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Application of Scanning Electron Microscope in Agriculture

Manoj V Parakhia

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ABOUT THE AUTHOR

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Contents

Sample preparation method for SEM analysis

* Collect the fresh sample (plant leaf, pollen, roots etc).
* Make 4% Glutaraldehyde in 2M Sodium cacodylate buffer.
* Sample fixation using 4% Glutaraldehyde at 4˚C for overnight.
* After fixation wash with buffer for 15 min.
* Serial dehydration using 30% , 50%, 70%, 80%, 90% and 100% Acetone solution for 15 min in each dilution.
* In 100% two times for 15 min.
* After dehydration dry in Hot air Oven at 38˚C for 30 min.
* Sample put in sputter coater for Gold-platinum coating (process current 10mAmp for 2 min).
* Analyze Sample in SEM.
* While in bacterial sample preparation the serial dehydration using triethylene glycol instead of Acetone. (10%, 20%, 30%...........100% for 15 min in each dilution).

Pollen structure of Important crops

Pollen grains are the powdery substances which are released from the anthers of flowers at the onset of pollination in seed plants. The anthers are sac-like structures on the top of the flower's stamens. They vary in grain size from the fine to the coarse and their size can range from 10 to 100 micrometres.

Pollen is a plant's male DNA that is transported to the female part of the flower to enable the plant to reproduce. Because pollen contains DNA, it can be used to change a plant's traits. Such changes can increase harvest production or help a plant survive in a specific environment.

Pollen types are identified by shape, size, and ornamentation of the outer wall. Pollen is usually round or oval but does come in other shapes. The outer wall contains pores and furrows, which allow shrinking and swelling. Changes in pollen size can be caused by changes in moisture within the pollen. Pollen surfaces can also be grooved or smooth.

All flowering plants create pollen, including grasses, trees and shrubs. Grasses and trees are the most common plants that spread pollen through the wind rather than with the help of insects.

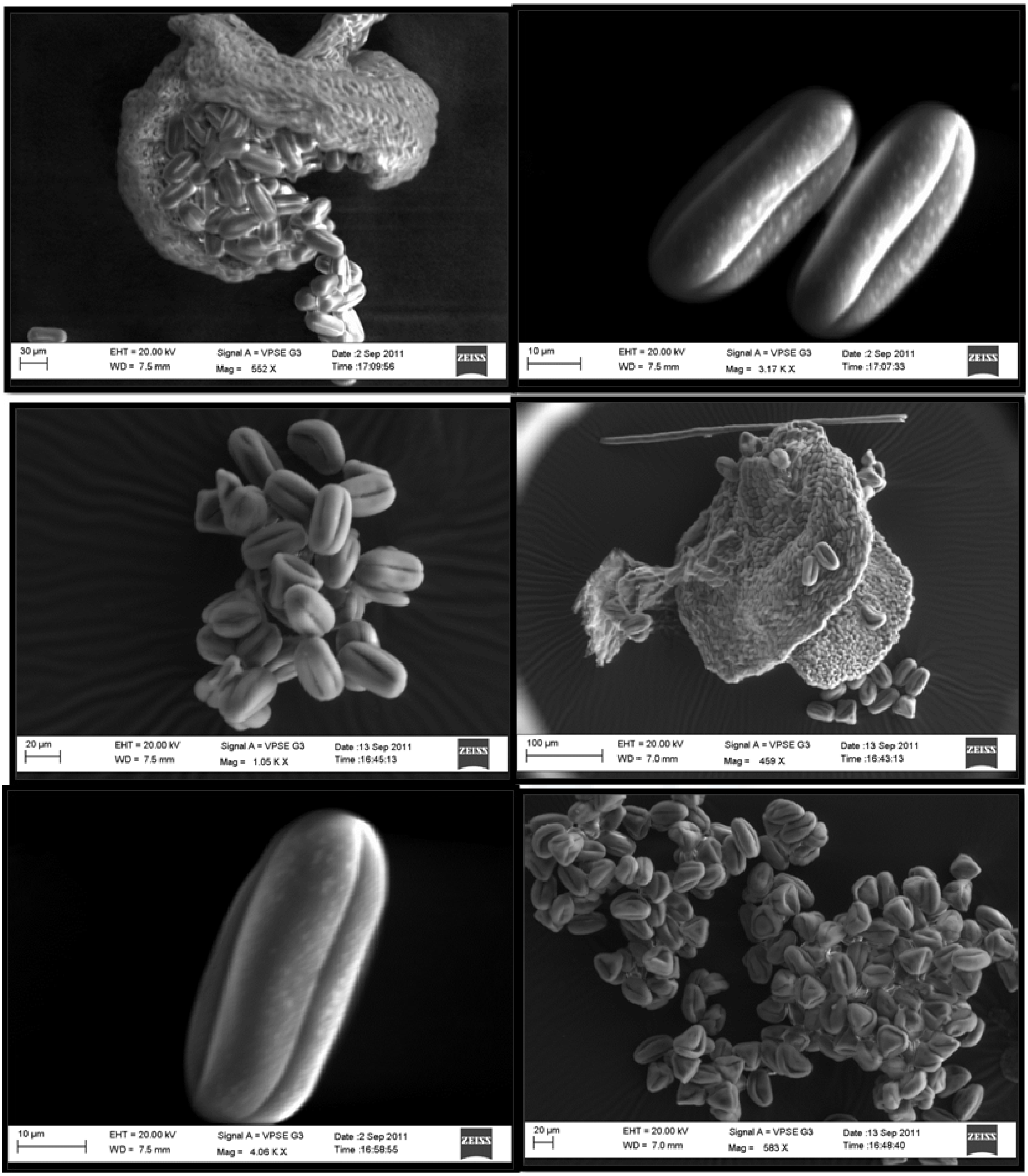
Based on pollen ultra structure we can be identified the family of plant. Pollen ultra structure is important for the phylogentic classification of plants. in future it is useful for the identification of plant.

Groundnut (*Arachis hypogaea L.*) Pollen

Scientific classification of Groundnut (*Arachis hypogaea L.*) as below

Kingdom	:	*Plantae*
Order	:	Fabales
Family	:	*Fabaceae*
Subfamily	:	*Faboideae*
Tribe	:	*Dalbergieae*
Genus	:	*Arachis*
Species	:	*A. hypogaea*

Groundnut was important oil crops in the world. India is the largest producer of groundnut. Gujarat is the important state for production of groundnut. the study of groundnut pollen grain is important for the crops improvement for biotic and abiotic stress tolerance. groundnut is the major crops of Saurashtra region of Gujarat.



Cotton (*Gossypium hirsutum L.*) Pollen

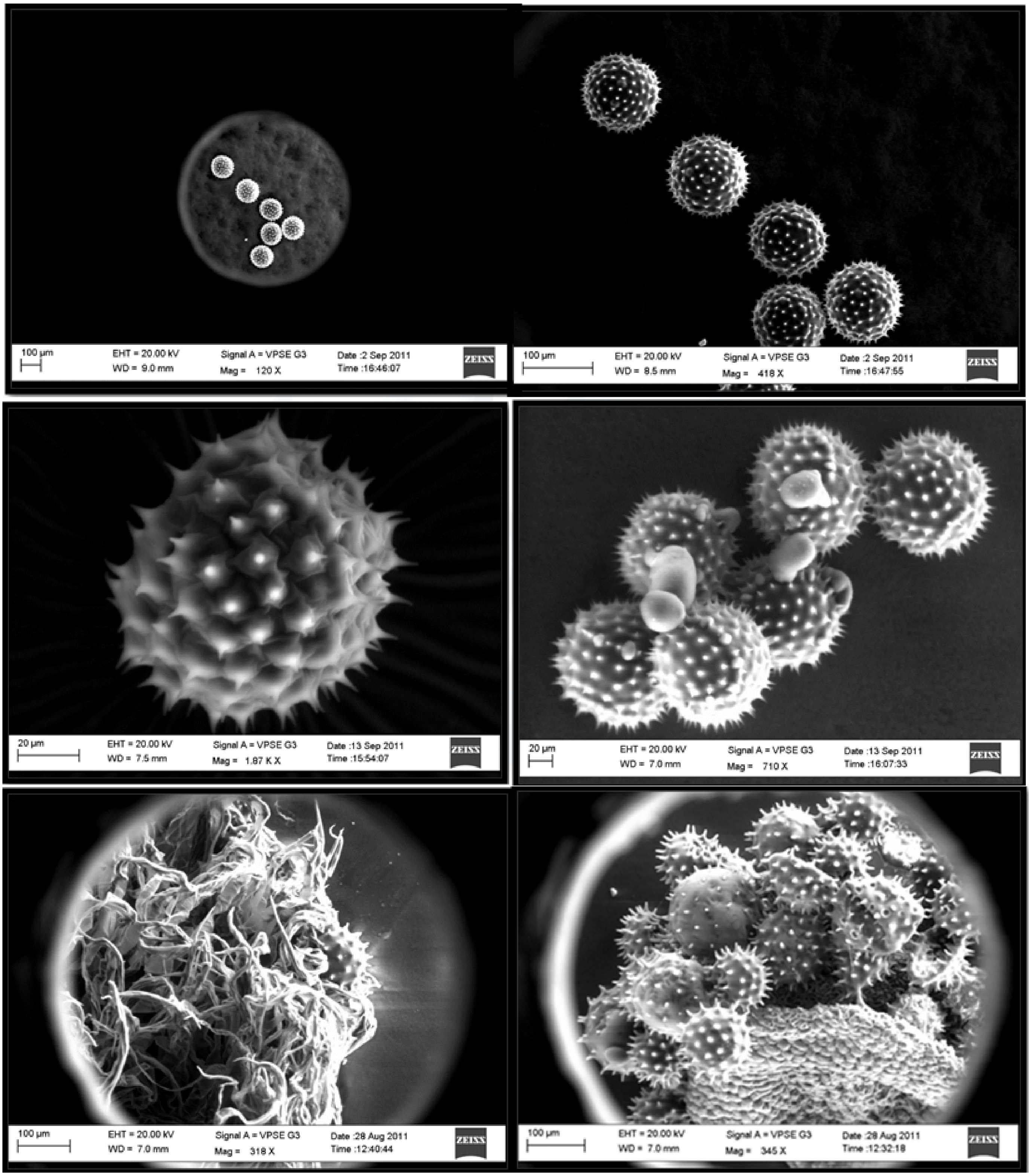
Scientific classification of Cotton (*Gossypium hirsutum L.*) as below

Kingdom	:	*Plantae*
Order	:	Malvales
Family	:	*Malvaceae*
Subfamily	:	*Malvoideae*
Tribe	:	*Gossypieae*
Genus	:	*Gossypium*
Species	:	*Gossypium arboreum L.*

Cotton is the most important fibre crop not only of India but of the entire world. It provides the basic raw material (cotton fibre) to cotton textile industry. India has the largest area under cotton cultivation in the world though she is the world's third largest producer of cotton after China and the USA. Currently it is grown over 6 per cent of the net sown area. Gujarat is the second largest cotton producing state of India.

Pollen of the Malvaceae is spherical, porate and echinate, with a remarkably thick inner wall (endexine).

SEM images of Cotton pollen at different magnification



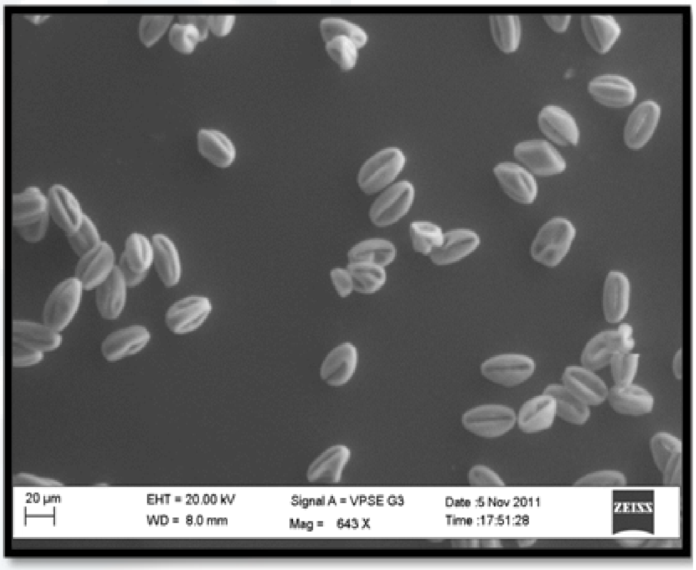
Castor (*Ricinus communis L.*) Pollen

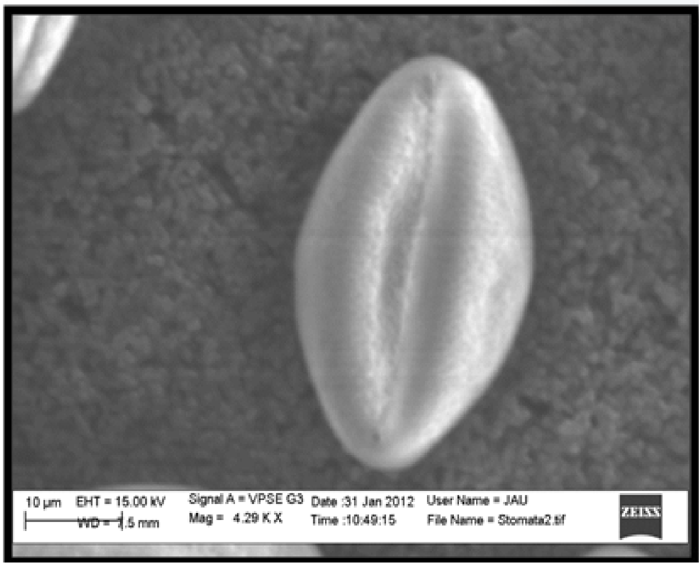
Scientific classification of Castor (*Ricinus communis*) as below

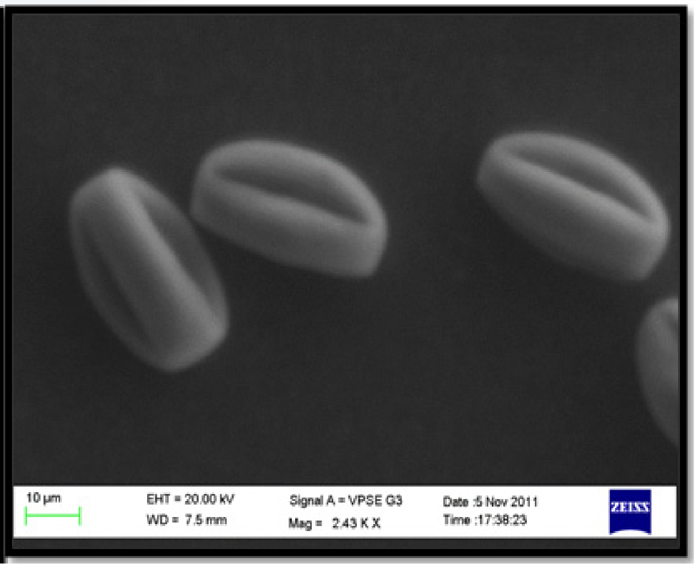
Kingdom	:	*Plantae*
Order	:	Malpighiales
Family	:	*Euphorbiaceae*
Subfamily	:	*Acalyphoideae*
Tribe	:	*Acalypheae*
Subtribe	:	*Ricininae*
Genus	:	*Ricinus*
Species	:	*R. communis*

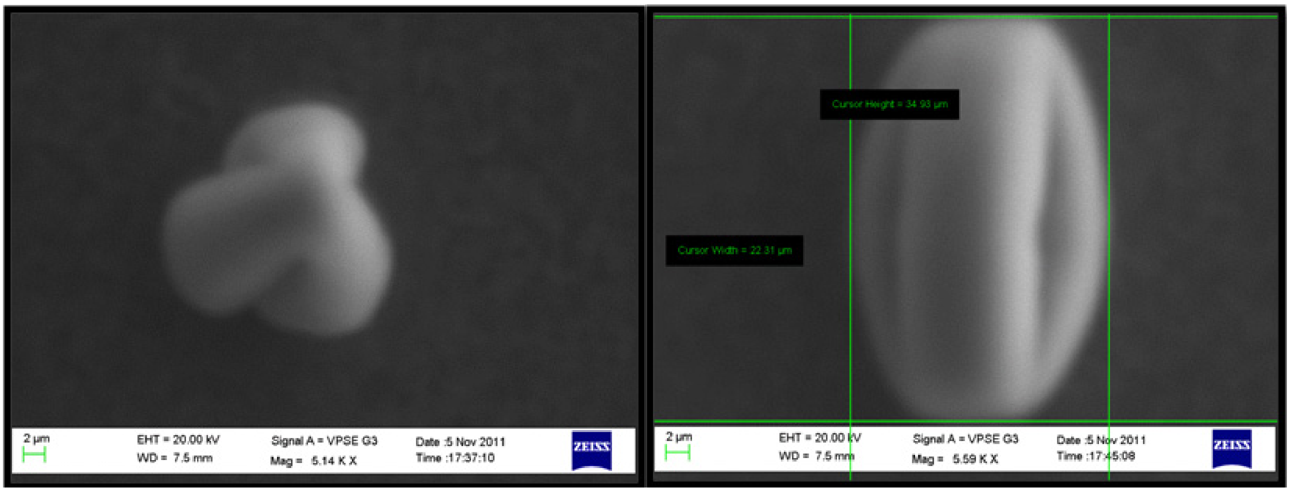
Castor is an important oilseed crop with great utilitarian value in industry, pharmaceutical and agricultural sectors. The seeds contain between 40% and 60% oil. Its oil is unique among vegetable oils because the oil is the only commercial source of a hydroxylated fatty acid. The presence of hydroxyl groups and double bonds in the ricinoleic acid imparts unique chemical and physical properties on castor oil that makes the oil a vital industrial raw material. In the last couple of years, demand for castor oil has kept increasing in the international market, assured by more than 700 uses, ranging from medicine and cosmetics to biodiesel, plastics and lubricants. The oil has advantages over petroleum base oils, especially at high and low temperatures because of its high boiling and low melting points.

