

Dolichandrone platycalyx: New entomophilous pollen—A report on pollen sensitization in allergic individuals

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ABSTRACT

Background: *Dolichandrone platycalyx*, commonly known as Nile trumpet tree, is believed to have originated in East Africa. However, this and the variants of this tree are found in Europe, Asia, and America (California and Florida). The tree mostly grows in tropical climates, but temperate species are also found. This study was designed to evaluate the allergenicity of *D. platycalyx*, one of the most common entomophilous avenue trees in Karnataka state, and to determine the pollen production of *D. platycalyx*.

Methods: All of the patients with allergic rhinitis and asthma attending a tertiary care center in South India during August 2007 to March 2008 underwent a detailed clinical evaluation and skin-prick testing to common allergens along with *D. platycalyx*. Control subjects without any symptoms of respiratory allergy also underwent skin testing. The pollen counts were determined for a mature unopened flower of *D. platycalyx*.

Results: A total of 317 subjects with respiratory allergy and 30 controls were included in the study. A significant percentage (16.1%) of patients evaluated were observed to be sensitive to *Dolichandrone* pollen extract by skin-prick testing, whereas none of the control subjects were found to be sensitized. *D. platycalyx* was the fourth most common sensitizer after *Parthenium hysterophorus*, *Prosopis juliflora*, and *Artemesia vulgaris*. *D. platycalyx* was found to be a moderate pollen producer at 66,000 pollens/flower.

Conclusion: Sensitization to *D. platycalyx* is common in subjects with respiratory allergies. The clinical relevance of this sensitization and other entomophilous plants needs additional study.

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Cases of respiratory allergy are increasing worldwide.^{1–7} Respiratory allergy may be caused by any biological particulate matter in the atmosphere such as pollen grains, fungal spores, trichomes, etc. They can sometimes cause respiratory disorders such as allergic rhinitis and allergic asthma. Natural pollen exposure worsens bronchial hyperresponsiveness and seasonal allergic rhinitis.⁸ Pollen grains not previously considered allergenic have been shown to cause allergies on evaluation.^{9–14} Therefore, there is a need to systematically study various pollen grains for their allergenicity. Many entomophilous types of pollen, not found in high quantities on aerobiological surveys and until now considered “allergy safe,” are now known to cause allergies. Common entomophilous plants, such as *Carica papaya* (papaya tree), *Moringa oleifera* (drumstick tree), *Tectona grandis* (teakwood), *Delonix regia* (Gulmohar), *Cassia siamea* (Siam cassia/red mohar) and *Cassia fistula* (Indian laburnum/golden shower),¹⁵ *Catharanthus roseus* (Sadaphuli/Vinca/Madagascar periwinkle),¹⁶ *Anacardium occidentale* (cashew),¹⁷ and *Helianthus annuus* (sunflower)¹⁸ are known to cause allergy. Development of sensitization is multifactorial and dependent on gene–environment interactions.¹⁹ Recent studies have shown that pollen fragmentation of large pollens caused by meteorological influences can lead to respirable pollen fragments and the phenomenon is common for both anemophilous and entomophilous plants.²⁰ The pollen fragments therefore increase the biogenic load in the atmosphere in a form that is not recognizable as pollen and can not be identified in microscopic analysis.²⁰ Trees planted widely in parks and gardens for their ornamental properties such as *Platanus orientalis* and related species can cause significant pollinosis symptoms and sensitization in the general population.²¹

Some of the most common flowering trees in Bangalore, viz., *Tabebuia argentea*, *Tabebuia rosea*, *Jacaranda mimosaeifolia*, and *Dolichandrone platycalyx*, have a very poor or no representation in the pollen calendar (according to an aeropalynological survey conducted in June–July 2008 by the authors using vertical cylinder pollen trap, unpublished data). All of the four trees are closely related and belong to the family Bignoniaceae. Among the four trees, only pollen grains of *J. mimosaeifolia* were trapped (3 pollens/cm²) in 2 months. The pollen grains of *Dolichandrone* were not trapped probably because of their entomophilous nature and large size. Taking into account that people in the vicinity of these plants are bound to inhale their pollen because of their widespread nature and abundance, one of the four plants, viz., *Dolichandrone* was chosen for evaluation of allergenicity. The other three trees are not as abundant as *Dolichandrone* and have a strict flowering season: February–April for *T. argentea*, *T. rosea*, and *J. mimosaeifolia*. Generally, a fully grown *D. platycalyx* flowers throughout the year.

In this study, we have sought to determine the pollen production of *D. platycalyx*, i.e., whether it is a low, moderate, or high pollen producer and if it is causing sensitization (through skin-prick tests) and thus could be an important aeroallergen.

