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Palynological Investigations of Cleome Viscosa Linn- A Medicinal Plant

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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. For the breeders the pollen is a discrete mobile male partner of the fertilization process in higher plants. It carries the genotype of one partner of the pollination process, which is in fact the process which the breeder manipulates. In the present study Palynological investigations of Cleome viscosa Linn.- an annual weedy herb medicinal plant was carried out. *Cleome viscosa* Linn. the whole plant and its parts like leaves, seeds and roots are widely used in traditional and folkloric system of medicine. In traditional systems of medicine, the plant is reported to possess beneficial effects as an anthelmintic, antiseptic, carminative, antiscorbutic, febrifuge, and cardiac stimulant.

In the present study, Pollen phenology, pollen morphology, pollen physiological studies like pollen production, viability, germination – in vitro and in –vivo etc., total pollen protein estimation, histochemical studies of germinated pollen grains of Cleome was investigated.

I. INTRODUCTION

Pollen is known to have a higher energy investment per gram of organic tissue than other plant parts. Pollen anemophilous species have lower calories contains than pollen of entomophilies species. In addition to its main function pollination and fertilization, pollen attracts and nurtures a variety of pollinators. Enhanced pollen energy contents could increase attractiveness to pollen consumers to cover their energy requirements (Agashe, 2006).

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 waste land. Cleome is an annual sticky herb with a strong penetrating odor and coated with a simple glandular hairs having immense medicinal importance. Leaves are 3-5 foliate gradually becomes shorter upwards. Flowers yellow in lax raceme. Flowering season is June to October. Seeds are brownish black when ripe, sub-globose with faint transverse lines. 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 (Rangari, 2008).

Whole plant contains glycoflavinone and navigenin glycoside a novel dipterpene lactone elemcolide. The seeds contain umbeliferon derivatives, Cleosandrin, Cleomiscosin and viscusic acid and viscosin. Roots possess kaempferol-4 methylether3-glucoronide, Betulinic acid, β-sistosterol (Chatterjee and Prakashi, 1991; Anonymous, 1992; Asolkar et.al., 1992). Whole plant is medicinally important. Bark is acrid, irritant, vesicant. Root is anthelmintic and vermifuge. The poultice of leaves is externally applied for wounds and ulcer (Dymack, 1890). Seeds are used as carminative, anthelmintic and stimulant. Along with all these qualities, the roots of Cleome viscosa reported as anticancerous in nature (Raychaudhari, 1991). With many of the properties pollen grains are allergenic in nature and causes dermal allergy (Nair et.al., 1986).

Kumar et al. (1988) have experimen¬tally proved that this plant is tolerant and resistant to salt and water stress, which is important for erosion control and, hence, is an ideal weed in both warm and cool environments. Apart from these advantag¬es, they have a unique role to play in plant commu-nity restoration, bloom quickly and sustain diverse insect pollinator, as well as herbivore communities. Cleome species are used as leafy vege¬tables in many regions Therefore, these species are im¬portant ecologically, medicinally and economically, and are essential constituents of tropical ecosystems by their interactions with local insects/animals and serve their part as constituents of biodiversity (Mali, 2010).

Fig-1 Cleome viscosa bush along

Fig-2 Cleome flower with

Plant germplasm resources are one of the most important renewable natural resources of the world. Increasing exploitation coupled with natural calamities has led to rapid dwindling of important plant species. Nearly 20,000 to 25,000 species of vascular plants are currently facing threat their existence. This necessitates urgent measures to conserve the plant wealth of ecosystem, species and gene pool levels, to enable sustained use for present and future generations by establishing pollen banks and germplasm resources centers.

The direct and indirect roles played by pollen in various spheres of applied biological research will be found useful in view of the fact that pollen is a material to work which seems to be providing an easier and even better means for experimentally controlling the genetic behaviors of the plants.

II. MATERIAL AND METHODS

Pollen phenology, pollen morphology, pollen physiological studies like pollen production, viability, germination – in vitro and in –vivo etc., pollen storage, total pollen protein estimation, Histochemical studies of germinated pollen grains of Cleome viscosa was carried out.

Pollen phenology- Anthesis took place nearly 6.00am on plant. Yellow coloured pollen dust deposited on anther. Pollen grains are monads, triangular, yellow, and slightly sticky.

Pollen morphology-The morphological analysis comprises fresh material collected from respective field and study was made with light microscopy and electron microscopy. Pollen morphology was studied by two method first by acetolysis method in which the pollen were subjected to the chemical treatment and the pollen become able to clear it surface and provides useful information in reaching conclusion of exine on variable taxonomy.

