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POLLEN VIABILITY STATUS IN SELECTED MEDICINAL PLANTS OF APOCYNACEAE FAMILY

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ABSTRACT
Information on pollen viability is a pre-requisite for carrying out a meaningful crop improvement programs. The present investigation on pollen viability was performed in the Department of Biological Sciences, M.G.C.G.V., Chitrakoot, Satna (M.P.). Five medicinal plants of Apocynaceae family (i.e. *Thevetia peruviana*, *Nerium indicum*, *Catharanthus roseus*, *Carissa carandus* and *Rauvolfia serpentina*) were used for the estimation of pollen viability. Acetocarmine staining method was applied to study the pollen viability. The average pollen viability was varied from 66% to 87%. The highest percentage (87%) of viable pollens was recorded in *Thevetia peruviana* which revealed that this genus has a normal meiosis and the lowest percentage of pollen viability (66%) was obtained in *Rauvolfia serpentina*. Among the selected species, *Thevetia peruviana*, *Catharanthus roseus* and *Carissa carandus* are distinguished for high pollen viability and can be successfully used as male parents in hybridization.

KEYWORDS: Pollen, Viability, Apocynaceae, Acetocarmine, Chitrakoot.

INTRODUCTION
The family Apocynaceae is the most important and largest families of angiosperm. The Apocynaceae family consists of approximately 5100 Species included in over 375 Genera.[1] It is a valuable family of flowering plants which contains herbs, shrubs and trees and commonly known as the dogbane or oleander family. Many species of the family are tall shrubs or trees found in tropical forests but some occur in tropical dry environments also.[2] The plants of the family Apocynaceae are native to India, China, Bangladesh, Pakistan and Sri Lanka.[3] Many species of the family have milky latex and some species are poisonous if ingested. The family carries significant importance in the field of medicine because of its species are used traditionally to treat various diseases such as diarrhea, diabetes, malaria, skin diseases and cancer chemotherapy.[4] Some selected medicinally important species of family Apocynaceae are as follows-

Catharanthus roseus commonly known as Sadabahar or Periwinkle is very popular for its high medicinal value and as one of the richest source of alkaloids.[5] It is originated from the islands of Madagascar but now it is occurred in almost every tropical and sub tropical parts of the world.[7] Alkaloids of the species *Catharanthus roseus* have a great medicinal importance for treating diabetes, asthma, malaria and menstrual problems. The leaf extract of the plant also applied to treat cancer and hypertension.[8]

Thevetia peruviana commonly known as Pili kaner or yellow oleander is an evergreen poisonous shrub and grown as an ornamentals in the parks and gardens.[9] It is indigenous to Mexico, South and Central America and naturally distributed in the tropical and sub tropical regions of the world containing Sri Lanka, Australia, India and China. In India, *Thevetia peruviana* is widely distributed throughout Gujarat, Delhi, West Bengal, Bihar, Uttar Pradesh, Madhya Pradesh and Rajasthan. Flowers and leaves are applied to cure malaria and asthma. The leaf extract of the species *Thevetia peruviana* is also applied for treating jaundice and intermitted fever.[11,12]

Nerium indicum generally known as kaner or Indian oleander is an evergreen shrub with milky latex.[13] It is distributed around the Philippines, Nepal, India and Bangladesh and now grown to produce flowers just as medicinal purposes throughout India, Japan and China. It is normally cultivated for its sweet smell of flowers in schools, gardens, homes, churches and road sides. It is useful in treating heart conditions, painful menstrual periods, asthma, leprosy, epilepsy, malaria, ringworm and venereal diseases.[14,15]

Rauvolfia serpentina commonly known as sarpgandha or Indian snake root is a valuable medicinal plant for treating hypertension.[16] It is native to tropical and sub tropical areas of the world, including Africa, Europe, Central and Southern America, Asia, Australia, India, Bangladesh, Malaysia and Sri Lanka.[17] It is widely grown in India's sub-Himalayan zone which stretches from Punjab to Sikkim and Bhutan. The roots of *Rauvolfia serpentina* have been used since the pre-Vedic period as a drug in India to treat fever and snake bites. Diarrhea, painful affections of bowels, dysentery and cholera have all been treated with the root extracts.[18]