Pollen of Castor are unique in shape and size. The size of pollen was found 20-25um in width and 32-35um height. The shape of pollen was oval. We have analyzed the pollen of different verities of castors and it has been found the similar shape and size. Castor is a temperature sensitive crops. Temperature affect the pollen formation means the development of male flowers.









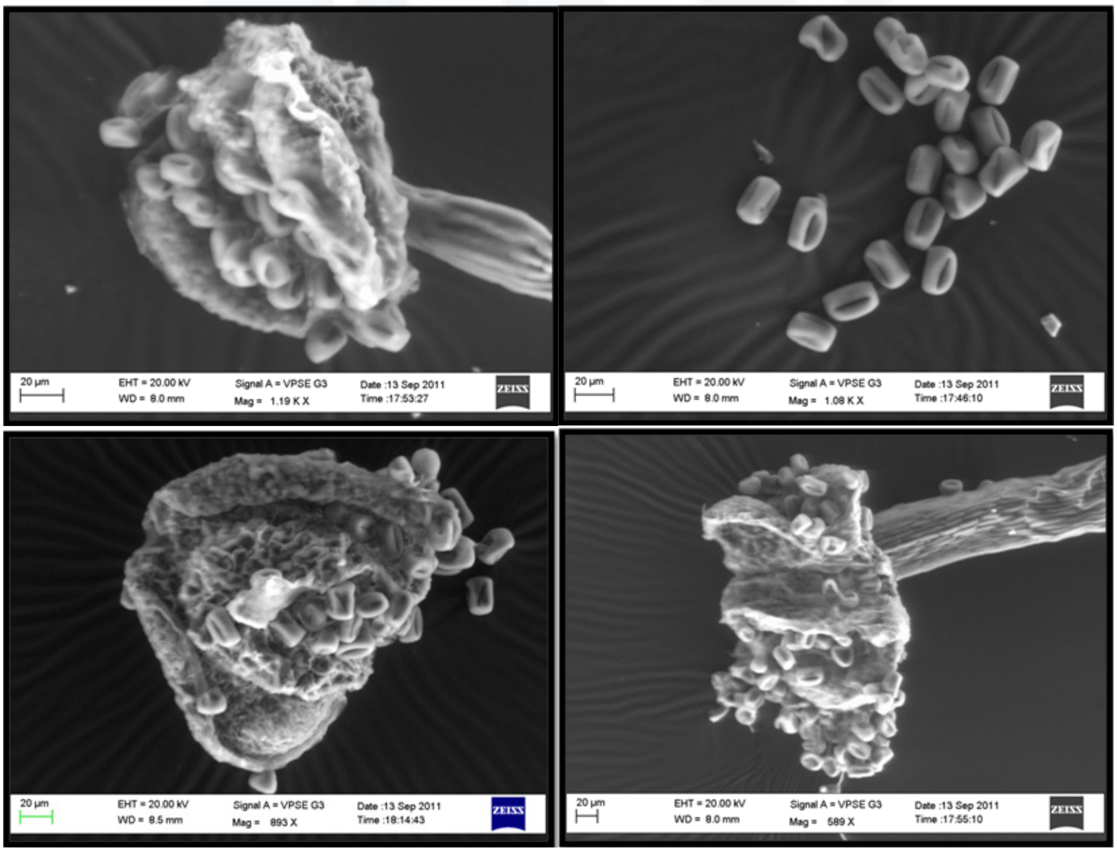
Maize (*Zea mays L.*) Pollen

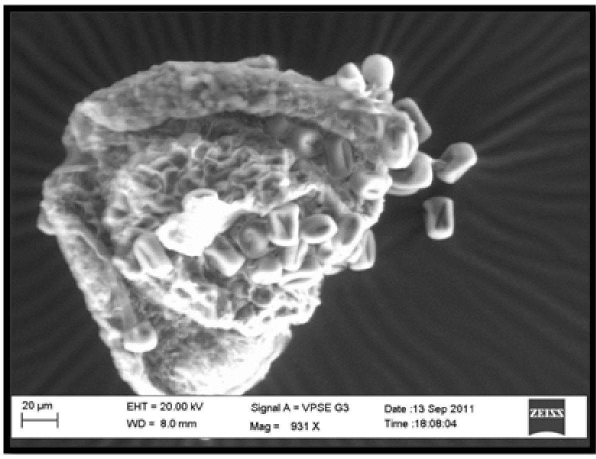
Scientific classification of Maize (Zea mays L.) as below

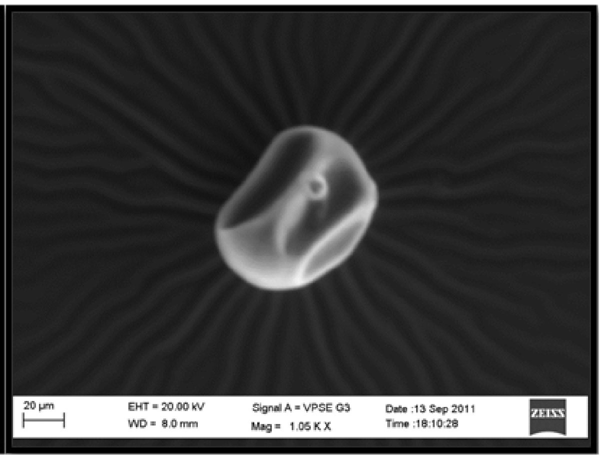
Kingdom	:	*Plantae*
Order	:	Poales
Family	:	*Poaceae*
Subfamily	:	*Panicoideae*
Tribe	:	*Andropogoneae*
Genus	:	Zea
Species	:	*Z. mays*
Subspecies	:	*Z. mays subsp. mays*

Maize (*Zea mays L.*) is one of the most versatile emerging crops having wider adaptability under varied agro-climatic conditions. Globally, maize is known as queen of cereals because it has the highest genetic yield potential among the cereals. It is cultivated on nearly 150 mha in about 160 countries having wider diversity of soil.

Poaceae, including maize, wheat, rice, barley, and sorghum, are the most economically important family in the world. Studies of anther development of *Poaceae* are important for both basic botanical research and agronomic applications.





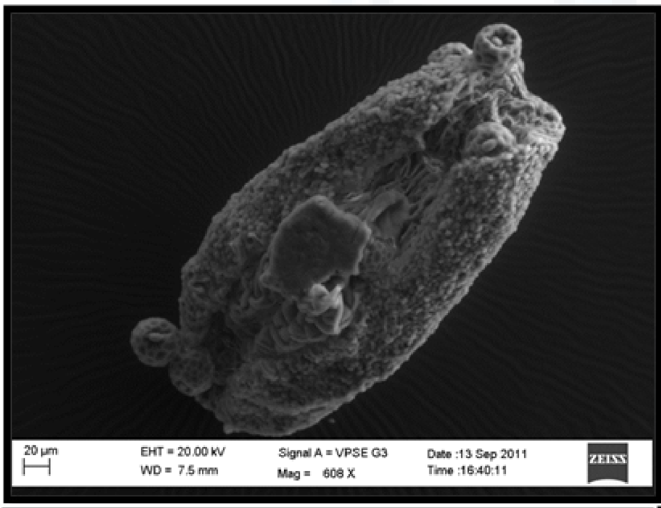


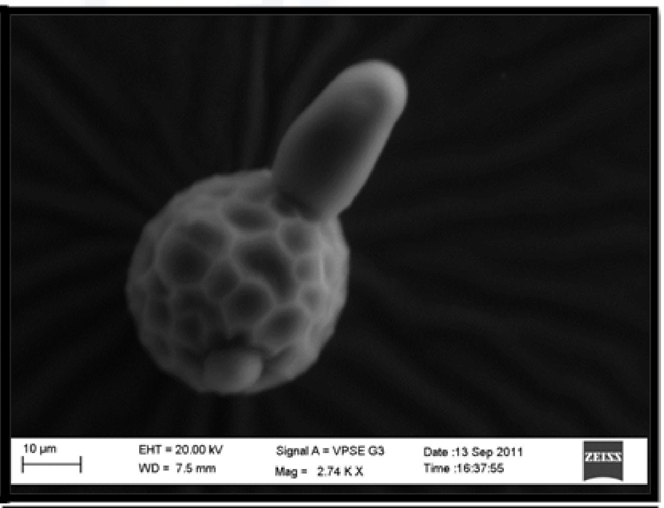
Mung bean (*Vigna radiata*) Pollen

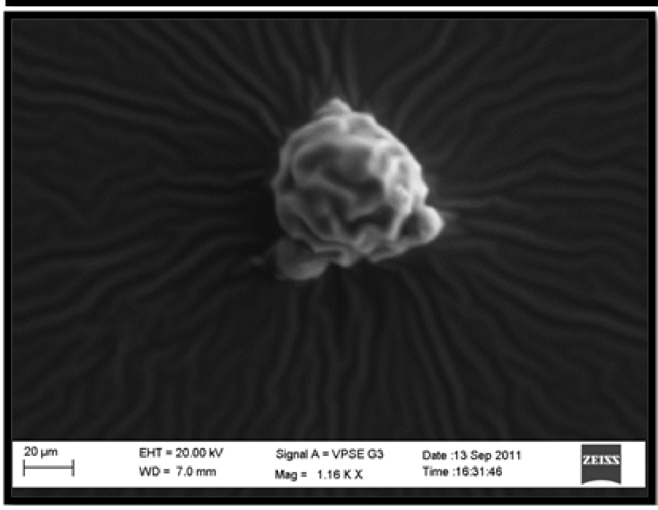
Classification of Mung bean (*Vigna radiata*) as below

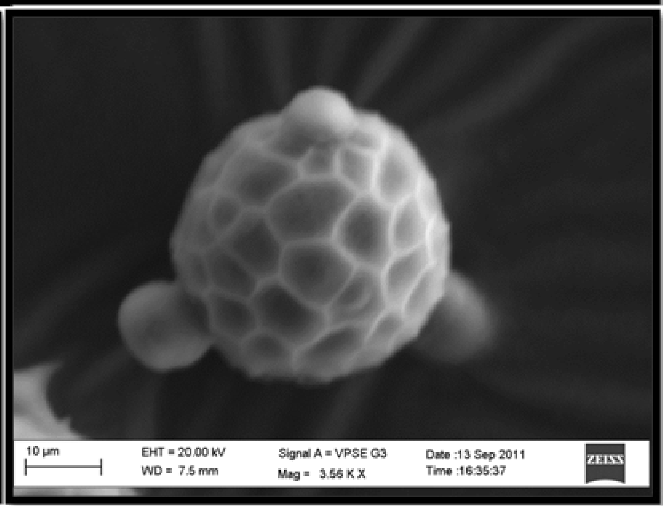
Kingdom	:	*Plantae*
Order	:	Fabales
Family	:	*Fabaceae*
Genus	:	*Vigna*
Species	:	*V. radiata*

Green gram is an important pulses crop in India and believed to be originated from India. It is short duration legume crop grown mostly as a fallow crop in rotation with rice. Similar to the leguminous pulses, green gram, enriches soil nitrogen content. It is grown mostly in Asian region traditionally while its cultivation has spread to Africa and Americas relatively in the recent times. More than 70%t of world's green gram production comes from India.





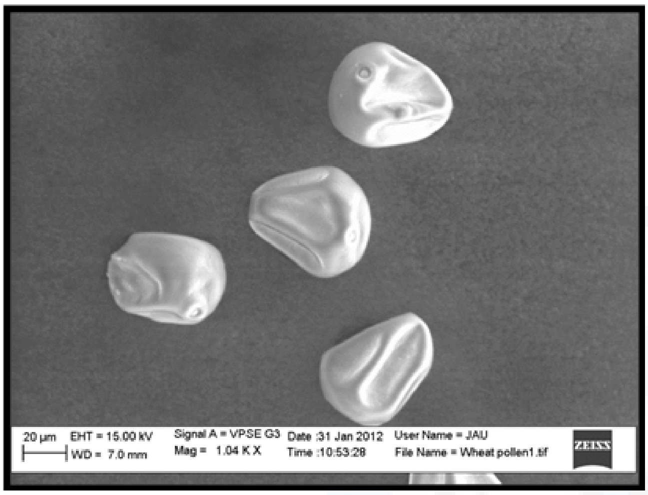




Wheat (*Triticum aestivum L.*) pollen

Scientific classification of Wheat (*Triticum aestivum L.*) as below

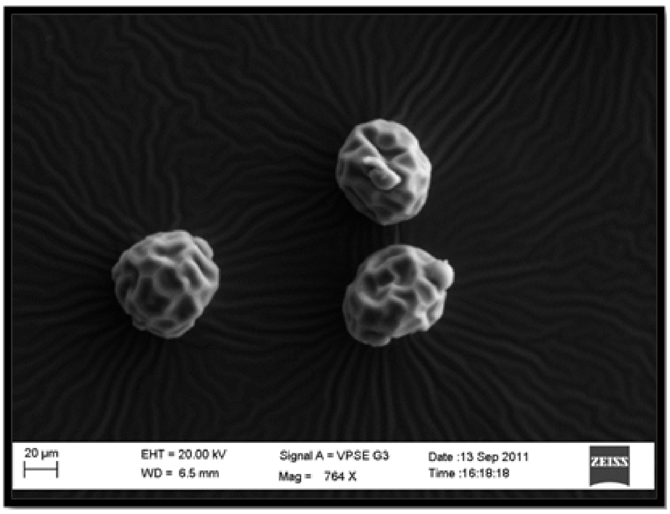
Kingdom	:	*Plantae*
Order	:	Poales
Family	:	*Poaceae*
Genus	:	*Triticum*
Species	:	*Triticum aestivum L.*



Cow pea (*Vigna unguiculata*) pollen

Scientific classification of Cow pea (*Vigna unguiculata*) as below

Kingdom	:	*Plantae*
Order	:	Fabales
Family	:	*Fabaceae*
Genus	:	*Vigna*
Species	:	*V. unguiculata*



Stomata

Stomata are miniature openings or pores in plant tissue that allow for gas exchange. Stomata are naturally found in plant leaves but can also be found in some stems. Guard cells surround stomata and function to open and close stomatal pores. Stomata allow a plant to take in carbon dioxide, which is needed for photosynthesis. They also facilitate to reduce water loss by closing when conditions are hot or dry. Stomata look like tiny mouths which open and close as they assist in transpiration.

