I J R B A T, Issue (XIII) Volume (I) Jan 2025: 5-11
A Double-Blind Peer Reviewed & Refereed Journal
OPEN ACCESS
e-ISSN 2347 – 517X
Original Article
INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
 www.ijrbat.in

EFFICACY OF ORGANIC SOLVENTS FOR STORING POLLEN GRAINS OF SOME MEDICINAL PLANTS

Jayshree Thaware
Department of Botany, S.K.Porwal College of Arts and Science and Commerce, Kamptee Dist-Nagpur
Corresponding Email :- jsthaware@gmail.com

Communicated :11.10.2024 | Revision: 21.10.2024 & 19.11.2024 Accepted: 23.11.2024 | Published: 30.01.2025

ABSTRACT:
Horticulturists and plant breeders have long been interested in crossing varieties species and even genera to produce new and improved types of plants better suited to human requirements. The pollen being a hereditary component is predominant in crop production programs. The disharmony in the flowering of the parent plants and their separation by distance are among the several problems faced during hybridization. In such a situation, pollen storage is a tool in a hand of plant breeders to preserve the viability of pollen and using them as per their need. In the present study, the efficacy of organic solvents like Benzene, Isopropyl alcohol, Chloroform, Acetone and Xylene for storing pollen grains of *Catharanthus, Allamanda, Datura, Brassica, Raphanus* and *Cleome* was checked. All the plants have many medicinal uses also they have potent anticancer properties in common has been assessed and compared with pollen stored under controlled temperature and humidity conditions. The results suggest that the use of organic solvents holds some promise only for short-term and not long-term storage. Xylene was not suitable for storing the pollen grains.

Keywords: - Pollen storage, Horticulturists, Organic Solvents, Humidity, Xylene.

INTRODUCTION :

Systematic research on pollen storage started at the end of the 19th century. There is a large number of crop species including vegetables, fibre and fruit crops forage and cereals for which pollen storage strategies are desirable. Genetic conservation through pollen storage is desirable for a variety of horticultural plant species. Since pollen is known to transmit important genetically heritable characteristics.

The life span of different pollen species varies between a few minutes to several years. Corn, Wheat, Barley and other grasses, pollen survives maximally for 24 hours, and pollen from deciduous fruit trees remains viable for a few days. Whereas pollens from wind-pollinated trees, like pine and dates can be stored for years or more. The viability of pollen depends on factors such as Humidity, Temperature, and Composition of the Gas of the atmosphere and may be modified by the nutritional side of the pollen-producing plant, virus infection or other pathogens. In general, pollen has a high degree of drought resistance, which could be explained by its high content of proline, an amino acid (Linsken, 1987).

Successful pollen storage is a very convenient tool in the hands of the breeders for improving trees by hybridization, occurring in different regions and also of those blooming in different seasons. The longevity of pollen grains enables the introduction of new characteristics in plants by using stored living pollens.

The humidity of the air and the temperature affect the storage of pollen grains of different plant species; where success has been obtained in storage through manipulation of temperature and humidity. It is a universal fact that the pollen of several species remains viable for longer periods at lower temperatures than at higher temperatures, and maximum longevity is obtained at low relative humidities (0-30%) (Rangari, 2008).

Pollen storage is the most efficient method to overcome barriers to hybridization between plants flowering at different times and or growing

http://doi.org/10.29369/ijrbat.2025.010.1.0002

in different regions as the pollen with this technique will be available whenever the female parent flowers. The most common method employed for pollen storage is the manipulation of temperature and humid conditions. Yet another method with considerable success is the storage of pollen at ultra-low temperatures by using dry ice (- 80˚ C), Liquid air (-180˚ C) or liquid nitrogen (- 190˚ C) However, both freeze-drying and storage at ultra-low temperatures are cumbersome and requires the use of sophisticated instruments. Thus, there has been a need for a simpler and more effective method for routine use. One such method which has shown some promise is that of storing pollen grains in organic solvents.

