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MAHUA FLOWER YEAST EXTRACT MEDIUM - A MORE EFFICIENT AFLATOXIN PRODUCING MEDIUM

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Mahua flower extract was evaluated for the growth of toxigenic strains of *Aspergillus flavus* and aflatoxin elaboration. Different concentrations of dried mahua flower, yeast extract, and different pH were tried to find out the best combination, which promoted optimum fungal growth and maximum aflatoxin production. 20% dried mahua flower, 2% yeast extract and 6.5 pH of the medium was found to be most conducive and produced maximum amount of aflatoxin, even more than that produced in commonly used SMKY medium.

Key Words : Mahua flower extract, Aflatoxin production.

Most of the toxigenic strains of *Aspergillus flavus* contaminate a wide range of food and feed commodities in different parts of the world (Hesseltine , 1974; Moreau, 1979; Bilgrami and Sinha, 1987). The amount and composition of aflatoxin produced vary with the substrates, environmental conditions and mould strain (Hesseltine *et al.*, 1966; Davis and Diner, 1969).

Different media, such as YES medium (Davis *et al.*, 1966), SMKY medium (Diener and Davis, 1966), Rice Flour Liquid medium (Misra and Sinha, 1979) and 0.7% YES + Salt medium (Mehan and Chohan, 1973) have been used to grow *Aspergillus flavus* and its toxin production.

Since Mahua Flower Agar medium served as a better growth medium (Saha and Singh, 1991) for wide range of fungi, it was screened for its efficacy in elaboration of aflatoxin by *Aspergillus flavus*. SMKY medium, considered as better medium promoting higher elaboration of toxin by *A. flavus* group was used as control.

MATERIALS AND METHODS

One kg of mahua flower was oven dried at 60°C for 72 hours, ground and packed in plastic bottles and kept in freeze. 5, 10, 15, 20 and 25 g of dried mahua flower (DMF) powder was mixed in 100 ml distilled water to make 5%, 10%, 15%, 20% and 25% dilution and boiled for 30 minutes. These were then filtered and final volume was made to 100 ml by adding distilled water, 0.7 g of yeast extract was added in DMF medium similar to SMKY medium.

In another experiment 0.7, 2.0, 3.0, 4.0 and 5.0 g of yeast extract was added to the 100 ml medium to find out the best combination of yeast extract in DMF medium.

In both the cases final pH of the medium was maintained at 6.5 by adding 0.1 NHCl or 1 N NaOH.

To observe the best suited pH for MFYE medium different pH i.e. 3.5, 4.0, 5.0, 6.0 and 6.5 were maintained in the medium to see the mycelial growth and aflatoxin production.

100 ml test medium was autoclaved in 250 ml flask (at 20 P.S.I. for 20 minutes) and were then inoculated with 2 ml spore suspension(61×10^4 conidia/ml) of toxigenic strain of *Aspergillus flavus*. Simultaneously 100 SMKY medium (Diener and Davis, 1966) was also inoculated with the same suspension and used as control. Three replicates were taken for each set. All sets were incubated at 28°C±2°C for 10 days and hand shaken twice daily.

Eight toxigenic strains of *A. flavus* having different potentials were also inoculated on MFYE medium (20% DMF, 2% yeast extract and pH 6.5) and SMKY medium to compare the growth of mycelium and aflatoxin elaboration in two media.

After incubation, aflatoxin was extracted using chloroform. Qualitative detection of aflatoxin was done on TLC plate using Toluene: Isoamyl alcohol : Methanol (90 : 30 : 20 v/v/v) solvent system (Reddy *et al.*, 1970). Quantitative estimation of aflatoxin was done by spotting the dissolved aflatoxin on high performance thin layer chromatographic (HPTLC) plate and the amount of aflatoxin measured densitometri-

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Table 1. Influence of dehydrated mahua flower (DMF) concentration on aflatoxin production by *Aspergillus flavus* growing in 0.7% yeast extract medium.

DMF g/100 ml	Mycelial dry weight g/100 ml	Aflatoxin (mg/100 ml)		
		B_1	G_1	Total B+G
5	1.5	0.5	0.9	1.4
10	2.8	0.7	1.2	1.9
15	3.1	1.9	2.4	4.3
20	3.2	2.3	2.8	5.1
25	3.5	2.0	2.6	4.6
SMKY	3.1	1.6	2.0	3.6

Table 1.1 Analysis of variance for mycelial dry weight and aflatoxin content at different DMF concentration.

Source of variation	D.F.	Mycelial dry wt.		Aflatoxin B_1		Aflatoxin G_1	
		M.S.S.	F. value	M.S.S.	F. value	M.S.S.	F.value
Concentrations (DMF)	5	1.541	69.781*	1.620	1171.084*	1.796	751.963*
Error	12	0.002		0.0013833		0.0023889	
Total	17						
C.D. at 5%			0.2640111		0.3644129		0.0869981

*P < 0.001

cally by Camag TLC scanner II with integrator SP-4290. The experimental data were analysed statistically by one way ANOVA to see the effect of concentration of DMF, yeast extract and pH on the production of aflatoxin and dry mycelial weight.