Plants that inhabit on land typically have thousands of stomata on the surfaces of their leaves. The majority of stomata are situated on the bottom of plant leaves reducing their exposure to heat and air current. In aquatic plants, stomata are located on the upper surface of the leaves. A stoma is surrounded by two types of specialized plant cells that differ from other plant epidermal cells. These cells are called guard cells and subsidiary cells. Guard cells are large crescent-shaped cells, two of which surround a stoma and are connected to at both ends. These cells enlarge and contract to open and close stomatal pores. Guard cells also contain chloroplasts, the light capturing organelles in plants. Subsidiary cells, also called accessory cells, surround and support guard cells. They act as a buffer between guard cells and epidermal cells, protecting epidermal cells against guard cell expansion. Subsidiary cells of different plant types exist in various shapes and sizes. They are also arranged differently with respect to their positioning around guard cells.

Function of Stomata

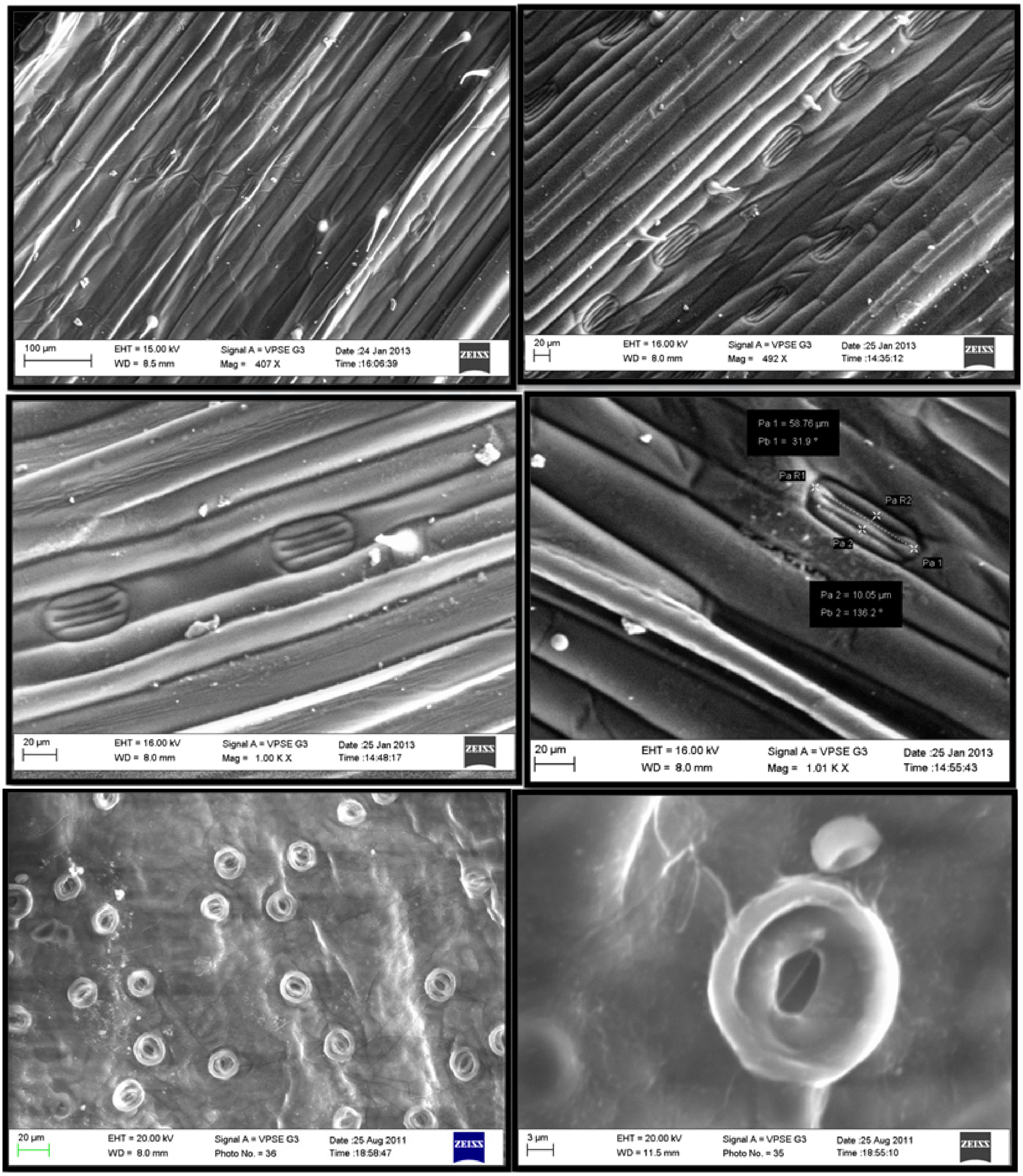
The two main functions of stomata are to allow for the uptake of carbon dioxide and to limit the loss of water due to evaporation. In many plants, stomata remain open during the day and closed at night. Stomata are open during the day because this is when photosynthesis typically occurs. In photosynthesis, plants use carbon dioxide, water, and sunlight to produce glucose, water, and oxygen. Glucose is used as a food source, while oxygen and water vapor escape through open stomata into the surrounding environment. Carbon dioxide needed for photosynthesis is obtain through open plant stomata. At night, when sunlight is no longer available and photosynthesis is not occurring, stomata close. This closure prevents water from escaping through open pores.

Stomata open and close

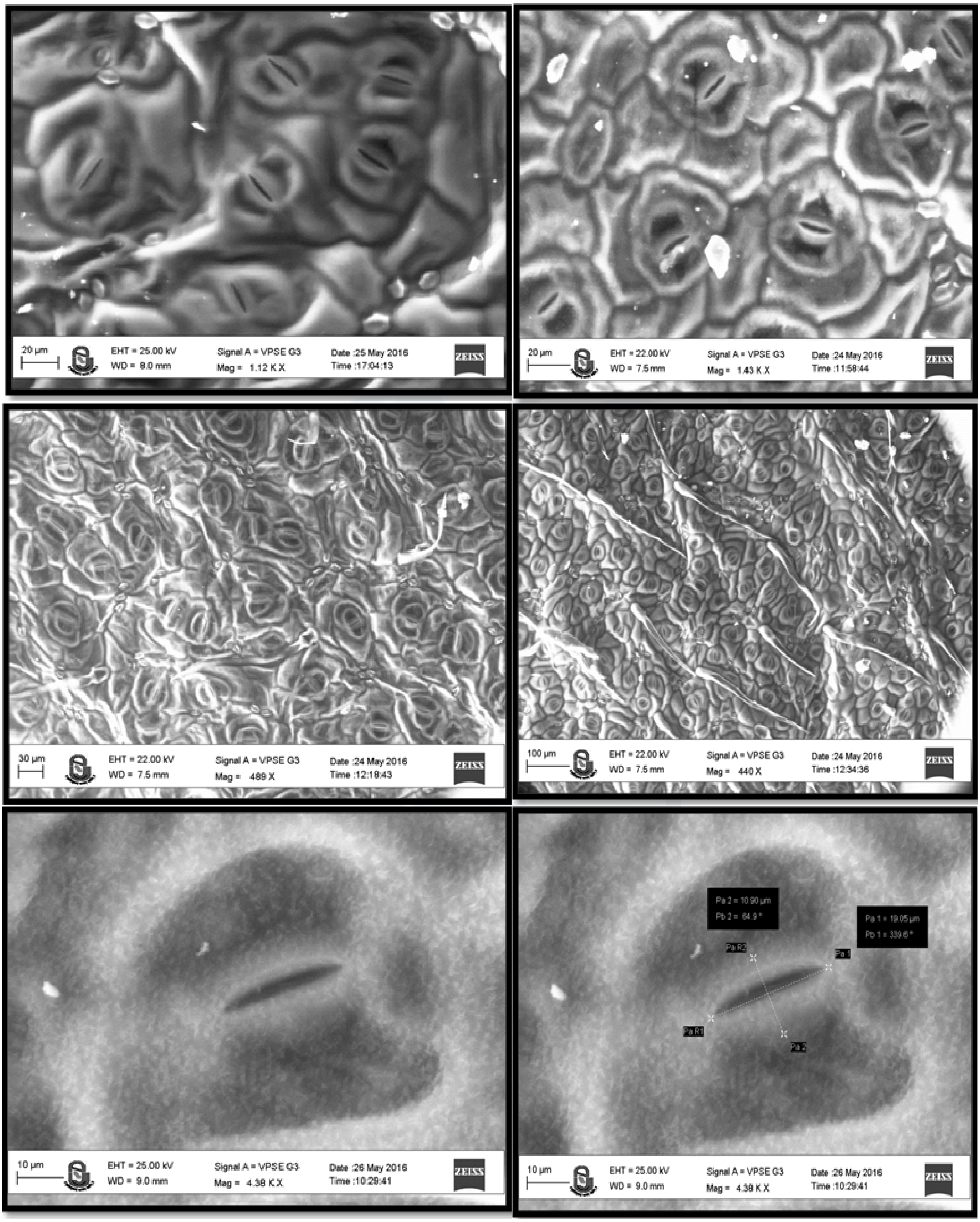
The opening and closing of stomata is regulated by factors such as light, plant carbon dioxide levels, and changes in environmental conditions. Humidity is an example of an environmental condition that regulates the opening or closing of stomata. When humidity conditions are optimal, stomata are open. Should humidity levels in the air around plant leaves decrease due to increased temperatures or windy conditions, more water vapour would diffuse from the plant into the air. Under such conditions, plants must close their stomata to prevent excess water loss.

Stomata open and close as a result of diffusion. Under hot and dry conditions, when water loss due to evaporation is high, stomata must close to prevent dehydration. Guard cells actively pump potassium ions (K+) out of the guard cells and into surrounding cells. This causes water in the enlarged guard cells to move osmotically from an area of low solute concentration (guard cells) to an area of high solute concentration (surrounding cells). The loss of water in the guard cells causes them to shrink. This shrinkage closes the stomatal pore.

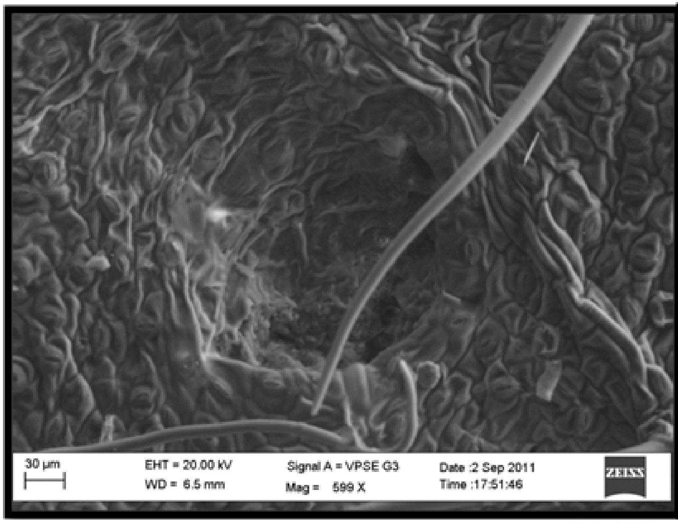
Stomata of wheat (*Triticum aestivum L.*) leaf.

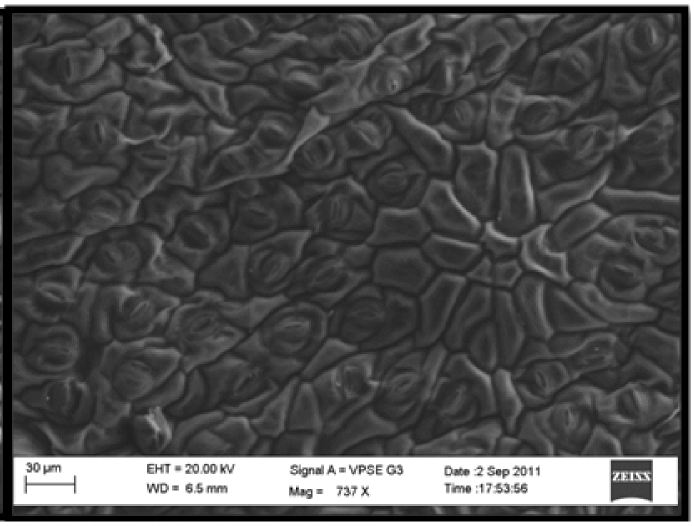


Stomata of groundnut leaf

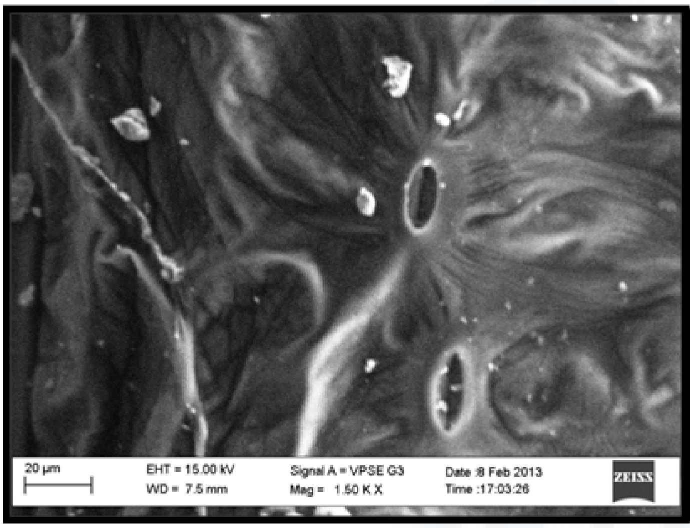


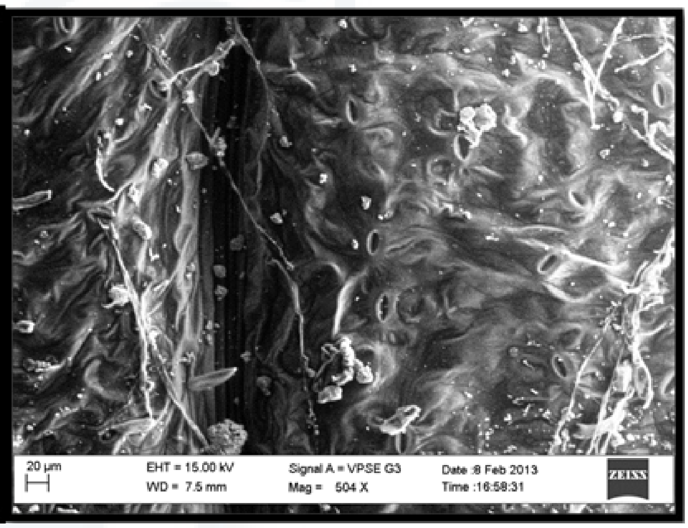
Stomata of cotton (*Gossypium hirsutum*) leaf



