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Morphological characterization and fertility of pollens of two economically important seed species from Apiaceae

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Abstract- Pollen morphology is a disciplinary area with the primary objective to describe the pollen grains by their characteristics that also defines different plant groups. On the other hand, pollen fertility indicates the pollen viability. This present work deals with study of pollen morphology and pollen fertility on two varieties of each, of *Foeniculum vulgare* Mill. and *Anethum graveolens* L.A. sowa., two very important economical crops belong to the family Apiaceae. The investigation was carried out under the light microscope with the aid of oculo-micrometre and stage micrometre.

Key words: Pollen grains, Pollen morphology, Pollen fertility, Apiaceae, *Anethum, Foeniculum*.

INTRODUCTION

The family Apiaceae is the 16th largest family of the flowering plants, comprising more than 3600-3780 species under more than 455 genera worldwide.[1] Several ethno-medicinally important horticultural crops belong to this aromatic family, are raised for seed spices, leaf spices and condiments. Fennel (*Foeniculum vulgare* Mill.) and Indian Dill (*Anethum graveolens* L.A. sowa) are two such economically important seed spices from this family cultivated in our country, makes us the largest producer, consumer and exporter in the world.[2] They are mostly grown in rabi season in arid and semi-arid part of India.[3,4] Both the plants are herbaceous, featured with compound umbel inflorescence, presents small yellow, radially symmetrical, hermaphrodite flowers arranged in umbels.[5,6] Our Indian Dill (*Anethum graveolens* L.A. sowa) popularly known as sowa due to the presence of dillapiole and is the most valuable medicinal herb of Apiaceae.[7] Rather than a commercial seed spice, it is used as a antispasmodic and carminative in pharmaceutical industry.[8] Where Fennel (*Foeniculum vulgare* Mill.) is considered one of the oldest medicinal plant in the world.[9] It is valuable for the different ether-essential oils and useful in disease related to chest, kidney, spleen and digestive system. It is also found that fennel is even used to stimulate lactation[10].

A major role of the pollens of these two plant species found in melissopalynology for the production of multiflora honeys, word widely, including India. Now, microspore or pollen grain is the first cell of male gametophyte of spermatophytes.[11] Pollens release after their maturation from anthers. Taxonomists use pollen grain as one of the important tools to generate different phylogenetic

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information about the species. Not only, here, in other disciplines like paleobotany, forensic, cytogenetics, medicine, knowledge of pollen plays crucial role.[12] The pollen grains show case a great variation of morphological characteristics, established by all genetic heredity and generally do not vary upon the environmental changes or events. Thus, they are very defined by their own way in each genera to species of different plant groups. Only the production can be increased or reduced depending on the environmental conditions.

OBJECTIVE OF RESEARCH

Study of pollen morphological characteristics of two varieties of Fennel (*Foeniculum vulgare*)-Ajmer Fennel-(AF-1), Ajmer Fennel-2 (AF-2) and two varieties of Indian Dill (*Anethum graveolens* L.A. sowa) Ajmer Dill-1 (AD-1), Ajmer Dill-2 (AD-2). Along with, the estimation of pollen fertility by stainability test of concerned four materials.

MATERIALS & METHODS

Plant Material: -The seeds of plants were brought from ICAR-National Research Centre on Seed Spices, Ajmer, Rajasthan, India. They were sown in pots and grown in Purulia, West Bengal, India.

Morphological Analysis and Viability Test: - A regular method to study the morphological characteristics and assess the pollen viability is by staining and direct count.[13] For this purpose, anthers were collected from open flowers at full flowering stage from the plants.[14] A cytological dye aceto-orcein was used to stain the fresh pollen grains.[15] Firstly, the anthers were taken, on a watch glass and warmed in 2% aceto-orcein for 2-4 minutes. Then on a clean slide, anthers were placed, teased with needle to uniform distribution of pollen grains and discard the debris. A drop of 45% acetic acid was added to the slide, warmed, covered with coverslip and squashed.

