7. Malhotra, O. P. and Indira, R., *Indian J. Biochem..*, 1969, **6**, 15.
8. Matsumoto, T., Yatazawa, M. and Yamamoto, Y., *Plant Cell Physiol.*, 1977a, **18**, 353.
9. Streeter, J. G., *Plant Physiol.*, 1979, **63**, 478.
10. Herridge, D. F., Atkins, C. A., Pate, J. S. and Rainbird, R. M., *Plant Physiol.*, 1978, **62**, 495.
11. Miflin, B. J. and Lea, P. J., In *The Biochemistry of plants*, (ed) B. J. Miflin, Academic Press, New York, 1980, vol 5 p. 169.
12. Fujihara, S. and Yamaguchi, M., *Plant Physiol.*, 1978a, **62**, 134.
13. Fujihara, S. and Yamaguchi, M., *Phytochem.*, 1978b, **17**, 1239.
14. Reinbothe, H. and Mothes, K., *Annu. Rev. Plant Physiol.*, 1962, **13**, 129.
15. Lea, P. J. and Miflin, B. J., In *The biochemistry of plants*, (ed) B. J. Miflin, Academic Press, New York, 1980, Vol. 5, p.569.
16. Thomas, R. J. and Schrader, L. E., *Plant Physiol.*, 1981b, **67**, 973.
17. Matsumoto, T., Yatazawa, M. and Yamamoto, Y., *Plant Cell Physiol.*, 1977b, **18**, 613.
18. Atkins, C. A., Rainbird, R. M. and Pate, J. S., *Z. Pflanzenphysiol.*, 1980, **97**, 249.
19. Luthra, Y. P., Sheoran, I. S. and Singh, R., *Photosynthetica*, 1983, **17**, 210.
20. Givan, C. V. In *The biochemistry of Plants*, (ed) B. J. Miflin, Academic Press, New York, 1980, vol. 5, p. 329.

CHEMICAL CHANGES IN DRY FRUITS DURING AFLATOXIN ELABORATION BY *ASPERGILLUS FLAVUS* LINK.EX FRIES

K. S. BILGRAMI, K. K. SINHA AND ANJANA SINGH
Post Graduate Department of Botany, Bhagalpur University, Bhagalpur 812 007, India.

Abstract

Maximum amount of aflatoxin B_1 (23.4 ppm) was elaborated on coconut by *Aspergillus flavus* as compared to almond, cashewnut, walnut and makhana. Important chemical changes were recorded during aflatoxin production in dry fruits. There was a significant loss in the quantity of total, reducing and non-reducing sugars as well as in ascorbic acid level. An increase in protein and phenol level was also noted.

Introduction

A. FLAVUS Link ex Fries is well known for its ability to elaborate aflatoxins. This group comprises both storage[1] and field fungi[2-4]. Being cosmopolitan, these fungi are capable of infecting a large number of food and feed commodities. Besides elaborating aflatoxins they also cause significant changes in the nutritional components of the associated substrates[5,6].

Dry fruits which are of substantial nutritive value are also very good sources for the growth of *A. flavus* and aflatoxin production. Natural occurrence of these mycotoxins is reported in some dry fruits[7,8]. There is, however, no information regarding the chemical changes occurring in dry fruits during aflatoxin production. This aspect is incorporated in the present communication.

Materials and Methods

Five dry fruits *viz.* almond (*Prunus amygdalus* Batsch.), cashewnut (*Anacardium occidentale* L.), walnut (*Juglans regia* L.), coconut (*Cocos nucifera* L.) and makhana (*Euryale ferox* Salisb.) were selected for this study. Dry fruits (25 g each) were soaked in distilled water for 1 hr in 150 ml Erlenmeyer flasks and were subsequently autoclaved for 10 min at 15 lbs p.s.i. On the following day these were inoculated separately with 0.5ml spore suspension (concentration approx. 3×10^6 spores/ml) of toxigenic and non-toxigenic

Table I

Aflatoxin production on dry fruits by A. flavus

Dry fruit	Amount of aflatoxin B_1 produced (ppm)
Coconut	23.4
Makhana	11.9
Almond	10.6
Cashewnut	9.3
Walnut	8.1

strains of *A. flavus*. Each set was run in triplicate and all these flasks were kept at 28 ± 2° C for 11 days. On the 12th day the infested fruits were dried at 60° C for 48 hr and powdered subsequently. Aflatoxins were extracted by the method employed by Jones[9] and estimated qualitatively on TLC plates using toluene: *iso*-amyl alcohol: methanol (90:32:2, v/v) solvent systems[10]. The aflatoxin B_1 was determined spectrophotometrically[11].