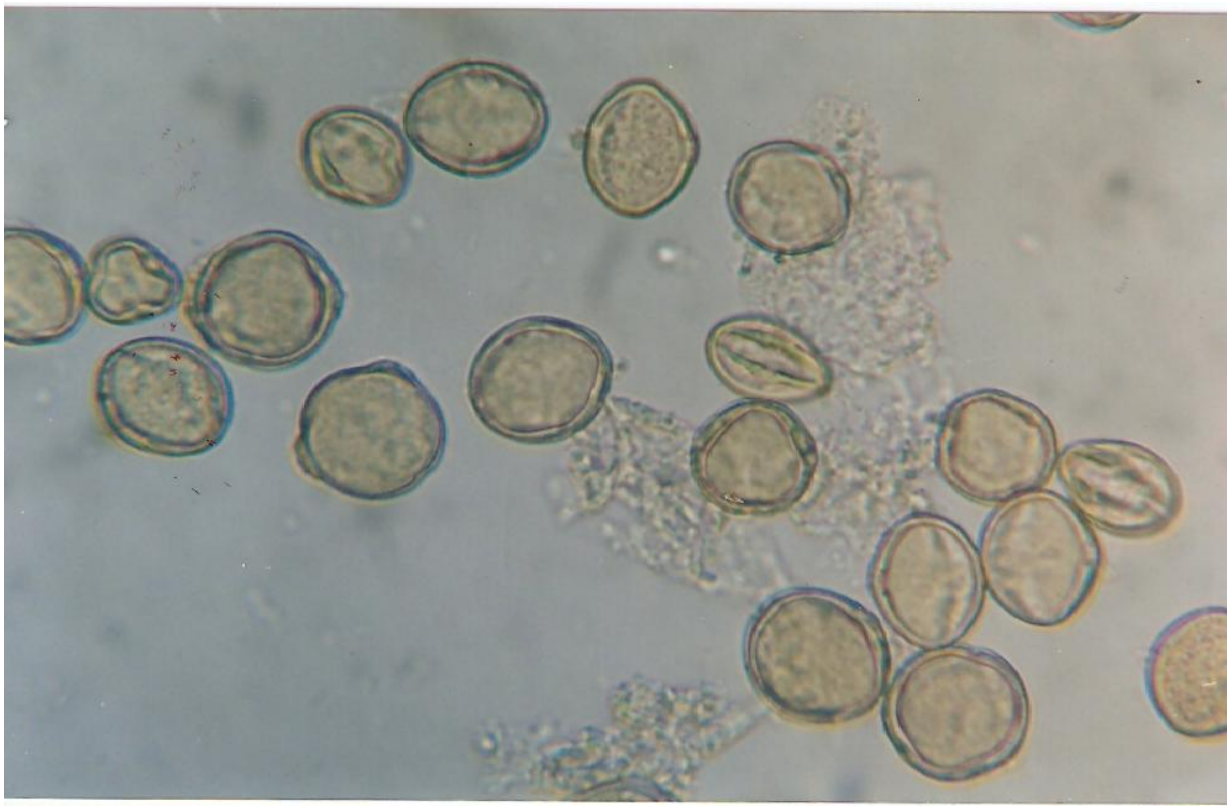


Fig-3 Acetolyzed pollen grains 400X

However, scanning electron micrographs serves to elucidate further detail of exine sculpture such as pores and colpi and the external surface of the pollen grains as well as diameter of pollen and measurement of the exine stratification.

Grain Trizonocolporate, polar axis 30 µm, equitorial axis 22 µm, grains prolate, colpi 28 µm in length, crassimarginate. Exine surface rough, finely reticulate, ora lolangate.

