Methodology:
The methodology would be involving

1. **Reference Slide Preparation:**

   **Survey site:-**
   
   The Jaipur is famous as "Gulabi Nagar" or Pink City in the world. It is situated in 77 degree east longitude and 27 degree latitude in the north-eastern part of the Rajasthan State. This is the capital city of the State. The present studies are confined to the survey of pollen flora and prepare reference slides for identification of the specific pollen in the exposed slides. Thus qualitative and quantitative evaluation of the pollens present in the air of Jaipur district.

   First of all a general field survey was made within the district. Extensive botanical study of the district was made. For the collection of polliniferous material, an intensive field survey of flora of Jaipur district was conducted, within the range of Jaipur district. Field trips of adjacent places were made frequently for collection purpose. Flowering periods, flower colours, size of flowers, pollination mechanism of collected plants were observed during collection.

   **Physiography of Jaipur District:** The soil of the area is sandy to sandy loams with very little organic matter and nutrients. The summer is marked by very high temperatures associated with dry, dusty winds. A wide range of temperature variation has
been recorded in Jaipur district. The temperature ranges from 4°C to 43°C, the maximum temperature recorded during the month of June and minimum during the month of December. In summers the maximum temperature reaches upto 45°C and minimum temperature 21°C. In winters the minimum temperature reaches upto 4°C.

**Survey:**

Periodic field trips to various parts of the city and the adjoining areas covering various seasons of the year were made. Plant collections for the present investigation, were made from the adjacent vegetation of the area of Jaipur district. Field trips were made several times in a month to different localities within the area marked for this study. The survey of the vegetation of Jaipur district was conducted.

In the field each polliniferous material was collected in separate polythene bags then brought to the laboratory and anthers or very small flowers were fixed in F.A.A. (Formaline 5%, acetic acid 25% and absolute alcohol 70%) in small vials. A plant specimen for herbarium was also maintained for reference and identification purpose. All the identification has been done in the "Herbarium" Department of Botany, University of Rajasthan, Jaipur. Reference slides were named of the specific species, after identification and confirmation.
Following places were surveyed in present study of Jaipur District, for the purpose of flora:
Ram Niwas Garden, Jaipur.
University Campus, University of Rajasthan, Jaipur.
Smritivan, JLN Marg, Jaipur.
Jhalana Reserve Forest, Jaipur.
Nehru Garden, Jaipur.
Jharkhandh, Jaipur.
Jhotwara.
Kalwar.
Khatipura.
Mansarower.
Jawahar Nagar, Jaipur.
Malaviya Nagar Etc.
Reference Slide:-

Reference slides of acetolysed pollen were prepared by the method suggested by Erdtman (1952).

The fixed anthers were crushed in glacial acetic acid in a test tube and passed through a sieve. The dispersion was collected in a glass centrifuge tube and centrifuged at 1500 rpm, the supernatant acid was removed and the residue was acetolysed by freshly formed acetolysing mixture constituting nine parts acetic anhydride and one part concentrated sulphuric acid and the centrifuge tube was placed on a water bath for 2 to 5 minutes. The pollen material was again centrifuged, washed carefully two or more times with 10% glycerine & distilled water. After centrifugation the pollen sediment was caught on glycerine jelly held on a needle. Then the jelly and crushed paraffin wax were placed on a microslide. Now it is melted and mounted by a cover slip. This is known as a "Acetolysis preparation'. By using this method two acetolysed slides (stained and unstained) and one unacetolysed slide were prepared.

Unacetolysed pollen preparations are useful for identification and comparison of the air borne pollen caught on the exposed slides as both have retained the protoplasm, while acetolysed preparations help in the study of pollen morphology (aperture, ornamentation etc.) in greater detail, because of the dissolution of the pollen protoplasm.
The morphological characters have been noticed under shape, size aperture, ornamentation, thickness of wall (exine and intine) etc. However, of these, the aperture characteristics are of primary importance.