Slides were examined under light microscope, equipped with DSLR camera and photographs were taken at 40X power. Morphological observation was done and measurements of 10 pollens of each variety were calculated with the help of ocular and stage micrometre. To determine pollen fertility at least 200 pollen grains of each variety were analysed. Pollens which are fully expanded, with intact exine and strongly coloured in red considered as fertile or viable, where those were abnormal in shape and remain unstained or very slightly stained were sterile or non-viable.[16,17]

RESULTS

Pollen morphology: - The various pollen morphological characters and measurements of different parameters of four materials have been tabulated in Table-1. All the pollen grains of AF-1, AF-2, AD-1 and AD-2 were exclusively isopolar and radially symmetrical. They were all monads, medium in size[18], 3-zonocolporate[19], prolate[20] and erectus. The polar caps were obtuse type. In case of shape, pollens of AF-1 and AF-2 were elliptical to slightly bone shaped, where pollens of AD-1 were clearly elliptical and AD-2 pollens were bone-shaped.

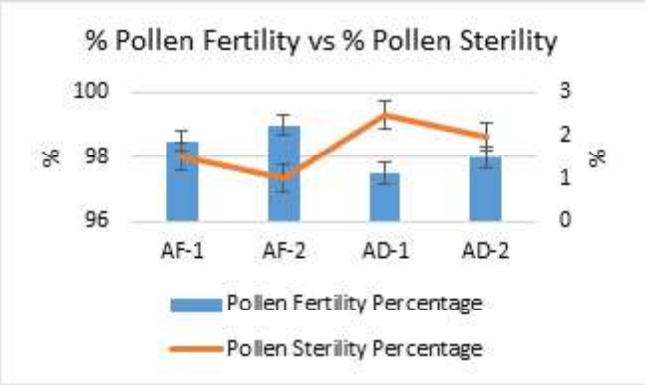
The polar diameter ranges from 29.25±0.6 µm to 35.37±0.67 µm. The longest pollen was observed in AF-2 while the smallest in AD-1. The equatorial diameter ranges from 18.33±0.56 µm to 20.12±0.39 µm. The highest equatorial diameter was found in AD-2 where the lowest was in AF-1. The P/E ratio of all pollen grains were showing ranges from 1.6±0.06 µm to 1.885±0.05 µm. That was below 2.0. At the polar view of the pollen of AD-2 was almost triangular with apertures inside.

Table 1- Pollen morphological measurements and characteristics of two varieties of Fennel–Ajmer Fennel-1 (AF-1), Ajmer Fennel-2 (AF-2) and two varieties of Dill – Ajmer Dill-1 (AD-1), Ajmer Dill-2(AD-2).

Species	Variety	Polar Diameter (P) (µm)	Equatorial Diameter (E) (µm)	P/E	Pollen Shape		Size	Unit	Pollen Class	Polarity	Symmetry	Outline	Polar Cap
					Equatorial View	Polar View							
***Foeniculum vulgare* Mill.**	AF-1	33.68 ±0.64	18.33 ±0.56	1.85 ±0.05 Erect	Prolate	–	Medium	Monad	3-Zonocolporate	Isopolar	Radially symmetrical	Elliptical to slightly bone shaped	Obtuse
	AF-2	35.37 ±0.67	19.42 ±0.55	1.83 ±0.06 Erect	Prolate	–	Medium	Monad	3-Zonocolporate	Isopolar	Radially symmetrical	Elliptical to slightly bone shaped	Obtuse
***Anethum graveolens* L.A. sowa**	AD-1	29.25 ±0.61	18.55 ±0.88	1.6 ±0.06 Erect	Prolate	–	Medium	Monad	3-Zonocolporate	Isopolar	Radially symmetrical	Elliptical	Obtuse
	AD-2	34.40 ±0.48	20.12 ±0.39	1.7 ±0.03 Erect	Prolate	Triangular with apertures inside	Medium	Monad	3-Zonocolporate	Isopolar	Radially symmetrical	Bone shaped	Obtuse

Table 2- Percentage of pollen fertility and sterility of two varieties of Fennel-Ajmer Fennel-1 (AF-1), Ajmer Fennel-2 (AF-2) and two varieties of Dill - Ajmer Dill-1 (AD-1), Ajmer Dill-2(AD-2).

