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Effects of Light, Temperature, and *p*H Value on Aflatoxin Production In Vitro

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In the complete absence of light, an isolate of *Aspergillus flavus* produced up to 170,000 pg aflatoxin per g, whereas the incubation of the fungus in light caused a reduction (35,000 pg/g) of the toxin synthesis.

The effects of the substrate, temperature and *p*H value on the growth of and aflatoxin production by *Aspergillus flavus* have been examined by several investigators (1-5). Since, to our knowledge, the effect of another environmental condition, namely that of light, on aflatoxin biosynthesis by this fungus has not been reported in the literature, we undertook such a study.

The isolate of *A. flavus* used in our work produced toxic material when determined by the rabbit skin (A. Z. Joffe, J. Invest. Dermatol., *in press*) and animal feeding (A. Z. Joffe, Mycopathol. Mycol. Appl., *in press*) tests. It was maintained routinely on Czapek's agar medium and was deposited in the stock culture collection of our department as isolate no. 7290. One loopful of spores of a 7-day-old culture grown in Erlenmeyer flasks with wheat substrate was used to inoculate 70 ml of sterile Czapek's liquid medium containing traces of $ZnSO_4 \cdot 7H_2O$. The media were adjusted to an initial *p*H of 4.0 and 7.4, respectively, in 150-ml Erlenmeyer flasks.

Incubation was carried out in five replicate flasks at 18, 24, 32, and 40 C, both in complete darkness and in normal, diffused laboratory light (with overnight illumination). At the end of an 8-day incubation period, the *p*H of the liquid was determined; the mycelium was collected and inactivated with 70% acetone. The aflatoxin in the residual clear liquid was extracted and quantitatively determined by the method of Pons et al. (6).

Average values of each of the five replicate flasks are given in Table 1. Negligible yields of aflatoxin were obtained at 18 and 40 C. The highest amounts of this substance by far were synthesized by our isolate at 24 C, which is in a good agreement with the data reported in the literature (7-9). Although Davis et al. (1) found that the initial *p*H of the medium did not affect the toxin production, our results show clearly that Czapek's medium produced 26 to 83 times more of the toxin when initially adjusted to *p*H 40 than at the initial *p*H of 7.4. The effect of light however, was most striking. The data show that light was deleterious to the aflatoxin formation, since in the complete absence of light a fivefold increase of the toxin (from 35,000 to 178,000 pg/g) as noted at 24 C and an initial *p*H of 4.0. The reason for this phototoxicity is unknown.

TABLE 1. *Aflatoxin yields formed by Aspergillus flavus under various environmental conditions*[a]

Temperature	Incubation conditions	Initial *p*H 4.0		Initial *p*H 7.4	
		Final *p*H	Yield	Final *p*H	Yield
18 C	Dark	4.9	5	7.3	16
	Light	4.1	5	7.5	7
24 C	Dark	6.2	178,000	6.2	6,720
	Light	6.1	35,000	6.2	800
32 C	Dark	6.4	4,940	6.4	190
	Light	6.3	3,850	6.2	45
40 C	Dark	7.0	12	6.6	20
	Light	6.7	9	6.5	14

[a] Averages of five replicates, expressed as picograms per gram.

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