BOTANICAL DESCRIPTION

D. platycalyx (*Markhamia lutea*) is an upright evergreen tree, 10–15 m high, with a narrow, irregular crown and long taproot. Bark is light brown with fine vertical fissures. Leaves are pinnately compound. The margins are thin and wavy. Each leaflet is up to 10 cm, wider at the tip and often with round outgrowths at the base. Flower buds are yellow–green and furry, splitting down one side as the flower emerges. Flowers are bright yellow, in showy terminal clusters, each trumpet shaped, 5–6 cm long, with five frilly lobes, the throat striped with orange–red. Fruits are very long, thin, brown capsules, up to 75 cm in length, hanging in clusters and tending to spiral. They split on the tree to release abundant seeds with transparent wings. The seeds are 2.0–2.5 cm long and whitish–yellow when mature (Figs. 1–4). Earlier, the genus was named *Markhamia lutea* after Sir Clement Markham, who introduced the famous quinine–yielding cinchona into India. The specific name, “*lutea*”, is Latin for golden–yellow.

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Figure 1. *Dolichandrone platycalyx* habit.



Figure 2. *Dolichandrone platycalyx* fruit.



Figure 3. *Dolichandrone platycalyx* flower.

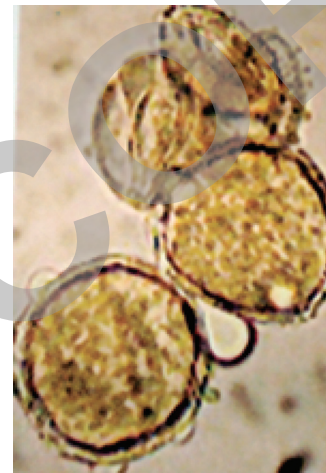


Figure 4. *Dolichandrone platycalyx* pollen.

METHODS

Skin-Prick Testing

This is a cross-sectional study, which was cleared by the local ethics committee and written informed consent was obtained from all participants. In children the consent was obtained from the parent. All patients presenting with allergic rhinitis and or asthma to a tertiary center during the period August 2007 to March 2008 were included. A total of 317 subjects aged 8–84 years underwent evaluation using a structured questionnaire and skin-prick testing to common aeroallergens and spirometry. The structured questionnaire collected data on demographics, a detailed clinical history including rhinitis and asthma. Skin-prick tests were performed using prick Lancetter (Hollister Stier, Spokane, WA) on the flexural aspect of the forearm according to standard guidelines²² using glycerinated allergen extracts from ALK Abello (Madrid, Spain) (1:10 to 1:20 w/v) and the following categories of allergens were evaluated: weeds (*Amaranthus*, *Artemisia*, *Chenopodium*, *Parthenium*, ragweed, *Xanthium*, and *Zea mays*), grasses (*Cynodon dactylon*), shrubs (*Morus alba* and *Helianthus*), trees (*Prosopis juliflora*, *Eucalyptus*, *Causarina*, *Cocos nucifera*, and *D. platycalyx*), fungi (*Aspergillus* and *Alternaria*), mites (house-dust mites), and insects (cockroach). Histamine phosphate (10 mg/mL; ALK Abello) and glycerinated saline (ALK Abello) were used as positive and negative control, respectively. The skin-prick test to the antigen was considered positive if the wheal size was >3 mm when compared with saline control. Fresh *Dolichandrone* extract was prepared for skin testing by Bioproducts and Diagnostics, Pvt., Ltd., Trivandrum, Kerala, India.

Table 1 Characteristics of the study population

Characteristic	No. of Subjects	Percentage (%)
Total study population	317	
Gender		
Male	189	59.6
Female	128	40.3
Age distribution		
<20 yr	79	24.9
21–40 yr	132	41.6
41–60 yr	72	22.8
>60 yr	34	10.7
Allergic disease		
Allergic rhinitis	153	48.3
Asthma	187	58.9
Allergic pharyngitis	166	52.3
Allergic conjunctivitis	120	37.8

Pollen Production

Mature, unopened flower buds of *D. platycalyx* were collected in the morning hours. All four anthers were removed using forceps and crushed gently with a glass rod in 50 drops (2 mL) of 50% glycerine, to release the pollen grains. This suspension was used for pollen counting in a hemocytometer. The process of counting was repeated in at least 10 drops of the pollen grain suspension. The average pollen count was determined (Procedure adapted from "Palynology and Its Applications" by Agashe²³).

Protocol for Allergen Extraction from Dolichandrone Pollen

A flowering twig with mature unopened flower buds was collected from the field, usually in the early morning hours. The macroscopic identification of the plant and the flower was done by comparing with the reference herbarium. The microscopic identification was done by studying the pollen morphology under the microscope.