Sl. No.	Species	Variety	Total No. of Pollen studied	Fertile Pollens	Pollen Fertility Percentage	Sterile Pollen	Pollen Sterility Percentage
1	*Foeniculum vulgare* Mill.	AF-1	200	197	98.5	3	1.5
		AF-2	200	198	99	2	1
2	*Anethum graveolens* L.A. sowa	AD-1	200	195	97.5	5	2.5
		AD-2	200	196	98	4	2



Graph 1- Percentage of pollen fertility and percentage of pollen sterility of two varieties of Fennel-Ajmer Fennel-1 (AF-1), Ajmer Fennel-2 (AF-2) and two varieties of Dill - Ajmer Dill-1 (AD-1), Ajmer Dill-2 (AD-2).

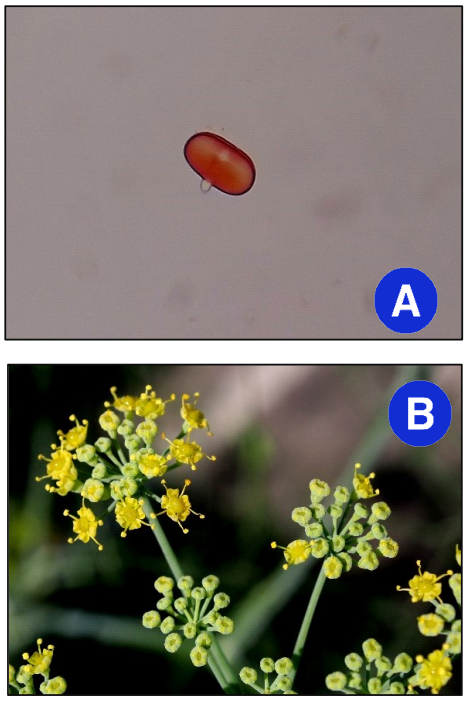


Fig. 1- *Foeniculum vulgare* Mill. Variety: Ajmer Fennel 1 (AF-1), A: Pollen grain in equatorial view (Fully stained, Germinated) B: Flowers on Umbels

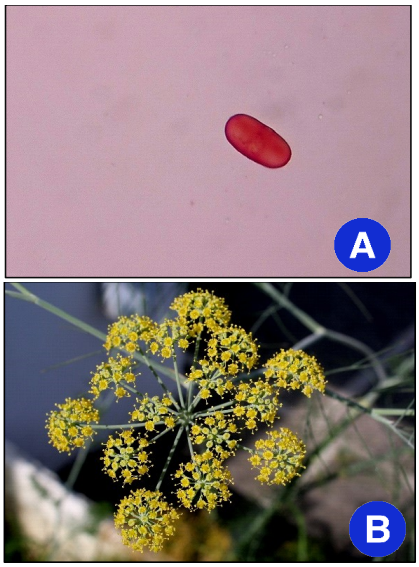


Fig. 2- *Foeniculum vulgare* Mill. Variety: Ajmer Fennel 2 (AF-2), A: Pollen grain in equatorial view (Fully stained), B: Flowers on Umbels

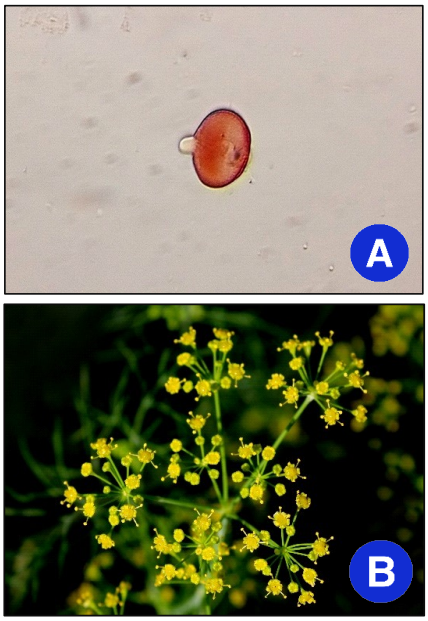


Fig. 3- *Anethum graveolens* L.A. sowa Variety: Ajmer Dill 1 (AD-1), A: Pollen grain in equatorial view (Fully stained, Germinated), B: Flowers on Umbel

