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POLLEN VIABILITY OF SOME TREES OF NORTH-WESTERN PUNJAB, INDIA

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ABSTRACT

Plants perform various vegetative and reproductive functions throughout the year in order to persist in their habitats. Pollen viability is the necessity of plants for their reproductive success. Thus, the study of pollen viability has attained much attention of the researchers as it also contributes to the studies like hybridization programs, evolutionary ecology, fruit breeding programs etc. The pollen viability varies from species to species. Different approaches including the staining methods have been used to study pollen viability of different plant species. This paper deals with the study of pollen viability of twenty five tree species of Amritsar using three stains on fresh and heat treated pollen (control) and to suggest suitable stain for each species studied. It is recommended that some type of control such as killed pollen should be used to check the potential of a dye to test pollen viability of a particular species.

KEYWORDS: Pollens, Viability, Trees, Stains

INTRODUCTION

Plants perform different functions such as maintenance of their biomass, vegetative growth and reproduction throughout the year. Since pollination is required in order for the plants to reproduce, it is important that the pollen used is viable. Pollen considered sterile is unable to complete the fertilization process and hence the reproduction. Thus, the viability of pollen is the necessity of plants for their reproductive success. Viability is defined as the ability to live, develop, or in the case of pollen, to germinate when conditions are favorable. Studies related to pollen viability have received a great concern and are essential for the reproductive success, conservation and management of different plant species (Lyra *et al.*, 2011).

The assessment of pollen viability is important in artificial pollination and breeding experiments (Rodriguez-Riano and Dafni, 2000). Generally three different approaches have been considered for evaluating pollen quality: *in vivo* which involve determining the number of pollen germinated on stigmas of emasculated flowers; *in vitro* which involve germinating the pollen on artificial media and pollen tube growth; and histochemical which are based on the ability of pollen to get stained (Abdul-Baki, 1992). Among different techniques available, staining methods are considered most suitable for routine screening of many samples as they are inexpensive, faster and easier than other methods such as pollen tube germination.

Many stains such as aceto-carmin, propione carmine, aniline blue, aceto-orcein, Alexander's stain, IKI (iodine+ potassium iodide), FDA (fluorescein diactate), NBT (p-nitro blue tetrazolium), MTT (2,5-diphenyl tetrazolium bromide) and TTC (2,3,5-triphenly tetrazolium chloride) have been used to determine the pollen viability of a range of plant species (Abdul-Baki, 1992; Oberie and Watson, 1953; Werner and Chang, 1981; Widrlechner *et al.*, 1983; Pearson and Harney, 1984; Lee *et al.*, 1985; Hecker and McClintock, 1989; Parfitt and Ganeshan, 1989; Bolat and Pirlak, 1999; Palma-Silva *et al.*, 2008; Sasikala *et al.*, 2009; Chaudhary *et al.*, 2010; Ge *et al.*, 2011; Silva *et al.*, 2011; Abdullateef *et al.*, 2012).

Some studies have shown that these stains also stain killed pollen of certain species (Rodriguez-Riano and Dafni, 2000; Parfitt and Ganeshan, 1989; Kapyla, 1991; Sedgley and Harbard, 1993; Khatum and Flowers, 1995). It is suggested that some type of control such as killed pollen should be used to check the potential of a dye to test pollen viability. If a dye also stains killed pollen, it must be avoided. Hence, it becomes important to use a dye which is able to differentiate between fresh and killed pollen. Rodriguez-Riano and Dafni (2000) determined the potential of four vital dyes to differentiate fresh pollen from pollen heated for 2 h and 24 h at 80°C (killed pollen).

