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Effect of Temperature on Aflatoxin Production in *Mucuna pruriens* Seeds

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This paper describes the effect of temperature on the level of aflatoxin production in *Mucuna pruriens* seeds. The highest level of aflatoxin B_1 (1.75 μg/g) was detected in the samples incubated at 25°C for three weeks. At 20, 30, and 35°C, aflatoxin levels were 0.30 to 0.56, 0.37 to 1.20, and 0.26 to 0.65 μg/g, respectively. The lowest concentration of aflatoxin B_1 (0.10 to 0.29 μg/g) was produced at 15°C.

The seeds of *Mucuna pruriens* (L.) DC, having astringent, aphrodisiac, alexipharmic, laxative, and antihelminthic properties, are also useful against scorpion sting, fever, and gonorrhea (4). During storage, as with cereals or grains, plant parts of medicinal significance are influenced by moisture contents of the substrates, relative humidity, and temperature, which ultimately play a vital role in the development of storage fungi. The moisture content of the *Mucuna* seeds is 17%, a level reported (11) to be favorable for growth of *Aspergillus flavus* and for aflatoxin production. Most of the fungi grow luxuriantly at between 20 and 30°C (1). *A. flavus* exhibits optimum growth between 36 and 38°C (range, 6 to 46°C) (7, 12). The optimum temperature for aflatoxin production by *A. flavus* ranges between 25 and 35°C (10).

Several earlier workers have reported (3, 5) the effect of temperature on aflatoxin production in various food and agricultural commodities. There are some reports indicating aflatoxin contamination in plant parts of medicinal value (9); however, the effect of temperature has not been studied so far. The present investigation, therefore, was carried out to observe the effect of temperature on aflatoxin production in *Mucuna* seeds, keeping their medicinal value in view.

A 100-g surface-sterilized (2% NaOCl, 10 min) seed sample was infected with a 10-ml inoculum (containing 2×10^3 spores per ml) of an *A. flavus* strain having aflatoxin B_1 and B_2 production potential in sterilized flasks. Flasks were incubated for 7, 14, 21, and 28 days in incubators at various temperatures, i.e., 15, 20, 25, 30, and 35°C. At 7-day intervals, the infected samples were washed with sterilized distilled water, oven dried at 60°C for 48 h, and powdered for aflatoxin analysis by a standard method (13). Aflatoxins were qualitatively detected on thin-layer chromatography plates with toluene-isoamyl alcohol-methanol (90:32:2, vol/vol/vol) as the solvent system (8), and aflatoxin B_1 was quantitated by a spectrophotometric procedure (6). The presence of aflatoxin was also confirmed chemically by derivatization with trifluoroacetic acid (15) and by spraying with 50% sulfuric acid.

The highest levels (0.67 to 1.75 μg/g) of aflatoxin B_1 were detected in the samples incubated at 25°C in week 3 of incubation (Table 1). However, the aflatoxin levels ranged from 0.30 to 0.56, 0.37 to 1.20, and 0.26 to 0.65 μg/g in the samples stored at 20, 30, and 35°C, respectively, within the same period. The lowest concentration (0.10 to 0.29 μg/g) of aflatoxin B_1 was produced at 15°C. However, no visible growth of fungus or aflatoxin production was observed at 10°C. In addition to temperature, the incubation period had a marked influence on aflatoxin production. At almost all temperatures, maximum production of aflatoxin B_1 was found in week 3 of incubation; thereafter, production decreased, indicating that some aflatoxin was either degraded or reabsorbed by the fungus (2). Earlier studies (3, 14) have also shown the highest levels of toxin production to be in peanuts stored at temperatures of 25 to 35°C for 21 days. The results also reflect that a temperature of 25°C is more suitable for production of aflatoxin B_1 in *Mucuna* seeds; thus, it may be inhibited to a considerable extent by controlling the temperature during storage.