Carissa carandus also known as karonda or christ's thorn is a common medicinal plant.[19] It is distributed all over India, Pakistan, Sri Lanka, Bangladesh, Java, and Nepal, in both tropical and sub tropical regions.[20] In India it is cultivated in Rajasthan, Madhya Pradesh, Maharashtra, Bihar, Orissa, West Bengal, Chhattisgarh, and Gujarat.[21] The leaves, fruits, roots and barks of *Carissa carandus* have also been used for herbal medicine to treat various diseases such as anorexia, diarrhea, malaria, intermittent fever, headache, cough, mouth ulcers and epilepsy.[19,22]

Pollen viability refers to a pollen grain's ability to deliver male gametes to a female gametophyte. This function varies from species to species after releasing of pollen from anther and its validity is used to determine its consistency. If pollen kept dry, it can retain its value for a long time.[23] Similarly Navel and Rulty (1937)[24] reported that pollen has the capacity of compatible fertilization even after long period of storage. Basic knowledge on the meiotic activities and assessment of pollen viability are useful for germplasm characterization and detection of biodiversity, genetic variability and evolutionary processes of the organisms.[25] Various exogenous and endogenous factors such as low temperature (15°C),[26] high temperature (40°C),[27] the flower's growth stage,[28] nutritional value of the plant,[29] agricultural pesticides and other chemicals[30,31] can affect pollen viability during plant maturation. Pollen viability which can be measured in-vitro using a test of pollen viability is very essential for fruit and seed development in flowering plants. Therefore, the pollen viability information for any species is important for both plant breeders and commercial growers.[32]

MATERIAL AND METHODS

This research work was conducted at M.G.C.G.V.'s Department of Biological Science, Chitrakoot, Satna (MP) from January 2019 to August 2019. To study the pollen viability of selected members of Apocynaceae family, flowers or flower buds were collected in the morning (from 05:00 AM to 07:00 AM), instantly after anthesis, and kept at high humidity in a closed vessel with a wet paper towel to avoid pollen dehydration. Flower buds were fixed in Carnoy's solution in the ratio of 3:1 ethanol: glacial acetic acid for 24 hours at room temperature and stored in 70% alcohol in a freezer at 3°C.

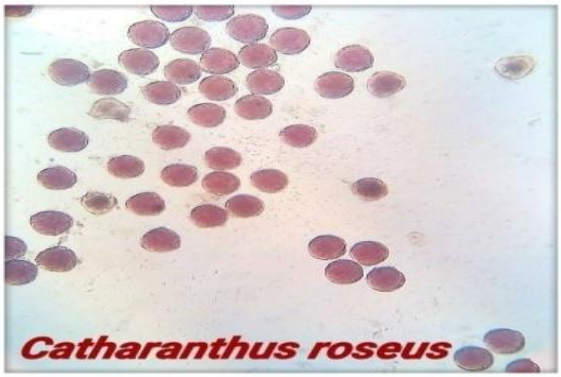
The pollen viability of selected medicinal plants of family Apocynaceae was determined by using acetocarmine staining process. The fresh anthers were crushed and the pollen from mature anthers were mixed in 1-2 drops of acetocarmine. Four slides were prepared for each selected medicinal plant and for each prepared slide five randomly selected areas were analyzed in an optical microscope. To determine the pollen viability, deeply stained or normal looking pollens were recorded as fertile or viable (it has the capacity to germinate) and colorless or shriveled pollen grains were regarded as non viable or sterile (it has no capability to germinate).

Formula used

$$\% \text{ Pollen viability} = \frac{\text{Number of viable pollen grains}}{\text{Total number of pollen grains analysed}} \times 100$$