A number of studies on pollen viability of different plant species are being carried out all over the world including India such as the works by (Chaudhary *et al.*, 2010; Silva *et al.*, 2011; John and Rao, 2005; Ahmad *et al.*, 2010; Kalkar and Neha, 2012; Koshy *et al.*, 2013) etc. Firmage and Dafni (2001) suggested that prior testing of several stains on a species in question should be done before final determinations are made. Hence, the present research was planned to study pollen viability of 25 tree species growing in Amritsar using three different stains viz. aceto-orcein, Lugol's solution and 2,3,5 triphenyl tetrazolium chloride on fresh and heat treated pollen.

MATERIALS AND METHODS

Study Area: The study area is district Amritsar (historically also known as *Ramdaspur* and colloquially as *Ambarsar*), located in northwestern part of the Punjab state (India) and lies between 31°28'30" to 32°03'15" north latitude and 74°29'30" to 75°24'15" east longitude. Total area of the district is 5056 sq. km with tropical dry deciduous type of vegetation (Champion and Seth, 1968). Natural vegetation is fragmented and is at present available only in narrow strips and patterns.

Plant Material: The whole area of Amritsar was surveyed and 25 different trees growing at different localities belonging to different families were selected. A number of mature flower buds of each tree were collected from three different sites for this study so as to get at least 1000 pollen from each site. Table 1 gives the family wise list of plant species with their family and leaf habit.

Table 1: Family Wise List of Plant Species Studied with their Leaf Habit

S. No.	Species	Family	Leaf Habit
1	Alstonia scholaris R. Br.	Apocynaceae	E
2	Heterophragma adenophyllum Seem.	Bignoniaceae	D
3	Jacaranda mimosifolia D. Don		D
4	Kigelia pinnata DC.		E
5	Tabebuia argentea (Bureau & K. Schum.) Britt.		D
6	Tecomella undulata (Sm.) Seem.		D
7	Bauhinia purpurea Linn.	Caesalpiniaceae	D
8	Bauhinia variegata Linn.		D
9	Cassia fistula Linn.		D
10	Cassia siamea Lam.		E
11	Emblica officinalis Gaertn.	Euphorbiaceae	D
12	Bombax ceiba Linn.	Malvaceae	D
13	Melia azedarach Linn.	Meliaceae	D
14	Swietenia mahagoni (L.) Jacq.		D
15	Acacia nilotica (Linn.) Delile	Mimosaceae	D
16	Prosopis juliflora DC.		D
17	Callistemon lanceolatus DC.	Myrtaceae	E
18	Eucalyptus longifolia Link & Otto		E
19	Butea monosperma (Lamk.) Kuntze	Papilionaceae	D
20	Dalbergia sissoo Roxb.		D
21	Erythrina crista-galli Linn.		D
22	Millettia ovalifolia Kurs.		D

Table 1: Contd.,

23	Citrus limon (Linn.) Burm. f.	Rutaceae	E
24	Murraya koenigii (Linn.) Spreng.		E
25	Pterospermum acerifolium Willd.	Sterculiaceae	D

E: Evergreen, **D:** Deciduous

Viability Tests: Three methods of staining were used to test pollen viability.

- **Aceto-Orcein:** It stains the chromatin material (Dionne and Spicer, 1958; Rudich *et al.*, 1977). 2 g of orcein powder was added to 45% acetic acid. The solution was boiled for 3-4 minutes, allowed to cool, filtered and stored in a dark bottle. To use, the stain was mixed with glycerin in 1 : 1 ratio.
- **Lugol's Solution:** It is used to detect starch content in the pollen. The Lugol's solution consists of iodine and potassium iodide, turning viable pollen into black color (Charles and Harris, 1972). 2 g of iodine and 4 g of Potassium Iodide were dissolved in 100 ml of distilled water. The solution was filtered and stored in refrigetator till further use.
- **2, 3, 5 Triphenyl Tetrazolium Chloride (TTC):** TTC is a redox indicator commonly used in biochemical experiments especially to indicate cellular respiration. It is used to differentiate between metabolically active and inactive tissues. The white compound (TTC) is enzymatically reduced to red TPF (1,3,5-triphenylformazan) in living tissues due to the activity of various dehydrogenases. It basically differentiates between living and non-living cells (Cook and Stanley, 1960). 0.1 g of TTC salt was added to 10 ml of saturated solution of sucrose in distilled water.

