I J R B A T, Issue (IX), Vol. III, Sept 2021: 54-58
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

POLLEN PRODUCTION STUDY BY HAEMOCYTOMETER METHOD IN *OXALIS CORNICULATA* & *TRIDAX PROCUMBENS*

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Communicated: 19.06.21 | Revision : 22.07.21 & 24.08.2021 Accepted: 29.08.2021 | Published: 30.09.2021

ABSTRACT: The pollen grains are smallest unit of the plants, which contain so many characters of taxonomic and phylogenetic importance. The shape and size of the pollen grains, germinal furrows and the number of germ pores are important taxonomic features, which are taken into consideration in classification of plants.Now a days study of pollen is an important area of research. Various pollen morphological features such as symmetry, shape, apertural pattern and exine configuration are very conservative features for the taxonomic assessment of the plant. Moreover some plants growing in the surroundings cause respiratory troubles or allergy in human beings. The pollen grains of which are responsible for allergy. Considering an immense importance of pollen grains with respect to pollen allergy the present work has been initiated for the research study. For the present research work weeds *Oxalis corniculata* (Oxalidaceae) and *Tridax procumbens* **(**Asteraceae) which are highly allergic with respect to pollen allergy are selected. Comparative study of pollen production of selected plants is studied by glycerin suspension method and haemocytometer method. In *Oxalis corniculata* pollen production by glycerin suspension method was found to be 80 whereas by haemocytometer method 217. In *Tridax procumbens* pollen production by glycerin suspension method was found to be 2044.5 whereas by haemocytometer method 314. The variation occurs in number of pollen grains produced per anther because pollen production is affected by distribution, habit of plant and difference in climatic conditions.

Key words: - Haemocytometer, Suspension

INTRODUCTION:

Palynology, the science of pollen obtained real impetus after the discovery of the microscope. This is logical because the pollen grains are extremely tiny particles comparable to dust particles which cannot be seen by the naked eye. Pollen grain come in an infinite variety of shapes with complex surface ornamentation and occurs on almost every surface in nature. Discovery of microscope by Robert Hooke in 1665 was a landmark in the development of science particularly palynology subsequent improvement in microscopy accelerated the study of pollen grains especially finer structure of pollen wall and its varied ornamentation patterns.

The pollen grains are smallest unit of the plants, which contain so many characters of taxonomic and phylogenetic importance. The shape and size of the pollen grains, germinal furrows and the number of germ pores are important taxonomic features, which are taken into consideration in classification of plants.

Now a days study of pollen is an important area of research. Various pollen morphological features such as symmetry, shape, apertural pattern and exine configuration are very conservative features for the taxonomic assessment of the plant. Moreover some plants growing in the surroundings cause respiratory

troubles or allergy in human beings. The pollen grains of which are responsible for allergy.

Pollen are ubiquitous in nature unlike other plant parts they are highly resist to decay they occur buried deep in rocks ground surface water and air indoor and outdoor including the upper atmosphere. Besides this pollen find their way through nasal and oral cavity to the digestive tract of humans and animal causing various degrees of discomfort. Pollen has the longest geological history as they are well preserved in rock as old as 400 million years. On account of these unique characters pollen and spores are often referred as nature's fingerprint of plants (Chandra, 1980).

Pollen biology encompasses pollen production, their transfer to the stigma or pollination and details of pollen pistil interaction leading to fertilization and seed set. Any break in these sequential events affects seed and fruits set. Pollen biological studies are a prerequisite for any program aimed at optimization and improvement of the yield of crop plant. Pollination ecology is also a part of pollen biology which involves the study of various aspects dealing with efficient pollination (Saoji, 1972). Pollen biotechnology is one of the techniques employed to study pollen biology for crop production and improvement. Pollen biotechnology is one of the most challenging areas of plants reproductive biology and plays an important role in crop improvement programs.