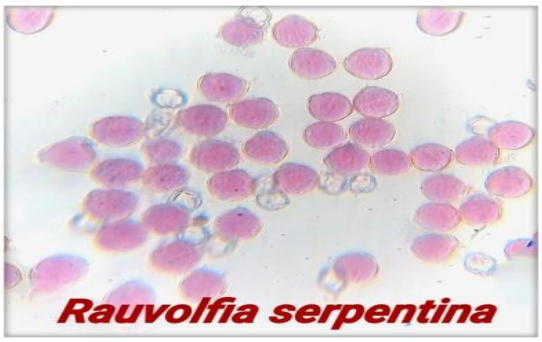
RESULTS AND DISCUSSION

Viability of pollen grains is essential for the vitality of the plant species and also for the efficient sexual reproduction. From a theoretical as well as practical standpoint, pollen viability estimates may be highly essential in some circumstances. The pollen grains produced by the selected medicinal plants are of high quality and their fertility (viable or fertile pollen grains) is greater than sterility (non viable or sterile pollen grains).[33] Pollen viability results obtained using acetocarmine method in selected medicinal plants of Apocynaceae family are presented in table 1 and illustrated in figure 1. The obtained results presented in table 1 reveals that pollen viability of selected medicinal plants varied significantly; when the pollen grains were stained with acetocarmine. The average pollen viability was varied from 66% to 87%. Similar observations have also been made by Bhat and Kudesia[34] and Singh et al[35] in different species of Solanaceae family. The percentage pollen viability was 87% for *Thevetia peruviana*, 83% for *Catharanthus roseus*, *Carissa carandus* had 82%, while *Nerium indicum* had 78% and *Rauvolfia serpentina* had lowest 66% pollen viability.



(A)

(B)



(C)

(D)

(E)

Figure 1: Showed viable (darkly stained) and non viable (light stained or empty) pollen grains of selected medicinal plants of Apocynaceae family (A). *Catharanthus roseus* (B). *Carissa carandus* (C). *Rauvolfia serpentina* (D). *Thevetia peruviana* (E). *Nerium indicum*.

The data obtained from this study indicated that the maximum pollen viability (87%) was observed in *Thevetia peruviana* followed by *Catharanthus roseus* (83%) and *Carissa carandus* (82%). Minimum percentage of pollen viability was obtained in *Rauvolfia serpentina* (66%). The pollen viability status of these selected medicinal plants tested was high. Data on pollen viability and pollen grain production are basic for reproductive biology and genetics breeding of these selected medicinal plants, ensuring safer cross breeding to develop new hybrids and increasing fertility.[36]

Table 1: Showing percentage pollen viability of selected medicinal plants of apocynaceae family.

S. No.	Local name	Botanical name	No. of pollen grains examined	No. of viable pollen grains	No. of non viable pollen grains	Percentage of pollen viability
1	Pili Kaner	*Thevetia peruviana*	100	87	13	87%
2	Sadabahar	*Catharanthus roseus*	100	83	17	83%
3	Karonda	*Carissa carandus*	100	82	18	82%
4	Kaner	*Nerium indicum*	100	78	22	78%
5	Sarpgandha	*Rauvolfia serpentina*	100	66	34	66%

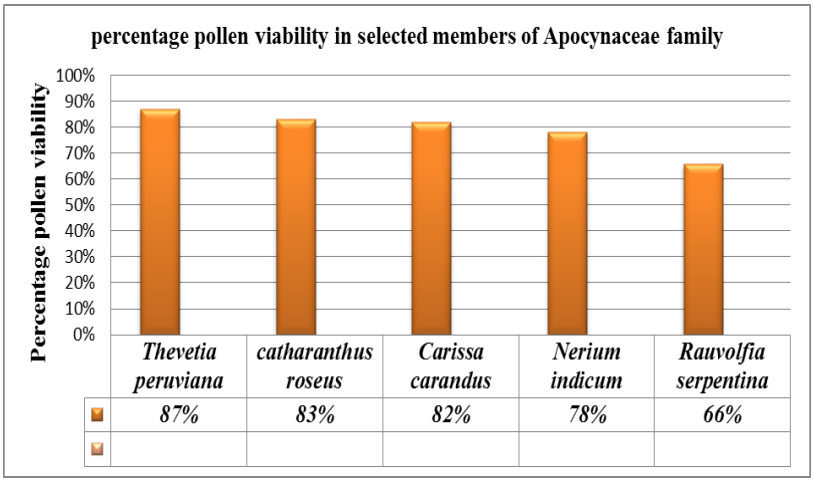


Figure 2: Showed percentage pollen viability in selected medicinal plants of apocynaceae family.

CONCLUSION

The staining of pollens with acetocarmine is an effective method for estimating the viability of selected medicinal plants of Apocynaceae family.[37] The evaluation on pollen viability is a pre requisite for carrying out a significant crop improvement programs, particularly when a wild source character is to be integrated into cultivated forms.[38] As per the present findings, the maximum pollen viability was recorded in *Thevetia peruviana* which revealed that this species has a normal meiosis and well adjusted to local environments. While the lowest percent of viability was reported in *Rauvolfia serpentina* revealed that this species might become scarce and endangered. Therefore, *Thevetia peruviana*, *Catharanthus roseus*, and *Carissa carandus* are characterized by maximum percentage of pollen viability and can be successfully used as male parents in hybridization programmes.

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