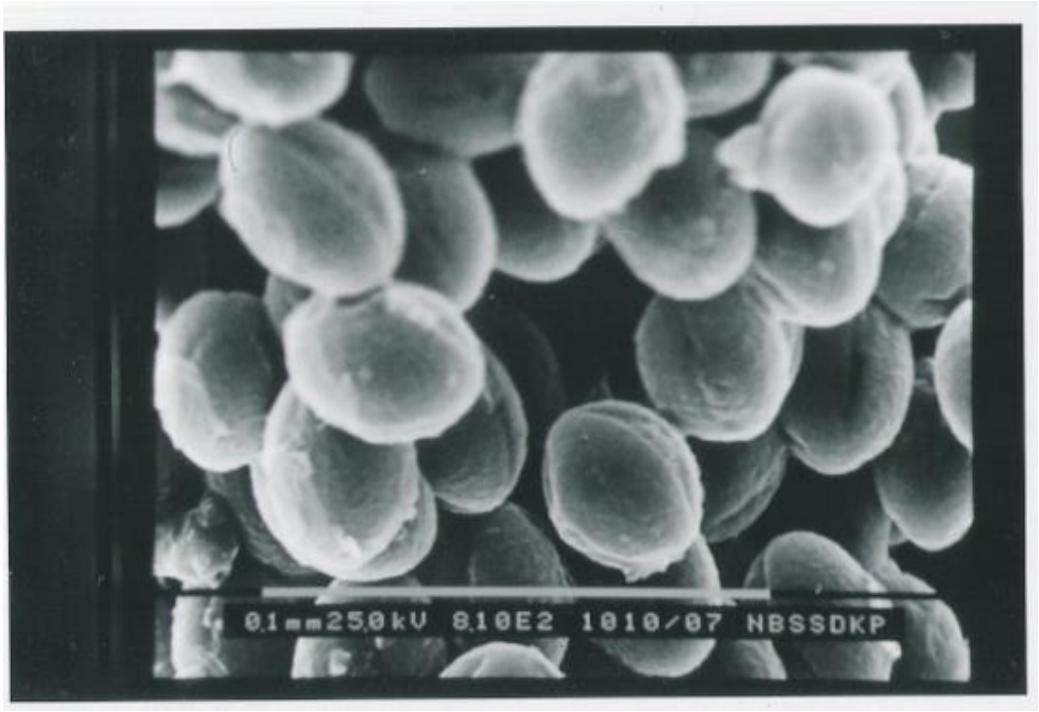


Fig-4 SEM image of Cleome Pollen 1000X

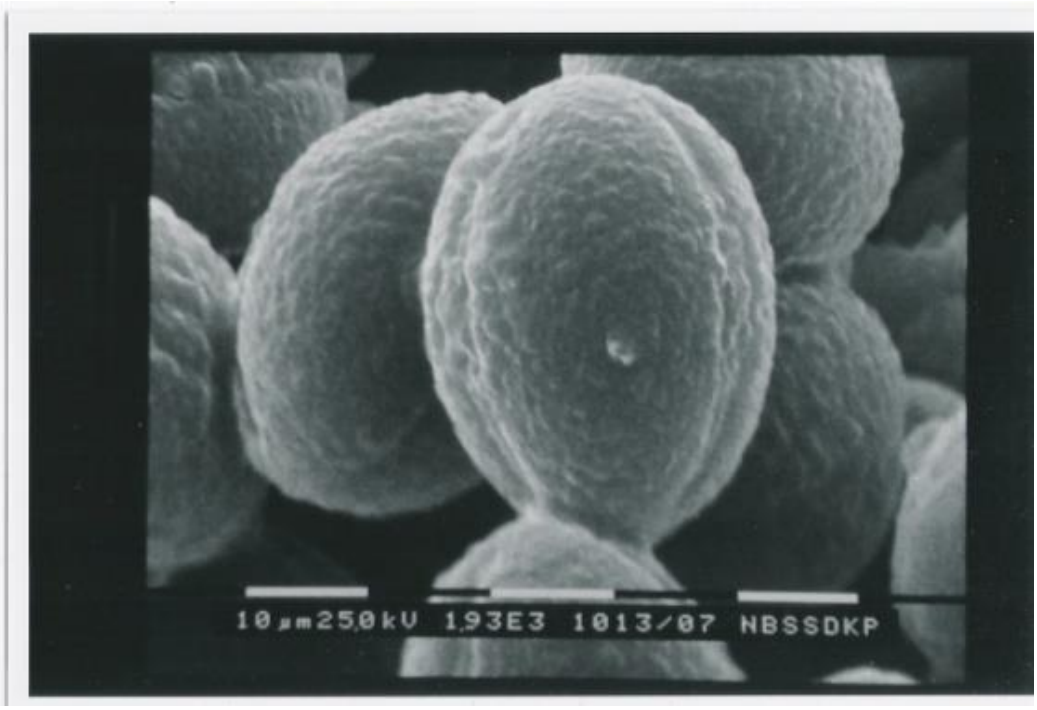


Fig-5 SEM Image of pollen in equatorial view 4000X

Pollen production- Pollen production was studied by two methods i) Simple method and ii) Haemocytometer method. It was noted that there were some differences in the total pollen output by both the method. Out of

the two the Haemocytometer method appears to be more accurate as the number of pollen is counted for 0.1 cubic mm. of the solution. Whereas, in simple method it is counted from 0.05 ml. of the solution. Counting becomes easier by Haemocytometer and it appears to be the best suited instrument for the evaluation of pollen grains as the pollen size is small. In case of Cleome, the 1, 02, 384 pollen per flower found whereas pollen per anther was found to be 4266.

