5. DISCUSSION

Airborne pollen grains are the most important natural sources of atmospheric biopollution concerned with inhalent allergy of human beings. In recent years many researchers have showed interest in aerobiological studies by considering its relevant in clinical and agriculture fields.

It is believed that after the exposure to pollen grain about 15 % of the world population are subjected to have developing allergic symptoms (Cadman, 1990). These symptoms usually include congestion, rhinorrhea, pruritus, sneezing, as well as recurrent post-nasal drip.

In view of their clinical implications, several surveys on airborne pollen were conducted in the Gangetic, peninsular and coastal regions of India (Chanda, 1978; Nair, et al. 1986 and Singh, 1984) and observed that patients from the hill stations are suffering from respiratory allergic disorders. Therefore, it was considered as imperative to carry out a research on pollen grains of Ooty and Coonoor, hill stations lie in Western Ghats of Tamil Nadu, to reveal their composition in the atmosphere and accordingly the present has taken its shape.

The present study covering two years from June 1993 to May 1995. The investigation deals with Botanical, Biochemical, Immunological and clinical aspects pertaining to the pollen flora of Ooty and Coonoor. It was hoped that
the results would be useful in the assessment of post immunotherapy treatment of patients with respiratory allergic disorders.

**Aerospora sampler**

In any aerobiological work, the apparatus employed to catch the airborne particles is important as each has its own virtues and limitations (Gregory, 1961).

The methods that are currently used for sampling airborne bioparticles are chiefly based on 3 principles, viz (i) Gravimetric (ii) Volumetric and (iii) Impaction.

Of these, gravimetric method is based on the principle that bioparticles settled down on surface due to gravitational force. The instrument is economical, simple and does not require power and thus more suited to Indian subcontinent. Therefore for the present analysis, the aeroscope - gravity slide method is used to study the airborne pollen grain at Ooty and Coonoor.

As pollen incidence vary from place to place and from day to day, various sampling devices were tested and by and large gravity slide method was found to be the most useful and universally followed for all practical purposes, as it is simple and economy, with reasonably good result (Durham, 1946).
The sampler was installed at a height of 10-15 m on the rooftop of the buildings and operated for a period of two years from June 1, 1993 to May 31, 1995. Standard microslides, smeared with glycerine jelly, were exposed at an angle of 45°. The slides were changed every day at 10 A.M in an interval of 24 hours, with its open end facing towards the wind currents for efficient catch.

**Phenological studies**

The incidence and dispersal of pollen in any given area depends upon a number of factors, of which the most primary are the flower forms in the ground flora. The pollen presentation mechanisms in the flowers is related to the type of pollination mechanisms, which in turn accounts for the dissemination of pollen grains (Faegri and Iversen, 1964).

Clinically important pollinosis is primarily due to flowering plants with wind dispersed pollens. Although these anemophilous form a minority of flowering plants, they produce prodigious amounts of pollen. Entomophilous (insect - pollinated) plants produce relatively few pollen grains. Plants having only a portion of their pollen grains airborne are termed amphiphilous. Their role in pollinosis is not clear (Bush, 1989).

There is a definite correlation between the abundance of plants, duration of their flowering, number of pollen grains produced, nature of pollen dissemination, their distribution in the air and the allergenic reaction due to these pollen types (Thommen, 1931).
In the above background, knowing the details of these plants and the phenomenon of flowering and related subjects on the vegetation of the area under investigation is a prime necessity.

The flowering period of commonly growing plant species is very helpful in aerobiological studies for confirming and identifying of morphologically similar airborne pollens (Mishra, 1992). More than 100 species were found to be exotic in the present study. Among this monocultured and some other plants were selected for the phenological observation. The phenological information consists of the common name, pollination group, frequency in the field and flowering period.

Vegetation

Generally, the Nilgiris district especially Ooty and Coonoor have diverse vegetation with mixture of angiosperms and gymnosperms besides ferns and fungi.

