Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/13695
DOI URL: http://dx.doi.org/10.21474/IJAR01/13695

*RESEARCH ARTICLE*

# ELECTRON MICROSCOPIC STUDIES OF SOME ANTICANCER PLANTS POLLEN GRAINS

**Dr. Jayshree Sandesh Thaware**
Assistant Professor and Head Department of Botany, S.K.Porwal College of Arts and Science and Commerce, Kamptee Dist-Nagpur (MS), India.

## *Manuscript Info*

***Manuscript History***
Received: 31 August 2021
Final Accepted: 30 September 2021
Published: October 2021

***Key words:-***
Palynology, Pollen Morphology, Ethno-Medicinal Plants, Anticancer Plant, Sem Microscopy

## *Abstract*

Pollen is appropriately referred by some as 'Golden dust' extremely valuable on account of their tremendous applications in science, industries and public health. No other plant part even though extremely tiny in size is packed with so much information and power. Similar to other plant parts, pollen characters are so varied that the classification system of plants can be built up entirely on the basis of pollen morphology.Palynology is the distinct branch of biology that deals with the dispersed microscopic tiny living and fossil entities including pollen grains, spores, algal and fungal fragments and others. An important aspect of Palynology is the Pollen morphology. The importance of Palynology in taxonomic and phylogenetic consideration of plants is well known. The changes occurring through hybridization and years of cultivation are reflected in pollen morphology. The scope and interest in the study of pollen morphology have widened with the advent of Scanning Electron Microscopy (SEM) and with regards to unipalynous taxa particularly the understanding of finer morphology is of fundamental importance. SEM gives a correct understanding of exine surface as the electron photographs of the surface replica of the exine provides the exact picture of the ornamentation pattern. The variation in the pollen morphological characters helps in the classification of plant taxa and their assessment of their phylogenetic relationship. In the present investigation, the pollen morphological studies were carried out of some ethnomedicinal plants like *Catharanthus roseus*, *Allamanda cathartica*, *Datura metel*, *Brassica juncea*, *Raphanus sativus* and *Cleome viscosa* pollen grains by Scanning electron microscopy. All that they possess anticancer characteristics in common.



## Introduction:-

Palynology has applications in various fields. Some of them are palynotaxonomy, plant evolution, plant breeding programme, biotechnology, the microbiology of water, soil and air, pharmaceutical industry, cosmetic industry, energy, food industry, forensic science, aerobiology, allergy, epidemiology, meteorology, fossil fuel exploration and biodiversity[1].

**Corresponding Author:- Dr. Jayshree Sandesh Thaware**
Address:- Assistant Professor and Head Department of Botany, S.K.Porwal College of Arts and Science and Commerce, Kamptee Dist-Nagpur (MS), India.

The importance of Palynology in taxonomic and phylogenetic consideration of plants is well known. The changes occurring through hybridization and years of cultivation are reflected in pollen morphology. The scope and interest in the study of pollen morphology have widened with the advent of Scanning Electron Microscopy (SEM) [2] and with regards to unipalynous taxa particularly the understanding of finer morphology is of fundamental importance. SEM gives correct understanding of the exine surface as the electron photographs of the surface replica of an exine provides the exact picture of the ornamentation pattern[3]. The variation in the pollen morphological characters helps in the classification of plant taxa and their assessment of their phylogenetic relationship[4]. Morphology of pollen has been categorized into five groups of characters in the order of their phylogenetic importance viz. apertures, exine orientation, exine strata, size and shape. The aperture shows variations in number, position and character, the various combinations of which make a sporomorph (referring to taxa), a definite entity. The exine ornamentation is comprised of projections, (spines etc) or depressions. The exine consists of stratified layers of which endexine is homogenous while the ectexine is heterogeneous, consisting of a basal layer, a radical rods or columella, and the outermost roof or tegillum, bearing the projection or the depression i.e. the microstructure and the micro sculpturing of the exine is of great importance. The sculpturing of the pollen grain is generally a fairly constant character and is an excellent means of requisition.

