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A SIMPLE AND RAPID DETECTION METHOD FOR AFLATOXIN USING POLYTHENE MINICOLUMN

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AFLATOXINS, the naturally occurring food toxicants produced by *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare have been reported to occur in a wide range of agricultural commodities[1]. Several outbreaks of the disease in animals and men due to aflatoxins have been reported from India[2] and elsewhere. The methods that are in wide use currently for detecting and quantitating aflatoxins are expensive and time consuming. To overcome this, several minicolumn methods have been developed during the last decade[4]. Jemmali[5] suggested the use of disposable cellulose tubing and a confirmatory test using 50% H_2SO_4. However, the cellulose tubing material is not always readily available and further it dissolves readily in 50% H_2SO_4. Hence, an attempt has been made to use minicolumn prepared out of locally available material for the detection and confirmation of aflatoxins.

Polythene tubing (12cm × 1cm) was prepared from an ordinary polythene sheet of 0.2 mm thickness. One end of the column was plugged with a small piece of cotton. Anhydrous sodium sulphate was poured into the column to a height of one cm and it was covered with neutral alumina (one cm.) activated at 110° C for 2 hr. Silica gel (column chromatography grade, 60–120 mesh, activated at 110° C for 1 hr) was added to a height 1 cm above alumina followed by thin layer chromatography grade silicagel to a height 6 cm. The top of the column was plugged with a piece of cotton. After a number of combinations tested in initial trials, the above described column materials were found to be most satisfactory.

Fifty gm lot of contaminated wheat sample was extracted with 100 ml acetone + water (85:15 v/v); 10 ml of the filtered extract was mixed with 10 ml of 20% aq. lead acetate solution to remove interfering fluorescent materials. The mixture was filtered and the filtrate was shaken vigorously with 3 ml of benzene (3 minutes) and allowed to stand and one ml from benzene layer was taken in a test tube and the packed column was inserted into the test tube and the ascending solution was allowed to reach TLC grade silicagel zone. The column was developed for 3 min in 5 ml. chloroform, acetonitrile, isopropanol mixture (93:5:2 v/v/v). The developed column was observed under long wave UV light. A blue fluorescent band just above the neutral alumina zone indicated the presence of aflatoxins. Aflatoxin contaminated rice and groundnut cake samples were also screened with this technique.

For confirmation of aflatoxin, the column was dipped in one ml of 20% H_2SO_4 in methanol or 10% HCl in ethanol for about a minute. The blue fluorescence turned to yellow confirming the presence of aflatoxins. Semiquantitation can also be done by comparing the column developed in sample extract with standard columns of different concentrations as described by Cucullu *et al*[3]. With this procedure aflatoxin could be detected upto 10 ppb level. The entire process of development and detection requires about five minutes.

However, aflatoxin has been shown to be absorbed to the extent of 7% after 15 min when it is in direct contact with polythene tube[6]. But, in the present method the aflatoxin does not come into direct contact with tubing as it passes through adsorbents and development is completed within 5 min. Thus this simple and rapid technique can be used conveniently at the source.