It was first demonstrated by Iwanami (1972 a, b) and Nakamura (1972) first demonstrated the use of different organic solvents in pollen storage. The organic solvents include benzene, petroleum, diethyl ether, acetone, chloroform etc. whose efficiency varies greatly for different plant species. Pollen grains stored in non-polar organic solvents like benzene, diethyl ether and cyclohexane retained viability and showed very little leaching of phospholipids, sugars and amino acids into the solvent. On the other hand, extensive leaching of substance and loss of viability was seen in polar organic solvents (Jain and Shivanna, 1989, 1990), thus establishing a correlation between the polarity of the solvents and their potency for pollen storage. Pollen grains stored in organic solvents have been shown to retain their fertilizing ability also (Thaware and Saoji, 2017). Analysis of the results of investigations done so far indicates that the organic solvents are potential only in short-term pollen storage. (Mishra and Shivanna, 1985).

MATERIAL AND METHODS :

a) Pollen Collection

Pollen grains of *Catharanthus roseus, Datura metel* and *Allamanda cathartica* were collected from the fresh flowers. The above said all plants have a unique property that the anthers get dehisced in the fully mature bud. Another convenient method for pollen collection was to excise mature anthers (just before anthesis) and allow them to dehisce under low humidity in desiccators. Anther debris is then removed by brush or forceps. These methods for collection of pollen grains in the laboratory were very convenient and economical because the loss of mass of pollen due to external factors gets minimised and the yield of pollen is enhanced.

In the present study the species like *Brassica juncea, Raphanus sativus* and *Cleome viscosa* it was convenient to excise inflorescence or flowering twigs the previous evening and it was kept overnight in the laboratory with their cut ends dipped in the water. By the following day the fully matured bud get opened and the anthers get dehisced and a gentle tap on such flower held over a watch glass sheds the pollen by this procedure it was ensured the availability of pollen irrespective of any change in weather conditions such as wind and rain in the previous night. The excised inflorescence of *Brassica* and *Raphanus* continued to produce fresh flower and viable pollen grains for 3 days. In case of *Cleome* the fresh pollen was there for 2 days. The pollen grains collected from this method immediately used in the storage experiments.

b) Storage and Preservation

Storage of pollen grains in organic solvent is probably the simplest method so far, the storage of pollen in organic solvents given by Iwanami (1962, 1971). The pollen grains were collected, in the morning time just after the dehiscence of anthers. The collected pollen grains were then dried over silica gel at 0°C, then 5ml of each solvent benzene, isopropyl alcohol, chloroform, acetone and xylene was taken in the glass vials separately, plugged tightly and kept in an ice container of refrigerator which has a temperature near about 4˚ C.

c) Prehumidification of the stored pollen

http://doi.org/10.29369/ijrbat.2025.010.1.0002

The stored pollen grains were removed from the glass vials at an interval of 48 hrs. with glass dropper on a slide. The organic solvent left on the slide was allowed to evaporate. The slides were then kept in a Petri dish lined with wet filter paper for humidification before culturing the pollen grains.

d) Viability test

The viability of the hydrated stored pollen was tested *in vitro* by culturing the pollen grains in standardized solution in which the different pollen grains shows maximum germination *in vitro* (Rangari,2008) by 'sitting drop technique'. For *Catharanthus, Allamanda* and *Brassica* 15% sucrose solution, For *Datura* Brewbaker's medium, for *Raphanus* and *Cleome* 20% sucrose solution was used to check the viability of stored pollen grains as these medium gave best results *in vitro* experiments. The % of germination and the viability of pollen in day and also in hours were monitored under the microscope.

RESULTS :

Effect of organic solvents on the viability of pollen grains of some medicinal plants pollen, in different organic solvents such as benzene, isopropyl alcohol, chloroform, acetone, and xylene at 4 ° C are given as follows;

I) *Catharanthus roseus L (G.) DON*

It was observed that the Pollen grain stored in Benzene refer could maintain their viability only for 35 days. There maximum germination of 70% and Maximum tube length 795 µm was observed. After 30 days of storage the viability declining. Isopropyl alcohol was found to be a good solvent for storage because in it pollen remained viable for 40 days but the % of germination was very less on 40th days i.e. 12% with maximum tube length 96.01 µm. The maximum germination 80 % and maximum tube length 550 µm was observed on 20th day of storage. Chloroform also found to be good solvents of storage of pollen as it could maintain 80% germination for 24 day. The germination % declining on 34th day .In acetone pollen grains was viable for 50 days with maximum length of 935 µm, after 30 days the viability starts declining. Xylene found to be very weak organic solvent as it only gives viability of 11 days with only 35% of germination with maximum length of 203 µm (Table 1; Graph 1 and 2).