In local descriptions of these hill stations, two very constrating physiognomic types are recognised: (1) forest and (2) grass lands.

The forests, locally called "shola", are almost invariably confined to the sheltered sites such as the valleys, glens, hollows, and depressions where moisture is good. It is an evergreen stunted forest with short bolded trees seldom higher than 15 m. The crowns are dense and the leaves coriaceous. The branches are covered with mosses, lichens and epiphytes. Woody climbers are
The strata are not clearly defined. The other formation - grassland, comprises grasses, herbs and shrubs mixed in varying proportions, and covers large areas of the hill tops.

Although, information on the vegetation of the Nilgiris district especially Ooty and Coonoor has been given by many Scientists (Bor, 1938; Fyson, 1915, 1921, 1932; Mathew, 1955b, 1969, 983; Gamble and Fischer, 1915-1936) no systematic phenological study on plant species that contribute pollen to the atmosphere is available.

In the present study, monoculture plants covered are the Acacia spp, Eucalyptus spp, Pinus spp, Cupressus spp. The other plants either cultivated for their food, fodder or naturally abundant.

**Present study**

- **Acacia** - July - December
- **Cupressus** - bloom during all months
- **Pinus** - February - April

The results observed on flowering period of these plants highly correlated with the other findings of various scientists.

- **Acacia** - Sept - February (Satheesh, 1992)
- **Cupressus** - Winter season (Barletta, *et al.* 1996)
  - In winter and early spring (Caiaffa, *et al.* 1993)
- **Pinus** - March to June (Armentia, *et al.* 1990)
The atmospheric pollen season in New Zealand commence with the flowering of *Pinus* together with *Acacia* was observed by Licitis, 1953. The flowering behaviour of these plants may change due to the variation of climatic and physiographic conditions.

**Airborne pollen incidence**

It is now well established that pollen grains are the Chief component of the aerospora. Pollen concentration in any atmosphere is naturally dependent on the following: (1) Local vegetation (2) Flowering behaviour of the plants (3) Pollen production and other characters (4) Pollination mechanism of the pollen such as wind, insect or both and water and (5) Meteorological factors such as temperature, rainfall, wind velocity, relative humidity etc.

Pollen production and pollen output of the plants varied from place to place, from season to season and from year to year depending on climate, altitude or latitude, affecting the incidence of pollen in the air (Akers, *et al.* 1979; Lacey, 1981; Nair, *et al.*, 1986).

Based on the above principles, the present study was carried out at Ooty and Coonoor, the two famous hill stations in the Nilgiris district of Tamil Nadu, located only at a distance of 19 km from each other. The vegetation does not show much variation. But the climate of Coonoor is milder than that of Ooty. These two locations were selected for the study because of the non report of the aerobiological and allergic studies.
Pollen grains

A total of twelve pollen types at Ooty and eleven pollen types at Coonoor were recorded and they belong to nine different families. Variation in the concentration of pollen grains was observed during the entire period of study and it was due to the blooming periods of various ground taxa and seasons.

During the entire period of study, total pollen counts at Ooty was higher than that of Coonoor (Figure 16). This might be due to the undisturbed thickness of the vegetation nearby area of Ooty.

*Cupressus* is observed as the most dominant pollen contributor in both locations. This result indicates the (1) High occurrence of *Cupressus* spp, in the vegetation and found to be in bloom during all months, (2) anemophilous nature of the pollen grains, and (3) the abundant occurrence of this genus nearer to the installation centre.

Among the various groups of plants analysed, trees contributed the most at both locations. The aeropalynological survey of various pollination groups of Ooty and Coonoor revealed that the Anemophilous plants contributed the largest number of pollen grains to the atmosphere of the respective stations and the studies conducted at other places had also revealed that Anemophilous taxa was the highest contributor of pollen to aerospora (Singh and Babu, 1980; Singh, 1985).
Meteorological data

The effect of meteorological parameters on pollen counts was studied in general by Singh and Babu (1980) at Delhi. It was reported that with the increase in temperature up to a certain level in a day, there is an increase in pollen counts. Rose and Davies (1969) and Nair, et al. (1986) have also obtained similar results, and further, they recognised that the positive correlation between atmospheric pollen concentration and temperature is due to "temperature inversion".