Slide Preparation: Anthers removed from the buds within 1 h of collection from the trees were placed on a clean slide. A drop of the stain was dropped on to anthers which were pierced with needle and slightly tapped with the help of flattened back of needle. This caused the release of pollen from the anther wall. The anther wall and other debris were completely removed, cover slip was placed on the stain and was tapped with the help of match stick. Air bubbles were removed and the cover slip was sealed with DPX. The slides were observed under the microscope immediately after preparation for checking pollen viability in case of aceto-orcein and Lugol's solution. But for the TTC stain, slides were placed in semi shade of sunlight for nearly 2 hours of incubation before observation. Fully stained pollen grains were recorded as viable and those partially stained or fully unstained or shrunken were counted as non-viable.

To check the stain's ability to stain only the viable pollen, the anthers were heated at 80°C for 5, 7 and 9 h. The heated anthers were used to stain the pollen with all the three dyes as mentioned above to check their ability to stain heat killed pollen.

Scoring: 1000 pollen were scored for each plant from each site for four different types of anthers viz fresh, 5 h, 7 h and 9 h heat treatment. The staining was performed in triplicates and the data are represented as mean ± standard deviation of percentage viability.

Calculations: The percentage pollen viability was calculated for each site individually as given below:

$$\text{Pollen viability (\%)} = \frac{\text{No. of viable pollen}}{\text{Total no. of pollen}} \times 100$$

The results were statistically analyzed using one way Analysis of Variance to check if there was any significant differences in pollen viability information between the stains for fresh pollen of each plant species for the three stains and two way Analysis of Variance was applied to see if there was any significant difference between the stains on fresh and heat treated anthers at 5% and 1% level of significance.

RESULTS AND DISCUSSIONS

The quantity and quality of the pollen produced by a plant is an important component of reproductive success. In this context, pollen viability is considered to be an important parameter of pollen quality (Dafni and Firmage, 2000). Several staining methods are advantageous as indicators of pollen viability, for being quicker and easier than trials with *in vitro* pollen germination.

A large number of dyes are generally used to stain pollen by many researchers without any type of control. Rodriguez-Riano and Dafni (2000) recommended using some type of control such as killed pollen to check the potential of a dye to test pollen viability before using it. Hence, we used three different types of stains on fresh and heat killed pollen of 25 tree species growing in Amritsar to indicate suitable stain for studying pollen viability of those species. The results of the present study are summarized in Table 2.

Table 2: Pollen Viability (Mean±S.D.) of Fresh and Heat Treated Pollen of Different Species Using Different Stains

