the same in descending manner in *n*-BuOH-HOAc-H_2O (4:1:5, top layer), *n*-BuOH-EtOH-H_2O (4:1:2) and phenol saturated with water. Sucrose was isolated from silica gel column using $CHCl_3$-EtOH (1:1).

Total acid concentrations (9.184 g/lit) of the exudate and that of dried sap (5.60 g/l) were determined by standard sodium hydroxide. Citric, malonic, succinic and fumaric acids in the dried exudate were separated one-dimensionally on a paper chromatogram with *n*-BuOH-HCOOH-H_2O (4:1:5). The dried paper was sprayed with bromothymol blue to get yellow spots. Acetic acid was identified in the distillate of the sap.

The exudate was extracted with chloroform to separate steroids and limonoids. The extract was concentrated, subjected to column chromatography over silica gel after the formation of slurry and the column was run with benzene, chloroform and methanol in the order of polarity. Fractions (1–5) eluted with benzene gave β-sitosterol, m.p. and m.m.p. 140–141°, $[\alpha]_D^{32} - 36°$ (conc. 0.4 g in $CHCl_3$). Acetylation with acetic anhydride and pyridine gave an acetate, m.p. 130–132°.

Further elution of the column with benzene (6–9 fractions) gave 24-methylenecycloartanol, m.p. 120–121, $[\alpha]_D^{32} + 45°$ (conc. 0.3 g in $CHCl_3$), IR (KBr) 3400, 1629, 1010, 890 cm^{-1}.

Fractions (10–12) eluted with benzene-chloroform (9:1) yielded nimbin which on crystallization from methanol melted at 203–205°, $[\alpha]_D^{32} + 168$ (conc. 1 g in $CHCl_3$) (lit.[4] m.p. 201 – 204°, $[\alpha]_D + 170°$).

Fractions (13–15) eluted with benzene-chloroform (4:1) on repeated chromatography on silica gel gave azadirone in gummy form, $[\alpha]_D^{32} + 23°$ (conc. 0.55 g in $CHCl_3$) (lit.[5] $[\alpha]_D + 26°$), λ_{max}^{MeOH} 225 nm (9,980), IR (KBr) 1745, 1685, 1240, 885 cm^{-1}.

The fractions (16–20) collected from benzene-chloroform (1:1) on crystallization from methanol gave gedunin, m.p. 216–217°, $[\alpha]_D^{32} - 40°$ (conc. 1 g in $CHCl_3$), (lit[5]. m.p. 218°, $[\alpha]_D - 44°$), λ_{max}^{MeOH} 225 nm (9200), IR (KBr) 1745, 1675, 885 cm^{-1}.

β-Sitosterol and 24-methylenecycloartanol were the only two steroids obtained from the neem exudate. Earlier, these steroids were isolated from the leaves and heartwood of this plant[6,7]. The limonoids nimbin, azadirone and gedunin might be responsible for the medical properties of the sap. TLC of the chloroform extract indicated that at least three other limonoids might be present in trace amounts which could not be separated. The tonic properties of the sap might be due to the presence of amino acids, proteins, carbohydrates and salts in sufficient amounts.

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LOSS OF TOXIGENICITY OF *ASPERGILLUS FLAVUS* STRAINS DURING SUBCULTURING – A GENETIC INTERPRETATION

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THE loss of toxigenic potentials of fungal strains on repeated subculture has been reported[1]. The present authors examined the toxigenic potentials of *Aspergillus flavus* strains which elaborate aflatoxins (a well-known carcinogen and mutagen). Four strains of *A. flavus* [MZ from maize seeds with 4600 ppb potential; W-1 from wheat sample-1 with 2800 ppb potential; W-2 from wheat sample-2 with 1800 ppb potential and MC from mustard cake with 920 ppb potential] were isolated and grown on SMKY liquid medium[2] at 28 ± 2°C. After seven days the medium was filtered and extracted with chloroform. Qualitative and quantitative estimations[3,4] of aflatoxin were carried out. The subculturing was repeated seven times at regular intervals of seven days and the amount of aflatoxin produced at the end of each incubation period (i.e. 7–49 days) was estimated.

The results showed gradual decline in the toxin production. This decrease was almost rectilinear and