RESULTS AND DISCUSSION

Maximum production of total aflatoxin (B + G) was recorded at 20% DMF concentration (5.1 mg/100 ml) which was significantly higher in comparison to SMKY medium where the production was only 3.6 mg/100 ml of the medium (Table 1.1). But mycelial growth increased with the increase in the concentration of DMF upto 25 per cent. (Table 1). Dried mahua flower contains about 90% sugar (mainly glucose, sucrose, fructose, rhamnose, arabinose and maltose), different minerals, vitamins and growth factors (Belavady and Balasubramanian, 1959). Davis *et al.* (1966) have recommended only 15-20% sugar in the medium for high level of aflatoxin production. In the present study also 20% dried mahua flower (contain approx 15-20% sugar) yielded the best result. Mateles and Adye (1965) had suggested glucose, sucrose, fructose and raffinose as the most productive carbon source for the growth of *Aspergillus flavus*. Thus mahua flower powder fulfilled the requirement of all the sugars required for the growth and elaboration of aflatoxin.

Table 2. Influence of yeast extract concentration on aflatoxin production by *Aspergillus flavus* growing in 20% dehydrated mahua flower (DMF) medium

DMF g/100 ml	Mycelial dry weight g/100 ml	Aflatoxin (mg/100 ml)		
		B_1	G_1	Total B+G
0.7	3.2	2.3	2.8	5.1
2.0	4.0	3.2	3.7	6.9
3.0	4.3	3.6	2.6	6.2
4.0	4.8	3.3	2.4	5.7
5.0	5.4	2.5	1.9	4.4
SMY	3.1	1.6	2.0	3.6

Table 2.1 Analysis of variance for mycelial dry weight and aflatoxin content at different concentrations of yeast extract.

Source of variation	D.F.	Mycelial dry wt.		Aflatoxin B_1		Aflatoxin G_1	
		M.S.S.	F. value	M.S.S.	F. value	M.S.S.	F.value
Concentrations (Yeast extract)	5	2.415	226.637*	1.689	348.247*	1.280	338.326*
Error	12	0.011		0.00485		0.0037833	
Total	17						
C.D. at 5%			0.186684		0.123%		0.1094828

*P < 0.001

Yeast extract also influenced the total aflatoxin (B + G) production (Table 2.1). In SMKY medium, 0.7 per cent yeast extract was added which might be forming the best combination with amino acids, minerals, vitamins and growth factors (Davis *et al.*, 1967). However, the concentration of yeast extract in mahua flower medium, at which maximum elaboration of total aflatoxin took place was 2% after which there was decrease in aflatoxin production. Two per cent yeast extract probably making the best combination with sugars and other ingredients (Table 2).

pH of the medium also showed influence on the growth and elaboration of aflatoxin (Table 3.1). Maximum fungal growth was recorded at pH 5 while total aflatoxin elaboration was maximum at 6.5 pH (Table 3). Similar result were earlier reported by Joffe and Lisker (1969) and Buchanan and Ayres (1975).

Aflatoxin elaboration was significantly higher in MFYE medium (DMF 20%; Yeast extract 2% & pH 6.5) than SMKY medium by all the eight test toxigenic

Table 3. Influence of pH on aflatoxin production by *Aspergillus flavus* growing in mahua flower yeast extract (MFYE) medium.

DMF g/100 ml	Mycelial dry weight g/100 ml	Aflatoxin (mg/100 ml) B_1	G_1	Total B+G
3.5	2.3	2.2	1.4	3.6
4.0	2.8	1.9	1.9	3.8
5.0	3.2	1.7	1.5	3.2
6.0	3.0	2.7	2.8	5.5
6.5	3.0	2.9	2.8	5.7
6.5 (SMKY)	2.8	1.8	2.0	3.0

Table 3.1 Analysis of variance for mycelial dry weight and aflatoxin content at different DMF concentration.

Source of variation	D.F.	Mycelial dry wt. M.S.S.	F. value	Aflatoxin B_1 M.S.S.	F. value	Aflatoxin G_1 M.S.S.	F.value
Concentrations (DMF)	5	0.281	62.983*	0.744	194.087*	1.124	424.151*
Error	12	0.004556		0.0038333		0.00265	
Total	17						
C.D. at 5%			0.118813		0.1102039		0.0916291

*P < 0.001

Table 4. Production of aflatoxin by selected eight isolates of *Aspergillus flavus* growing in mahua flower yeast extract (MFYE) medium and SMKY medium.

Isolates	Mycelial dry weight g/100 ml		Aflatoxin (mg/100 ml) B_1		G_1		Total B + G	
	MFYE	SMKY	MFYE	SMKY	MFYE	SMKY	MFYE	SMKY
UDB-1	3.1	2.9	2.4	2.1	2.5	2.0	4.9	4.1
UDB-4	3.0	3.2	2.9	2.6	2.9	2.2	5.8	4.8
UDB-6	3.1	2.8	2.8	2.3	3.0	1.9	5.8	4.2
UDB-9	3.1	3.0	2.2	1.8	2.4	1.6	4.6	3.4
UDB-68	2.8	3.0	2.2	1.9	2.3	1.7	4.5	3.6
UDB-76	2.7	2.8	2.6	2.3	2.8	2.0	5.4	4.3
UDB-83	3.0	2.9	2.3	2.0	2.6	1.8	4.9	3.8
UDB-110	3.0	3.4	3.0	2.6	3.1	3.0	6.1	5.6

isolates of *A. flavus* (Table-4). Mateles and Adye (1965) have suggested that zinc, iron, manganese are also required as trace elements for better production of aflatoxin. Mahua flower though devoid of zinc and manganese, contains iron and many other trace elements such as calcium, phosphorus, magnesium and vitamins which might be compensating the absence of zinc and manganese. Thus greater production of aflatoxin in MFYE medium in comparison to SMKY medium could be due to the cumulative effect of sugars, minerals, vitamins and growth factors. Thus, mahua flower yeast extract medium can be used in the laboratory for aflatoxin elaboration.

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