Pollen viability- Pollen viability by stainability technique was studied using 2, 3, 5,-triphenyl tetrazolium chloride which is a vital stain used for the viability of tissues. The test is based on the presence of functional enzyme which converts the colorless solution of 2, 3, 5-triphenyl tetrazolium chloride into the insoluble red Triphenyl formazon. The viability of the pollen by TTC in the present study was 98.23%.

In-vitro germination study- Pollen viability by pollen germination in vitro was carried out by 'Hanging Drop Technique'. The fresh material was sown in various artificial culture mediums like Sucrose, Boric acid along with sucrose and in Brewbaker's medium and observations were noted after 24 hours, so that they grow to their maximum limits. The different media used for pollen culture were standardized by series of experiments. The different media used for studies were sucrose solution (from 5% to 40%), Boric acid medium with sucrose as a basal medium (from 10 ppm,25 ppm,50 ppm,100 ppm,200 ppm, 300 ppm, 500 ppm and 700 ppm) and in Brewbaker's medium which also known as 'Calcium complex'.

i) Sucrose

Cleome viscosa pollen grains showed vigorous germination in all the grades of sucrose except 35 and 40 percent were they do not showed any germination. In the remaining grades, i.e. from 5 % to 30 % pollen showed the effective germination. The maximum germination was noticed in 15 percent of sucrose which was 97.67 percent with maximum tube length of 1489 microns. The average tube length varies from 1289 to 1423 micron. The Cleome pollen showed population effect.

During daily experiments when Cleome pollen was subjected to germination, in 15 percent sucrose they showed variation in germination from 91 to 97.67 percent with maximum tube length range from 1388 to 1489 microns. The pollen tubes grow straight and no bulging at tip of tube was recorded. No bursting of pollen tube and pollen content was recorded.

ii) Boric acid with sucrose

Cleome pollen grows well in the lower concentration of Boric acid with 15 percent sucrose as a basal medium. In 10 ppm the pollen showed 78 percent germination, in 25 ppm pollen showed 82 percent germination, in 50 ppm boric acid 89 percent germination recorded with bursting of pollen tubes. In 100 ppm of boric acid maximum percent of germination was recorded i.e. 94 percent. From 200 ppm onwards the germination percent decreases with the increase in the bursting of pollen contents. The maximum pollen tube length was noticed in 100 ppm of boric acid with 15 percent sucrose that was found to be 1378 microns. In certain cases the bulging at the tip portion recorded. The average pollen tube ranges between 689 to 1178 microns.

When daily experiment carried out with 100 ppm boric acid with 15 percent sucrose the pollen grains of Cleome showed variation in maximum germination from 89 to 94 percent and maximum tube length varies from 1172 to 1378 microns.

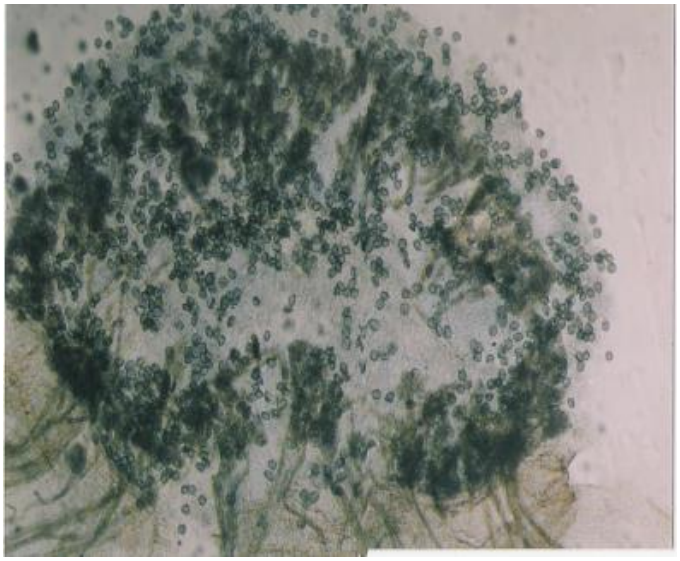
iii) Brewbaker's medium

In Brewbaker's medium, Cleome pollen grains showed good germination like sucrose. It showed 96 percent maximum germination and the percentage varies from 90 to 96. The maximum length recorded in the Brewbaker's medium was found to be 1549 microns highest than any other medium. The average pollen tube length varies from 1179 to 1346 microns.

