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POLLEN GRAINS: A POSSIBLE SOURCE OF FUNGAL INFESTATION IN SEEDS OF *CEDRUS DEODARA* LOUDON

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CEDRUS DEODARA is an important tree species which is well-known for its valuable timber. Its multiplication chiefly depends upon its seed setting. Despite heavy pollination, the seed setting is low and a good percentage of seeds is sterile. Besides other environmental and physical factors, the mycoflora may also be responsible for this seed sterility. Holmes and Burzewicz[1] observed many fungi during routine testing of conifer seeds, the most frequent being *Penicillium* spp.

One of the most important sources of fungal infestation in seeds while they are in the course of development on the tree may be pollen grain which is unfortunately almost overlooked. Since pollen can easily be contaminated due to their high starch content, when mature either at male cone or during pollination which is of anemophilous type, they subsequently carry their contamination to the ovules and ultimately to the seeds. Therefore, the present investigation was planned to study the mycoflora associated with pollen grains and seeds of *C. deodara* at hilly areas of Nainital where the trees are widely grown.

Fresh, mature, male and female (unopened) cones were collected from the trees in sterilized polythene bags (for two years) and soon brought to the laboratory. Pollen grains were collected from the mature male cones on sterilized glazed paper and the seeds were collected by mechanically opening the female cone scales with the help of a sterilized scalpel. Dilution plate and direct plate techniques were applied for the isolation of associated fungi. The seeds with coat removed and twice washed with sterilized water were also screened.

The results are given in table 1. In all, 16 fungi were isolated, of which 14 were associated with pollen grains, 12 with whole seeds and only 7 with coat removed seeds. Five fungi were isolated from pollen grains, whole seeds as well as from the coat removed seeds.

Table 1 *Fungal infestation of pollen grains and seeds of C. deodara*

Fungi isolated	From pollen grains	From seeds: Whole seeds	From seeds: Coat removed seeds
Mucor luteus (Linn.) Schipper	+	+	−
Rhizopus nigricans Ehrenb.	+	+	+
Aspergillus flavus Link	+	+	+
A. fumigatus Fresenius	+	+	+
A. niger Tieghem	+	+	−
A. pulvinus Kwon & Fennell	+	+	−
Penicillium chrysogenum Thom	+	+	+
P. nigricans (Bainier) Thom	+	+	−
P. oxalicum Currie and Thom	+	+	+
P. purpurogenum Stoll	+	−	−
P. simplicissimum (Oudem) Thom	+	+	−
Alternaria alternata (Fr) Keissler	+	−	+
Cladosporium cladosporioides (Fr.) de Veries	+	−	+
Fusarium moniliforme Sheld.	−	+	−
Trichoderma viride Pers. ex. Gray	+	−	−
Mycelia sterilia	−	+	−

+, present; −, absent.

It is clear from table 1 that the mycoflora which reaches the seeds is carried by pollen grains because the fungi which were isolated from the pollen grains, {some of them (10)} were also present on the seed surfaces and some (7) inside the seeds of which the coat has been removed. It is due to the fact that pollen grains are sticky in nature and pollination is of anemophilous type; therefore, the spores of aeromycoflora can easily be adhered to the surface of pollen grains which in turn can carry them to the ultimate site (i.e. ovule) and finally to the seeds. After the pollination the scales of female cones again close rendering the cone compact.

The ovule is the most protected part of female gametophyte and it is not possible for many fungi to survive even after they reached there through pollen grains; this may be the reason for the lesser number of fungi in the coat removed seeds. The number of fungi on the surface of seeds was higher than that inside of the seeds because pollen grains could also be trapped in the space between the scales and ovules since all pollens that take part in the pollination do not necessarily take part in fertilization. However, there were two fungi, viz., *Fusarium*

moniliforme and a member of mycelia sterilia isolated, which were absent in pollen grains. These fungi possibly might have come with the wind when the scales were open for the pollination. The two fungi which were present in pollen grains but absent in the seeds, could not find the base for their establishment in the latter.

The presence of fungi in the seeds of compact cones which were not apparently mechanically injured, is a moot question. Gibson[2] reported that the seed coat of most of the conifers is resistant against invasion. These facts also favour the conclusion that the pollen grains are vectors for fungi at the time of pollination and fertilization.

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CYTOLOGICAL STUDIES IN *APONOGETON SATARENSIS* RAGHAVAN, KULKARNI AND YADAV

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GENUS *Aponogeton* L. is represented in India by five species. Asiatic species of *Aponogeton* are characterized by bisexual flowers and one spiked inflorescence only. However, a two-spiked, dioecious *Aponogeton* species, viz., *Aponogeton satarensis* Raghavan, Kulkarni and Yadav is of special interest from cytological standpoint[1]. Sharma and Chatterjee[2] reported $2n=76$ in *A. natans*. Moreover, other Indian species of *Aponogeton* still remain unexplored from this aspect of study. With this view in mind the present investigation was undertaken.

Plants collected from type locality and surroundings of Satara district (MS) are maintained in the gardens of Botany Department of this University. The karyotypic studies from root tips were performed after pretreatment with *p*-dichlorobenzene and then by applying normal aceto-orcein technique. For classification of karyotype asymmetry the scheme of Stebbins[3] was used and the nomenclature recommended by Levan *et al*[4] for centromeric position was adopted. For meiotic studies also, normal aceto-orcein technique was followed. Photomicrographs were taken from temporary preparations using mfAks system of JENAVAL Carl Zeiss microscope.

Somatic chromosome number of *A. satarensis* is $2n=26$ (figure 1). Chromosomes are in general short (2.30 μ–1.12 μ). On critical examination of the karyotype the following types of chromosomes were categorized.

Type A: One pair of long chromosomes (2.30 μ) with one constriction in the median (m) region.
Type B: Four pairs of comparatively long chromosomes (2 μ) with a constriction in the median (M) region.
Type C: Five pairs of medium size chromosomes (1.86 μ) with one constriction in the median (m) region.
Type D: Two pairs of short chromosomes (1.72 μ–1.58 μ) with one constriction in the median (M) region.
Type E: One pair of very short chromosomes (1.12 μ) with one constriction in median (M) region.

Thus karyotype formula of *A. satarensis* is represented as:

$$K:2n:26:2A^{m}+8B^{M}+10C^{m}+4D^{M}+2E^{M}.$$

The idiogram of species is represented in figure 2. Karyotype analysis has been done in both the male and the female plants of this species and no sharp differences have been observed in male and female plants.

During meiosis, the formation of 13 distinct bivalents at diakinesis was observed (figure 3). Smallest pair of chromosomes showed association with that of largest chromosomes in diakinesis, while metaphase-I was with normal orientation (figure 4). Generally the arrangement of microspores in tetrad was isobilateral, rarely decussate.

The present study is the first record of the diploid chromosome number, karyotype morphology and meiotic behaviour of *A. satarensis*. *Aponogeton distachyon* and *A. fenestralis* have shown[5,6] $n=8$, whereas in Indian species *A. natans* $2n=76$ was reported[2]. It is also interesting to note here that there is no significant difference in chromosome size (2.30 μ–1.12 μ) of *A. natans* and *A. satarensis*. It seems, therefore, that there is a definite indication of polyploidy and aneuploidy in evolution with a probable base number $n=13$. However, investigation