The outer pollen wall, or exine, is more structurally complex than any other plant cell wall, comprising several distinct layers, each with its own organizational pattern. Since the elucidation of the basic events of pollen wall ontogeny using electron microscopy in the 1970s, knowledge of their developmental genetics has increased enormously. However, self-assembly processes that are not under direct genetic control also play an important role in pollen wall patterning [5].

The use of plant products, as medicine, is inherent in the Ayurveda, the ancient Indian Health Care System. Several Ayurveda preparations employ a combination of several species of plants for preservation or cure of diseases. Modern medicine utilizes the experience of the ancient practice of herbal medicine and has worked out helpful drug regulations [6].

Plants have had several uses throughout history. Medicinal plants constitute vast, undocumented and overexploited economic recourses and also they are the principal health care resources for the majority of the world's population serving as an alternative system to allopathic. Today their position is quite dismal. Lots of germplasm have been depleted and we cannot afford to lose more[7].

Conservation of these medicinal plants needs an integrated approach. A multidisciplinary action & close collaborations among planners, administrators, scientists, developmental agencies, user industries & growers required. Extinction of these precious plants may be very costly[8].

Pollen morphological studies of some of the ethnomedicinal plants like *Catharanthus roseus., Allamanda cathartica, Datura metel, Brassica juncea, Raphanus sativus and Cleome viscosa* plants having their anticancer properties in common was undertaken.

***Catharanthus roseus* L. (G) Don**

The Madagascan periwinkle, Catharanthus roseus G. Don., has been variously designated Vinca rosea L. It is probably indigenous to Madagascar but is now widely distributed throughout the warm regions and is much cultivated as an ornamental. Periwinkle has had the reputation of a being magic plant, for its beautiful flowers and leaves endowed with a unique chemical compound that has a high medicinal value. True to its name, derived from the Latin word 'vincere' that means 'to overcome'. It has been found to be a useful remedy for not only treating headache, diarrohea and bleeding gums but also for improving memory, lowering blood sugar and blood pressure and even combating cancer. Periwinkle is the most important antileukemic plant. There are at least nine reportedly 'antitumour' compounds present in this plant, namely leurosine, perivine, quercetin, reserpine, serpentine, β-sitosterol, ursolic acid, vinblastine and vincristine[9]. Periwinkle alkaloids are used in the treatment of both malignant and non malignant diseases. Madagascan periwinkle is source of indole alkaloids, vinblastine and vincristine. These alkaloids are called 'spindle poisons' and they have proven to be very effective in chemotherapy treatments for leukemia and Hodgkin's disease (lymph node and spleen cancer). They cause the dissolution (depolymerisation) of protein microtubules which make up mitotic spindles in dividing cells. This effective stop the tumor cells from dividing, thus causing remission of cancer. After treatment of these periwinkle alkaloids, there is 90% chance of the survival[10].

USA the world's largest user of this plant's raw material, a single firm that has the patent to manufacture Vinblastine and Vincristine sulphate has been consuming more than 1000 tons of leaves of the plant annually. The Vincristine is reported to be extracted from pollen also [11].

Rosy periwinkle or Madagascar periwinkle is used for Chromium supplementation, certain kinds of leukaemia, human neoplasm and circulatory disorders.

***Allamanda cathartica* L. Var. *grandiflora* Linn. (fig.2)**

Allamanda cathartica linn. is a common garden plant and commonly grown for its bright, large, showy, yellow coloured flowers and shiny dark green, ovate or lanceolate foliage and commonly known as 'Golden trumpet'. This genus is native to Brazil but now widespread.

The plant has milky sap and is considered poisonous. The whole plant body bears an iridoid lactone 'Allamandin' which is highly cathartic in nature (hence name) leaves are very cathartic and bears alkaloid and Glucoside. Trease and Evans recorded the anticancerous activity of Allamandin which is present in the form of latex in the plant[12].