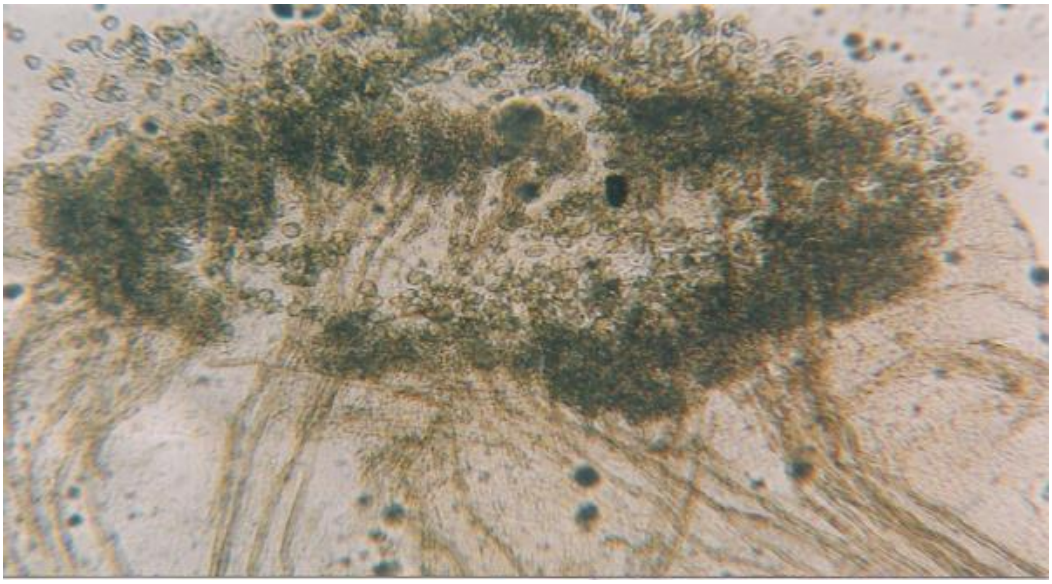
In vivo germination study- The breeding system of an angiosperm is multidimensional and covers, among other aspects, sequential processes such as pollen delivery, pollination, and pollen germination on the stigma, pollen tube growth down the style, fertilization of the ovules, seed development and dispersal of the seeds. Out of this broad field, the pollen germination and the competition of the tubes in the transmitting tract plays very important role in the fertilization that we studied in the laboratory by studying the stigmas.

In vivo studies was conducted on the stigma with some portion of styles on the first, second, third day of the anthesis till the drooping stage. All days stigma showed the germination. 10 stigmas in each category was studied and maximum 88% germination was found in Cleome viscosa and maximum pollen tube length was found on second day with 1995 microns in length. The number of pollens retained on stigma on drooping stage was also large i.e. 2947 pollens per stigma.

Day 1 stigma



Day 2 stigma



Drooping stage stigma

Fig- 6 In –vivo germination of pollens on stigma

1.	Period after opening of flower.	Day 1	Day 2	Day 3	Dropping
2.	Total number of stigma's observed.	10	10	10	10
3.	Number of stigma's showing germination.	7	8	10	10
4.	Mean number of pollen retained on stigma.	2835	2947	2872	2298
5.	Mean number of pollen germinated on stigma	1234	1667	1845	2024
6.	% of germinated pollen.	44	56	64	88
7.	Average tube length in microns.	708	1557	1180	1288
8.	Maximum tube length in microns.	1273	1995	1287	1353

Table 1: In vivo pollen germination on stigma

Pollen storage- The longevity of the pollen is governed by a number of factors. Temperature, Relative Humidity, light and the time of blooming govern the longevity of the pollen. Of special significance temperature & relative humidity and their effects are interdependent. Here, in the present study, the storage of pollen grains was studied using different parameters such as providing the different temperature as well as the relative humidity. Pollen storage in different organic solvents at 4°C was also studied as a different parameter.). Low temperatures are generally found suitable for long term storage. At room temperature & 100% RH pollen lost viability within 24 hrs. Relative humidity between 0 – 20% had been reported to retain viability for several days. In the light of at available evidences, it is obvious that temperature alone cannot be the ideal storage condition, unless coupled with suitable levels of relative humidity.

The artificial maintenance of the viability and fertilizing ability of pollen over a long period is an important problem from both the theoretical and practical point of view. The pollen longevity of different species varies between minutes and years depending primarily on the taxonomic status of the plant & abiotic environmental conditions.