All these phenomena were correlated with the results obtained in this study. During the periods of higher temperature, lower rainfall and minimum wind velocity, a higher pollen percentage was observed. Such results are similar to the findings of Bianchi, et al (1959). The effect of climatic factors on pollen counts in the atmosphere was reported from a number of centres in India (Nair, et al. 1986; Singh, et al. 1987; Sinha and Mishra, 1988; Singh, et al. 1988).

Biochemical studies

The study of the pollen chemistry is paramount important because pollen grains which carrying male genetic material, are responsible for causing certain forms of allergic manifestations in human beings.

Therefore, a preliminary biochemical investigation of the Acacia, Cupressus and Pinus in PBS (pH 7.2) was analysed for their protein content,
total nitrogen content, non protein content, aminoacids content, carbohydrates content. The crude protein was characterized in SDS-Page. This may helpful for diagnosis and therapy of the patients.

The most of allergenic extracts are prepared from the raw materials of pollen grains. They are crude and contain a large number of proteins. Out of these only some of them may be allergenic and associated with specific biological activity of the extract. Besides protein, many other components like nonspecific irritants, polysaccharides, pigments, degraded proteins, histamine etc., are present in the extracts. Till today only crude extracts are used for diagnosis and immunotherapy of respiratory disorders. The lack of highly specific potent antigen is the limiting factor to effective immunotherapy. The standardization of allergenic extracts for diagnosis and treatment has been a major problem (Singh and Malik, 1992).

**Protein**

According to Stanley and Linskens (1974) most of the pollen allergens are water soluble which enhances their degree of diffusion in mucoid tissues and increase the reactive charge groups available on dissociated molecules. Considering the above view the soluble protein was estimated in the present study by adopting modified Lowry's method and Micro-Kjeldahl method.
Total nitrogen and non-protein nitrogen

Total nitrogen and non-protein nitrogen were estimated by Micro-Kjeldahl method. Nair, et al. (1986) also estimated the total nitrogen content of the pollen by Micro-Kjeldahl method. But Baer, et al. (1969) could not find any relationship between the total nitrogen or protein content and the biological potency of the allergens.

Total Free Amino Acids

The total free amino acids of pollen extracts were estimated by using colorimeter. According to Laserna and Manalo (1968), the allergenic activity of the pollen might be linked with the amino acid content of the grains rather than other substances.

Total Free Soluble Sugars

The total soluble sugars present in the extracts were determined by the phenol-sulphuric acid method (Dubois, et al. 1956). The protein - carbohydrate ratio are also presented in table no.14.

Lunden (1956) reviewed the pollen chemistry from 1918 till 1955 and described the various pollen constituents viz., protein and aminoacids, carbohydrates, lipids, vitamins and hormones, enzymes and coenzymes, pigments, inorganic constituents and miscellaneous.
The chemical analysis of pollen grains of various species were done by various scientists to ascertain their allergenic nature by adopting various methods (Chanda and Ganquly, 1976; Chanda, et al. 1975; Agarwal and Nair 1989; Datta, et al. 1992; Mandal, et al. 1993).

In the present investigation Cupressus pollen extract showed a very low content in all the biochemical parameters analysed followed by Pinus. In contrary Acacia pollen showed the highest value in all the biochemical parameters.

The difference between the various biochemical parameters analysed may be responsible for controlling the variation in the degree of allergenicity of the pollen antigens analysed.

**Methods for Determination of Extract Composition**

The composition of allergenic extracts can be determined by using several techniques.

1. Technique for determination of extract composition.
Proteins denatured

Polyacrylamide gel electrophoresis with sodium dodecylsulphate (SDS-page), is the easy and reproducible qualitative technique. The proteins are separated according to size, but only after denaturation.