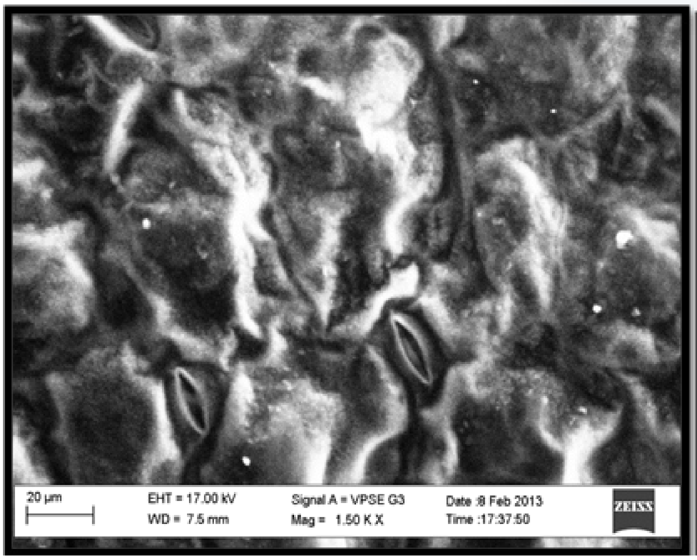


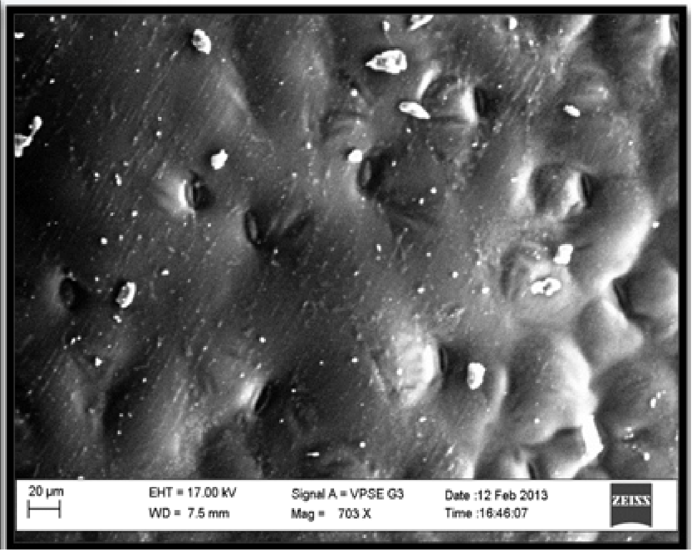
Stomata of coriander (*Coriandrum sativum*) leaf





Stomata of fenugreek (*Trigonella foenum-graecum*) leaf

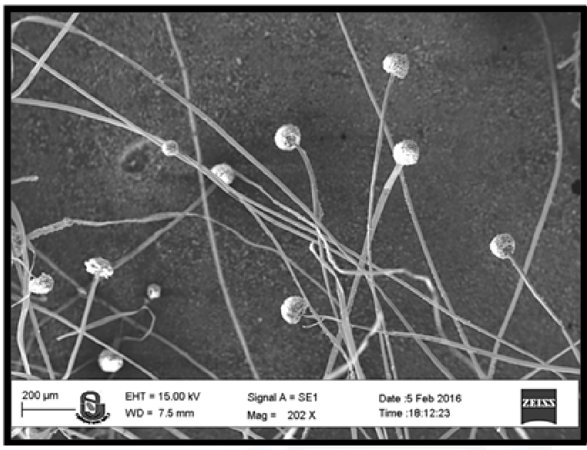


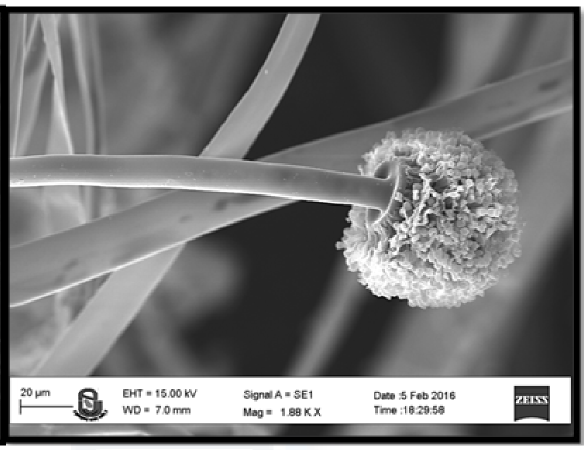


Fungi Structures

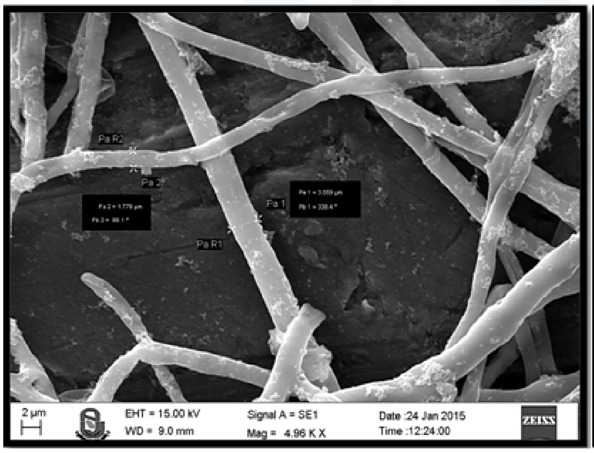
Fungi are spore-forming, non-chlorophytic, eukaryotic (cells having true nuclei) organisms and most of the true fungi are filamentous and branched. Most of the over 100,000 species of fungi are saprophytes. However, over 20,000 species of fungi are parasites and cause disease in crops and plants (USEPA 2005). Fungal parasites are by far the most prevalent plant pathogenic organism. All plants are attacked by one species or another of phytopathogenic fungi. Individual species of fungi can parasitize one or many different kinds of plants. To understand the structure of fungi is important for the identification of specific fungi. SEM overcome the limit of light microscope like size and shape of spores, type of filaments, arrangement of filaments. based on the structural studied classify the fungi according to its species . Some of the fungi have micro structure that we cannot study using light microscope while using SEM we can study the ultra structure of fungi.

SEM images of Plant pathogenic fungi.



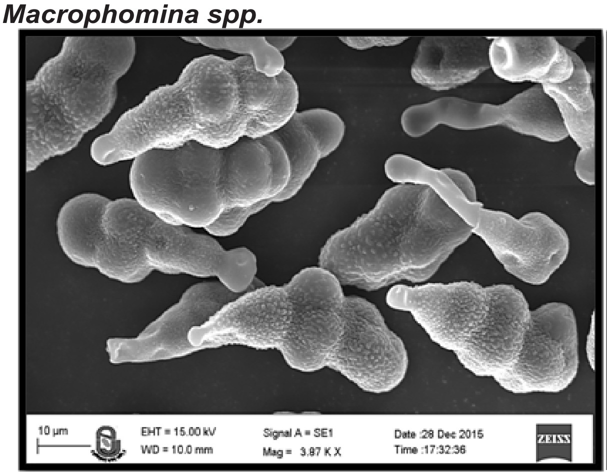


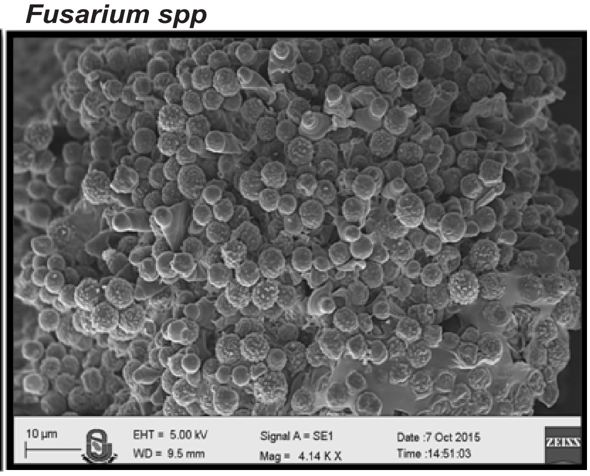
Aspergillus spp.



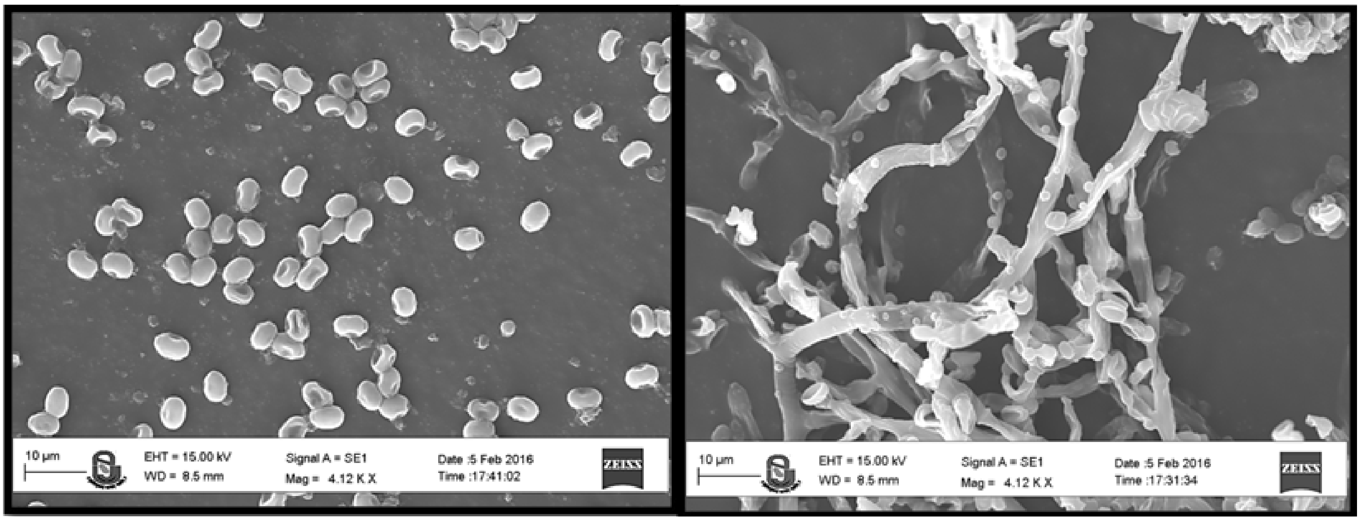
Macrophomina spp.

Fusarium spp

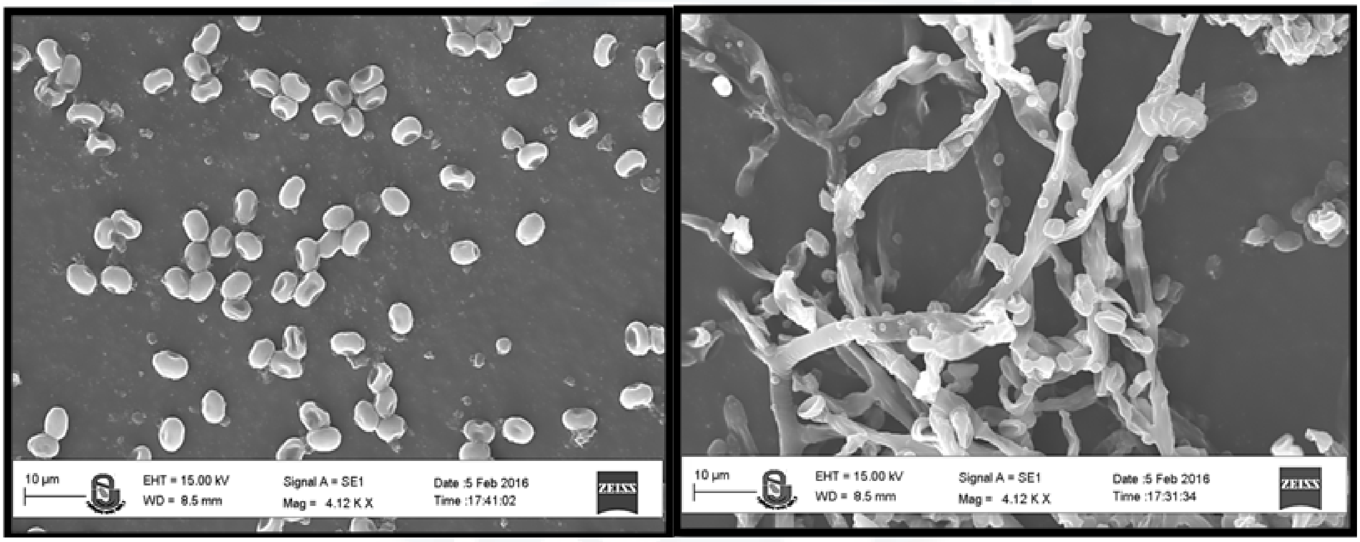




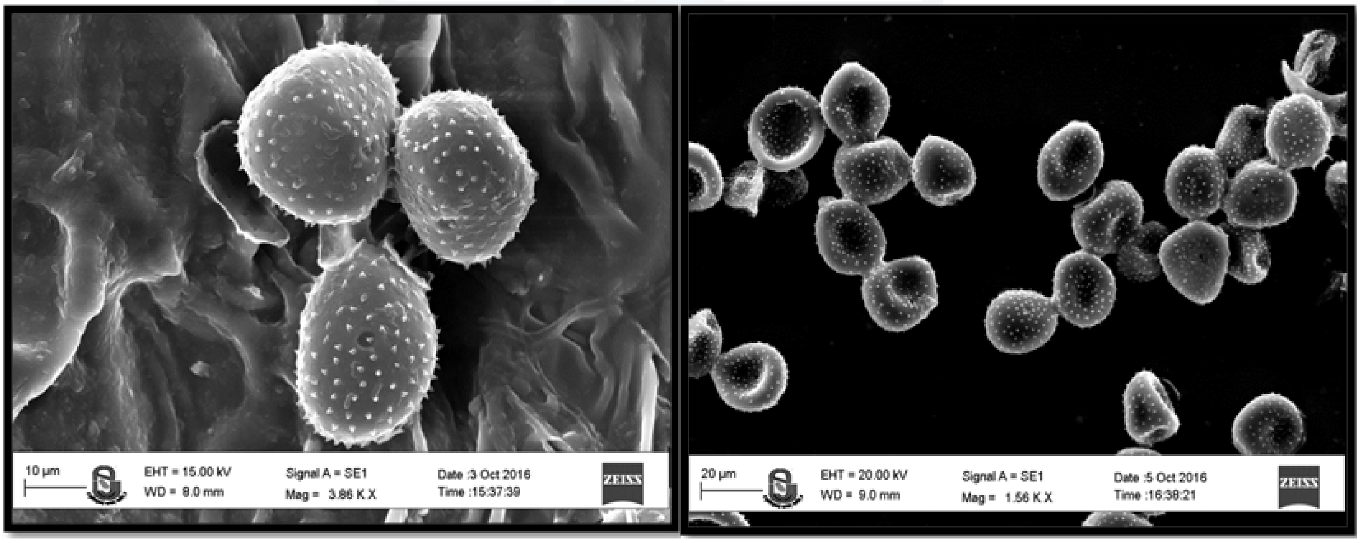
Cercospora arachidicola



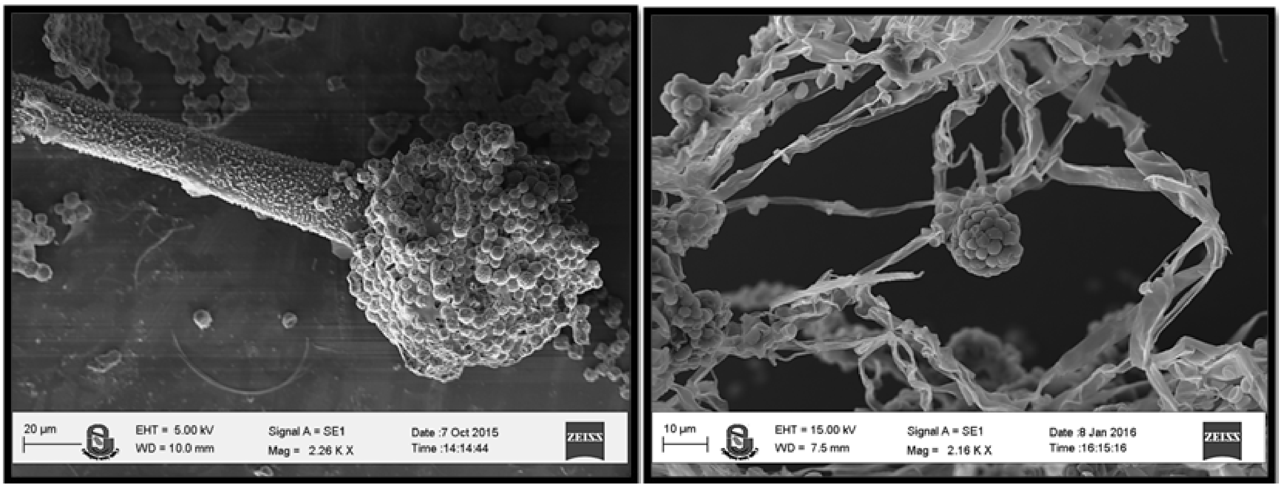
Sclerotium rolfsii



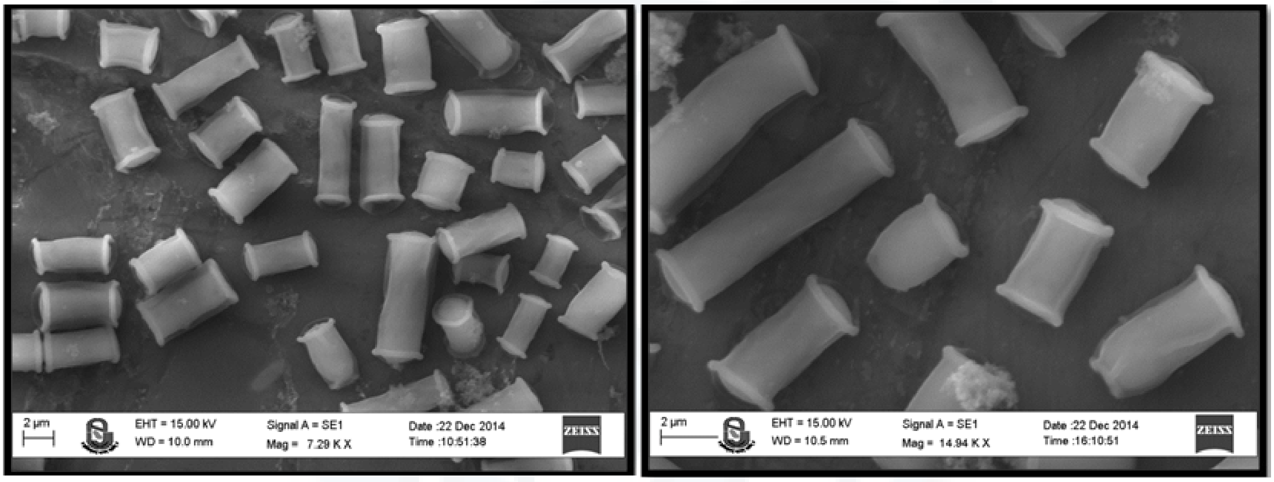
Trichoderma spp.