S. No.	Name of the Plant	Family	Pollen Viability (%)			
			Fresh	Heat Treated for		
				5 h	7 h	9 h
1	*Alstonia scholaris* R. Br.	Apocynaceae				
	Aceto-orcein		**95.57±2.21**	0.00±0.00	0.00±0.00	0.00±0.00
	Lugol's solution		85.33±3.51	10.70±0.30	0.00±0.00	0.00±0.00
	TTC		95.97±3.65	36.27±3.29	10.50±0.95	0.00±0.00
2	*Heterophragma adenophyllum* Seem.	Bignoniaceae				
	Aceto-orcein		72.80±4.61	39.87±1.42	0.00±0.00	0.00±0.00
	Lugol's solution		67.83±1.40	23.30±2.87	0.00±0.00	0.00±0.00
	TTC		**67.67±4.59**	0.00±0.00	0.00±0.00	0.00±0.00
3	*Jacaranda mimosifolia* D. Don					
	Aceto-orcein		98.43±0.25	36.43±0.83	11.77±0.81	0.00±0.00
	Lugol's solution		92.57±1.86	24.27±0.81	4.67±0.50	0.00±0.00
	TTC		**64.17±1.96**	0.00±0.00	0.00±0.00	0.00±0.00
4	*Kigelia pinnata* DC.					
	Aceto-orcein		75.30±4.51	10.50±1.04	0.00±0.00	0.00±0.00
	Lugol's solution		**24.97±2.61**	0.00±0.00	0.00±0.00	0.00±0.00
	TTC		53.97±1.76	20.90±2.21	0.00±0.00	0.00±0.00
5	*Tabebuia argentea* (Bureau & K. Schum.) Britt.					
	Aceto-orcein		57.50±1.66	43.27±1.46	11.37±0.93	0.00±0.00
	Lugol's solution		47.00±2.43	24.23±1.39	5.53±0.74	0.00±0.00
	TTC		**78.30±0.66**	0.00±0.00	0.00±0.00	0.00±0.00
6	*Tecomella undulata* (Sm.) Seem.					
	Aceto-orcein		72.00±2.07	62.10±2.33	25.47±3.79	0.00±0.00
	Lugol's solution		82.57±1.76	53.30±1.08	10.53±0.67	0.00±0.00
	TTC		**12.37±1.99**	0.00±0.00	0.00±0.00	0.00±0.00
7	*Bauhinia purpurea* Linn.	Caesalpiniaceae				

Table 2: Contd.,

8	**Aceto-orcein**		**88.87±1.94**	10.87±0.61	0.00±0.00	0.00±0.00
	Lugol's solution		80.53±1.50	10.77±1.07	0.00±0.00	0.00±0.00
	TTC		78.93±3.52	34.30±0.96	0.00±0.00	0.00±0.00
	Bauhinia variegata Linn.					
9	Aceto-orcein		69.17±2.93	0.33±0.58	0.00±0.00	0.00±0.00
	Lugol's solution		**77.93±3.42**	0.00±0.00	0.00±0.00	0.00±0.00
	TTC		72.20±2.32	0.00±0.00	0.00±0.00	0.00±0.00
	Cassia fistula Linn.					
	Aceto-orcein		97.53±0.61	70.73±1.30	11.80±1.06	0.00±0.00
	Lugol's solution		**96.77±0.60**	0.00±0.00	0.00±0.00	0.00±0.00
	TTC		95.80±2.99	77.37±1.07	0.00±0.00	0.00±0.00
10	*Cassia siamea* Lam.					
	Aceto-orcein		81.73±9.83	37.57±4.85	0.00±0.00	0.00±0.00
	Lugol's solution		74.40±3.67	23.43±0.75	0.00±0.00	0.00±0.00
	TTC		**79.67±2.97**	0.00±0.00	0.00±0.00	0.00±0.00
11	*Emblica officinalis* Gaertn.	Euphorbiaceae				
	Aceto-orcein		86.63±2.30	25.30±3.76	0.00±0.00	0.00±0.00
	Lugol's solution		**88.20±0.66**	17.80±2.19	0.00±0.00	0.00±0.00
	TTC		83.97±3.26	62.10±2.33	25.47±3.79	0.00±0.00
12	*Bombax ceiba* Linn.	Malvaceae				
	Aceto-orcein		**96.40±2.02**	0.00±0.00	0.00±0.00	0.00±0.00
	Lugol's solution		26.87±1.62	17.70±0.63	12.27±0.50	0.00±0.00
	TTC		26.57±1.79	16.13±0.83	10.70±0.44	0.00±0.00
13	*Melia azedarach* Linn.	Meliaceae				
	Aceto-orcein		**96.30±0.79**	0.00±0.00	0.00±0.00	0.00±0.00
	Lugol's solution		97.80±0.72	86.60±1.78	50.37±1.82	0.00±0.00
	TTC		95.27±0.64	0.00±0.00	0.00±0.00	0.00±0.00
14	*Swietenia mahagoni* (L.) Jacq.					
	Aceto-orcein		83.97±3.26	8.20±0.36	0.00±0.00	0.00±0.00
	Lugol's solution		86.40±3.67	9.50±0.75	0.00±0.00	0.00±0.00
	TTC		**76.57±2.06**	0.00±0.00	0.00±0.00	0.00±0.00
15	*Acacia nilotica (*Linn.) Delile	Mimosaceae				
	Aceto-orcein		84.90±2.61	34.07±1.17	0.00±0.00	0.00±0.00
	Lugol's solution		26.33±2.25	8.20±0.63	0.00±0.00	0.00±0.00
	TTC		**68.83±3.97**	0.00±0.00	0.00±0.00	0.00±0.00
16	*Prosopis juliflora* DC.					
	Aceto-orcein		96.97±0.51	18.13±0.31	12.10±1.08	0.00±0.00
	Lugol's solution		92.23±2.40	14.63±1.96	0.00±0.00	0.00±0.00
	TTC		**65.50±1.81**	0.00±0.00	0.00±0.00	0.00±0.00
17	*Callistemon lanceolatus* DC.	Myrtaceae				
	Aceto-orcein		82.87±3.13	40.37±1.26	0.00±0.00	0.00±0.00
	Lugol's solution		79.13±2.21	22.67±1.72	0.00±0.00	0.00±0.00
	TTC		**78.67±3.02**	0.00±0.00	0.00±0.00	0.00±0.00
18	*Eucalyptus longifolia* Link & Otto					
	Aceto-orcein		**90.30±1.47**	0.00±0.00	0.00±0.00	0.00±0.00
	Lugol's solution		22.00±0.10	3.57±0.67	0.00±0.00	0.00±0.00
	TTC		92.07±2.06	71.37±1.21	20.17±1.56	0.00±0.00
19	*Butea monosperma* (Lamk.) Kuntze	Papilionaceae				
	Aceto-orcein		82.57±1.70	65.03±4.04	43.87±4.03	23.20±1.80
	Lugol's solution		73.07±3.26	51.97±1.63	23.27±1.90	0.00±0.00
	TTC		**73.43±0.75**	0.00±0.00	0.00±0.00	0.00±0.00