In the present study crude pollen protein of Acacia, Cupressus, Eucalyptus and Pinus were analysed for their protein by SDS-page. Their molecular weight ranged from 30 K d to 97 K d.

Thaka (1989) fractionated the crude allergen extract of Prosopis juliflora (Mimosaceae) by SDS-page. The molecular weight of the pollen ranged from 81,000-13,000. Acacia (Mimosaceae) antigens also showed similarity with SDS-page ranging of the above species in the same family.

Cornford, et al. 1990 found the banding pattern of leached pollen proteins (SDS-page) of Pinus radiata ranging from 76 K d to 26 K d. The immunoblot analysis of the Pinus radiata showed the five detectable protein bands viz., 82 K d, 67 K d, 54 K d, 44 K d and 38 K d. In the present study also Pinus antigen showed the correlation with the above findings.

Fernandez, et al. 1993 has obtained 13 bands, by SDS-page, ranging from the molecular weight, 94 K d to 14 K d by immunoblot. The result
obtained from the present study are similar to the above study despite using crude allergen.

Barletta, et al. 1996 have determined the molecular weight of Cupressus arizonica and Cupressus sempervirens pollen by SDS-page ranging from 97 K d to 14 K d. This result is similar to the present Cupressus antigen molecular weight ranges.

**Immuno Diffusion**

Immuno diffusion technique of Ouchterlony (1962) was adopted for the immunodiffusion analysis. The polyclonal antibodies which were raised in the rabbits for Acacia, Pinus and Cupressus were used. Immunoprecipitates were observed for all the three antigens. This test was the qualitative conformational rather than quantitative.

**Rocket Immunoelectrophoresis**

In rocket immunoelectrophoresis (RIE), the area of the precipitate (subsidiarily the height) formed by electrophoresing antigen into the monospecific antibody containing gel is proportional (within certain limits) to the antigen concentration. However, RIE are dependent on monospecific antibodies. The production of these antibodies required purification of the corresponding allergen before immunization of animal.
Rocket immunoprecipitate was observed by the RIE technique for the *Cupressus* antigen against their polyclonal antibodies. *Cupressus* antibodies showed the cross reaction to the *Acacia* antigen. From this it was confirmed that *Acacia* and *Cupressus* pollen may have some form of similarity.

Many scientists adopted the RIE, CRIE, CIEI, TCIE, FRIE, FRRIE and Western blot techniques for the monoclonal antibody analysis (Ipsen, *et al.* 1991).

**Skin Test**

Dependent on allergic volunteers.

The most widely used technique for verification of the *in vivo* activity of an allergenic extract, is skin testing. This technique is based on a panel of (volunteering) patients who are allergic to the allergens in consideration. The major problem with skin testing is the selection of patients; the group must represent the average allergic patient and should be defined by other methods.

Skin test has been the diagnostic method of choice for demonstrating immediate hypersensitivity (Nelson, 1993). The allergenic potential of *Acacia, Alnus, Asteraceae, Amaranthaceae, Cupressaceae, Eucalyptus, Poaceae* and *Prunus* were well established in India as well as in other countries (Singh, 1985; Spieksma, 1990). In the above background the following tests were conducted in rabbits and human volunteers.
Sensitized rabbits were used for the ID skin tests. Results revealed that *Cupressus* and *Acacia* produced higher percentage of allergic reaction than the *Pinus* allergen.

**Patch Test**

It has been conducted on 50 patients for three antigens. This technique is adopted only because of its less riskyness. Ueda, *et al.* 1992 studied the patch test reaction to eight kinds of flowers and fourteen kinds of pesticides. He observed the negative reaction except to chlorothalonil and trifonine (pesticide). The findings of present study also highly correlated with the above results with the observation of highly negative reaction to the allergens.