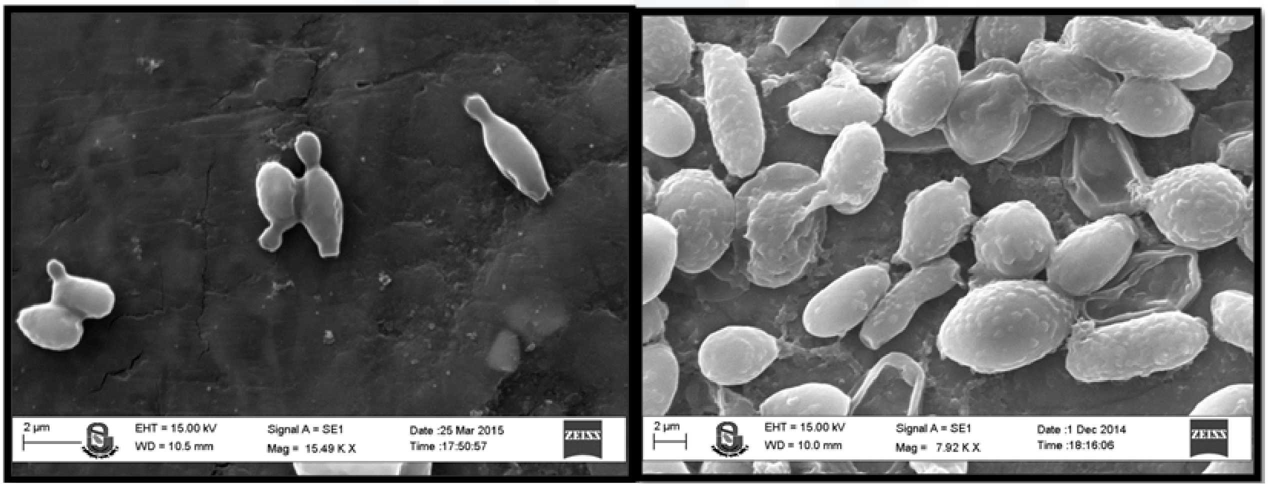
***Puccinia arachidis* (Peanut rust)**



Penicillium spp.



Fungi

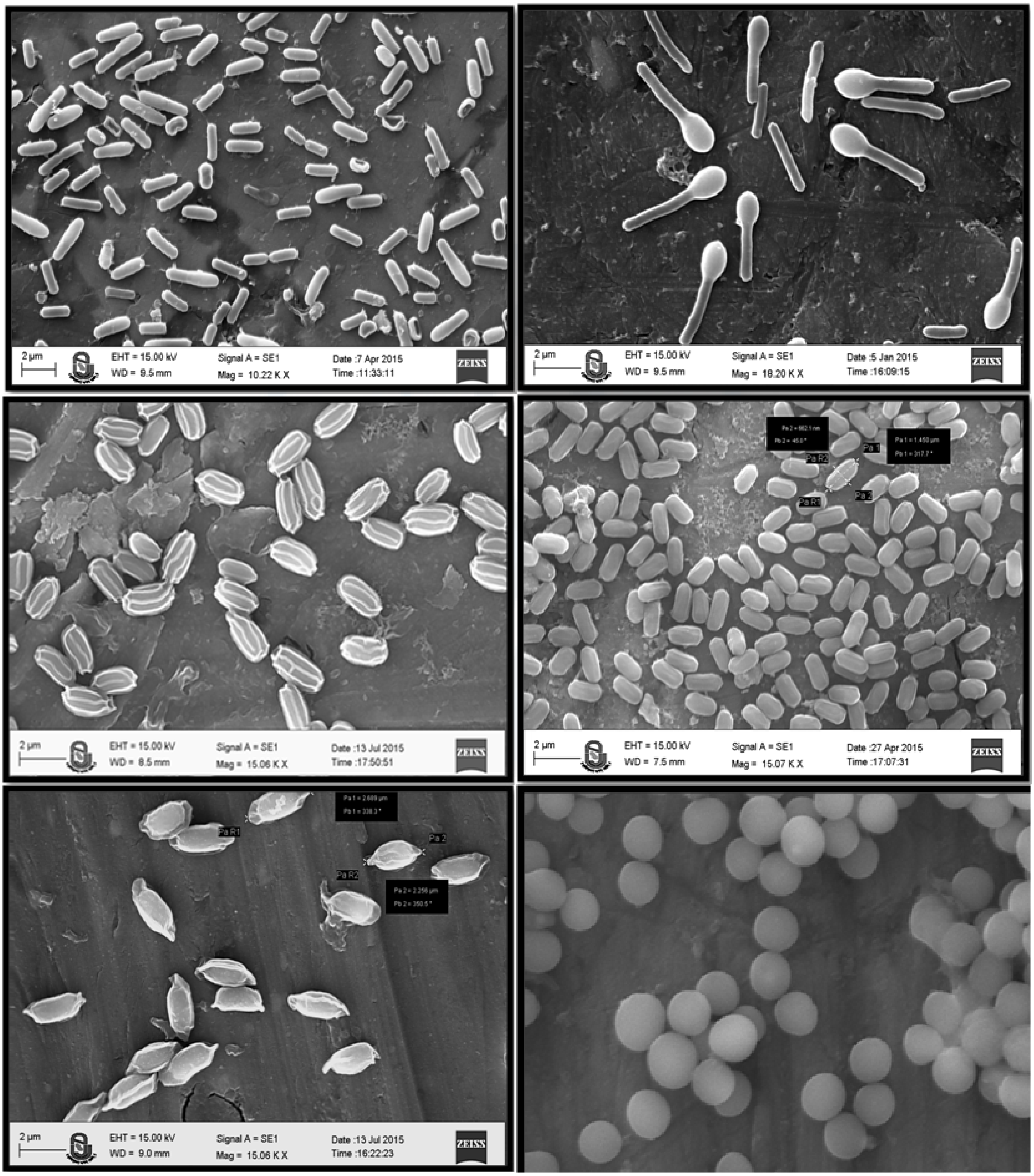


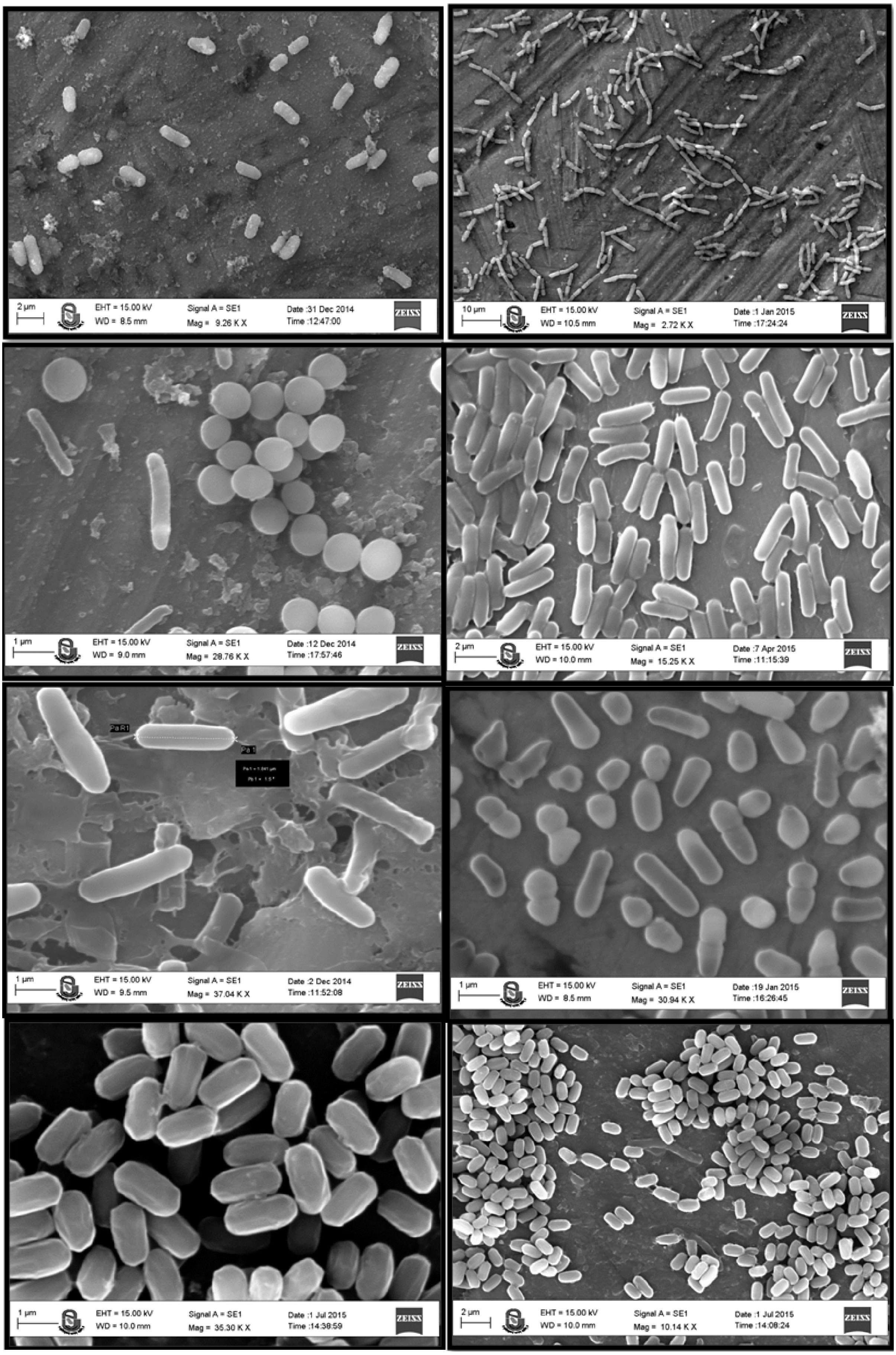
Yeast

Bacterial Structure

Bacteria are present everywhere: in the soil, deep in the rocks, in all bodies of water, in the atmosphere, including the clouds, and also on and inside other living organisms. Their effects on higher life forms are known for only a limited number of bacterial species. Some are harmful - pathogenic - causing diseases in plants or in animals including humans, and some are useful to humans either as “probiotic” bacteria protecting health (Karpa 2006), or industrially by participating in the production of various commodities. The size of bacteria 2-5um so in light microscope we not saw clear structure of bacteria. Using scanning electron microscope we can be studied the detail structure of bacteria. Bacteria are of different shape and size like *cocci, bacillus, vibrio*, spiral shape etc. Bacteria live inside the plant called endophytes. Study of endophytes possible with the help of SEM.

SEM images of Different bacteria isolated for plant and soil.




2 µm
EHT = 15.00 kV
WD = 8.5 mm
Signal A = SE1
Mag = 9.26 K X
Date :31 Dec 2014
Time :12:47:00
ZEISS
10 µm
EHT = 15.00 kV
WD = 10.5 mm
Signal A = SE1
Mag = 2.72 K X
Date :1 Jan 2015
Time :17:24:24
ZEISS
1 µm
EHT = 15.00 kV
WD = 9.0 mm
Signal A = SE1
Mag = 28.76 K X
Date :12 Dec 2014
Time :17:57:46
ZEISS
2 µm
EHT = 15.00 kV
WD = 10.0 mm
Signal A = SE1
Mag = 15.25 K X
Date :7 Apr 2015
Time :11:15:39
ZEISS
1 µm
EHT = 15.00 kV
WD = 9.5 mm
Signal A = SE1
Mag = 37.04 K X
Date :2 Dec 2014
Time :11:52:08
ZEISS
1 µm
EHT = 15.00 kV
WD = 8.5 mm
Signal A = SE1
Mag = 30.94 K X
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1 µm
EHT = 15.00 kV
WD = 10.0 mm
Signal A = SE1
Mag = 35.30 K X
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Time :14:38:59
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2 µm
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Mag = 10.14 K X
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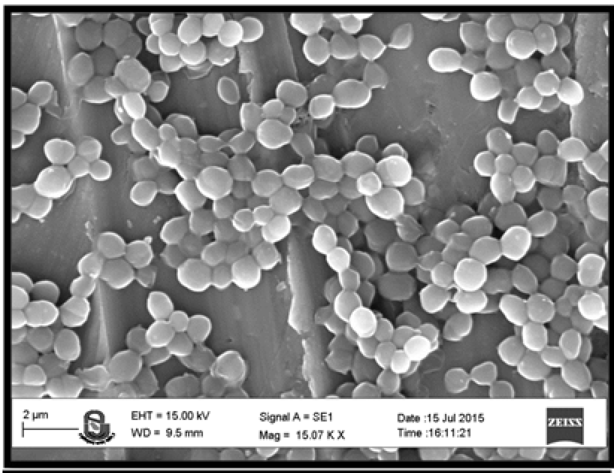
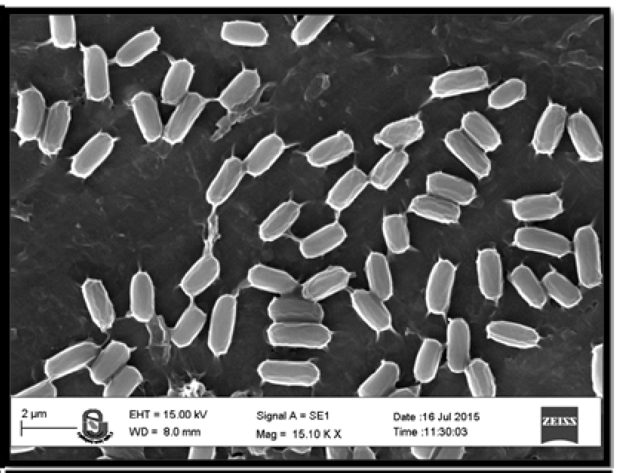
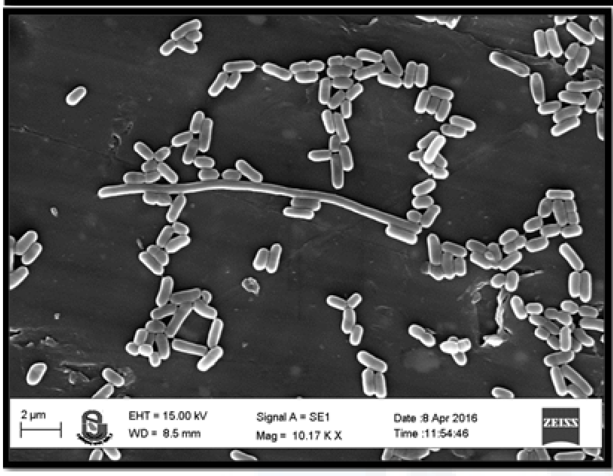
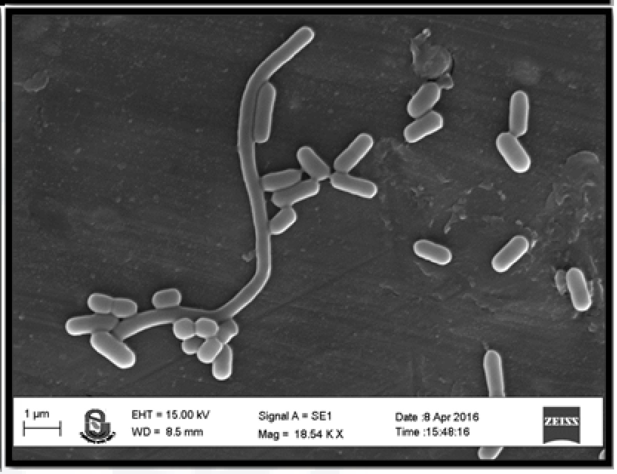