Table 2: Contd.,

20	*Dalbergia sissoo* Roxb.					
	Aceto-orcein		75.33±2.85	14.57±0.67	0.00±0.00	0.00±0.00
	Lugol's solution		76.97±4.18	15.13±1.30	0.00±0.00	0.00±0.00
	TTC		**70.50±1.68**	0.00±0.00	0.00±0.00	0.00±0.00
21	*Erythrina crista-galli* Linn.					
	Aceto-orcein		88.07±1.17	58.80±1.25	27.23±1.27	11.77±2.97
	Lugol's solution		92.13±2.21	48.77±1.20	18.50±1.67	6.57±1.39
	TTC		**86.17±2.12**	0.00±0.00	0.00±0.00	0.00±0.00
22	*Millettia ovalifolia* Kurs.					
	Aceto-orcein		82.03±5.01	25.43±1.12	0.00±0.00	0.00±0.00
	Lugol's solution		90.8±1.39	11.37±1.17	0.00±0.00	0.00±0.00
	TTC		**77.90±0.89**	0.00±0.00	0.00±0.00	0.00±0.00
23	*Citrus limon* (Linn.) Burm. f.	Rutaceae				
	Aceto-orcein		**83.87±2.48**	0.00±0.00	0.00±0.00	0.00±0.00
	Lugol's solution		78.77±1.80	22.77±1.20	0.00±0.00	0.00±0.00
	TTC		69.17±1.55	33.6±4.92	26.57±1.34	0.00±0.00
24	*Murraya koenigii* (Linn.) Spreng.					
	Aceto-orcein		85.67±2.74	53.63±1.34	23.6±1.20	0.00±0.00
	Lugol's solution		82.17±6.21	47.33±1.00	13.23±1.06	0.00±0.00
	TTC		**85.10±3.28**	0.00±0.00	0.00±0.00	0.00±0.00
25	*Pterospermum acerifolium* Willd.	Sterculiaceae				
	Aceto-orcein		**90.37±0.45**	10.37±1.88	0.00±0.00	0.00±0.00
	Lugol's solution		89.77±0.51	46.37±1.70	20.13±1.34	0.00±0.00
	TTC		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

