purpurea* respectively. In *Cassia occidentalis* and *Abrus precatorius* seeds were rounded. The Colour of the seed varied significantly from dark brown, olive green, gray, creamer and red with black at the apex in *Cassia siamea*, *Cassia occidentalis*, *Tephrosia villosa*, *Tephrosia purpurea* and *Abrus precatorius* respectively. The texture of the seeds was found smooth in all taxa except *Tephrosia villosa* where it was rough. The apex of the seed was recorded acute for *Cassia siamea* and *Cassia occidentalis* while it was rounded in *Tephrosia villosa*, *Tephrosia purpurea* and *Abrus precatorius*. Oblique to round seed base in *Cassia siamea*, truncate base in *Tephrosia villosa* and rounded base in *Cassia occidentalis*, *Tephrosia purpurea* and *Abrus precatorius* were recorded. Hilum was found visible in all the seed samples studied. Hilum shape was rounded in all seeds except *Cassia siamea* and *Abrus precatorius*. Narrowly elliptical and widely elliptical hilum shapes were found in *Cassia siamea* and *Abrus precatorius* respectively. In *Cassia siamea* and *Cassia occidentalis* apical hilum position was observed while in the rest of the seeds examined it was sub-apical. Hilum poles were found rounded in all the seed samples studied.

Table 3: Qualitative characters of examined taxa.

Species Character	<i>Cassia siamea</i>	<i>Cassia occidentalis</i>	<i>Tephrosia villosa</i>	<i>Tephrosia purpurea</i>	<i>Abrus precatorius</i>
Shape	Elliptical	Rounded	Kidney	Oblong	Rounded
Colour	Dark brown	Olive green	Gray	Creamer	Red with black at the apex
Texture	Smooth	Smooth	Rough	Smooth	Smooth
Apex	Acute	Acute	Rounded	Rounded	Rounded
Base	Oblique to round	Rounded	Truncate	Rounded	Rounded
Hilum visibility	Visible	Visible	Visible	Visible	Visible
Hilum shape	Narrowly elliptical	Rounded	Rounded	Rounded	Widely elliptical
Hilum position	Apical	Apical	Sub-apical	Sub-apical	Sub-apical
Hilum poles	Rounded	Rounded	Rounded	Rounded	Rounded

Micromorphology of seed i.e., testa topography patterns was examined by using a scanning electron microscope (SEM). Testa topography as viewed under SEM showed three basic patterns levigate (smooth), rugulate (irregularly roughened) and multi-reticulate pattern (primary and secondary ridges). Terminologies used to describe testa topography patterns are that of Lersten (1981). Levigate testa pattern was observed in *Cassia siamea* and *Abrus precatorius*. In the seeds of *Cassia occidentalis* and *Tephrosia purpurea* rugulate pattern was noted. Multi-reticulate pattern testa was recorded only in *Tephrosia villosa*.

Table 4. Seed testa topography pattern observed.

Species	Testa topography patterns observed
<i>Cassia siamea</i>	Levigate
<i>Cassia occidentalis</i>	Rugulate
<i>Tephrosia villosa</i>	Multi-reticulate
<i>Tephrosia purpurea</i>	Rugulate
<i>Abrus precatorius</i>	Levigate

Many studies in certain Fabaceae genera and other families on the micromorphology of seeds showed that seed topography features can be used for distinguishing taxa including Kaya & Dirmenci, 2012 and Venora, Grillo, Ravalli, & Cremonini, 2009. According to Taia (2004) SEM studies of seed testa helps to identify the minute taxonomically significant structures in seed coat patterns in seed coat patterns which might enable us

to define species characteristics. Skvortsov and Rusanovitch (1974) examined SEM of the seed-coat surface in *Epilobium* and showed that seed spermoderm patterns (seed coat topography) are genetically determined. Thus, such patterns are the main source of intra- or interspecific variation.