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FOLIAR STOMATAL DEVELOPMENT IN THREE SPP. OF *ARISTOLOCHIA* L

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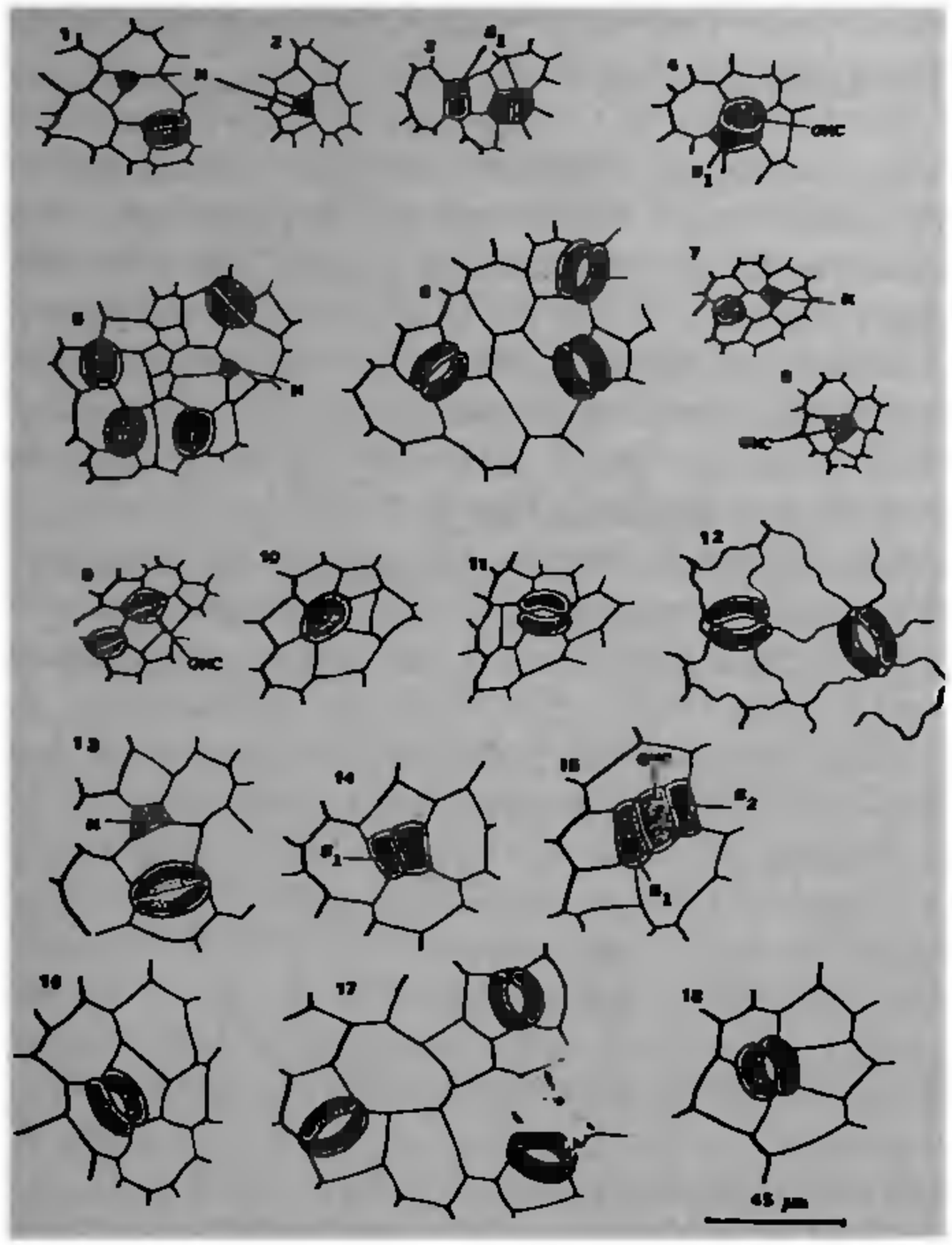
STRUCTURE and development of stomata in several families and plants have been studied by various workers[1-8]. But there seems to be no epidermal and stomatal study in the family Aristolochiaceae, except what is mentioned by Metcalfe and Chalk[9]. In order to fill this gap, an epidermal study on five species of *Aristolochia* L. was undertaken earlier[10] The present paper describes the development of stomata in three species of *Aristolochia* namely *A. bracteata* Retz., *A. indica* L., and *A. Leuconeura* Lindl.

Leaves of *A. bracteata* were collected from Vishakapatnam (Andhra Pradesh), *A. indica* from Changanacherry (Kerala) and *A. leuconeura* from Bangalore (Karnataka) and Trivandrum (Kerala). Epidermal peels from young as well as old leaves were stained in alcoholic safranin and mounted in 20% glycerine.

The leaf of *A. bracteata* is amphistomatic while the leaves of the other two species are hypostomatic. Stomata in the family Aristolochiaceae have earlier been described as anomocytic[9]. But our study reveals the predominance of paracytic stomata in *A. leuconeura* along with a few anomocytic stomata[10]. The anomocytic stomata are often surrounded by 4 or 5 subsidiary cells and rarely by 6 cells (figures 6 & 12).

The stomatal meristemoids in all the species are scattered irregularly among the epidermal cells and can be distinguished from the latter by their smaller size and deep staining cytoplasm. They are usually squarish, rectangular or triangular in shape (figures 1,2,7 & 13).



Figures. 1-18. Development of stomata in *Aristolochia* leaves. **1-6.** *A. bracteata.*, **7-12.** *A. indica.*, **13-18.** *A. leuconeura.* (GMC-gaurd cell mother cell, M – stomatal meristemoid, S_1 and S_2 – subsidiary initials).

The development of anomocytic stomata in the investigated species follows two different patterns. In *A. bracteata* each meristem undergoes a vertical division forming two cells, one slightly smaller than the other (figure 3). The smaller one forms the first subsidiary cell (s_1) while the larger one directly functions as the gaurd cell mother cell (GMC), which is enlarged, assumes lenticular shape (figure 4) and divides into two equal-sized cells by a longitudinal wall (figure 5). These cells elongate, develop an intervening pore and form two bean shaped gaurd cells (figure 6). The remaining cells are formed from the surrounding epidermal cells. Since the stomatal meristemoid as well as the surrounding epidermal cells constitute the formation of stomatal complex, the development is mesoperigenous.

Anomocytic stomata in *A. indica* and *A. leuconeura* develops perigenously. In these species the stomatal meristemoids directly function as the GMC,