Extraction

The active allergenic substances from the defatted powder were extracted with slightly alkaline buffer. The extraction was performed in 1:50 concentration for 72 hours with occasional shaking. After the extraction, the active ingredients were separated by clarification. The purity and identification of the pollen material was done according to the Cour and Laublier method and the standardization of allergenic extracts is by biopotency test.²⁴

Sterilization

The allergenic extracts are thermolabile and, hence, the sterilization was effected by filtration through Millipore filters. Sterility tests for both aerobic and anaerobic bacteria and molds were performed on filtered extract for 14 days in the biochemical oxygen demand incubator at ambient temperature.

RESULTS

A total of 317 atopic patients were included in the study. The demographic characteristics of the subjects are presented in Table 1. The mean age of the study population was 34.55 (SD, 18.60 years) years and the male–female ratio was 1.5:1 (Table 1). Aeroallergen sensitivity of the common pollens tested is presented in Table 2. Among the allergens tested, house-dust mites (72%) were the most common allergen sensitized, followed by mosquitoes (51.4%) and cockroaches (48.2%). Among the pollens, *Parthenium hysterophorus* was the most common (18.29%) and *P. juliflora* was the most common tree pollen (17.66%). Sensitivity to *D. platycalyx* was found to be

Table 2 Aeroallergen sensitivity of some of the pollen tested

Pollens	No. Tested Positive	Percentage of Positivity (%)
<i>Parthenium hysterophorus</i>	58	18.29
<i>Prosopis juliflora</i>	56	17.66
<i>Artemesia vulgaris</i>	53	16.71
<i>Dolichandrone platycalyx</i>	51	16.08
<i>Ambrosia artemisiifolia</i>	49	15.45
<i>Amaranthus spinosus</i>	47	14.82
<i>Chenopodium pollen</i>	47	14.82
<i>Ailanthus excelsa</i>	47	14.82
<i>Zea mays</i>	45	14.19
<i>Cynodon dactylon</i>	42	13.24
<i>Brassica nigra</i>	41	12.93
<i>Xanthium strumarium</i>	40	12.61
<i>Cocos Pulmosa</i>	37	11.67
<i>Helianthus anuus</i>	37	11.67
<i>Ricinus communis</i>	36	11.35
<i>Sorghum vulgare</i>	35	11.04
<i>Casuarina equisetifolia</i>	34	10.72
<i>Triticum vulgare</i>	25	7.88
<i>Morus Alba</i>	24	7.57
<i>Eucalyptus globulus</i>	21	6.62
House dust mite	229	72
Mosquito	162	51.4
Cockroach	153	48.2

16.08%. No differences were noted on comparison of pollen sensitization according to gender. Thirty controls without any respiratory allergy were tested for sensitization to *D. platycalyx* and no sensitization to *D. platycalyx* could be found in any of these subjects.

The flowers of *D. platycalyx* have four didynamous divaricate stamens (Fig 3). The pollen grains were found to be round to elliptical in shape, reticulate, and tricolporate, between 40–45 μm in size (Fig 4). *D. platycalyx* was found to be a moderate pollen producer with a pollen productivity of 66,000 pollens/flower.

DISCUSSION

Generally, it is believed by aerobiologists and allergists that only pollen grains of anemophilous plants cause allergic manifestation.^{17,25} Studies clearly indicate that entomophilous pollen although present in a smaller percentage in the atmosphere should not be neglected.^{16,17} Most of these pollen grains are heavy and pollen production is less in comparison with pollen of anemophilous plants (wind pollinated). However, it is noticed that in some entomophilous plants, there is immense pollen production and the pollen grains are abundant in the immediate vicinity of plants,²³ *Dolichandrone* being one such plant. The reason why *Dolichandrone* pollen grains are not usually caught on the slide during aerobiological surveys could be because the vertical cylinder air sampler is placed at a higher level, e.g., a terrace of a building at a height of 7–10 m from the ground level, and because the pollen grains are heavy (40 μm) they may not have been carried the distance.

One of the most important considerations to explain the high rate of positivity to *Dolichandrone* observed in our study could be cross-reactivity between members of the family and between other closely related trees. The members of the Bignoniaceae family consist of 120 genera and many of the trees are known to cause allergic contact dermatitis because of the presence of chemicals such as lapachol, deoxylapachol, and lapachone.²⁶ A case of occupational asthma due to the inhalation of sawdust of the *Tabebuia* tree, related to *Dolichandrone*, has been reported.²⁷ An aerobiological survey in Karachi has shown low levels of pollens of the Bignoniaceae family in the atmosphere.²⁸ Cross-reactivity between members of the family has indi-

cated clinically relevancy, as shown by the immunoblot and inhibition studies for *P. orientalis*, which cross-reacts with *Platanus acerifolia* and *Platanus occidentalis*.²¹ A close relationship between the amino acids of Scrophulariaceae and Bignoniaceae family has been reported,²⁹ although the allergenic potential of many of these pollens and the potential for cross-reactivity are still to be elucidated in clinical studies. Other common avenue entomophilous trees in the Bignoniaceae family, *Kigelia pinnata*, *Spathodea campanulata*, and *Tecoma stans*, need to be investigated for their allergic and cross-reactive potential with *Dolichandrone*. An investigation into the sensitizing potential of various ornamental plants, which included a member of the Scrophulariaceae family, *Antirrhinum majus* (Snap dragon), revealed a high rate of sensitization (20–33%).³⁰