Changes in the quantity of total and reducing sugars were estimated by standard methods [12,13]. The non-reducing sugars were calculated by subtracting the value of reducing sugars from total sugars. Protein contents were estimated colorimetrically[14] and ascorbic acid was determined by the method of Roe and Kuether[15]. Changes in phenol contents were recorded following the method of Singh *et al*[16].

Mean values of all the chemical constituents along with their standard deviations were calculated and are recorded in table 2. *T* test value has also been calculated for their per cent significance.

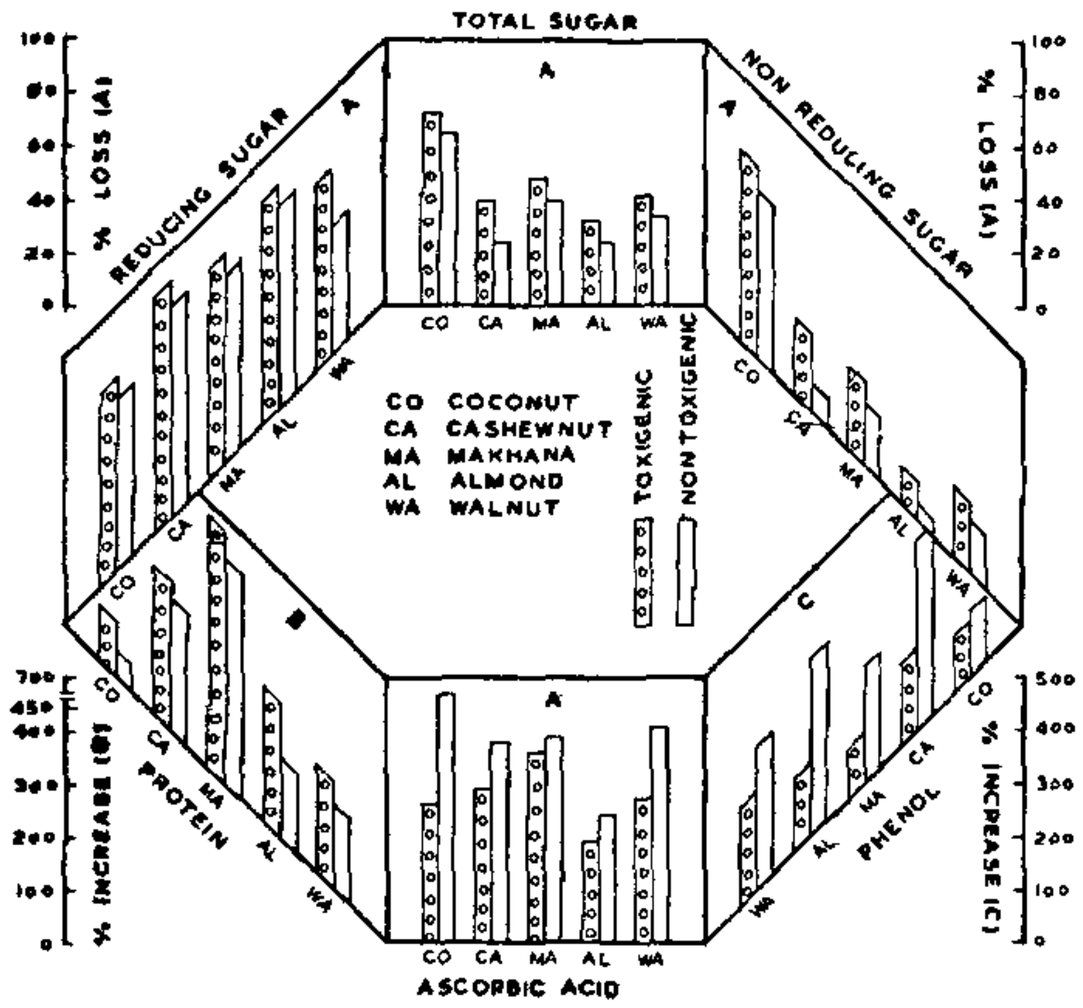

Figure 1. Chemical changes in dry fruits during aflatoxin production.