***Datura metel* Linn. Var. *fastuosa***

Datura metel linn. is one of the medicinally important members of the Solanaceae family found on wasteland along with the roadside. Branches tinged with purple corolla purplish outside, white within. Widely cultivated and naturalized in the tropics. It occurs throughout India and is occasionally grown in gardens. Seeds and leaves are sources of hyoscine. All the plant parts contain alkaloids. Percentages of alkaloids in different parts of the plants are, in leaves and stem 0.12%, roots 0.1%, fruits 0.20%. The alkaloid content varies with altitude and season of the year. *Datura metel* is an indigenous weed of India. Ancient Hindu physicians used the herb in digestive troubles. In Vedic period, people used to smoke *Datura* seeds to get relief from asthma during cold season. In the Ayurveda system of medicine, the herb is the good intoxicant as the leaves contain the alkaloid 'Hyoscine' and 'Hyoscyamine', 'atropine' is one main. The British pharmacopoeia has recommended the dried leaves in whooping cough and severe attacks of asthma. The fruit of *Datura* is used as a specific medicine for malarial fever. Seeds contain alkaloid like Daturine, Atropine and Scopolamine which is found to be poisoned at larger doses. Leaves and seeds are reported to have anticancerous properties. Decoctions of leaves are used in painful tumors.

***Brassica juncea* Linn. Czern and Coss**

*Brassica juncea* is commonly known as 'Indian Mustard' which is a very important genus of the family Brassicaceae. It is an annual herb and cultivated for its oilseeds. It is distributed in India and China and commercially cultivated for its essential oil contents. The oil content of the seeds is usually 30-38 % and the volatile oil content varies from 2.5 to 2.9 %. It is reported to contain Allyl isothiocyanate and related compounds among which crotonyl isothiocynates has been identified. It possesses rubefacient, vacicant, emetic and preservatives properties. Indian mustard is used in pickles and curries. It is also used for making soap and for burning and as a lubricant.

***Raphanus sativus* Linn**

The raddish, *Raphanus sativus*, is an annual herb that belongs to the Mustard family Brassicaceae. It is specially grown for its fleshy roots, which vary in size from a few grams to kgs. The radish was developed from a wild plant that grew in the cooler regions of Asia; it is spread in the Mediterranean region before the Greek era and was introduced to India in 16 $^{th}$ century. They are eaten raw in a salad. Winter varieties are eaten cooked.

Roots are acrid, bitter, appetizing, digestive, diuretic, laxative, and antibacterial which contain glucoside, enzymes and methyl mercaptan. The leaves are antibacterial, diuretic, and laxative. Seeds are expectorant diuretic, carminative useful in cough, stagnancy and paralysis. Seeds of *Raphanus* yield non drying fatty oils suitable for soap making also for edible purposes and as illuminant. In Japan, hydrogenated oil is used in the manufacture of crayons. Seeds cake is rich in protein and used as manure and after removal of isothiocynate it is used as foodstuff.

***Cleome viscosa* Linn**

Cleome viscosa linn. Commonly known as 'Dog Mustard' due to its yellow flowers. It is a common weed found throughout the plains of India, found abundantly on open and west land. *Cleome* is an annual sticky herb with strong penetrating odour and coated with a simple glandular hairs having immense medicinal importance. Oil extracted from seeds is said to be used for culinary purpose in some area. The oil is having a property of killing

worms in intestine and also expels gases from bowels. Leaf juice along with common salt relieve headache when applied on forehead. .the plant is used as a medicine for curing ear diseases and joint pains.

The whole plant is medicinally important. Bark is acrid, irritant, vesccant. The root is anthelmintic and vermifuge. The poultice of leaves is externally applied for wounds and ulcer[13]. Seeds are used as carminative and stimulant. Along with all these qualities, the roots of Cleome viscosa are reported as anticancerous in nature[14]. With many of the properties, pollen grains are allergenic in nature and causes dermal allergy[15].

Various bioactive compounds possessing the properties of an antioxidant, antibacterial, antifungal, ant mutagenic and anticancerous have been characterized and isolated from different parts of plants[16]**.** Some studies in recent years have reported the occurrence of such bioactive compounds in pollen grains as well.

The field of microscopy is in a period of rapid evolution, with its core technologies and techniques becoming ever more sensitive and diverse. These have made new advances possible across a wide swath of research disciplines, demonstrating that microscopy is as important now as it has ever been[17]**.**

Considering this, the present study was planned to examine the ultrastructure of pollen grains by Scanning Electron Microscopy (SEM).