TABLE 1. Effect of temperature on aflatoxin production in *Mucuna* seeds[a]

Incubation period (wk)	Aflatoxin B_1 level (μg/g) after incubation at:				
	15°C	20°C	25°C	30°C	35°C
1	0.10	0.30	0.67	0.37	0.26
2	0.29	0.45	1.55	0.82	0.31
3	0.21	0.56	1.75	1.20	0.65
4	0.15	0.41	1.15	0.45	0.54

[a] The effect of the incubation period on aflatoxin production was significant only at the 5% level, whereas temperature was significant at both the 5 and 1% levels.

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LITERATURE CITED

1. **Detroy, R. W., E. B. Lillehoj, and A. Ciegler.** 1971. Aflatoxin and related compounds, p. 3–178. *In* A. Ciegler, S. Kadis, and S. J. Ajl (ed.), Microbial toxins, vol. 6. Academic Press, Inc. New York.
2. **Diener, U. L., and N. D. Davis.** 1966. Aflatoxin production by isolates of *Aspergillus flavus*. Phytopathology **56**:1390–1393.
3. **Diener, U. L., and N. D. Davis.** 1970. Limiting temperature and relative humidity for aflatoxin production by *Aspergillus flavus* in stored peanuts. J. Am. Oil Chem. Soc. **47**:347–351.
4. **Dymock, W., C. J. H. Warden, and D. Hooper.** 1978. Pharmacographia Indica: a history of principal drugs of vegetable origin met with in British India, vol. 3, p. 176–178. International Specialized Book Service, Beaverton, Oreg.
5. **Mall, O. P., and H. M. Pateria.** 1983. Effect of temperature on biodeterioration and aflatoxin production in groundnut seeds, p. 275–281. *In* K. S. Bilgrami (ed.), Mycotoxins in food and feed. Proceedings of All India Symposium. Allied Press, Bhagalpur, India.

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6. **Nabney, J., and B. F. Nesbitt.** 1965. A spectrophotometric method for determining the aflatoxins. Analyst **90**:155–160.
7. **Northolt, M. D., and L. B. Bullerman.** 1982. Prevention of mould growth and toxin production through control of environmental conditions. J. Food Prot. **45**:519–526.
8. **Reddy, T. V., L. Viswanathan, and T. A. Venkitasubramanian.** 1970. Thin-layer chromatography of aflatoxins. Anal. Biochem. **38**:568–571.
9. **Roy, A. K., K. K. Sinha, and H. K. Chourasia.** 1988. Aflatoxin contamination of some common drug plants. Appl. Environ. Microbiol. **54**:842–843.
10. **Sauer, D. B.** 1986. Conditions that affect growth of *A. flavus* and production of aflatoxin in stored maize, p. 41. *In* M. S. Zuber, E. B. Lillehoj, and B. L. Renfro (ed.), Aflatoxin in maize. A proceedings of the workshop. CIMMYT, ElBatan, Mexico.
11. **Sauer, D. B., and R. Burroughs.** 1980. Fungal growth, aflatoxin production and moisture equilibrium in mixtures of wet and dry corn. Phytopathology **70**:516–521.
12. **Schindler, A. F., J. G. Palmer, and W. V. Eisenberg.** 1967. Aflatoxin production by *Aspergillus flavus* as related to various temperatures. Appl. Microbiol. **15**:1006–1009.
13. **Seitz, L. M., and M. E. Mohr.** 1977. A new method for quantitation of aflatoxin in corn. Cereal Chem. **54**:179–183.
14. **Sorenson, W. G., C. W. Hesseltine, and O. L. Shotwell.** 1967. Effect of temperature on production of aflatoxin on rice by *Aspergillus flavus*. Mycopathol. Mycol. Appl. **33**:49–55.
15. **Stack, M. E., and A. E. Pohland.** 1975. Collaborative study of a method for chemical confirmation of the identity of aflatoxin. J. Assoc. Off. Agric. Chem. **58**:110–113.