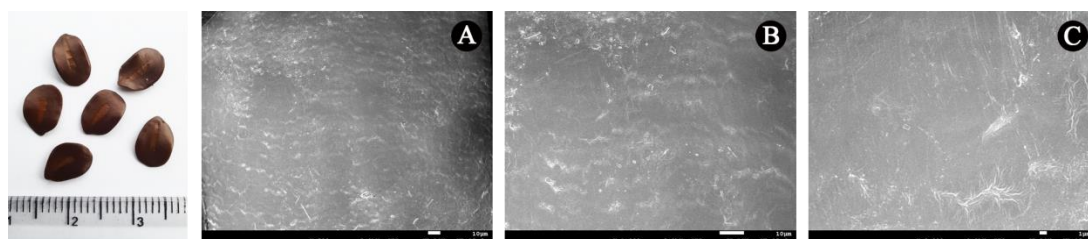


Fig.4- *Cassia siamea* - SEM photographs of seed (A, B and C).

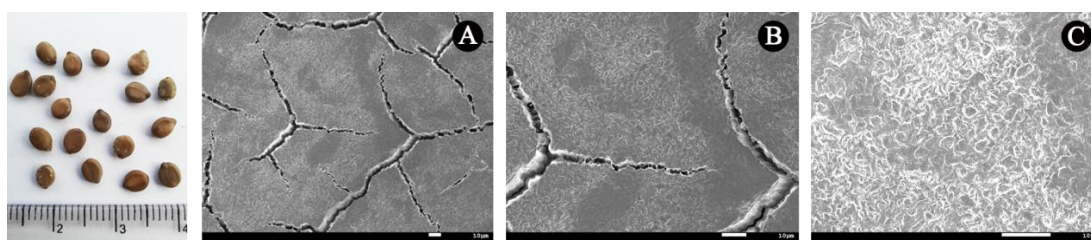


Fig.5- *Cassia occidentalis* - SEM photographs of seed (A, B and C).

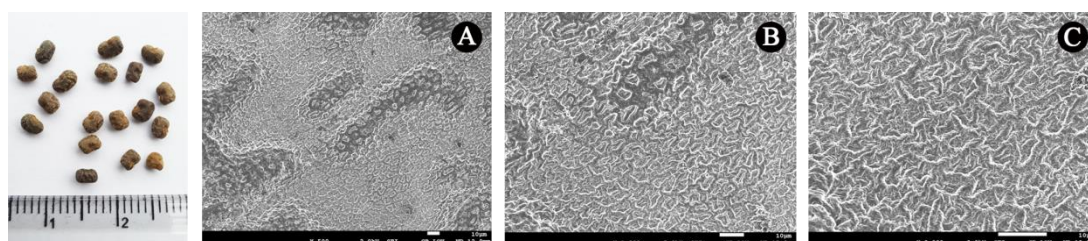


Fig.6- *Tephrosia villosa* - SEM photographs of seed (A, B and C).

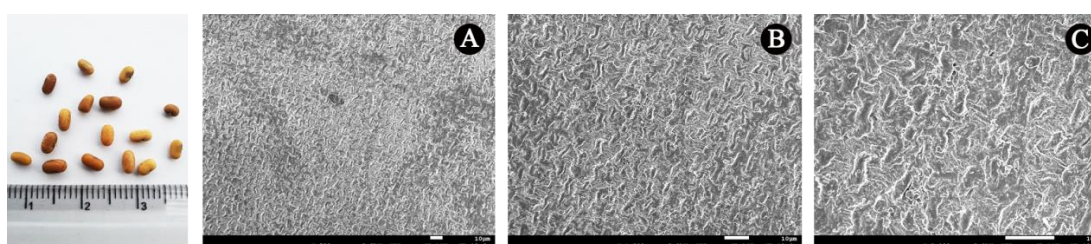


Fig.7- *Tephrosia purpurea* - SEM photographs of seed (A, B and C).

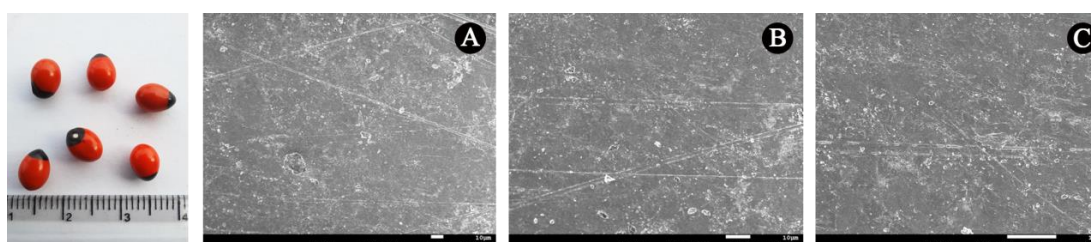


Fig.8- *Abrus precatorius* - SEM photographs of seed (A, B and C).