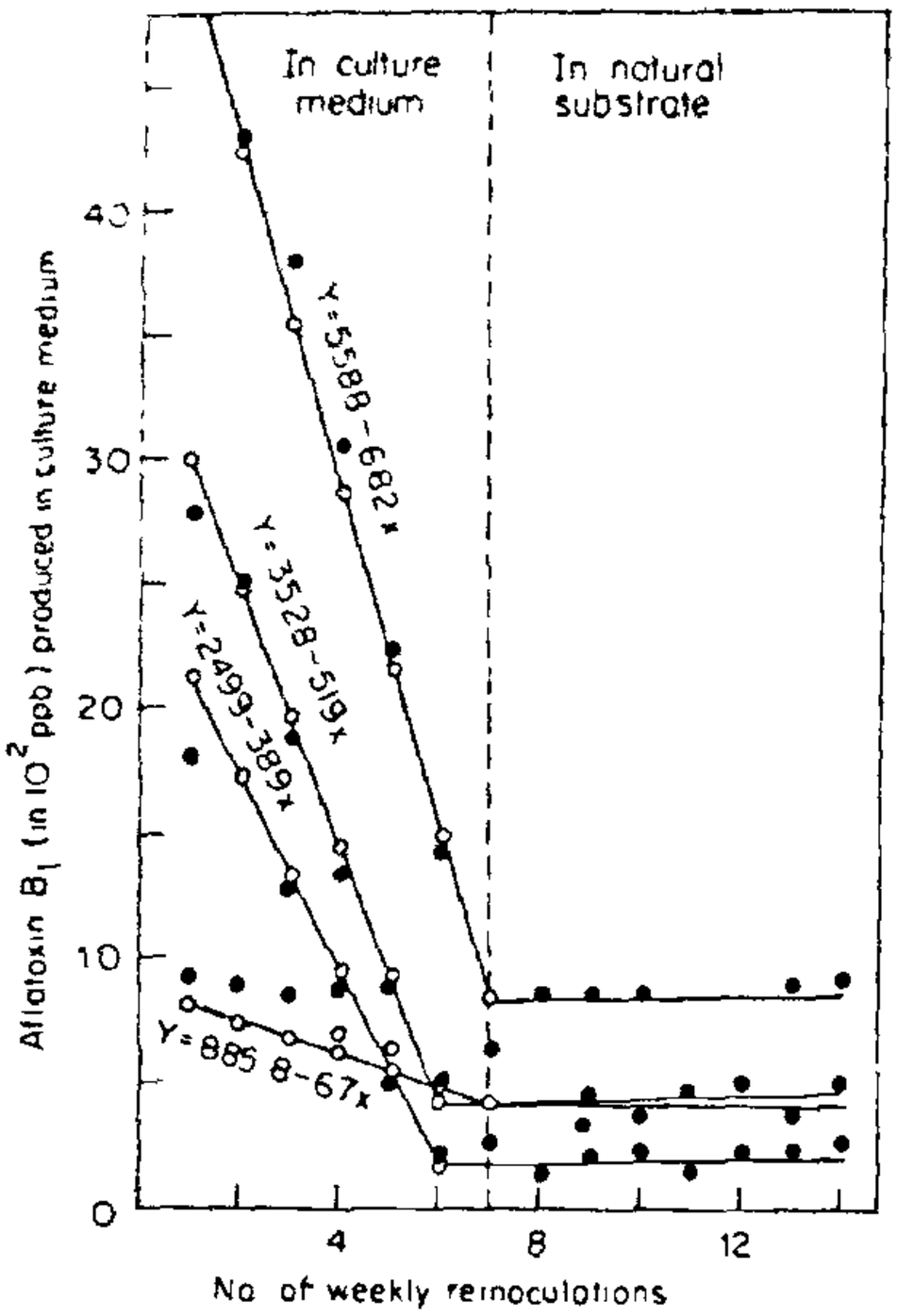

Figure 1. Graph showing loss in aflatoxigenicity at different stages of subculturing.

is represented by lines of regression (figure 1). After seventh week the amount of toxin decreased to its lowest level about 800 ppb in the strain having the maximum toxicity, and 200 ppb in the strain with minimum toxicity. The rate of decrease in the toxin elaboration was highest (682.X) and lowest (67.X) in the strains with maximum and minimum toxigenicity respectively. Reinoculations on natural substrates (maize, wheat and mustard cake) failed to revive or induce any increase in the toxin production[5].

Since sexual reproduction is not known in the fungus, the decrease in aflatoxin during subculturing (SMKY medium) cannot be attributed to any negative selection of toxin elaborating genes. Fall in toxigenic potentials during subculturing and its non-restoration can possibly be associated with the regulation of toxin elaborating genes in the fungus. Aflatoxin elaboration seems to be one such adaptation, which is induced in those strains of *A. flavus* which have to face the competitive and stressed conditions in nature. This acquired character gradually fades out when the fungus is transferred to culture media which are nutritionally rich without any competition for food. The loss of toxigenicity upon reversion to substrate medium may be due to genic assimilation of acquired character[6]. According to this a phenotypic character which initially is produced only in response to some environmental influence, is taken over by the genotype, so that phenotype is formed even in the absence of factors which caused it to appear earlier.

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POWDERY MILDEW OF *MOMORDICA COCHINCHINENSIS*

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MOMORDICA COCHINCHINENSIS Spreng. is a cucurbitaceous vegetable grown extensively in North-Eastern India. A powdery mildew was noticed during the summer (May–June) of 1986, affecting plants grown in the experimental plots of this School and in the cultivator's fields as well.

The pathogen infects all the green parts of the plant viz. leaves, stems and tendrils. Powdery growth first appears on the upper surface of the lower leaves as small (2–4 mm diam), scattered, white, almost circular colonies which eventually coalesce to form big patches covering the entire leaf surface. The disease was more prevalent on the older leaves. The colour of the colony gradually changes from white to yellowish-brown after aging. In severe infection the plants exhibited apparently talcum-powder-like appearance from a distance. Such plants showed reduction in leaf size, which became chlorotic and senescent, and finally died.