The study reveals that different plant species show different percentage of pollen viability with different stains for both fresh and heat treated pollen. This means that we should not rely on a single stain to test pollen viability of all species and that preliminary testing (using heat treated pollen) should be done to find the suitability of the stain on each species to be considered.

The suitable stain for each plant species (shown in bold in Table 2) is the one that did not stain (not even a single) killed pollen after 5h heat treatment. It was observed that for 23/25 tree species studied, at least one stain was such that it did not stain pollen obtained from anthers after heat treatment for 5 h. For the remaining two species *Bauhinia purpurea* Linn. and *Emblica officinalis* Gaertn., loss of pollen viability was observed after 7 h of heat treatment.

Among the three stains used in the present study, TTC was considered suitable for 14 out of 25 species studied as it did not stain heat killed pollen after 5 h of treatment. Similarly aceto-orein was considered suitable for 7 species and TTC only for 4 species (Table 3). Aceto-orcein did also stain pollen of two species (*Butea monosperma* (Lamk.) Kuntze and *Erythrina crista-galli* Linn.) even after 9 h of treatment. Similarly, Lugol's solution stained pollen of *Erythrina crista-galli* Linn. even after 9h of treatment, which means that these stains should not be considered for testing pollen viability of respective species mentioned above.

The differentiation between the viable and the non-viable pollen was purely on the basis of color differentiation with yellow pollen as non-viable and darkly stained (Dark Red for aceto-orcein and TTC or Black for Lugol's solution) as viable pollen. Representative photomicrographs of pollen stained with these three stains are shown in Figure 1.

Table 3: Suitability of Stain for Different Tree Species Studied

S. No.	Stain	Suitability for Plant Species
1	Aceto-orcein	*Alstonia scholaris*, *Bauhinia purpurea*, *Bombax ceiba*, *Citrus limon*, *Eucalyptus longifolia*, *Melia azedarach*, and *Pterospermum acerifolium*.
2	Lugol's Solution	*Bauhinia variegata*, *Cassia fistula*, *Emblica officinalis* and *Kigelia pinnata.*
3	TTC	*Acacia nilotica*, *Butea monosperma*, *Callistemon lanceolatus*, *Cassia siamea*, *Dalbergia sissoo*, *Erythrina crista-galli*, *Heterophragma adenophyllum*, *Jacaranda mimosifolia*, *Millettia ovalifolia*, *Murraya koenigii*, *Prosopis juliflora*, *Swietenia mahagoni*, *Tabebuia argentea* and *Tecomella undulata*.

The results of One way Analysis of Variance (Table 4) indicate that the differences in pollen viability using three different stains were significantly variable for 13 out of 25 plant species studied at 1% level of significance and for 5 species at 5% level of significance.