**ID-Skin Test**

Almost all allergists adopted one or other form of skin test technique on the local patients. The present study has been carried out on 50 patients and ID skin tests were performed on the forearms. *Acacia, Cupressus* and *Pinus* antigens were tested with the positive and negative controls. The three antigens were chosen for the study because of their antigenicity, abundance and the highly monoculture nature of the plants in the locations.

**Acacia Pollen Allergy**

*Acacia* is one of the introduced monocultured plants in the Nilgiris district and it is one of the pollen contributors to the Ooty and Coonoor
atmosphere. Even though the occurrence of these plants is very high in these areas, the pollen frequency in the atmosphere is less due to the amphiphilous nature of pollen grains.

The surveying of literatures indicated that allergenicity due to some insect pollinated type like Acacia are reported to be very less.


Ariano, et al. 1991 observed respiratory allergy to the pollen of mimosa (Acacia floribunda) in mediterranean area of Italy and France. The incidence of sensitivity is only 1.2 % among population of atotics but 31 % in the floriculturists.

The results of the present study also reveal the allergenicity of Acacia pollen same like as the above studies indicated. The lower occurrence of these pollen grains, heavier and amphiphilous nature of pollen grains might be the reasons for the lower percentage of allergy among the patients when compared with other allergens.
**Cupressus Pollen Allergy**

*Cupressus* is the extensively monocultured exotic plants in the Nilgiris district. *Cupressus* was the highest pollen contributor to the Ooty and Coonoor atmosphere. It is a anemophilous plant and produce enormous quantum of pollen grains. Cupressaceae pollen allergy on human was reported for the first time more than the years ago (Ordman, 1945).

The mountain cedar *Juniperus sabinoidea* a member of *Cupressus* family, is reported to be a major allergen source in central Texas, New Mexico, and northern Mexico (Fein, *et al.* 1962; Pence, *et al.* 1976; Ramirez, 1984). Studies conducted in South Africa four decades ago have proved that *Cupressus* was responsible for precipitating allergic symptoms as reported by Ordman, 1963; 1964; 1970).

The airborne pollen of *Cupressus* family have been recognized as a cause for respiratory allergy for many years in the mediterranean area, especially in Israel, (Tas, 1965; Keynan, *et al.* 1991) in Southern France (Bousquet, *et al.* 1984; Panzani, *et al.* 1986) and in Italy (Auteri, *et al.* 1989).

The pollinosis of Cupressceae had also been investigated in California, (Yoo, *et al.* 1975) and in Australia, (Ford, *et al.* 1991).

*Cupressus* pollens have been studied in detail from a biochemical point of view (Gross, *et al.* 1978; Ford, *et al.* 1991). Ford, identified the *Cupressus sempervirens* antigens by protein blotting techniques. The study provides basis
for the production of standardized safe and effective C. Sempervirens pollen extract applicable to diagnosis and therapy of cypress pollen allergy.

In Japan incidence pollen related allergy is due to a member of allied family of Taxoidanceae, the cedar Cryptomeria japonica (Yasueda, et al. 1983; Panzani, et al. 1986 and Ishizaka, et al. 1987). Caramiello, et al. 1991 examined 1343 patients in Piedmont (Itlay) and observed 15 patients were positive to Cupressus sempervirens.

Allergic rhinitis due to cypress pollen is a well recognized clinical condition. Cimignoli, et al. 1992 characterized the Cupressus sempervirens pollen by western blotting method. The Ig E of patients known to be cypress sensitive cross-react with the polypeptide antigen of Cupressus sempervirens obtained by SDS-paging.

The allergenic airborne pollen Cupressaceae was surveyed by Caiaffa, et al. 1993 in Southern Italy for 8 years.

Cross reactivity between Cupressus sempervirens and callistis glaucohylla pollen proteins and striking similarities in the Ig E recognition band patterns of two pollens was observed by Pham et al. 1994.

Mari, et al. 1996 found out that the four commercial and two in house Cupressus sempervirens pollen extracts showed low cutaneous reactivity.
Barletta, et al. 1996 determined that the *Cupressus arizonica* and *Cupressus sempervirens* extracts are highly cross-reactive at the Ig E level.