## Material and Methods:-

**For Scanning Electron Microscopy**

Since the investigation of the microscope, about 380 years back, there has been a continuous striving to improve the resolving power in order to observe the microstructure .In 1938, Borries and Ruska gave the first account of the technical electron microscope, which they designed for the Siemens Company in Berlin. Later on, scanning electron microscopy (SEM) has been established as a powerful technique for investigating the special structure in condensed matter. This method has found wide applications in materials science, biology and medicines [18].

The idea of scanning microscopy was extended to any kind of probe movable to two dimensions for eg. Scanning with a laser beam [19], with an acoustic beam [20] or with a mechanical micro contact[21]. However, due to highly developed technology for generating and manipulating a sharply focused electron beam, scanning so far has been found perhaps the widest application in scanning microscopy.

Scanning electron microscope differs from conventional transmission electron microscope in that the electron beam, instead of passing through an ultra thin specimen scans the surface of an opaque specimen. The scattered electron together with the secondary electron emitted by the specimen itself is then amplified and forms an image of the surface in strong relief on the face of a cathode ray tube. Although the resolution of the scanning microscope is at high as that of the transmission microscope, its usable depth of focus is much greater and its specimens are simpler to prepare. It is, therefore, possible to examine a large number of specimens in a relatively short period.

Present SEM work was carried out on Philips SEM 515 unit in SRS Division, National Burow of Soil Survey and Land Use Planning, Nagpur. In the present work scanning electron micrograph of all the dried pollen grains were taken on the surface of the stab. All the pollen grains are coated with a thin layer of gold (250 A° to 350°) to avoid the charging of electrons with the help of the BIO-RAD Polaron Division, SEM coating system. The major advantage in making use of this instrument is that it has three electromagnetic condenser lenses with an electromagnetic mini lenses and ultra fine probes at low accelerating voltage.

The descriptive terminology follows Bhattacharya et. al. [22], Punt et al. [23] and Halbritter et. al.[24].

## Observations:-

***Catharanthus Roseus***

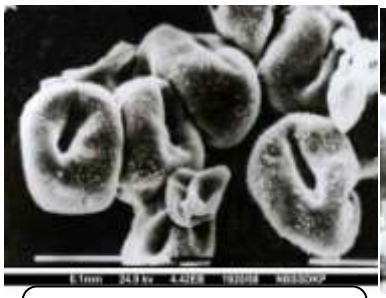
Pollen grains are Trizonocolporate; alternately with the colpi are present 3 deep pits ‘distinct’, prolate; polar axis 48 µ long; the equitorial diameter 36 µ; ora lolangate; long axis of ora 1.6 µ; exine irregularly pitted; exine stratification obscure. The fused state of sexinous elements is noteworthy. The tectum is developed on the distal portion of the fibrillar mat. The sole (foot layer) and the endexine are developed on tri-lamellated structures formed on the surface of the plasma membrane.

*Catharanthus* pollen in Equatorial view 3,800X

*Catharanthus* pollen with polar view 3,800X

***Allamanda Cathartica***

Trizonocolporate; subprolate, subspheroidal, polar diameter 58 µ ranges from 45-75 µ; equitorial diameter 49 µ; exine surface with free or fused granular elements of different size and an uneven irregularly thickened basal stratum (foot layer). Grains are more or less flattened colpi very deep and broad.



Equatorial views with short deep colpi 1,000X

Equatorial view 1, 500X

Magnified view of apertural region 6,000X

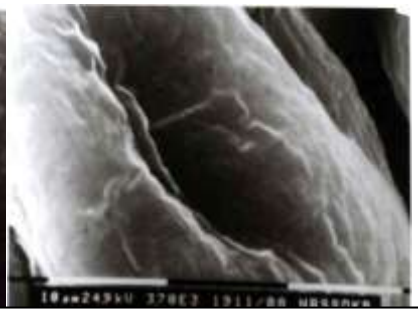
***Datura metel* var. *fastuosa***

Tricolporate prolate; amb speroidal, subprolate; exine striate reticulate, faintly granular, polar diameter 61 µ; equitorial diameter 49 µ; colpi slightly crassimarginate.