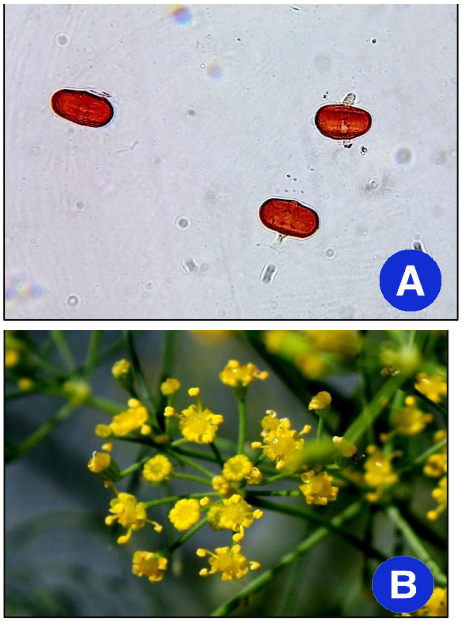


Fig. 4- ***Anethum graveolens*** **L.A. sowa Variety: Ajmer Dill 2 (AD-2), A: Pollen grain in equatorial view (Fully stained, Germinated), B: Flowers on Umbel**

Fig. 5- ***Anethum graveolens*** **L.A. sowa Variety: Ajmer Dill 2 (AD-2), A: Pollen grain in polar view**

Pollen Fertility: -The results of fertility through stainability observed in fresh pollen by aceto-orcein have shown a high pollen fertility in all four varieties. The data has been tabulated in Table -2. The highest pollen fertility percentage was shown in AF-2 i.e., 99%, where the lowest percentage of pollen fertility was found in AD-1 i.e., 97.5%. The pollen fertility- sterility percentage has been showing in Graph-1 to simplify understanding.

DISCUSSION

On the view of palynological contribution to the family Apiaceae, the most extensive and interdisciplinary studies were done by Cerceau-Larrival (1959, 1962, 1963, 1965, 1967a, 1967b, 1971, 1973, 1981). Where the study of pollen by Ertdtman (1943, 1952) and Erdtman et. al. (1961, 1963) has provided us the basic categories with enlisted features to describe a pollen grain very easily and scientifically. A glossary on terminologies of pollen grains and spores by W. Punt et. al. (2006) has become the widely accepted reference guide to the scientists to prepare accurate description for their study materials.[21]

In this investigation, we have found that the pollens of four varieties showed a greater uniformity with very slight difference in their shape and size, presents almost similar external morphology, supported the stenopalynous characteristics of pollen grains of Apiaceae.[14,20] The pollens were having longer polar axis than the equatorial axis, resulted P/E ratio always below 2.0, which reflects the erectus nature of the grains.[22] Now, the natural tendency of an elongated pollen is to lay parallel in a microscopic slide. Therefore, it has become very difficult or even tends to impossible to get the polar view.[23] Where Cerceau (1962) and Punt (1984) mentioned in their literature that polar view of Apiaceae pollens were very important for differentiation. Fortunately, during our experimental studies, we have found the polar view of the pollen of AD-2. The term viability has been defined as “having the capacity to live, grow, germinate or develop”. In this experiment we have used aceto-orcein method to test the fertility of pollens. Here, it has considered, that the aceto-carmine or aceto-orcein stainability method is the simplest technique for pollen viability analysis. It is a less sensitive test. It ensures the presence of cytoplasm in the pollen.[24] In our study, even germinating pollens have been also observed confirmed that the pollens were fully viable. It has marked a high fertility percentage over sterility, gives us he idea, why our country has been leading on the production of these crops.

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