TABLE 2

Changes in the level of sugars, protein, ascorbic acid and phenol in dry fruits during aflatoxin elaboration

Constituent	Amount %					
	Control fruits		*A. flavus* infested fruits		*t* test value	
	0 day	11 day	Toxigenic	Non-toxigenic	AFT	AFNT
			ALMOND			
Total sugar	1.96 ±0.026	1.82 ±0.002	1.22 ±0.045	1.38 ±0.036	21.43*	19.13*
Reducing sugar	0.56 ±0.03	0.49 ±0.026	0.07 ±0.026	0.11 ±0.036	20.00*	14.61*
Non-red. sugar	1.40 ±0.036	1.33 ±0.085	1.15 ±0.026	1.27 ±0.02	4.73**	1.67*
Protein	0.67 ±0.026	0.72 ±0.02	2.21 ±0.03	1.57 ±0.02	76.81*	59.37*
Ascorbic acid	0.137 ±0.008	0.120 ±0.002	0.075 ±0.004	0.062 ±0.003	2.17	29.00*
Phenol	0.075 ±0.004	0.080 ±0.008	0.190 ±0.002	0.350 ±0.004	22.45*	33.33*
			WALNUT			
Total sugar	1.30 ±0.026	1.20 ±0.036	0.70 ±0.045	0.80 ±0.026	13.15*	15.69*
Reducing sugar	0.19 ±0.026	0.18 ±0.036	0.05 ±0.045	0.09 ±0.026	6.04*	3.07**

contd., next page

TABLE 2 (continuation).

Non-red. sugar	1.11 ±0.036	1.02 ±0.01	0.65 ±0.02	0.71 ±0.02	29.13*	23.53*
Protein	0.62 ±0.02	0.54 ±0.045	1.67 ±0.05	1.44 ±0.036	28.97*	26.78*
Ascorbic acid	0.150 ±0.008	0.132 ±0.002	0.062 ±0.008	0.023 ±0.008	14.28*	27.43*
Phenol	0.080 ±0.002	0.092 ±0.008	0.270 ±0.004	0.340 ±0.004	29.97*	27.62*
			CASHEWNUT			
Total sugar	1.40 ±0.026	1.24 ±0.045	0.74 ±0.02	0.94 ±0.03	17.48*	5.45*
Reducing sugar	0.25 ±0.026	0.22 ±0.026	0.02 ±0.03	0.04 ±0.008	13.16*	11.32*
Non-red. sugar	1.15 ±0.036	1.02 ±0.002	0.72 ±0.026	0.90 ±0.02	32.89*	10.53*
Protein	0.49 ±0.02	0.45 ±0.026	1.71 ±0.036	1.60 ±0.02	49.41*	60.53*
Ascorbic acid	0.237 ±0.004	0.215 ±0.001	0.090 ±0.008	0.050 ±0.003	42.92*	116.67*
Phenol	0.065 ±0.002	0.075 ±0.006	0.190 ±0.003	0.350 ±0.002	30.26*	76.39*
			COCONUT			
Total sugar	2.80 ±0.02	2.38 ±0.03	0.62 ±0.026	0.83 ±0.03	76.52*	60.42*
Reducing sugar	0.83 ±0.026	0.79 ±0.02	0.20 ±0.026	0.26 ±0.02	31.05*	33.12*
Non-red. sugar	1.97 ±0.036	1.59 ±0.03	0.42 ±0.026	0.57 ±0.02	50.87*	49.27*
Protein	0.86 ±0.026	0.79 ±0.036	1.69 ±0.05	1.21 ±0.036	25.00*	14.48*
Ascorbic acid	0.20 ±0.036	0.186 ±0.002	0.087 ±0.004	0.012 ±0.002	15.00*	108.75*
Phenol	0.110 ±0.001	0.120 ±0.008	0.25 ±0.03	0.270 ±0.026	7.22*	9.43*
			MAKHANA			
Total sugar	11.20 ±0.02	10.40 ±0.036	5.40 ±0.036	6.10 ±0.02	170.65*	183.76*
Reducing sugar	3.70 ±0.056	3.54 ±0.03	0.72 ±0.03	0.96 ±0.03	115.10*	105.30*
Non-red. sugar	7.50 ±0.04	6.86 ±0.01	4.680 ±0.026	5.140 ±0.026	134.57*	106.17*
Protein	0.97 ±0.02	0.900 ±0.026	7.09 ±0.05	4.730 ±0.033	196.50*	188.67*
Ascorbic acid	1.05 ±0.03	0.980 ±0.002	0.275 ±0.004	0.212 ±0.002	275.39*	480.00*
Phenol	0.195 ±0.004	0.210 ±0.004	0.415 ±0.003	0.670 ±0.008	30.59*	69.13*

AFT = *A. flavus* toxigenic AFNT = *A. flavus* non-toxigenic
* $P<0.01$ ** $P<0.05$

RESULTS

Toxigenic strain of *A. flavus* was able to elaborate aflatoxins on all the dry fruits under study; however, their amount varied with the nature of the substrates (table 1). Coconut was the best substrate (23.4 ppm) whereas elaboration on other dry fruits was less than 50% of that on coconut.