*Datura metel* has only acolpate and monocolpate pollen types indicating that it is a primitive dicotyledon. [27] Datura metel pollen grains also have a characteristic thick wall and the highest mean diameter amongst the pollen grains studied in the present investigation.

Pollens with distinct deep colpi 3200X

Single pollen in Equatorial view 2000X



Magnified equatorial views of slightly crassimarginate colpus 6,000X

***Brassica Juncea***

Pollen is tricolpate, subprolate to prolate with coarsely reticulate exine ornamentation. Polar axis 32.46 µm, equatorial axis 22.8 µm. Exine thickness 1.40 µm Sexine thickness 1.20 µm (tectum 0.35 µm, baculum.0.85 µm) width of nexine 0.20µm. Exine surface is reticulate foveolate, brochi almost uniform, Lumina irregular, circular or oval .Colpus length is 21 µm colpi wider at equator and narrower towards either poles, muri thick 0.83 µm is raised due to wavy deposition of exinous materials .

Pollen grains in low magnification 1,250X

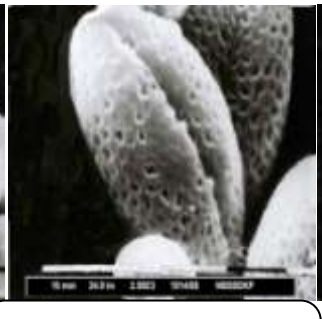
Single Pollen with elongated apertures 4000X

Single pollen with colpus and reticulate ornamentations 6000X

***Raphanus sativus***

Grains are 3 zonocolpate, Polar axis 33 µm, equitorial diameter 24 µm colpus length 30 µm. Grains subprolate, sexine reticulate. Exine thickness is 1.20 µm, colpus 7.11 µm length×2.11 µm width, lumina irregular-nearly rounded. Lumen is successively smaller towards polar area, ungustimurate. The thickness of muri is 4.4 µm; muri ornamented at places.

Pollen grains in low magnification 1,250X

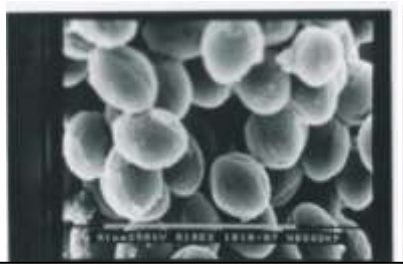


Single pollen with colpus and reticulate ornamentations 6000X

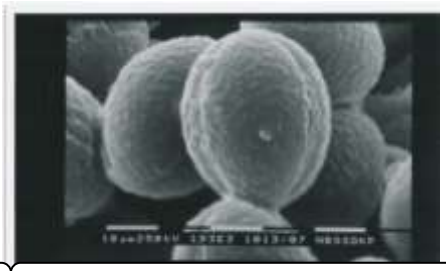
Magnified views of equatorial and polar view 4,000X

***Cleome Viscosa***

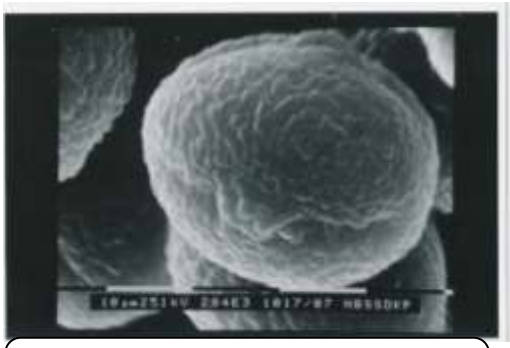
Grain Trizonocolporate, spheroidal, prolate, polar axis 30 µm, equatorial axis 22 µm, grains prolate, colpi longicolpate 28 µm in length, triangular, crassimarginate. Exine surface rough, finely or sculpturing obscurely reticulate and 2.6 µm thick, sexine thinner than nexine, ora lolangate.