SEM coupled Energy Dispersive X-Ray Spectroscopy (EDS) based determination of the elemental composition of studied seeds (Table-5; Fig. 9-13) showed noticeable disparity. In all the specimens Carbon (C) was detected with ± 50 wt% followed by Oxygen (O) with more than 40 wt%. Calcium (Ca) and Potassium (K) were traced in all the samples with wt% between 1.30- 4.00. Magnesium (Mg) was detected in two species *Tephrosia villosa* and *Tephrosia purpurea* having 1.40 wt% and 0.50 wt% respectively. Silicon (Si) is noted only in *Tephrosia villosa* contributing 0.60 wt%. The elemental composition of the studied seeds showed detectable variation in their wt%. This can be used as the basis for the discrimination of taxa. This finding indicates that studied seeds are reservoirs of essential elements and provide with a unique element wt%.

Table 5. Comparative Wt % of detected elements in EDS spectra of examined seeds.

Species	Wt % of Detected elements						Total
	C	O	Ca	K	Mg	Si	
<i>Cassia siamea</i>	51.3	45.5	2.7	0.5	--	--	100
<i>Cassia occidentalis</i>	53.3	43.8	1.60	1.30	--	--	100
<i>Tephrosia villosa</i>	49.2	41.9	4.00	2.90	1.40	0.60	100
<i>Tephrosia purpurea</i>	52.2	40.9	3.00	3.40	0.50	--	100
<i>Abrus precatorius</i>	53.9	43.3	1.30	1.50	--	--	100

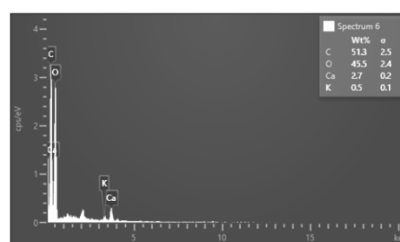


Fig.9- EDS spectrum of *Cassia siamea*.

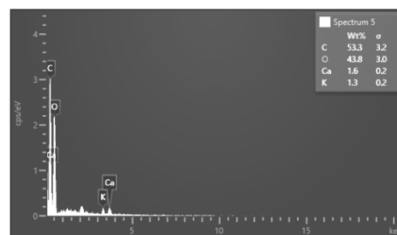


Fig.10- EDS spectrum of *Cassia occidentalis*.

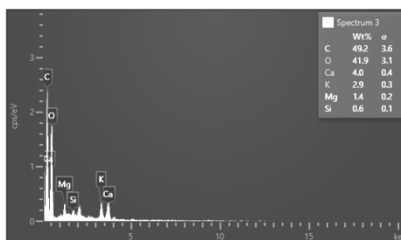


Fig.11- EDS spectrum of *Tephrosia villosa*.

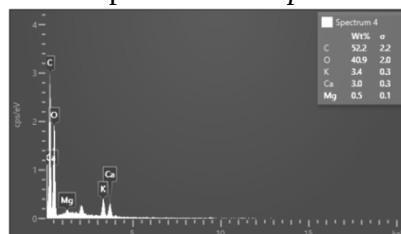


Fig.12- EDS spectrum *Tephrosia purpurea*.

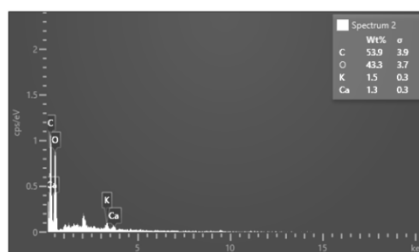


Fig.13- EDS spectrum *Abrus precatorius*.

The present investigation suggests the use of Morphological and SEM-based seed coat topography patterns for solving taxonomic problems. The seeds bear diversity in shape, dimensions and seed coat topography pattern to be characteristic of each species. Thus, this study has shown that the seeds carry an adequate amount of information about seed characters in Leguminosae that helps in the delimitation of species.

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