There was a sharp decline in the level of total, reducing and non-reducing sugars of all the dry fruits during *A. flavus* infestation (table 2, figure 1) which was more pronounced when infestation was by toxigenic strains *i.e.*, during aflatoxin production. Loss in ascorbic acid content, on the contrary, was more sharp during infestation by non-toxigenic strain of *A. flavus*. The protein and phenol levels of dry fruits increased during infestation of *A. flavus* (table 2, figure 1). There was greater accumulation of protein in dry fruits infested by the toxigenic strain of *A. flavus*. whereas phenol concentration was higher when infestation was by the non-toxigenic strain.

Changes in the nutritional components due to *A. flavus* infestation were statistically significant.

DISCUSSION

Rich fatty acid and carbohydrate contents or some toxin-stabilizing factor in coconut are attributed as important factors responsible for the elaboration of high concentration of aflatoxin on this substrate. Other dry fruits were also good substrates for aflatoxin elaboration. Most of the higher fatty acids formed by the breakdown of the lipids present in these natural substrates are known to have significant influence on aflatoxin production by the toxigenic strain of *A. flavus*[18]

Toxigenic strains of *A. flavus* utilized greater quantity of sugar during its metabolism as compared to non-toxigenic strain. This is possibly due to the presence of more glycolytic enzymes in the toxigenic strains which hydrolyse sugar molecules to simpler forms like pyruvate, which is one of the precursors for aflatoxin biosynthesis[18].

Increase in protein concentration of the dry fruits during infestation by *A. flavus* was quite distinct. Earlier reports[19,20] also indicate that protein concentration of the substrates may be increased due to fungal infestation. While working with peanut seeds, Cherry *et al*[21] also found an increase in protein concentration due to an aflatoxin-producing strain of *A. parasiticus*. Initially they attributed such increase to mycelial tissues present on the surface and inside the seeds, but subsequently, using gel electrophoretic techniques they finally confirmed protein accumulation due to aflatoxin production[22].

Although there is no evidence of the direct role of ascorbic acid or phenol in aflatoxin biosynthesis[23], changes in their amount may be due to fungal infestation. The degree of increase or decrease depends upon the nature of *A. flavus* strain. On the basis of these results, it is clear that toxigenic strain of *A. flavus* not only produces aflatoxins inside the associated substrates but also causes considerable loss in the nutritive value by changing the concentrations of the vital components.

ACKNOWLEDGEMENT

The authors thank UGC New Delhi for financial assistance.

25 January 1983; Revised 3 May 1983

1. Christensen, C. M. and Kaufmann, H. H., *Grain storage: The role of fungi in quality loss.* University of Minn. Press, Minneapolis, 1969.
2. Anderson, H. W., Nehring, C. W. and Wichser, W. R., *J. Agric. Food Chem.*, 1975, **23**, 775.
3. Shotwell, O. L., *J. Am. Oil Chem.*, 1977, **54**, 216A.
4. Bilgrami, K. S., Prasad, T., Misra, R. S. and Sinha, K. K., *Indian Phytopathol.*, 1981, **34**, 67.
5. Bilgrami, K. S., Prasad, T., Misra, R. S. and Sinha, K. K., *Biol. Bull. India*, 1979, **1**, 9.
6. Singh, Anjana and Sinha, K. K., *Curr. Sci.*, 1982, **51**, 841.
7. Anonymous, Final Technical Report (DST), Dept. of Food Technol., T. N. Agric. Univ., Coimbatore, 1980.
8. Singh, Anjana, *Proc. Symp. Mycotoxins in food and feed*, Bhagalpur, 1983.
9. Jones, B. D., *Methods of aflaxotin analysis*, G 70, Tropical Products Institute, London, 1972.
10. Reddy, T. V., Vishwanathan, L. and Venkitasubramanian, T. A., *Anal. Biochem.*, 1970, **38**, 568. 568.
11. Nabney, J. and Nesbitt, B. F., *Analyst*, 1965, **90**, 155.
12. Dubois, M., Gills, K. A., Hamilton, J. K., Robers, P. A. and Smith, F, *Methods in microbiology*, (eds. J. R. Norris and D. W. Ribbons), Academic Press, New York, 1956.
13. Plummer, D. T., *An introduction to practical biochemistry*, T.M.H. Pub. Co., Bombay, 1971.
14. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, A. J., *J. Biol. Chem.*, 1951, **193**, 265.
15. Roe, J. H. and Kuether, C. H., *Hawk's physiological chemistry*. (ed. B. L. Oser). Tata McGraw Hill, Bombay and New Delhi, 1943.