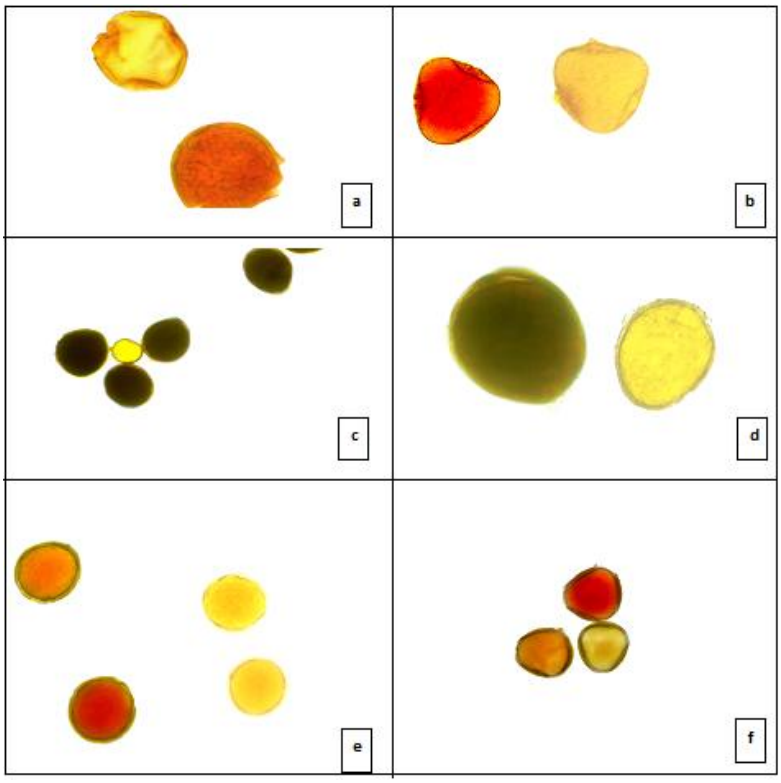

Figure 1: **Pollens of *Bauhinia purpurea* (a) and *Bombax ceiba* (b) Stained with Aceto-Orcein; *Cassia fistula* (c) And *Kigelia pinnata* (d) Stained with Lugol's Solution; *Tabebuia argentea* (e) And *Tecomella undulata* (f) Stained with TTC (Magnification1000X)**

Table 4: Results of One Way Analysis of Variance

S. No.	Name of Species	F Ratio (Calculated)
1	*Pterospermum acerifolium* Willd.	52150.34**
2	*Eucalyptus longifolia* Link & Otto	2236.54**
3	*Bombax ceiba* Linn.	1464.56**
4	*Tecomella undulata* (Sm.) Seem.	1139.36**
5	*Jacaranda mimosifolia* D. Don	411.26**
6	*Acacia nilotica* (Linn.) Delile	298.32**
7	*Prosopis juliflora* DC.	278.39**
8	*Tabebuia argentea* (Bureau & K. Schum.) Britt.	250.42**
9	*Kigelia pinnata* DC.	189.73**
10	*Citrus limon* (Linn.) Burm. f.	42.53**
11	*Butea monosperma* (Lamk.) Kuntze	18.50**
12	*Bauhinia purpurea* Linn.	13.88**
13	*Millettia ovalifolia* Kurs.	13.61**

Table 4: Contd.,

14	*Alstonia scholaris* R. Br.	10.72*
15	*Melia azedarach* Linn.	9.34*
16	*Swietenia mahagoni* (L.) Jacq.	8.34*
17	*Erythrina crista-galli* Linn.	7.76*
18	*Bauhinia variegata* Linn.	6.98*
19	*Dalbergia sissoo* Roxb.	3.58
20	*Emblica officinalis* Gaertn.	2.52
21	*Callistemon lanceolatus* DC.	2.00
22	*Heterophragma adenophyllum* Seem.	1.73
23	*Cassia siamea* Lam.	1.08
24	*Cassia fistula* Linn.	0.70
25	*Murraya koenigii* (Linn.) Spreng.	0.56

* Significance at p≤ 0.05.

** Significance at p≤ 0.01.

Table 5 gives the results of Two way Analysis of Variance which showed that variation in results due to different stains used were statistically significant (at p≤ 0.01) for all the species studied, whereas for the heat treatment, the results were significant (at either p≤ 0.01 or p≤ 0.05) for all species except *Bauhinia purpurea* Linn. and *B. variegata* Linn. For combination of stain and heat treatment, variation in results on pollen viability for five species (*Jacaranda mimosifolia* D. Don, Bauhinia purpurea Linn., *Bauhinia variegata* Linn., *Swietenia mahagoni* (L.) Jacq. and *Dalbergia sissoo* Roxb.) were not statistically significant. Most significant results were obtained with *Pterospermum acerifolium* Willd., followed by *Melia azedarach* Linn. and *Bombax ceiba* Linn.