These reference slides are stored and used for pollen identification.

2. **Pollen Identification:**

Pollen is a fine to coarse powder containing microgametophytes produce male gametes (sperm cells).

The individual pollen grains are small enough to require magnification to see detail.

The pollen wall protects the sperm nucleus while the pollen grain is moving from the anther to the stigma, it protects the vital genetic material from drying out and solar radiation.

The pollen grain surface is covered with waxes and proteins, which are held in place by structures called sculpture elements on the surface of the grain.

The outer pollen wall prevents the pollen grain from shrinking and crushing the genetic material during desiccation and it is composed of two layers.

These two layers are the tectum and the foot layer, which is just above the intine. The tectum and foot layer are separated by a region called the columella, which is composed of strengthening rods.
The outer wall is constructed with a resistant biopolymer called sporopollenin. The pollen tube passes through the wall by way of structures called apertures.

Pollen apertures are any modification of the wall of the pollen grain.

These modifications include thinning, ridges and pores, they serve as an exit for the pollen contents and allow shrinking and swelling of the grain caused by changes in moisture content.

The elongated apertures/ furrows in the pollen grain are called colpi (s.colpus) which along with pores, are a chief criteria for the identifying pollen classes.

Pollen grains may have furrows, the orientation of which (relative to the original tetrad of microspores) classify the pollen as colpate or sulcate.

The number of furrows or pores helps classify the flowering plants, with eudicots having three colpi (tricolpate), and other groups having one sulcus.

Except in the case of some submerged aquatic plants, the mature pollen-grain has a double wall, a thin delicate wall of unaltered cellulose (the endospore or intine) and a tough outer cuticularized exospore or exine.

The exine often bears spines or warts, or is variously sculptured, and the character of the markings is often of value for identifying genus, species, or even cultivar or individual.
In some flowering plants, germination of the pollen grain often begins before it leaves the microsporangium, with the generative cell forming the two sperm cells.

The pollen grains range in size between 5 and 200 microns, mostly 10-100.

The fresh pollen grain includes three main parts: innermost living cell and inner wall layer, inane and outer wall layer exine.

The exine is divided into two layers called endexine and ektexine according to Faegri and Iversen.
Fig 1

The ektexine has a three-layered structure. As seen in Fig. 1a there is a lowermost foot-layer covered by a more or less dense carpet of small granules called columellae.
The columellae are in some cases fused together as a roof or rectum. The pollen grains can according to their possession of a roof or not be lactate, tinware or semitectate.

The positions of different features on the pollen grain are described with terms of a sphere or ellipsoid e.g. poles, polar axis, polar view, equator, equatorial plane, equatorial view, meridian and so on (Fig. 1b).

The outer shape of the grains varies, the main types being spherical, compressed — oblate, and long, drawnout — prolate (Fig. 1c).

Two types of apertures exist, through which the pollen tube emerges. Round apertures are called pores, and furrows are named colpi.

The pollen keys used for identification are based on the number of apertures and their position on the surface of the grain. In the pollen key of Faegri and Iversen 24 different groups are included, of which the ten most common are described below:

1. Saccate or vesiculate pollen grains — a central body with two bladders;
   conifers, pine (Pinus),
   spruce (Picea), fir (Abies)

2. Inaperturate — no obvious openings but provided with a weak zone of the exine, where the pollen grain splits or breaks;
   Juniper (Juniperus), yewtree (Taxus)
sedges (Cyperaceae, on which false apertures lacunae occur)
3. Monoporate — one pore, surrounded by a thickening, called annulus
grass (Gramineae)
4. Triporate — three pores, birch (Beluga), hazel (Corylus). S. Periporate — more than three pores distributed over the surface plantain (Plantago), goose foot family (Chenopodiaceae), pimpernel family (Caryophyllaceae).
5. Stephanoporate — more than three pores (usually 4-6) arranged around the equator elm (litmus), alder (Alnus), hornbeam (Carpinus).
6. Tricolpate — with three colpi oak (Quercus), ash (Fraxinus), willow (Salix), buttercup family (Ranunculaceae).
9. Tricolporate — three colpi and three pores lime (Tilia), beech (Fagus), composites (Compositae tubuliflorae), umbellifers (Umbelliferae), leguminose family (Leguminosae).
10. Fenestrate — windows arranged in a geometrical pattern, echinate composites (Compositae liguliflorae).
11. Tetrads — four pollen grains stuck together, each grain tricolporate or monoporate, heather (Ericaceae), reed mace (Typha latifolia).
Some of the Important Pollens are as:

**Acacia sp.**: bilateral pollen cluster, in polar view circular to elliptical; elongated elliptical in equatorial view (extremely seldom seen from this edge). The polyad consists of 16 almost cubed monads. Size: polar axis: 44.9 (42.5-47.5) µm x 48.8 (45.5-52.4) µm (in polar view), single monads sized 13.5 (11.9-14.8)µm. Apertures: colporate, apertures hardly visible by way of light microscopy. The exine shows a more or less quadrangular laesio on the distal pole of each monad. Pollen wall: very thin, psilate exine, thin intine, pollen wall thickened to about 1-2 µm at the distal edges and the outer vertices.

**Brassica sp.:** rounded triangular in polar view, circular to elliptical in equatorial view. Size: polar axis: 22.8 (21-24) µm, equatorial axis 24.0 (23-25) µm. Size can vary within cultivars, - oil seed rape pollen is usually clearly bigger. Apertures: tricolpate, colpi rounded at their end. Colpi often covered with fine granules. Pollen wall: reticulate exine, with more or less constant width of meshes, scarcely becoming smaller towards the colpi (as opposed to the reticulate tricolporate Salix-species).
**Eucalyptus sp.**: in polar view triangular, seldom more or less circular, elliptical in equatorial view. size: polar axis: 13.7 (12.9-14.8) µm, equatorial axis: 17.6 (16.8-19.8) µm. apertures: tri-, sometimes tetra-zono-colporate, syncolpate, pori ca. 3 µm in diameter, colpi narrow, fusing in the polar area thus forming a central triangle. Pollen wall: thin, psilate exine, often thickened around the pori. Thin intine, forming convex, approx. 4-7 µm wide onci. Pollen wall about 1 µm thick.

**Asteraceae**: rounded triangular in polar view, circulate-ovate in equatorial view size: polar axis: 35.2 (34-36) µm, equatorial axis 38.9 (37-41)µm. apertures: tricolporate pollen Pollen wall: exine thin, echinate, with fine up to 5 µm long spines. Intine protrudes underneath the pori.

**Morus alba**: circular size: polar axis: 18.5 (16.8-19.8) µm equatorial axis: 19.8 (17.8-21.8) µm. apertures: diporate, seldom triporate pollen, apertures up to 3 µm wide and clearly visible opercula. Pollen wall: thin, psilate or scabrate exine, thin intine, with 4-6µm wide onci.
Poaceae: rounded spheroidal to ovoid in equatorial view size: 22 \( \mu \text{m} \) -122\( \mu \text{m} \). apertures: monoporate pollen

Parthenium:

Family Asteraceae is the eurypalynous family with various aperture types. Family Asteraceae is the largest Angiospermic family comprising twenty species, both wild and cultivated. In most of the genera pollen are 3-zonocolporate. However, zonoporate aperture have been noticed in Sonchus asper and in xanthium indicum. Ectine ornamentation marks the difference in various groups. Among zonoporate grains, in Sonchus as per size of pore 6 \( \mu \text{m} \) and height of spinule 3 \( \mu \text{m} \), while in xanthium indicum it is 4.5x7.5 / \( \mu \text{m} \) and 1.5 \( \mu \text{m} \) respectively. In most of the genera ectine ornamentation is spinate or spinulate.