16. Singh, M., Singh, S. S. and Sanwal, G. G., *Indian J. Exp. Biol.*, 1978, **16**, 712.
17. Arseculeratne, S. N., Desilva, L. M, Wijesundra, S. and Bandunantha, C. H. S. R., *Appl. Microbiol.*, 1969, **18**, 88.
18. Maggon, K. K., Gupta, S. K. and Venkitasubramanian, T. A., *Bacteriol. Rev.*, 1977, **41**, 822.
19. Vidhyasekaran, P., Ramodoss, N., Ranganathan, K. and Krishnaswamy, V., *Indian Phytopathol*, 1973, **26**, 736.
20. Prasad, T., Bilgrami, K. S., Roy, A. K. and Jamaluddin, *Seed Res.*, 1976, **4**, 191.
21. Cherry, J. P., Mayne, R. Y. and Ory, R. L., *Physiol. Plant Pathol.*, 1974, **4**, 425.
22. Cherry, J. P., Young, C. T. and Beuchat, L. R., *Can. J. Bot.*, 1975, **53**, 2639.
23. Uraguchi, K. and Yamazaki, M., *Toxicology, biochemistry and pathology of mycotoxins*, John Wiley, New York, 1978.
23. Uraguchi, K. and Yamazaki, M., *Toxicology, biochemistry and pathology of mycotoxins*, John Wiley, New York, 1978.

ANGIOSPERM POLLEN—A SYSTEM FOR CELL DIFFERENTIATION

A. RASHID

Department of Botany, University of Delhi, Delhi 110 007, India.

INTRODUCTION

THE pollen of an angiospermous plant in its dual potential of forming male gametes and regeneration into an entire plant is an ideal system for studies on cell differentiation. The potential of pollen to produce male gametes has been known for a long time and accordingly a great deal of information is available about the differentiation of pollen into an androgenic/gametophytic cell, which has been reviewed frequently[1-3]. By contrast, not so long ago, was discovered the potential of pollen to undergo embryo/tissue formation in anther cultures of *Datura*[4] and consequently information about the differentiation of pollen into an embryogenic/sporophytic cell is meagre. Nevertheless, since the sporophytes produced by the pollen are haploid they are of immense importance in the improvement of crop plants. Therefore, the topic is not only of academic interest but also of applied value.

DIFFERENTIATION OF POLLEN INTO AN ANDROGENIC/GAMETOPHYTIC CELL

A mature pollen has an elaborate endoplasmic reticulum with an abundance of ribosomes and is equipped with messengers for germination and gamete formation[5]. This cytoplasmic constitution, which is unique to pollen, is a fine example of structure-function relationship. For this a number of differentiation processes are contributory, the details of which are yet to be delineated. At present what is known, with some degree of certainty, is two crucial steps of differentiation (a) inception of pollen as microspore mother cell and (b) the maturation of microspores into gametophytic cell.

During the differentiation of microspore mother cell, accompanying the process of meiosis which is primarily a nuclear phenomenon, a series of extranuclear changes take place in its cytoplasm which are interpreted to represent the transition from the sporophyte to the gametophyte. That this transition is marked by cell reorganization was noticed even by earlier workers[6,7], when based on light microscopic studies they recorded a loss in stainability of cell cytoplasm. Later this was found to be due to reduction in the level of RNA[8]. These observations have been substantiated by further studies[9]. However, a better characterisation of these details has been possible by subcellular studies. The salient features of cell reorganization are: (a) reduction of ribosome population, (b) alteration in nature and disposition of membranes; the plate-like profiles are replaced by circular ones, and (c) regression of organelles; in plastids there occurs the regression of lamellae and the mitochondria become electron dense and lose their cristae. These details of cell reorganization are seen in *Tradescantia*[10], *Lilium* and *Cosmos*[11-14] and *Nicotiana*[15].

Nevertheless, of late, it is increasingly realised that during the differentiation of microspore mother cell the elimination of sporophytic information is incomplete and some membrane-protected enclaves of cytoplasm do pass through the pollen mother cell[13-15]. Therefore, the rationale behind the different events of