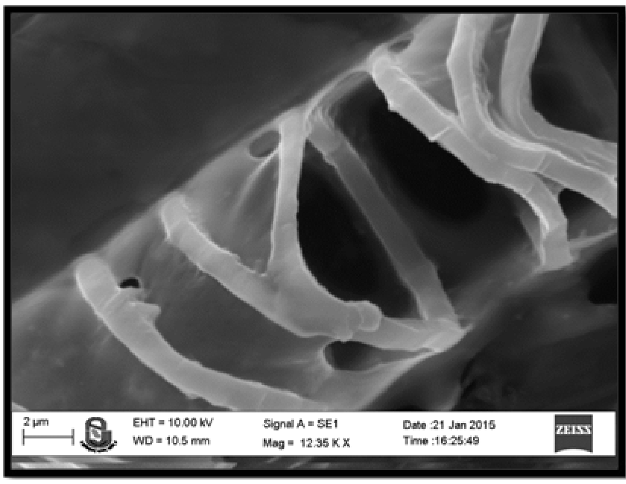


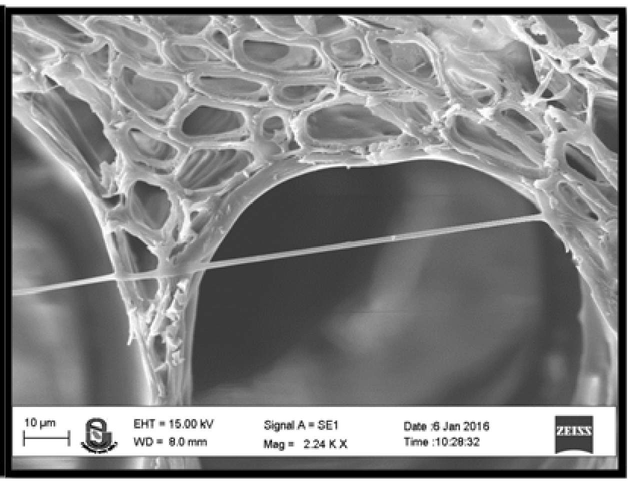
Mycorrhizae (Isolation from root of vegetables) Ecto

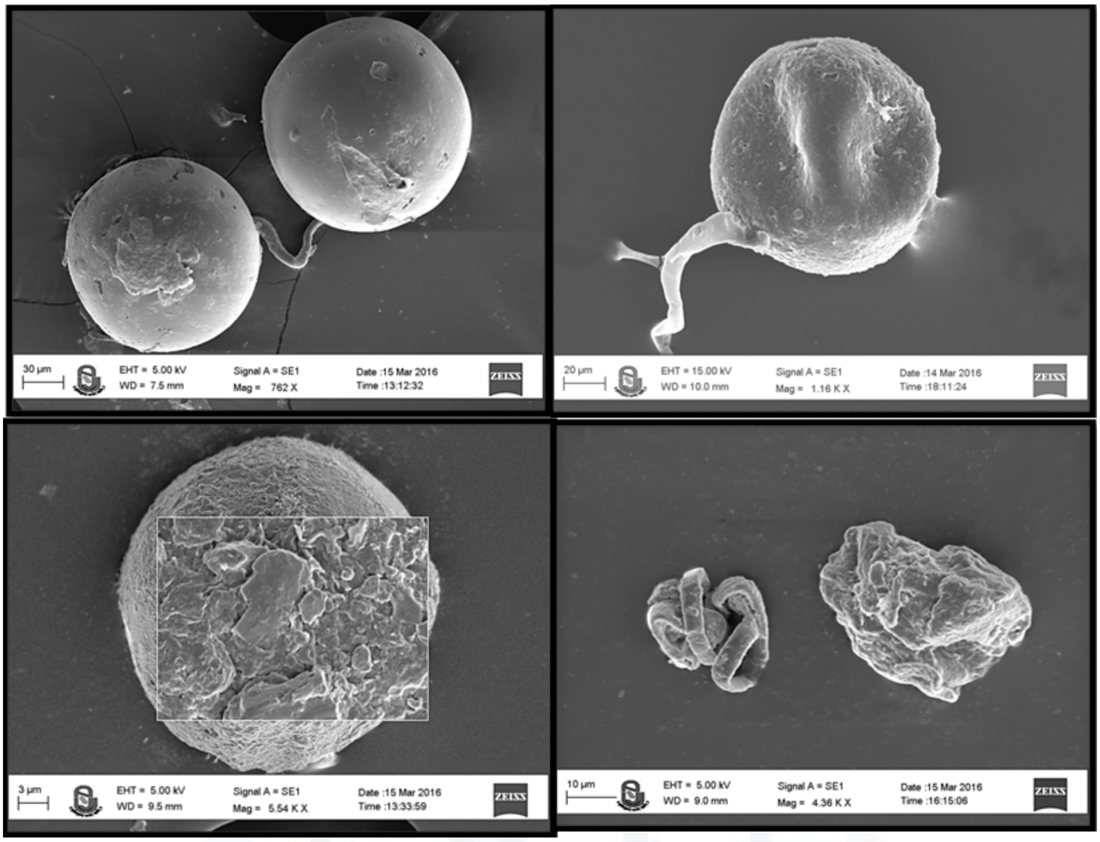
Myco" - "rhiza" literally means "fungus" - "root" and describes the mutually beneficial relationship between the plant and root fungus. These specialized fungi colonize plant roots and extend far into the soil. Mycorrhizal fungal filaments in the soil are truly extensions of root systems and are more effective in nutrient and water absorption than the roots themselves. More than 90 percent of plant species in natural areas form a symbiotic relationship with the beneficial mycorrhizal fungi.

The structural study of *mycorrhize* will be used to identification of mycorrhizae in specific plant species. It also useful for the study of plant-mycorrhizae interaction and based on study we can develop a Biofertilizer for the specific crops.

Mycorrhize was not studied extensively or not explore in agriculture as per the requirement to develop biofertilizer. Mycorrhize studied using SEM provide the detail structure of it and interaction with roots.

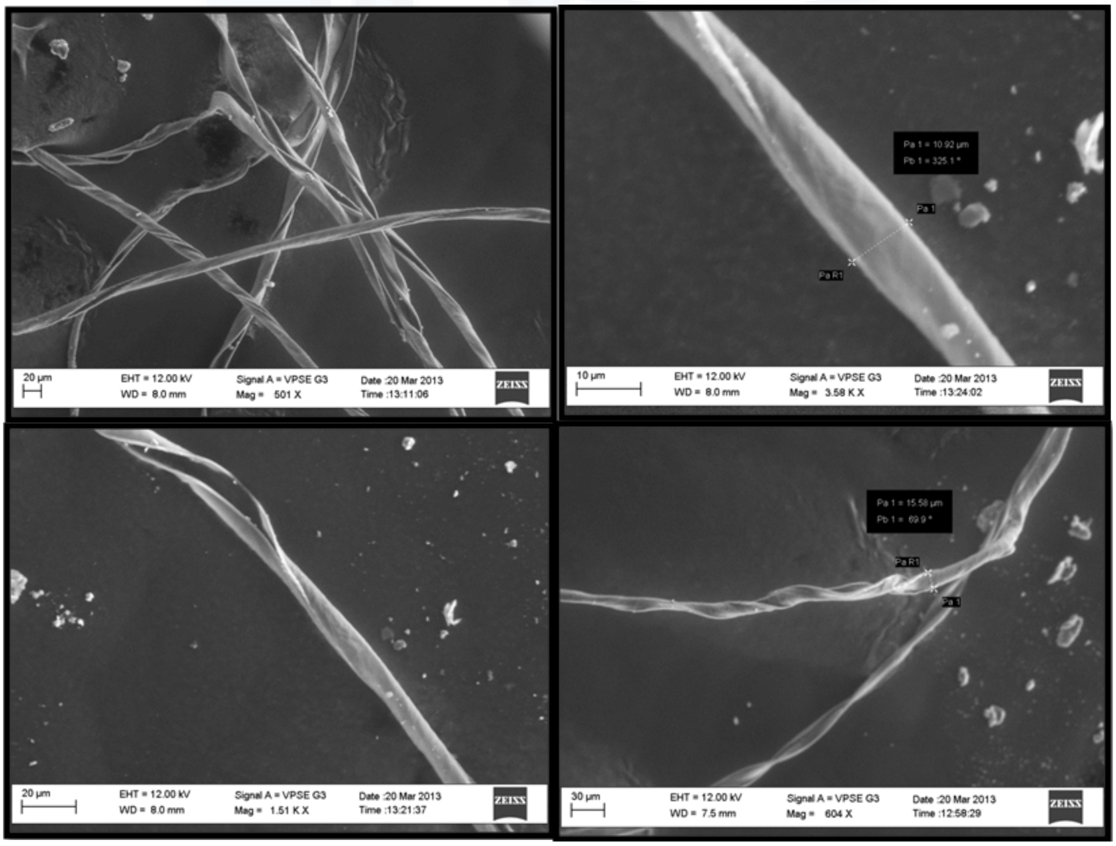


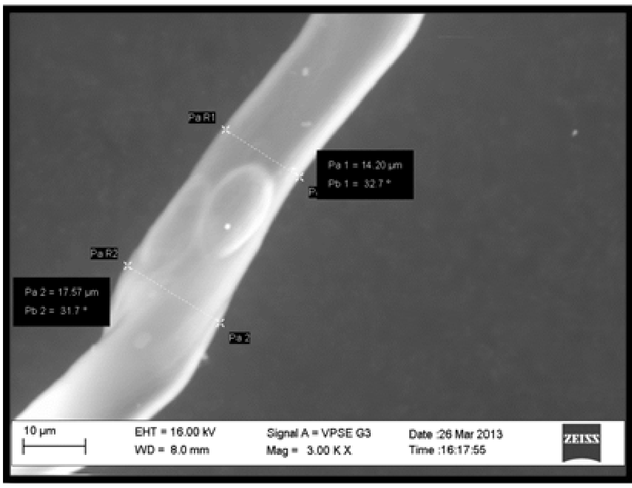


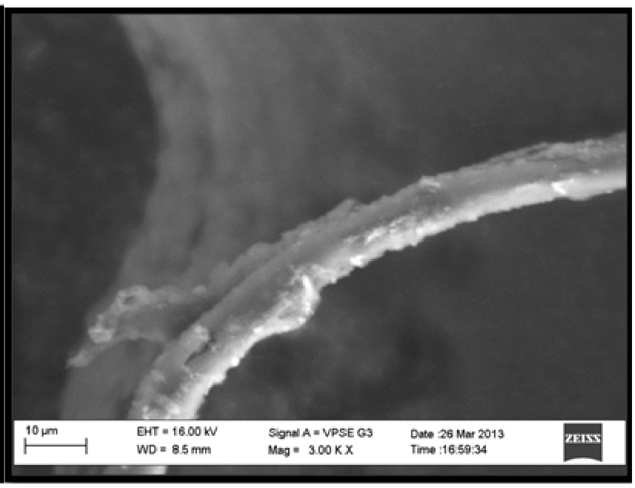


Cotton Fibers

Extensively use of cotton fibers in textiles industry it require to check the quality of fibers. Visually we can check the quality but the detail quality check of fibers by studying ultra-structure of cotton fibers.





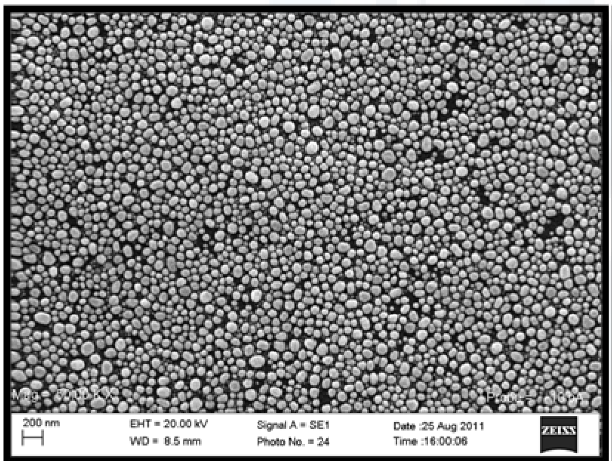


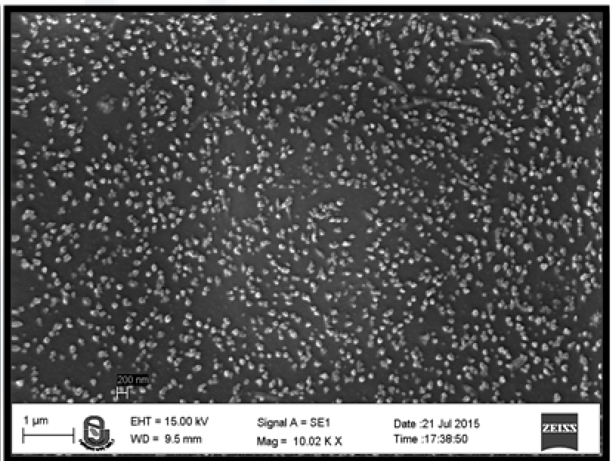
Nanoparticals

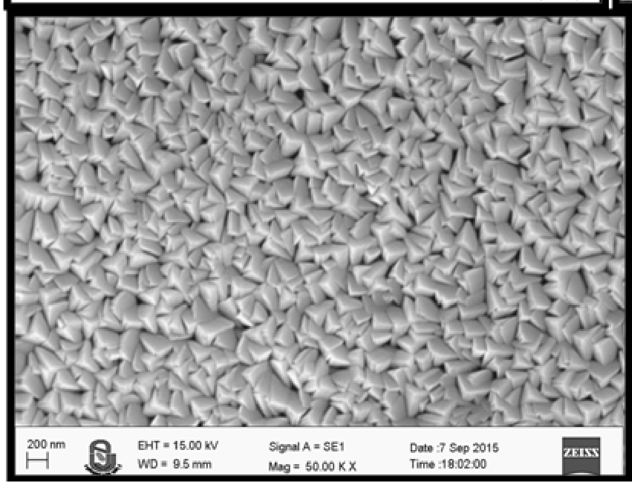
Nanotechnology is at the forefront of novel materials development, from colloidal gold nanoparticles for targeted cancer treatment through to carbon nanotubes in renewable energy capture. Despite the diversity in application, the performance of all nanoparticle technology is defined by the same physical properties, including particle size, size distribution, shape, and surface features.