II) *Allamanda cathartica Linn. var. grandiflora*

Allamanda pollen showed maximum viability of 42 days with maximum percentage germination in Chloroform with maximum tube length of 859 µm. Benzene showed 32 days viability with maximum tube length of 792 µm. 732 µm length was found in Isopropyl alcohol with maximum germination of 79%.the least maximum percent of germination was found in Xylene i.e. 35 with 13% viability and 228 µm length. In Acetone 26 days viability was recorded with 65 % of germination with maximum tube length of 697 µm (Table 2; Graph 1 and 2).

III) *Datura metel Linn. var fastuosa*

In case of ***Datura***, maximum viability was found of 45 days with maximum tube length of 360 µm with 52% germination in Chloroform. The maximum tube length was found in Benzene which was 405 µm. In case of Isopropyl alcohol, Viability was found to be 41 days with maximum pollen maximum tube length of 367 and maximum germination of 38 %. 255 µm Maximum tube lengths were found in Acetone which showed 38 days viability with 28 % of germination. Xylene showed least maximum percent of germination of 28 with only 8 days of viability with maximum tube length of 96 µm (Table 3; Graph 1 and 2).

IV) *Brassica juncea Linn Czern. and Coss*

The maximum pollen maximum tube length and highest maximum percent of germination was found in Chloroform which was 284 µm and 25 days respectively. Benzene showed 23 days viability with 34% of germination and maximum tube length of 246 µm. In case of Isopropyl

http://doi.org/10.29369/ijrbat.2025.010.1.0002

alcohol, 168 µm. maximum tube lengths were found with maximum 26 % germination. In Xylene only 6 days viability was found with 102 µm maximum length. Acetone showed 17 days viability with 31 % of germination and maximum tube length of 178 µm (Table 4; Graph 1 and 2).

V) Raphanus sativus Linn.

In case of *Raphanus*, Acetone showed 10 days viability with 14% of germination with maximum pollen maximum tube length of 133 µm. Benzene showed13 days viability with 23 % of germination with maximum tube length of 534 µm. Isopropyl alcohol showed only 8 days viability with 18 % germination and maximum tube length were 423 µm.8 days viability was also found in Chloroform showing 21 % of germination and 396 µm length. Here also, in case of Xylene, only 5 days pollen found viable with 5% of germination and 76 µm. maximum tube lengths (Table 5; Graph 1 and 2).

***VI) Cleome viscosa* Linn.**

Cleome pollen showed good viability and maximum tube length in all the organic solvents except Xylene. The maximum viability was found in case of Benzene, which was 65 days with maximum germination of 95 % with maximum length of 1305 µm. In Isopropyl alcohol, 88 % of germination was found with maximum tube length of 865 µm with 48 days viability. 59 days of viability was found in case of Chloroform with maximum percent of germination of 91 % and maximum pollen maximum tube length of 958 µm. Acetone showed 61 days of viability and 83% of germination with 1116 µm maximum length of pollen tube. Xylene showed the least maximum percent of germination of 62 and viability of 18 days (Table 6; Graph 1 and 2).

All the plants studied over here have anticancer properties in common.

Pollen grains stored in organic solvents, which maintained viability, showed very little leaching of sugars, free amino acids and phospholipids into the solvents (Jain and Shivanna 1987a, b), whereas the solvents which did not retain viability resulted in extensive leaching of these components. Thus, organic solvents favourable for storage do not remove membrane phospholipids; and consequently do not permit leakage of cyto¬plasmic components and maintain pollen viability.

Irrespective of the storage conditions, there was a positive and significant correlation between the loss of membrane integrity (indicated by the reduc¬tion in total and individual phospholipids, particularly phosphotidyl choline) in the stored pollen and loss of pollen viability (Shivanna and Tondon, 2020). In the light of these studies, deficiency of respiratory substrates or inactivation of enzymes/growth substances as causal factors for the loss of pollen viability is no more tenable.