Although it is generally believed that plants with higher pollen productivity and anemophilous plants are more likely to cause allergic manifestations, it may not be always the case; plants with very low pollen productivity also induce allergic reactions.²⁵ A variety of entomophilous plants and trees that can cause allergic manifestations can be low pollen producers such as *Alstonia scholaris* (5175 pollens/flower) and *Azadirachta indica* (6220 pollen per flower); moderate pollen producers such as *Dalbergia sissoo* (11,475 pollens/flower), *Lagerstroemia speciosa* (77,175 pollens/flower), or *M. alba* (24,924 pollens/flower); or high pollen producers such as *Ailanthus excelsa* (1,635,180 pollens/flower), *C. siamea* (537,036 pollens/flower), or *Argemone mexicana* (463,800 pollens/flower). The frequency of allergenicity of these low, moderate, and high pollen-producing entomophilous plants and trees were 0.62–0.71% for *A. scholaris*, 1.11% for *Azadirachta indica*, 0.24–0.38% for *Dalbergia sissoo*, 2.12–3.02% for *L. speciosa*, 2.57% for *M. alba*, 2.96% for *A. excelsa*, 0.65% for *C. siamea*, and 1.59% for *A. mexicana*.

D. platycalyx has no strict flowering season, flowering for most of the year. Flowering is profuse during April–July, i.e., the rainy season. At 66,000 pollens/flower, it could be an important source of aeroallergen, especially around its vicinity and also because it is a common avenue tree in South India. In addition, sometimes, heavy wind and profuse pollination activity during the rainy season are also responsible for increasing pollen load of *D. platycalyx*, in the atmosphere, resulting in significant exposure in the general population. Cross-reactivity could be an important reason for the high rate of sensitization to *D. platycalyx* in our study (16.08%). High frequency of sensitization for entomophilous trees has been observed by Chakraborty *et al.*³¹ for *C. papaya* (27.8% showed +1 level reaction and 5.6% showed +2 level reaction), Ghosh *et al.*¹⁶ for *C. roseus* (29.8% showed positivity and 7.1% showed $\geq +2$ level reaction), and Fernandes and Mesquita¹⁷ for *A. occidentale* (29.8% showed $\geq +2$ level reaction). Considering sensitization to both anemophilous and entomophilous plants and trees, *D. platycalyx* was the fourth most common among all pollens (Table 2) and is the second most common tree causing sensitization in our population. Our study reveals that entomophilous pollen, although not caught on the slide during aeropalynological surveys, can be a health hazard to already susceptible individuals.

CONCLUSION

Considering the foregoing data, the present study regarding pollen of an entomophilous species such as *D. platycalyx* and its clinical relevance as a common sensitizer assumes great significance. It is worth investigating the sensitizing potential of all the plants and trees that are entomophilous, because they could fill the gap in the list of unknown aeroallergens. Variants of *D. platycalyx* are found in California and Florida in the United States, *Markhamia hillebrandii*, for which there is no data on allergenicity. The same is true with species of *Catalpa* and *Campsis*, which belong to the family Bignoniaceae. *Catalpa* is a genus comprising 11 species of trees and shrubs and are natives of East Africa and North and South America, *Catalpa bignonioides* being the most popular species cultivated. The bark of *Carya ovata* is known to cause contact dermatitis, because of the chemical

deoxylapachol. *Campsis radicans* is native to the southeastern United States, in which its flowers cause contact dermatitis. It would be interesting to evaluate for allergenic cross-reactivity of these species with *D. platycalyx*. Additional studies of other commonly present avenue trees in Bangalore such as *J. mimosaeifolia* and *T. argentia*, which belong to the same family as *D. platycalyx*, could confirm whether they are equally common sensitizers and their cross-reactivity between the species. It is observed that these two plants have a strict flowering season from February to April, unlike *D. platycalyx*. More detailed studies are required to understand the clinical relevance of sensitization to *D. platycalyx*. Future studies to characterize antigens of *D. platycalyx* and bronchial challenges are necessary to confirm its relevance in asthma.

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