The immunoprint and the crossed radio immuno electrophoresis revealed common antigenicity between *Cupressus* and Thuja pollen (Guerin, et al. 1996).

The cupressaceae pollen allergy was recorded in the Madrid area - Spain (Caballero, et al. 1996).

Besides earlier studies, the present study also showed the importance of *Cupressus* pollen grains and their allergenicity.

The clinical analysis conducted in the present study confirmed the allergenicity *Cupressus* antigen and it showed a higher percentage of allergic reaction than with other antigens tested. It is also cross reactive with *Acacia* antigens. Almost all the findings in the present study highly correlate with the findings of various scientists.

*Pinus* Pollen Allergy

*Pinus* is the most important tree in Nilgiris district. It is the major component of the exotic forests. *Pinus* was the second maximum pollen contributor to the Ooty atmosphere and fourth major contributor to the Coonoor atmosphere. *Pinus* species is a wind pollinated plant and produce enormous quantum of pollen.
At present, a few studies on pine pollen allergy are available. Some authors maintain that this pollen is allergenic because of its large size. Others believe it to be an allergen that must be taken into account when it is found predominantly in the patients environment, even though the clinical manifestations of sensitization to this allergen are mild (Rowe, 1939; Newmark and Itkin, 1967; Harris and German, 1985). Pinus pollen allergy was observed by many scientists (Wodehouse, 1965; Higuchi, et al. 1977). Pine nut allergy was found by Santos and Unger, 1958 and Fine 1987.

Like other pollens, Pinus pollen also play an important role in the etiology of respiratory allergic disease in USA and Canada. (Collins - Williams, et al. 1971; Lewis, 1984). The incidence of Pine pollen allergy (Pinus radiata) was shown by Harris and German in 1985 in Sanfrancisco also.

Singh, et al. (1987) reported that Pinus roxburgi produced markedly positive skin reactions in 16.9% patients from hills. Galan, et al. (1989), recorded that 2.9% pine pollen allergy was found among atopic population in Spain. Armentia, et al. (1990). reviewed three cases of pine pollen allergy at Spain.

Enzyme - immunoassay inhibition studies revealed that leached Pinus radiata pollen protein could partially inhibit serum Ig E binding to ryegrass RAST discs thus providing preliminary evidences for allergen cross-reactivity between these two unrelated species (Cornford, et al. 1990).
Fountain & Cornford, *et al.* (1991) found that the allergenic protein of *Pinus radiata* partially cross-react with that of rye grass pollen. *Pinus radiata* pollen also leaches low molecular weight compounds (benzoic acid and 4-hydroxy benzoic acid) which are known to possess adverse pharmacological effect on a small proportion of the population.

The occurrence of airborne *Pinus sylvestris* was continuously monitored with Burkard traps at five locations in Finland over a period of 10 years (1982-1991) by Pessi and Pulkkinen, 1994.

Applying the postulates proposed by Thommen, pine pollen satisfy the following necessary criteria to be considered an antigen responsible for hay fever (*Armentia, et al.* 1990).

1. The pine tree produces pollen,
2. This pollen can be widely distributed in the environment, where, it can constitute the most predominant pollen,
3. The pollen can be produced in large quantity,
4. The pollen can be carried by air and
5. The pollen can be allergenic.

From the above studies, and the hypothesis and from the present investigation it was concluded that, even though the incidence of reactivity to pine pollen is low, it could be valid to perform skin and *in vitro* test to *Pinus* pollen in patients with allergic symptoms who live in pine forest regions.
As the earlier studies, the present study also confirmed the low allergenicity of *Pinus* pollen grains. It may be due to the large size and hydrophobicity of the grain. The large size of *Pinus* pollen with their distinctive air bladder, not allowing greater penetration into the respiratory tract of the susceptible population. A second possibility may be due to the release of lower allergenic contents when they come in contact with susceptible than the *Cupressus* and *Acacia* pollen allergens.