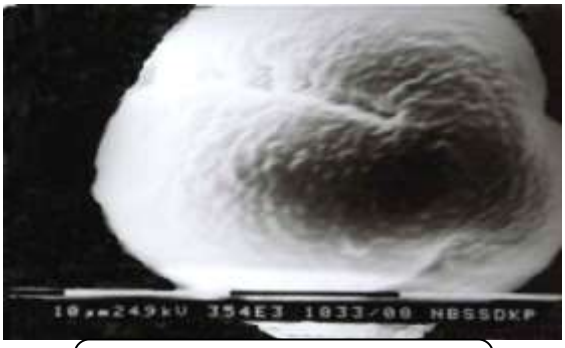
Pollen grains in low magnification 1,000X



Single Pollen with colpi and pore 3000X



Equatorial views with rough protruded aperture 3,800X



Pollen with tangential polar view 4.500X

## Discussion:-

Palynology, in recent years, has attracted the attention of workers of different disciplines on an account of its numerous applications to problems of Plant Taxonomy, Palaeobotany, Genetics, Geology, Medical and Agricultural Sciences.

Pollen acts as a biological protector of male sperm and is covered by an outer cell wall polymer called the exine, which consists of durable sporopollenin. Despite the astonishingly divergent structure of the exine across taxa, the developmental processes of its formation surprisingly do not vary, which suggests the preservation of a common molecular mechanism. The precise molecular mechanisms underlying pollen exine patterning remain highly elusive, but they appear to be dependent on at least three major developmental processes: primexine formation, callose wall formation, and sporopollenin synthesis[25].

The exine of angiosperm pollen grains exhibits remarkable diversity in their apertures and their surface sculpturing, which is often elaborate in its patterning. As is well known the diversity found between one taxon and another is often of taxonomic significance. Many different factors may have been responsible for the origin of diversity. The factors which are important in any particular case may have been quite different at different stages during evolution[26].

The sculpturing pattern on the exine surface has been of great help in the identification and delimitation of taxa especially, at a generic and lower level. The details of the exine are such that it can be used in the way that finger prints are used for identification of the criminals. [29] Recent techniques like SEM have opened up new vistas in understanding these structures because of their potentialities to disclose new dimensions of plant surfaces. These ornamentation patterns along with the aperture characters have provided the most stable and consistent characters for taxonomy and have been of decisive value in complex group[27].

## Conclusions:-

The morphology of pollen grains as, size, shape, exine thickness, its ornamentation, especially lumen and muri characters and colpi are very important characters of pollen grains and can be used for identification and differentiate between the species belong to the same genera but this characters cannot be used as a tool for taxonomical clusters for the different tribes.

This study gives important morphological characters of pollen grain for some species for the first time because some species are endemic for the study area and others are examined for the first time, this increase the number of studied species of the family at the global level. Also, the comparison between the common species with other studies gives more detail and indicates the taxonomical importance of pollen characters.

These applied aspects of Palynology have resolved themselves into practical feasibility mainly because of the characteristic morphology of the exine which is considered dependable to reveal information on the evolution and

interrelationship of plants. The cultivated plants alone provide examples of the uniqueness of the pollen walls and of the application of pollen morphology in plant taxonomy and evolution.

Pollen biotechnology is a very advanced technique which is highly promising, through which the genes of interest could be obtained directly. But due to the technological constraints it is not possible or up to the mark yet. Palynological studies of these medicinal plant suggests to technology to genetic improvement of several medicinal plants. So, the pollen is found to optimize the crop yield to the preparation of anticancerous drugs from the plant origin.