CONCLUSION:

In present study, it is found that lower the storage temperature longer is the pollen viability. Xylene is not suitable for storing the pollen grains. We can conclude that, the viability of pollen grains stored in organic solvents seems to be determined largely by the effect of the organic solvents on pollen phospholipids composition, which in turn affects membrane integrity and consequently pollen viability.

REFERENCES:

Iwanami, Y. (1972 a) Viability of pollen grains in organic solvents. Botanique 3: 61 – 68.

Iwanami, Y. (1972 b) Retaining the viability of Camellia japonica pollen in various organic solvents. Plant cell. Physiol. 13: 1139 – 1141.

Iwanami, Y. and Nukamura, N. (1972) Stain Technol. 47: 137 – 139.

Jain, A. & Shivanna K. R. (1990) Membrane state and pollen viability during storage in organic solvents. Proceedings of International Congress Plant Physiology, New De lhi In: Plant physiol & Biochem. S. K. Sinha, P. V. Sane, S. C. Bhargava, & P. K. Agrawal (Eds.) pp – 1341 – 1349

http://doi.org/10.29369/ijrbat.2025.010.1.0002

Jain, A. and Shivanna K. R. (1989) Loss of viability during storage is associated with changes in membrane phospholipids Phytochemistry. 28:999-1002.

Rangari, J. (2008) Palynological studies in plants with anticancer properties. Ph.D.thesis, RTMNU, Nagpur.

Shivanna, K.R. and Tondon, R. (2020) Developmental biology of dispersed pollen grains. Int. J. Dev. Biol. 64: 7-19.

Thaware, J and Saoji A. (2017) Pollen storage studies in Brassica juncea L.Czern and Coss. IJRBAT Special Issue (2) Volume IV : 990-995.

https://www.biologydiscussion.com/palynology/pollen-storage-in-plants-methods-and-significance/64594

http://doi.org/10.29369/ijrbat.2025.010.1.0002

Table 1: Pollen viability of *Catharanthus roseus* pollen in different organic solvents

	Organic solvent	Viability (in days)	Maximum percent of germination	Maximum tube length (in µm)
1	Benzene	35	70	795
2	Isopropyl alcohol	40	80	550
3	Chloroform	24	80	508
4	Acetone	50	83	935
6	Xylene	11	35	203

Table 2: Pollen viability of *Allamanda cathartica* in different organic solvents

	Organic solvent	Viability (in days)	Maximum percent of germination	Maximum tube length (in µm)
1	Benzene	32	88	792
2	Isopropyl alcohol	39	79	732
3	Chloroform	42	92	859
4	Acetone	26	65	697
5	Xylene	13	35	228

Table 3: Pollen viability of *Datura metel* in different organic solvents

	Organic solvent	Viability (in days)	Maximum percent of germination	Maximum tube length (in µm)
1	Benzene	32	46	705
2	Isopropyl alcohol	41	38	867
3	Chloroform	45	52	935
4	Acetone	38	28	855
5	Xylene	8	19	196

Table 4: Pollen viability of *Brassica juncea* in different organic solvents

	Organic solvent	Viability (in days)	Maximum percent of germination	Maximum tube length (in µm)
1	Benzene	23	34	246
2	Isopropyl alcohol	18	26	168
3	Chloroform	25	28	284
4	Acetone	17	31	178
6	Xylene	6	16	102