Among 3-zonocolporate grains spines are very small in xanthium indicum. Generally members are spherical to sub-spherical in shape. Well developed spines are present in most of the members. Further, classification can be made on the basis of size of pollen, pore and length of spine. Parthenium is 3 Zono colporate spinulate. The grains are spherical in shape, size ranges from 21 x 21 \( \mu \text{m} \) to 54 x 58 \( \mu \text{m} \) in diameter. Parthenium pollen grains are oblate-spheroidal to prolate-spheroidal; the amb triangular, 3-4 lobate and 3-4 colporate. The sexine is generally thick, tectate, and has long spines. The intine is thin
but slightly thickened below the apertures. Parthenium has pollen grains of the Baccharis-type.

3. **Sampling device for collecting Pollen:**

![Burkard Spore trap diagram](image)

**Figure:2 Burkard Spore trap showing (a) Principal exterior parts; (b) 7 day lid assembly with drum; (c) 24-hr lid assembly with slide.**

Aerobiological sampling was carried out to monitor the qualitatively and quantitative prevalence of aeroallergens through Burkard 24 hr. spore trap system, which is a type of suction sampler. Coloured photo in Fig:3.

This is based on the Hirst spore trap (Hirst 1952, Gregory 1973). It is a compact unit with built-in pump, designed to sample airborne particles, such as fungus spores and pollens for 24 hrs periods. Air is drawn in at 10 liters/min through a...
standard orifice size 2 x 14 mm. and airborne particles are deposited on a glass slide coated with adhesive mountant, in our study we used Glycerine jelly as a mountant.

**Glycerine jelly preparation:**

Reagents used - 20 g gelatin, 70 ml water, 60 ml glycerin (also known as Glycerol) and 2.4 g phenols and Safranine stain 2 drops (to stain all the pollens pink without changing the natural colors of fungal spore)

Method of preparation-

- Boil water and measure 70 ml of the boiled water and add to gelatin.
- Boil this mixture once again and mix thoroughly.
- Add glycerine and phenol to above mixture and keep steering to mix.
- Add drops of stain and mix again to give a uniform colour to the mountant.
4. Method of Collection:

The spore trap system was loaded with a glass slide on which Glycerin jelly is coated. This slide is placed in the sampler and the trapping of the aerobiological material takes place from one end to other at a speed of around 2mm/hr, covering the whole slide in 24hr. The date of slide is noted on the top of the slide and also a small arrow is put on the side of the date, the arrow indicates the direction in which the slide moves and thus the air gets struck on the glycerine jelly in 24hr.

The site selected for the entrapment of the air spora was the building of Asthma Bhawan situated at Vidyadhar nagar, Jaipur. Asthma bhawan is on the outskirt of the old city and is on the north western side of it. It is girdled by Aravali ranges, on the north and north eastern side, which have the forest vegetation. North – North West and West side of Asthma bhawan are mostly less populated and have open fields. The areas from West – South and South – East have thickly populated city areas. The trapping apparatus was installed at a height of 13 meter from ground and 24hrs of pollen sample was collected from 12:00 noon to 12:00 noon.
5. **Analysis of air samples:**

Samples were analyzed by direct microscopy. Identification of airborne pollen done by comparing them with the corresponding pollen in the reference slides prepared. Number and distribution of apertures and various patterns of ornamentation of exine to be considered as the chief characters employed for the identification of atmospheric pollen, as confirmed from standard literature for pollen identification by Erdtman (1952); Faegri and Iverson (1964).

6. **Pollen counting:** The counting has been performed on a daily basis using British Aerobiological Federation manual (Guide to trapping and counting).

Mean daily and hourly pollen concentrations are expressed as grains per cubic metre of air.
7. **Skin Prick Test Data collection:**

The Skin Prick Testing data has been taken from the OPD of Asthma Bhawan on monthly basis.

The data of patients belonging to Jaipur was separated out from the total patients conducted SPT.

This selected data has been entered on Xcel sheets on date wise basis for each month.

The result was calculated on the monthly total of all sheets.