The pollen of Cleome viscosa was subjected to various RH levels like,0%,10%,20%,30% and 100% RH on room temperature (32ºC) as well as on freeze temperature (4 ºC). Their storability was checked by germinability of pollen grains by germinating in artificial growth media in which they showed maximum germination in vitro. Here in the present study 15% sucrose solution was used as a basal medium.

Cleome showed good storage results from 0 %RH to 100% RH in both the condition at room temperature as well as on freeze temperature. On the room temperature, the storage days vary from 57 days to 2 days in 0 % RH to 100% RH respectively. In 0% RH 57 day's storage was recorded with 94 percent germination and 945 micron tube length. In 30% RH 17 days viability was recorded. In 100 % Rh pollen can be stored up to 2 days with 5 percent germination and 40 microns tube length. On freeze temperature, here also in all the RH level, pollen of Cleome showed, viability ranging from 64 days to 4 days. In 0% RH 64 day's storage was recorded with 95% germination and 1123 micron tube length. In 10% RH 52 days with 68% germination and 943 microns length and 36 days storage with 46% germination and 465 microns tube length observed (Table 2).

No. of days storage	Temp.	% of relative humidity									
		0% RH		10% RH		20% RH		30% RH		70% RH	
		% of Ger.	PT (□)	% of Ger.	PT (□)	% of Ger.	PT (□)	% of Ger.	PT (□)	% of Ger.	PT (□)
2	Room	94	945	63	845	38	521	40	348	05	40
	Freeze	95	1123	68	945	46	465	33	175	13	99
4	Room	87	735	56	635	23	463	23	129	--	--
	Freeze	89	1085	61	813	37	325	26	93	03	27
15	Room	63	779	43	670	18	379	15	45	--	--
	Freeze	68	978	53	735	30	270	12	56	00	00
17	Room	65	693	45	439	15	314	03	12	--	--
	Freeze	63	735	56	625	28	185	08	36	--	--
21	Room	48	678	36	348	09	39	01	08	--	--
	Freeze	59	539	52	420	23	122	02	18	--	--
22	Room	52	563	30	273	01	11	00	00	--	--
	Freeze	56	598	44	329	15	89	00	00	--	--
30	Room	45	439	22	118	00	00	00	--	--	--
	Freeze	49	440	49	269	08	65	--	--	--	--
36	Room	32	375	11	90	--	--	--	--	--	--
	Freeze	45	332	35	149	03	14	--	--	--	--
42	Room	36	239	05	25	--	--	--	--	--	--
	Freeze	37	275	28	117	00	00	--	--	--	--
48	Room	22	114	00	00	--	--	--	--	--	--
	Freeze	24	158	12	42	--	--	--	--	--	--
52	Room	11	89	--	--	--	--	--	--	--	--
	Freeze	17	93	04	36	--	--	--	--	--	--
57	Room	03	21	--	--	--	--	--	--	--	--
	Freeze	13	49	00	00	--	--	--	--	--	--
64	Room	00	00	--	--	--	--	--	--	--	--
	Freeze	02	25	00	00	--	--	--	--	--	--
66	Room	00	00	--	--	--	--	--	--	--	--
	Freeze	00	00	--	--	--	--	--	--	--	--

Table 2: Effect of Temperature and Relative Humidity on Storage of pollen grain

Pollen viability in various organic solvents was studied, like Benzene, Isopropyl alcohol, Chlorophyll, Acetone and Xylene. In the present investigation Xylene was proved to be a bad solvent for storing the pollen grains. Isopropyl alcohol and chloroform showed maximum viability i.e. 48 and 59 days Overall these solvents appear to be good for storing pollen for short term.

	Organic solvent	Viability (in days)	Maximum percent of germination	Maximum tube length (in μ)
1	Benzene	65	95	1305
2	Isopropyl alcohol	48	88	865
3	Chloroform	59	91	958
4	Acetone	61	83	1116
5	Xylene	18	62	356

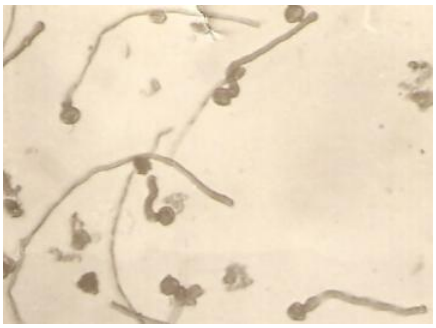
Table 3: Pollen viability of Cleome viscosa in different organic solvents

The major cause of the loss of viability during storage appears to be the deficiency of metabolites due to the continued metabolic activity of the pollen is going on at the much reduced rate had found that a higher moisture & temperature level reduces pollen quality by increasing metabolic rates & promoting microbial activities. Pollen storage conditions that maintain fertility increases the efficiency of handling breeding & genetics material of any plant species.