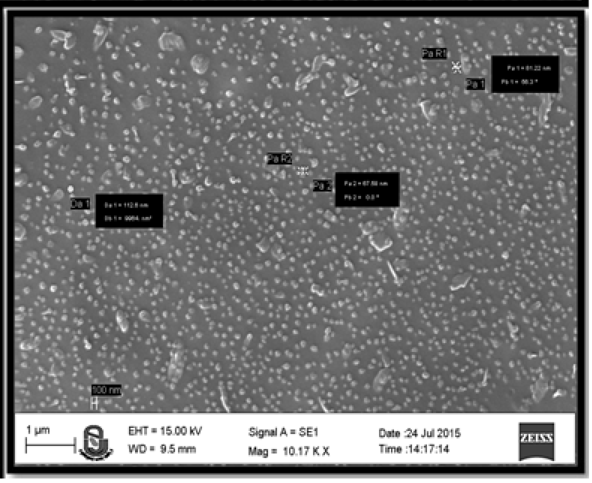
Nanoparticle size characterization therefore forms an important step in nanotechnology R&D and QC. Within the development toolkit, electron microscopy is one of the most powerful methods for determining these critical performance defining attributes.

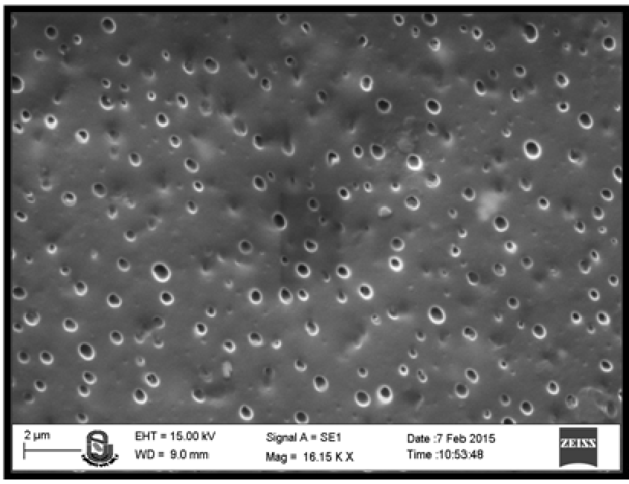
SEM is capable to provide a reliable characterization of the morphology of nanoparticles both (i) as a screening method for accompanying characterization “close” to the nanoparticles manufacturer and (ii) as a metrological tool for the evaluation of the shape and size distribution.

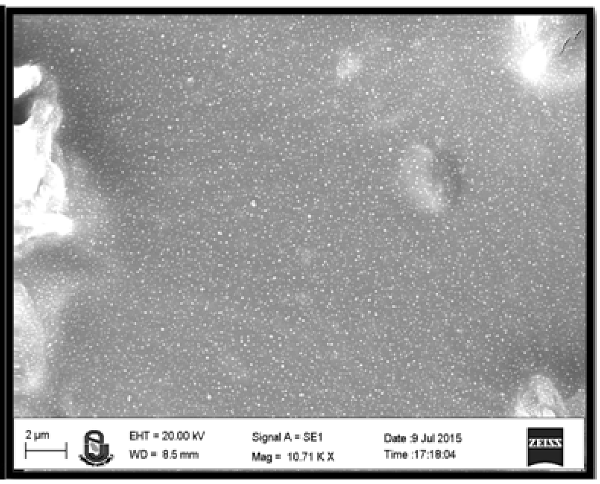






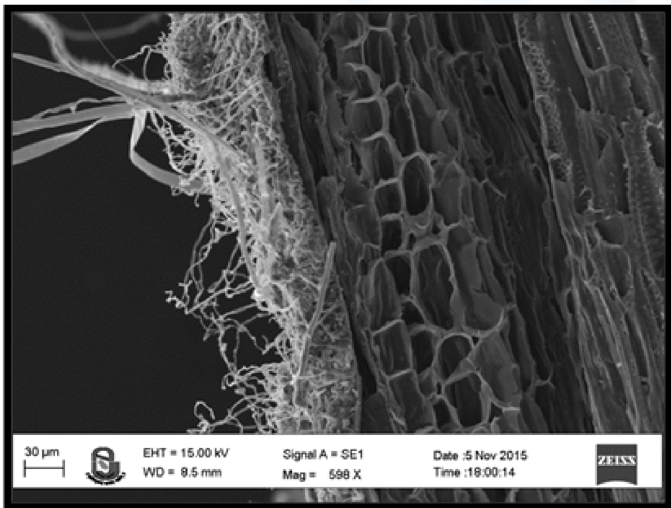


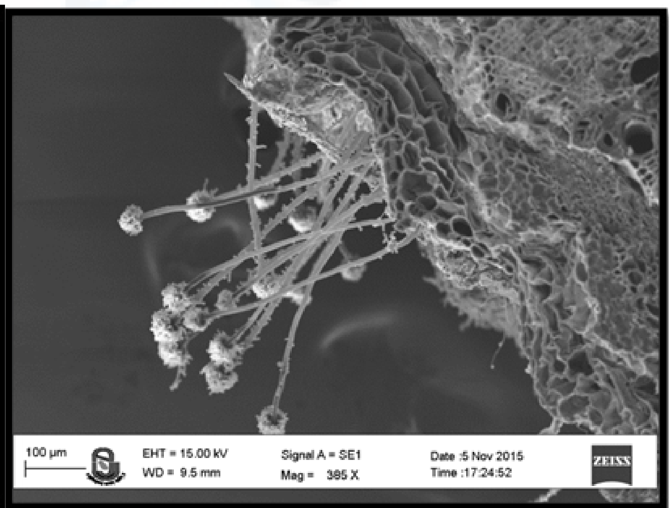


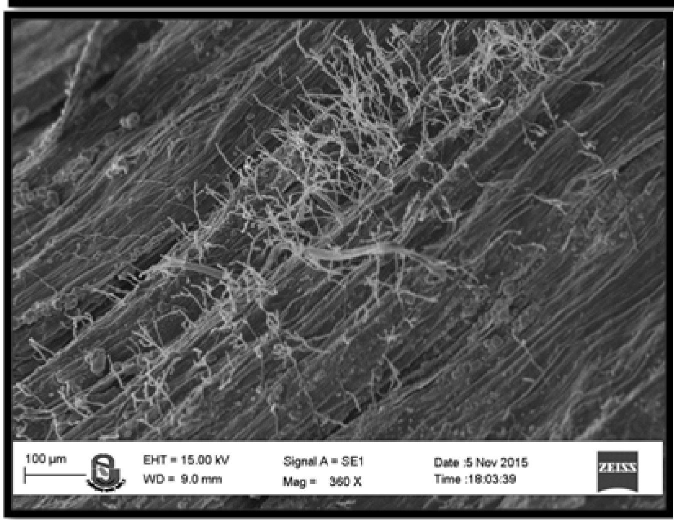


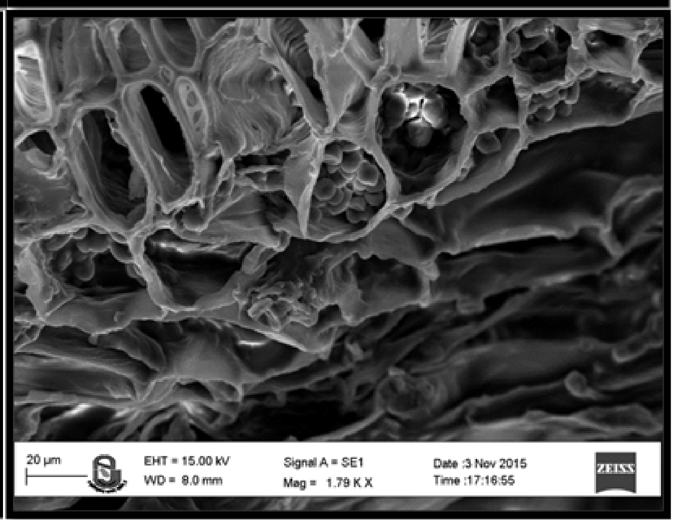
Plant-Fungi Interaction

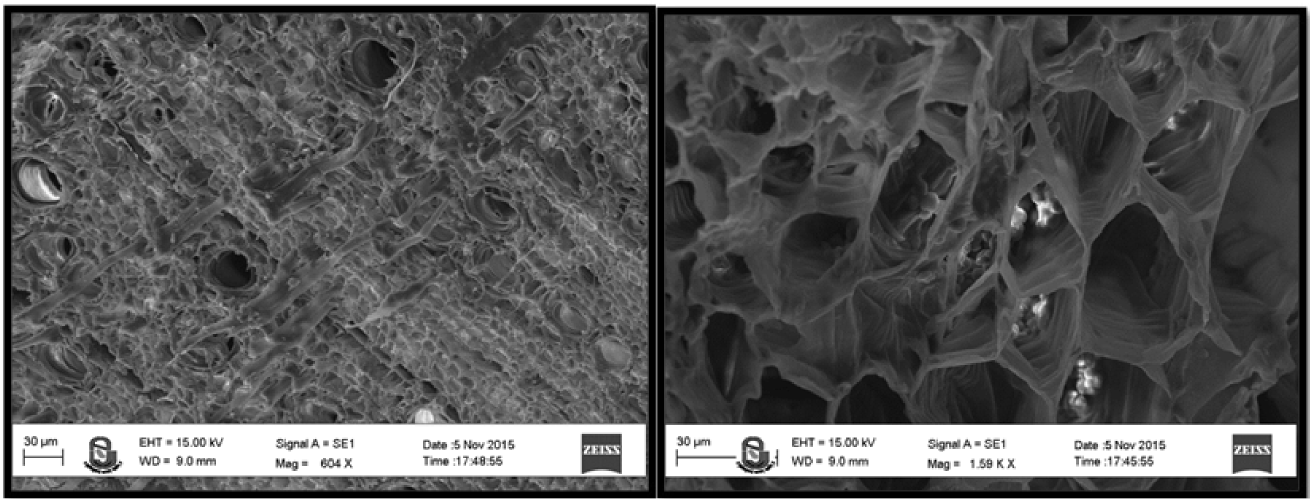
Fungal plant pathogens are major threats to food security worldwide. Fungi interact with plant two ways beneficial as well as harmful. Study of that interaction used to identification of specific pathogen and understanding the process of establishment of infection. How the fungal pathogen enter inside the plant and where it's colonize. Once we know the infection process than it will be used to development of methods or techniques for prevention if disease.







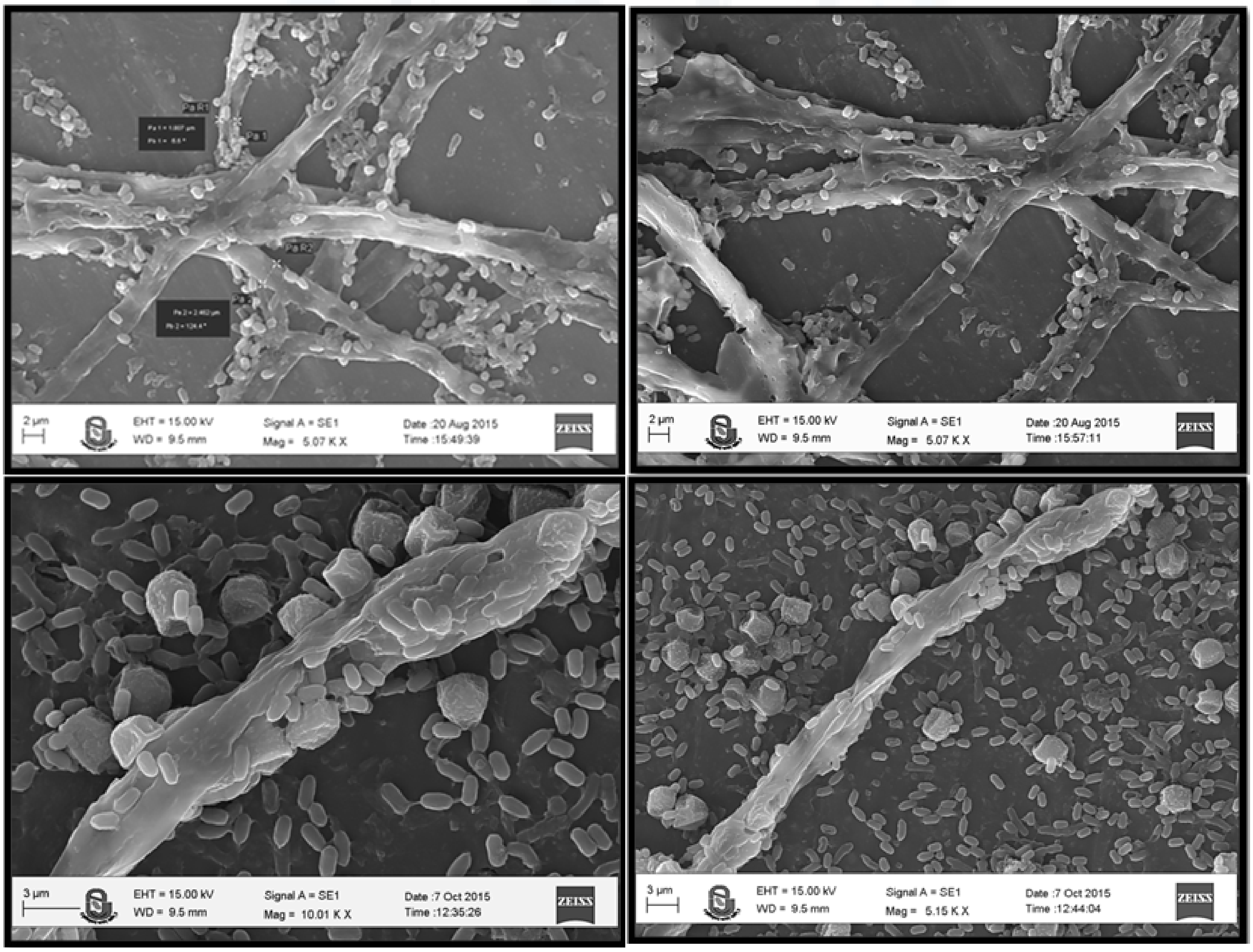


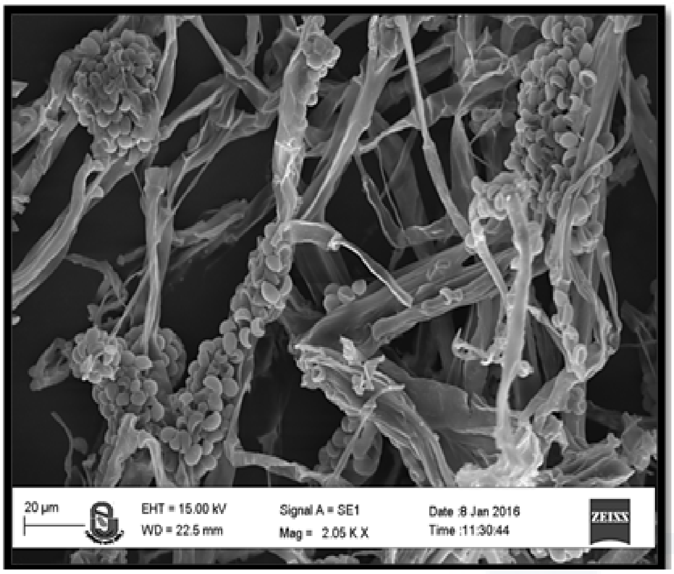


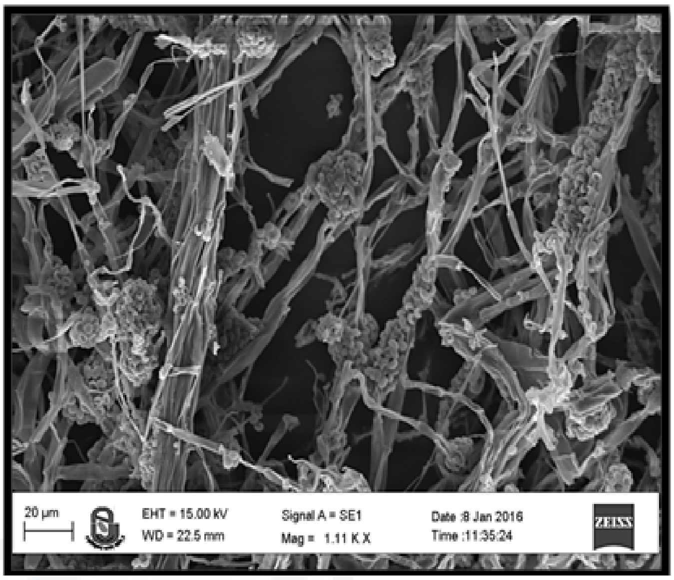
Bacteria-Fungi Interaction

Bacteria and fungi can form a range of physical associations that depend on various modes of molecular communication for their development and functioning. These bacterial-fungal interactions often result in changes to the pathogenicity or the nutritional influence of one or both partners toward plants or animals. They can also result in unique contributions to biogeochemical cycles and biotechnological processes. Thus, the interactions between bacteria and fungi are of central importance to numerous biological questions in agriculture, forestry, environmental science, food production, and medicine. Interaction study will be used to develop new biocontrol agent against plant disease.