## References:-


1. Agashe, S. N. (2006). Palynology and its Application. Oxford IBH Publishing Co. Pvt. Ltd., New Delhi. pp. v - vii.
2. Iwano, M., Wada. M., Morilay Shiba, H., Takayuma, S and Isogai, A. (1999). X-ray micro analysis of Papillar cells and pollen grains in the pollination process in Brassica using a variable pressure scanning electron microscope. Journal of Electron Microscopy. 48(6) 909.
3. Ayyangar, K. Rangaswamy (1970).Symposium of crop Palynology, Palynological bulletin 6:14-16
4. Faegri, K. and Iverson, J. (1950). Text book of modern Pollen analysis. Copenhagen.
5. Blackmore S, Wortley AH, Skvarla JJ, Rowley JR.(2007). Pollen wall development in flowering plants. New Phytol. 174(3):483-98.
6. Chopra, R. N., Chopra, I. C. and Handa, K. L. (1956). Utilization of Indian medicinal & allied plants. - A Review. Science & Culture 24: 59.
7. Rangari, J.G. (2008). Palynological studies in plants with anticancer properties. Ph.D. Thesis, Nagpur University, Nagpur.
8. Anand, M. (2000). Significance of Medicinal Plants. Agriculture Today Vol. 3: 33-35.
9. Kaufman, P.B., Cseke, L.J., S. Duke, J.A., Brielmann, H.L. (1999).Natural Products from Plants. CRC Press, Boca Raton, Florida33431. 193.
10. Bswas, R.C. (1973). Studies on the alkaloids of Apocynaceae plants. Science and Culture. 39:6. 259-262.
11. Jayshree Thaware. (2012). *Screening of Pollen of Catharanthus roseus L.-An Anticancer plant*. International Journal of Drug Discovery and Herbal Research. Vol.2 (2):403-407.
12. Trease, G.E. and Evans,W.C.. (1978). Text Book of Pharmacognosy (11th Edition), Bailiere, Tindall and Cassell, London.475.
13. Dymack, W. (1890). Pharmacographia indica, Humdard foundation, Nizamabad, Karachi,Pakistan. Vol. II: 131-136.
14. Raychoudhary, S.P. (1991). Recent Advances in Medicinal Aromtic and Spice Crops (Vol. I). Today and Tomorrow Publisher, New Delhi. : 63-72.
15. Nair,P.K.K., Joshi, A.P. ,Gangal, S.V. (1986). Airborne pollen spores and other plant materials of India- A survey. CSIR centre for Biochemical, Delhi. 1986. 41, 56, 181.
16. G. Kroyer and N. Hegedus, (2001). Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. Innovative Food Science & Emerging Technologies, Vol. 2( 3): 171–174
17. Azzouz, M.,Binny C. (2013). Microscopy: Progress and Prospects. J. of Microscopy and Ultrastructure(1): 63-64.
18. Pal, S.G. (1978). Frontiers of Scanning Microscopy in Biology. Everyman's Science, Vol.XIII. (5) :134-140.
19. Northrop, G.A. and Wolfe, J.P. (1980). 1979. Phy. Rev. Lett. 43 (1979)1424 and Phy a Rev. Bull. 6196
20. Heiserman, J. ,Rugar,D. and Quate, C.F. (1980). J.Acoust. Soc. Amer. 67: 1629.
21. Binnig, G., Rohrer, H., Gerber, C.H. Weibel, E. (1980). Appl. Phys. Lett. 40 (1982) 178 and 50 (1983) 120.
22. Bhattacharya, K., Majumdar, M.R.,Bhattacharya, S.G. (2006). A Textbook of Palynology. New Central Book Agency, (P) Ltd, Kolkata, India.
23. Punt, W.; Hoen, P.P.; Blackmore, S.; Nilsson, S.; Le Thomas, A. (2007). Glossary of pollen and spore terminology. Rev. Palaeobot. Palynol. 143, 1–81.
24. Halbritter, H.; Hess, U.S.; Grímsson, F.; Weber, M.; Zetter, R.; Hesse, M.; Buchner, R.; Svojtka, M.; Frosch-Radivo, A. (2018). Illustrated Pollen Terminology, 2nd ed.; Springer: Vienna, Austria, 3–415.
25. Adedeji, O., & Akinniyi, T. (2015). Pollen Morphology of Some Species in the Family Solanaceae. *Journal of Advanced Laboratory Research in Biology*, *6*(4): 124-128.
26. Ariizumi T, Toriyama K. (2011). Genetic regulation of sporopollenin synthesis and pollen exine development. Annu Rev Plant Biol. 62:437-60.
27. Punt (1986). Functional factors influencing pollen forms. Pollen and Spores: Forms and functions. Linnaean Society of London; 97-102.