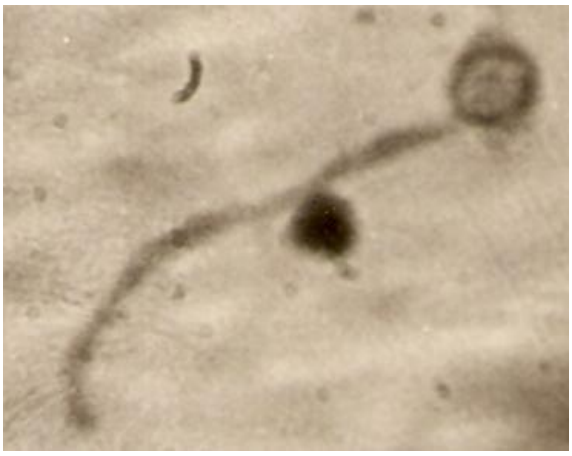
Free amino acids estimation- Although the amino acids contained in pollen grains have been studied as a part of pollen pathology here we studied from the pharmacological point of view. Changes in free amino acid pattern and amino acid composition of pollen grains were recorded while studying pollen chemistry. Free amino acids are always found in relatively large amount. Altogether 14 different free amino acids were separated and identified by chromatographic methods.

Protein estimation- Proteins are the essential metabolic substance for activation of pollen, following germination were accompanied by the initiation of protein synthesis. The localization of proteins and enzymes has shown that the proteins occur most prominently in the cellulosic intine near the pore & in the cavities of exine. Protein synthesized or activated in the germination stage of pollen tube are apparently required for the early stage of tube development. 30.12% total pollen protein was estimated spectrophotometrically.

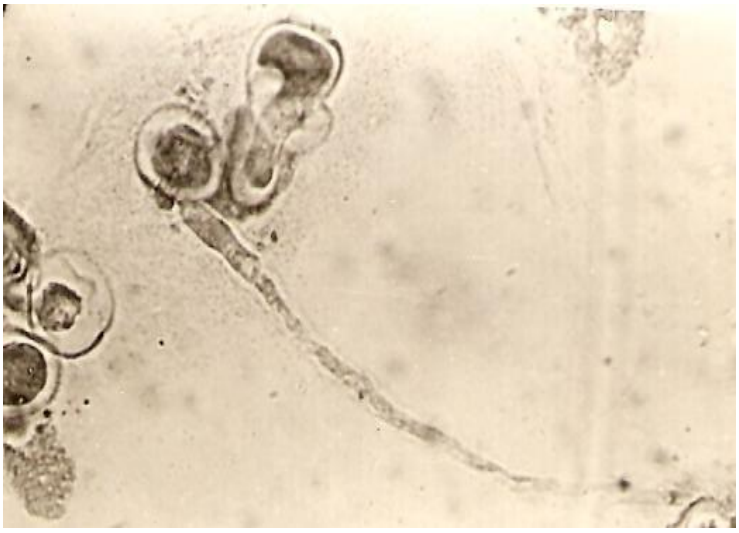
Histochemical studies of germinated pollen grains- In the light of the present work of Histochemical analysis of these anticancerous plants, it can be said that pollen is equipped itself at the time of shedding to carry out, its metabolism during the active phase of germination as evident from rich localization of reserve metabolites. It is also apparent that the pollen tube is the site for active metabolism in the tube.



Polysaccharides early stage



Pectic substances late



Pollen grain and pollen tube with ascorbic acid after five hours

Cytochemical substances	Time Interval (in hours)	Pollen grain	Pollen wall	Pollen tube content		Pollen tube wall
				Lower half	Upper half	
polysaccharides	1	Present	Positive	Rich	Rich	Positive
	2	Rich	Positive	Rich	Rich	Positive
	3	Rich	Positive	Present	Rich	Positive
	4	Intense	Positive	Present	Rich	Positive
	8	Intense	Positive	Present	Present at Tip.	Positive
Cellulose	1	Absent	Negative	Absent	Absent	Negative
	2	Absent	Negative	Absent	Absent	Negative
	3	Absent	Negative	Absent	Absent	Negative
	4	Absent	Negative	Absent	Absent	Negative
	8	Absent	Negative	Absent	Absent	Negative
	1	Absent	Negative	Absent	Absent	Negative