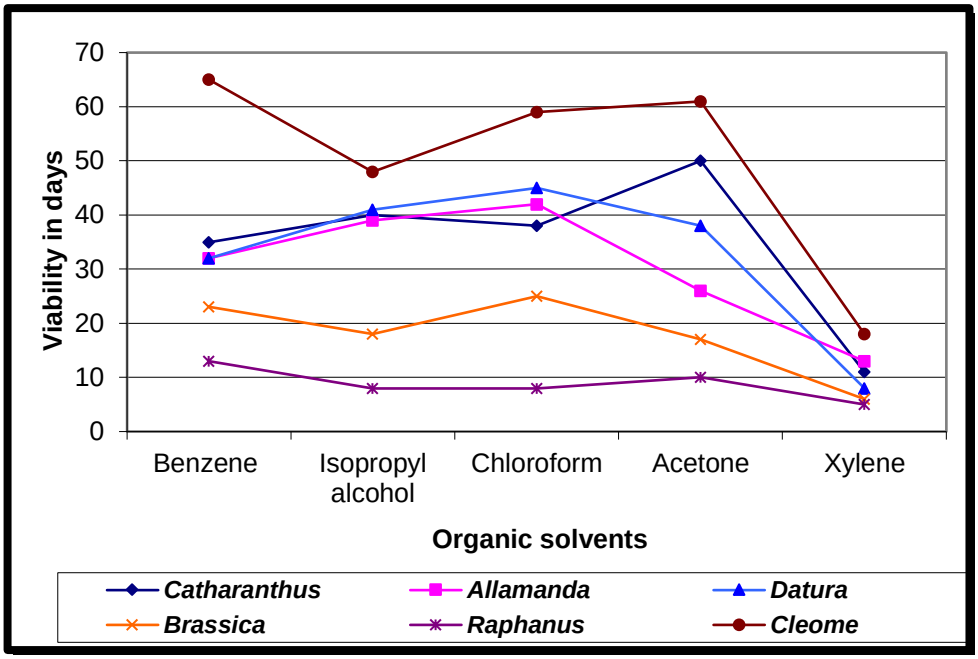
Table 5 : Pollen viability of *Raphanus sativus* in different organic solvents

	Organic solvent	Viability (in days)	Maximum percent of germination	Maximum tube length (in µm)
1	Benzene	13	23	534
2	Isopropyl alcohol	8	18	423
3	Chloroform	8	21	396
4	Acetone	10	14	133
6	Xylene	5	5	76

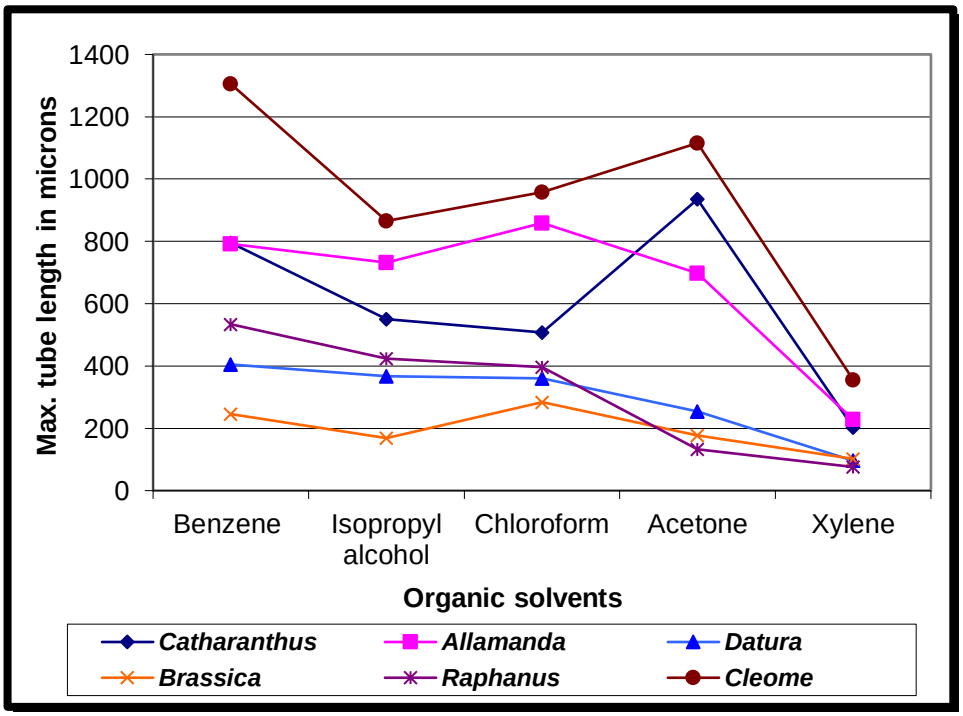
Table 6 : Pollen viability of *Cleome viscosa* in different organic solvents

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

http://doi.org/10.29369/ijrbat.2025.010.1.0002



Graph 1: Pollen viability (in days) in different organic solvents of medicinal plants



Graph 2: Max. tube length of pollen (in µm) in different organic solvent

http://doi.org/10.29369/ijrbat.2025.010.1.0002