Considering an immense importance of pollen grains with respect to pollen allergy the present work has been initiated for the research study. For the present research work weeds *Oxalis corniculata* (Oxalidaceae) and *Tridax procumbence* **(**Asteraceae) which are highly allergic with respect to pollen allergy are selected. Pollen production of selected plants is studied by glycerin suspension method and haemocytometer method.

MATERIALS AND METHODS:

Collection of pollen grains and identification of plants species

Fresh flowers of selected plant species *Oxalis corniculata* and *Tridax procumbens* were collected from H.P.T Arts and R.Y.K Science college campus Nasik and identified with the help of flora of Nasik district.

Pollen production

a) Glycerin suspension method -

All dehisced anthers from a fully mature bud were crushed in minimum quantity of 50% glycerin making to total volume 10 ml by addition of more glycerin. Standardization of the dropper was made by confirmation that 20 drops of the suspension from it make up the volume of 1 ml. one drop each of the suspension was transferred to 3 slides and each one was covered by cover glass. The number of pollen grains in this area was counted from the mean of which the total number of pollen grains per flower was calculated. Thus 10 buds of each plant were utilized for pollen production.

b) Haemocytometer method -

In this method Spencer Bright line Haemocytometer widely used for blood counts, was used to estimate pollen output. Using this method the number of pollen grains per anther was calculated as suggested.

From a single plot 20 samples of anthers each were collected. The flower buds were taken from all parts of the plot. Anthers were transferred to cotton plugged glass test tube well before the dehiscence. Care was taken to avoid any injury to anthers during collection. The anthers were crushed in 2.5 ml of water inside the test tube. All the pollen grains came out of the anthers. The contents of the test tube were shaken thoroughly and 2 drops were pippeted out and placed one each in two counting chambers of Haemocytometer. The number of pollen grains in each of the eight corner squares was counted. This was repeated four times for each suspension and each such repetition formed a 'Sub-sample'.

The average number of pollen grains per square was calculated from which the number of pollen grains per anther could be found out.

For reasonably accurate estimate ten samples each with 3 sub samples were studied for glycerin suspension method and 10 samples with 4 sub samples each were studied for Haemocytometer method.

RESULT & DISCUSSION:

Review of the available literature indicated that the methods of the study pollen production in flowers have been constantly improved upon since the year 1956. Knowlton (1935) Estimated that pollen output by allowing one anther to dehisce on a glass slide ruled into squares and counted all the pollen grains. He found it very cumbersome and suggested the use of the Haemocytometer. Erdtman (1943) cited the studies of Pohl (1937) who determined the number of grains per anther by suspending the contents of anthers in water all counting all the pollen grains in a fractional portion of the suspension.

Erdtman (1943) also referred to studies, he made, which involved the use of suitable chemicals to dissolve the structure of anthers except exine of the pollen grains. The exine were then counted by using a counting chamber. Vaish (1973) studied pollen production in *Lab – Lab niger* by teasing anthers on the slide and counting the number of pollen grains. Nair (1973) have adopted a method in which anther was crushed in 50 drops of glycerin followed by counting of pollen grains in a single drop of dispersion. Trivedi (1975) followed the same method in case of *Cassia tora* and *Arachis hypogea*. Pollen production studies in *Datura metal* var *fastuosa, Vinca, Argemone, Ricinus, Carica papaya* and *Sesbania granciflora* reported by same method (Saoji, 1972 and Saoji, 1979).

Ganguly *et al* (1961) have followed a different method where in a little quantity of rectified spirit was added to a known number of dehisced anthers and taking each drop, pollen number was counted.Chandra *et a*l (1980) in Malvaceae recorded increase in number of anthers from 25-29, results in more pollen production per flower while lesser pollen production occurs when anther number is decreased. Thus the density of pollen production depends upon number of anthers per flower. Oberole (1952) employed the Haemocytometer for estimation of pollen output and standardized the method. They recommended a suspension size of ten samples of 10 undehisced anthers each and four sub samples for taking counts in each sample of 100 anthers. Nagarjun *et al* (1972) followed the same method prescribed by Oberole (1952) with slight modification.