Cytochemical substances	Time Interval (in hours)	Pollen grain	Pollen wall	Pollen tube content		Pollen tube wall
				Lower half	Upper half	
Starch	2	Absent	Negative	Absent	Absent	Negative
	3	Absent	Negative	Absent	Absent	Negative
	4	Absent	Negative	Absent	Absent	Negative
	8	Absent	Negative	Absent	Absent	Negative
Pectic substances	1	Rich	Positive	Present	Present	Positive
	2	Rich	Positive	Absent	Absent	Positive
	3	Present	Positive	Absent	Absent	Positive
	4	Present	Positive	Absent	Absent	Positive
	8	Present	Positive	absent	Absent	positive
Lignin	1	Absent	Negative	absent	Absent	Negative
	2	Absent	Negative	absent	Absent	Negative
	3	Absent	Negative	absent	Absent	Negative
	4	Absent	Negative	absent	Absent	Negative
	8	Absent	Negative	absent	Absent	Negative
Lipids	1	Intense	Positive	Rich	Rich	Positive
	2	Intense	Positive	Rich	Rich	Positive
	3	Rich	Positive	Rich	Rich	Positive
	4	Rich	Positive	Rich	Present	Negative
	8	Rich	positive	Rich	Present	Negative
Protein	1	Rich	positive	Rich	Rich	Positive
	2	Rich	Positive	Rich	Rich	Positive
	3	Rich	Positive	present	Present	Negative
	4	Rich	Positive	present	Present	Negative
	8	Rich	Positive	present	present	Negative
DNA	1	Rich	Positive	Absent	Absent	Negative
	2	Rich	Positive	Absent	Absent	Negative
	3	Rich	Positive	Absent	Absent	Negative
	4	Rich	Positive	Absent	Absent	Negative
	8	Rich	Positive	Absent	Present in few tubes as dark spot in tip.	Negative
	1	Present	Positive	Absent	Absent	Negative

Cytochemical substances	Time Interval (in hours)	Pollen grain	Pollen wall	Pollen tube content		Pollen tube wall
				Lower half	Upper half	
RNA	2	Present	Positive	Absent	Absent	Negative
	3	Present	Positive	Absent	Absent	Negative
	4	Present	Positive	Absent	Absent	Negative
	8	Present	Positive	Absent	Absent	Negative
Ascorbic acid	1	Rich	Negative	Present	Present	Negative
	2	Rich	Negative	Present	Present	Negative
	3	Rich	Negative	Present	Present	Negative
	4	Rich	Negative	Present	Present	Negative
	8	Rich	Negative	Present	Present	Negative

Table 4: Distribution of cytochemical substances in germinated pollen of Cleome viscosa.

III. CONCLUSIONS

We can say that there are many opportunities regarding the Palynological work of medicinal plants;

i) The pollen is used as a convenient experimental system in genetic investigations, directed towards plant improvement. Pollen are irradiated to induce desired mutational variations and to overcome intraspecific incompatibility or to remove other fertilization barriers.

ii) The direct and indirect roles played by pollen in various spheres of applied biological research will be found useful in view of the fact that pollen is a material to work which seems to be providing an easier and even better means for experimentally controlling the genetic behaviors of the plants.

iii) In the eye of biochemist the pollen is a sac full of enzymes and substrates, locked up in compartments and filled with some types of cell organelles. In fact, if one carefully looks, one can find in pollen of different plant species nearly all physiologically important classes of substances, not only carbohydrates, proteins but all types of lipids, growth hormones, vitamins, pigments, sterols etc.

iv) India, with its vast biodiversity and potential for commercial exploitation, could become a world leader in the supply of raw material for the phytopharmaceutical industry.

v) By drawing up a comprehensive strategy for the cultivation and conservation of medicinal plants in league with the forest department, many threats outlined earlier could turn into opportunities for successful commercial exploitation without tampering with the interest of the communities involved in the collection of plants.

vi) India is among the traditional producer and exporter of several medicinal plants. Lack of basic information on different parameter of crop productivity is a limiting factor in this group of plants. There is therefore need for intensive agricultural studies leading to the genetic improvement and cultivation methods for expansion of area under medicine and medicinal plants.

vii) Plant germplasm resources are one of the most important renewable natural resources of the world. Increasing exploitation coupled with natural calamities has led to rapid dwindling of important plant species. Nearly 20,000 to 25,000 species of vascular plants are currently facing threat their existence. This necessitates urgent measures to conserve the plant wealth of ecosystem, species and gene pool levels, to

enable sustained use for present and future generations by establishing pollen banks and germplasm resources centers.

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