SEM images of fungi-bacteria interaction during the antagonist activity.







Actinomycetes

The actinomycetes are a group of most filamentous bacteria, which are similar to fungi, the morphology, reproduction and growth in culture media solids and liquids, are prokaryotes that mineralize organic matter generally true fungi and bacteria do not degrade.

Microscopic and microbiological analysis of soil and fertilizer indicates that Actinomycetes are of lesser impact than bacteria and fungi but are involved in:

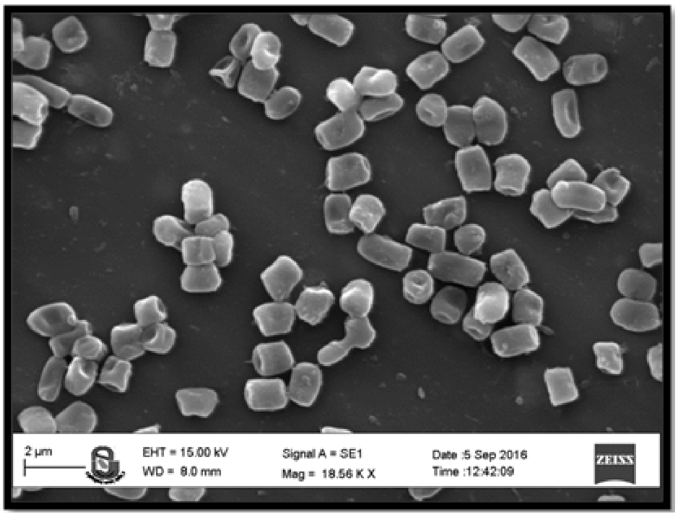
a. Mineralize some polysaccharides resistant plant and animal tissue, but not immediately respond to organic carbon enrichment, not compete with real bacteria or fungi with simple carbohydrates, but if the compounds are resistant to mineralization.

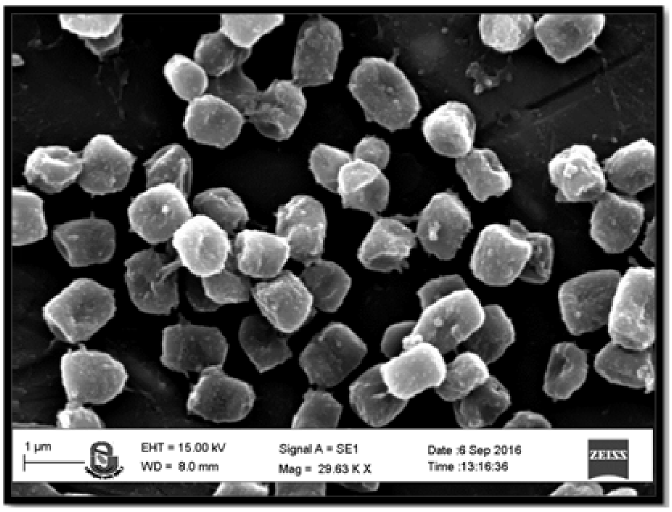
b. Participate in the formation of humus by the oxidation of plant debris and leaves from the organic portion of soil, some kinds of Ac to grow in culture synthesize complex molecules that are essential in the fraction of humus in soil.

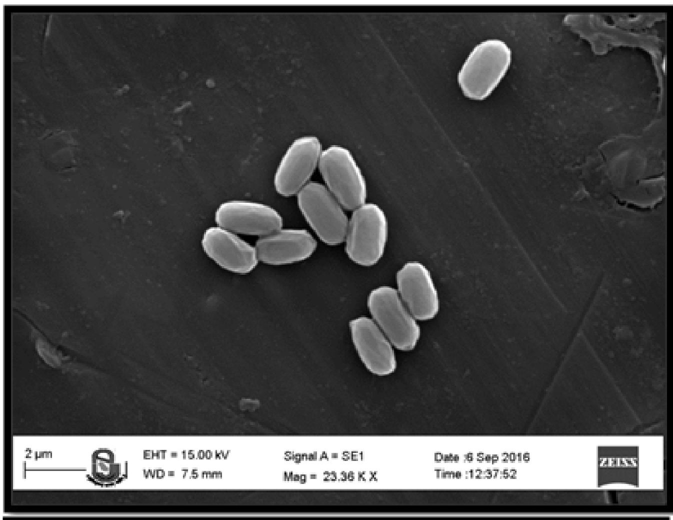
c. Perform the mineralization of green manure, straw and manure at high temperatures which dominate the genre as Thermoactinomyces, Streptomyces, the surface of these materials may be white or gray by the activity typical of that group.

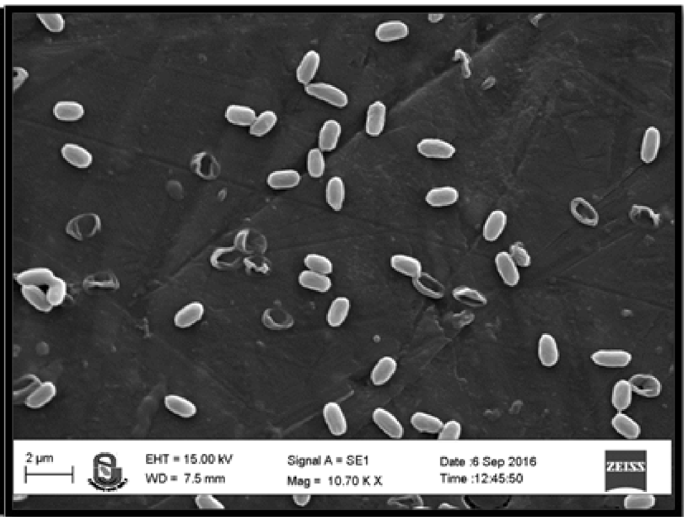
d. Plant pathogens, Streptomyces scabies and S. ipomoeae are pathogens, causing scab of potato, sweetpotato and smallpox.

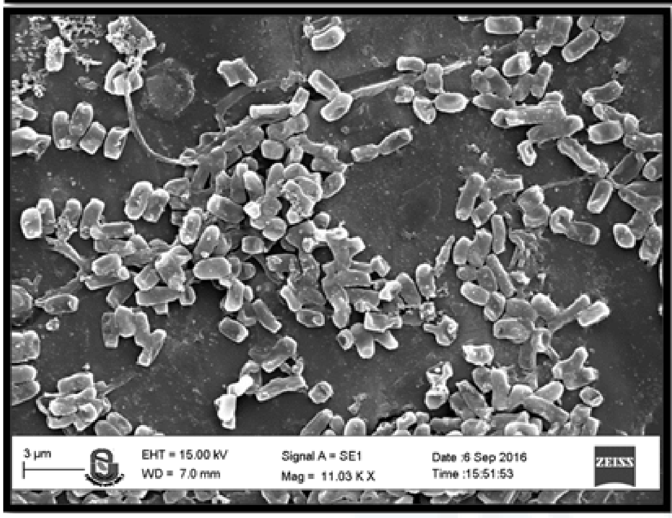
e. There are those who associate with N2 fixing trees in plant roots and Frankia to solve problems of reforestation and land degradation, with actions such as the plant growth promoting bacteria with potential use as biological inoculants [1-5].

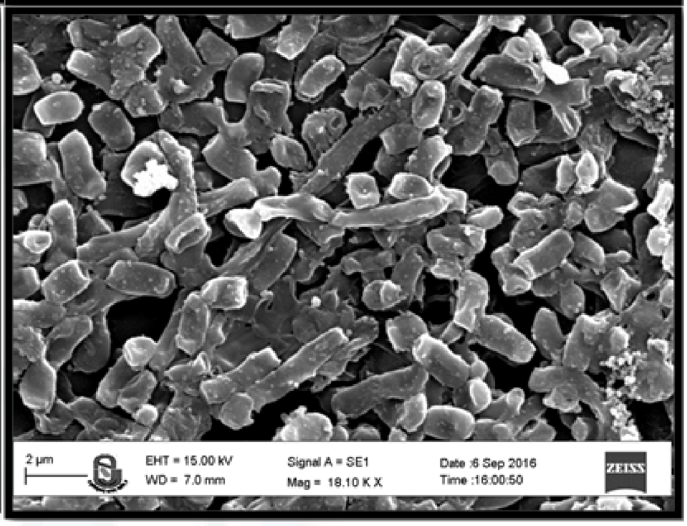




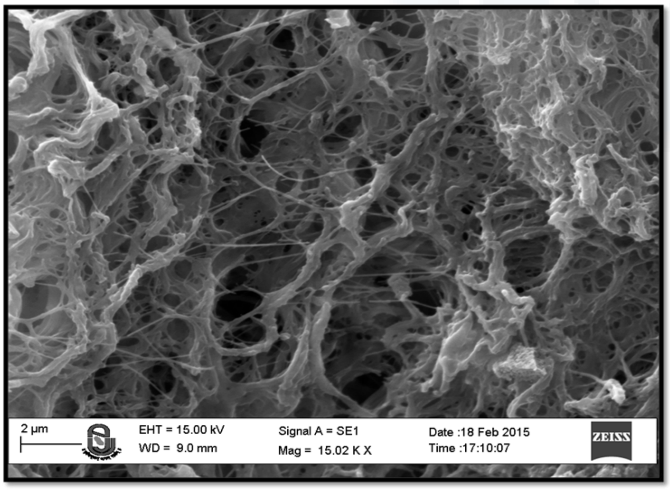


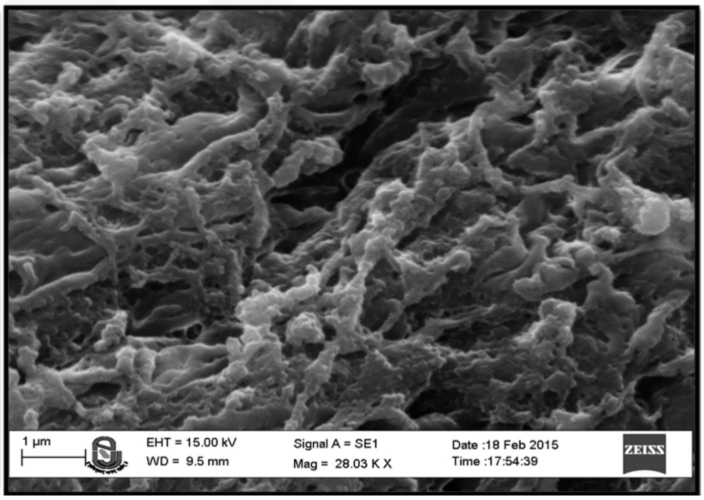






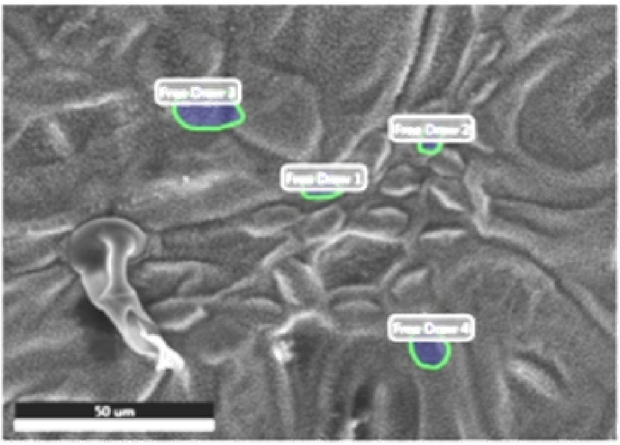
Animal Tissue



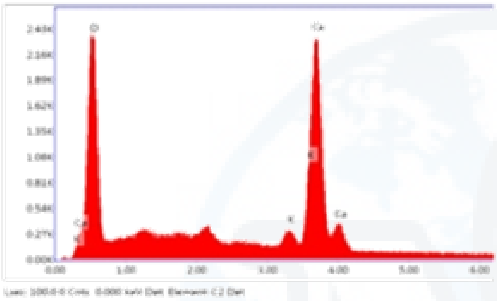


Elemental Analysis of Groundnut's Leaf through EDAX TEAM

Elemental detector which is fit in SEM use for elemental analysis of sample. This is mainly useful for the characterization of nano particles.

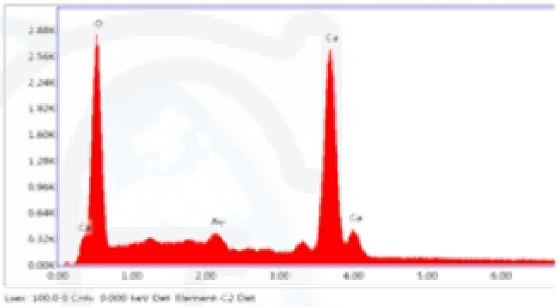


Free Draw 1



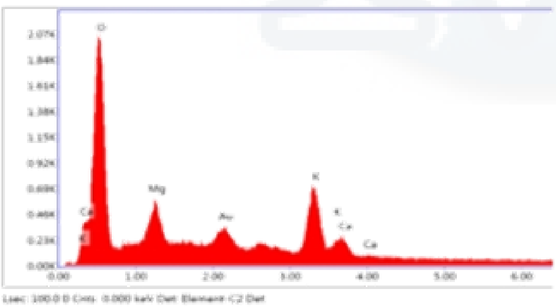
Element	Weight %	Atomic %
OK	59.68	78.68
KK	1.98	1.07
CaK	38.42	20.25

Free Draw 2



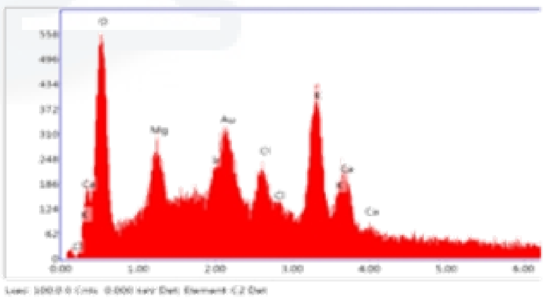
Element	Weight %	Atomic %
OK	59.44	79.11
KK	1.55	0.17
CaK	39.01	20.72

Free Draw 3



Element	Weight %	Atomic %
OK	64.71	81.72
MgK	7.61	6.33
AuM	5.53	0.57
KK	17.30	8.94
CaK	4.84	2.44

Free Draw4



Element	Weight %	Atomic %
OK	44.91	40.12
MgK	3.97	4.07
ClK	5.74	4.07
KK	20.83	13.31
CaK	10.75	6.70

References

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5. http://www.azonano.com/article.aspx?articleID=4118