From the results it was evident that there are some differences in the total pollen output by both the methods, though they are not major. The Haemocytometer method appears to be more accurate (Table – 3 & 4) as the number of pollen grains is counted for 0.1 cubic mm of the solution. In the glycerin suspension method the pollen count is made from single drop (0.05 ml). It was observed that the total pollen output was little more when Haemocytometer was used.

However due to ruled area counting becomes easier by Haemocytometer and it appears to be the best suited instrument for evaluation of pollen output except for the plants whose pollen size is comparatively bigger.

In the present investigation, results of pollen production by Haemocytometer method indicates that the slight variation occurs in pollen grains produced per anther to that reported by Gupta (1991) in *P. hysterophorus.* Maheshwari (1966) reported that *P. hysterophorus* produces an average of 624 million pollen grains per plant. Agashe (2006) also reported the pollen production 9600 per staminate flower by haemocytometer method in *P. hysterophorus* from Bangalore.

In *Oxalis corniculata* pollen production by glycerin suspension method was found to be 80

whereas by haemocytometer method 217. In *Tridax procumbens* pollen production by glycerin suspension method was found to be 2044.5 whereas by haemocytometer method 314 (Table 1 – 4).

The variation occurs in number of pollen grains produced per anther because pollen production is affected by distribution, habit of plant and difference in climatic conditions.

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Table 1 - Pollen production by glycerin suspension method in *Oxalis corniculata*

S.No.	Total no of pollen grain 20 drop			Mean no of pollen grain / drop	Total No of Pollen grain / inflorescence	Average
	1	2	3			
1	9	10	12	10.33	206.6	217.92
2	10	8	13	10.33	206.6	
3	14	12	10	12	240	
4	9	8	12	9.66	193.2	
5	15	10	13	12.66	253.2	
6	11	14	9	11.33	226.6	
7	8	13	11	10.66	213.2	
8	9	12	14	11.66	233.2	
9	7	15	9	10.33	206.6	
10	10	13	7	10	200	

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e-ISSN 2347 – 517X
Original Article

Table 2 - Pollen production by glycerin suspension method in *Tridax procumbens*

S. No.	Total no of pollen grain 20 drop			Mean no of pollen grain / drop	Total No of Pollen grain / inflorescence	Average
	1	2	3			
1	144	98	157	133	2660	2044.5
2	121	155	137	137.66	2753.2	
3	89	117	75	93.66	1873.2	
4	95	122	77	98	1960	
5	90	75	86	83.66	1673.2	
6	133	68	124	108.33	2166.6	
7	140	79	98	105.66	2113.2	
8	96	88	57	80.33	1606.6	
9	128	76	61	88.33	1766.6	
10	93	78	110	93.66	1873.2	

Table 3 - Pollen production by haemocytometer method in *Oxalis corniculata*

S. No.	Total no of pollen grain counted in each four sub-sample				Total no. of pollen grain / sample	Total No of Pollen grain / inflorescence	Average
	1	2	3	4			
1	0	1	0	0	1	20	82
2	2	0	0	1	3	60	
3	1	0	3	0	4	80	
4	1	4	0	1	6	120	
5	2	1	0	2	5	100	
6	0	2	1	0	3	60	
7	4	0	1	0	5	100	
8	1	0	2	0	3	60	
9	0	2	3	1	6	120	
10	3	1	1	0	5	100	

Table 4 - Pollen production by haemocytometer method in *Tridax procumbens*

S. No.	Total no of pollen grain counted in each four sub-sample				Total no. of pollen grain / sample	Total No of Pollen grain / inflorescence	Average
	1	2	3	4			
1	8	8	3	2	21	420	314
2	5	9	7	3	24	480	
3	6	10	1	2	19	380	
4	1	2	4	1	8	160	
5	6	3	1	2	12	240	
6	2	4	3	1	10	200	
7	4	7	4	2	17	340	
8	5	3	2	3	13	260	
9	9	2	8	1	20	400	
10	1	5	6	1	13	260	