Table 5: Results of Two Way Analysis of Variance

S. No.	Name of Species	F Ratio (Calculated)		
		Stain	Heat Treatment	Stain x Heat Treatment
1	*Alstonia scholaris* R. Br.	3829.69**	96.19*	70.56*
2	*Heterophragma adenophyllum* Seem.	1158.62**	83.59*	51.59*
3	*Jacaranda mimosifolia* D. Don	13015.51**	1393.45**	10.58
4	*Kigelia pinnata* DC.	1248.43**	261.07**	110.02**
5	*Tabebuia argentea* (Bureau & K. Schum.) Britt.	3024.44**	162.31**	825.98**
6	*Tecomella undulata* (Sm.) Seem.	444.67**	2517.85**	55.82*
7	*Bauhinia purpurea* Linn.	10027.55**	13.88	13.88
8	*Bauhinia variegata* Linn.	5557.93**	6.45	7.36
9	*Cassia fistula* Linn.	4841.94**	1317.49**	1332.88**
10	*Cassia siamea* Lam.	641.27**	24.78*	22.35*
11	*Emblica officinalis* Gaertn.	5316.84**	47.52*	81.84*
12	*Bombax ceiba* Linn.	3659.84**	758.01**	2040.09**
13	*Melia azedarach* Linn.	26113.54**	4988.24**	4544.55**
14	*Swietenia mahagoni* (L.) Jacq.	5434.74**	32.63*	0.11
15	*Acacia nilotica (*Linn.) Delile	1938.29**	552.17**	202.30**
16	*Prosopis juliflora* DC.	11174.43**	480.87**	36.95*
17	*Callistemon lanceolatus* DC.	3340.58**	157.78**	105.65**
18	*Eucalyptus longifolia* Link & Otto	6033.49**	5140.36**	1803.26**
19	*Butea monosperma* (Lamk.) Kuntze	1139.08**	393.29**	266.49**
20	*Dalbergia sissoo* Roxb.	3658.84**	41.59*	8.39
21	*Erythrina crista-galli* Linn.	5488.68**	730.86**	573.33**
22	*Millettia ovalifolia* Kurs.	4388.06**	71.48*	46.86*
23	*Citrus limon* (Linn.) Burm. f.	2469.17**	26.91*	141.48**
24	*Murraya koenigii* (Linn.) Spreng.	1161.21**	125.83**	134.52**
25	*Pterospermum acerifolium* Willd.	33832.59**	13629.31**	8884.23**

* Significance at p≤ 0.05

** Significance at p≤ 0.01

Pollen and thus pollination play fundamental role in fertilization and consequently seed and fruit set in spermatophytes (Asma, 2008), deficiency in pollen production and performance could have direct effects on seed formation, seed viability and seed germination. Pollen viability is a significant determinant of whether in a population there will be enough regeneration through sexual reproduction to ensure the survival of that species. Plants with low pollen viability may become scarcer and could become endangered. In this context, pollen viability is considered to be an important parameter of pollen quality. Among different methods being used to test pollen viability, staining methods are widely used for being easier, quicker and reliable. But most of the studies using different stains/ dyes have been carried out without any type of control.

Rodriguez-Riano and Dafni (2000) for the first time recommended use of some kind of control (such as killed pollen) to check the potential of a dye to test pollen viability before using it. For example, one should not use a stain/dye if it also stains killed pollen. Similarly in our study we have revealed suitability of different stain for different tree species. Hence selection of a stain to be used for testing pollen viability of a particular plant species is very important to obtain reliable results.

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