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Factors influencing zearalenone production by *Fusarium moniliforme*

K. NARASIMHA RAO, G. SHYAM PRASAD, S. GIRISHAM and S.M. REDDY*
Department of Microbiology, Kakatiya University, Warangal 506 009

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Zearalenone (ZEA), was first isolated and characterized from cultures of *F. graminearum* NRRL 230 obtained from corn and implicated in a field out break of hyperestrogenism in pigs in the United States by Stob, *et al*. (6). The most important producer of ZEA is *F. graminearum*, which frequently is referred to incorrectly in the literature as *F. roseum* (3). Some other species of *Fusarium* that produce ZEA are *F. culmorum* (5), *F. crookwellense* (7) *F. sporotrichioides* and *F. equiseti* (3). World wide occurrence of zearalenone in cereals and its co-occurrence along with trichothecenes particularly deoxynivalenol has been reported by Tanaka *et al*. (8). Pittet (4) has excellently discussed the natural contamination of cereals by zearalenone. However, only limited information is available on the factors influencing production of zearalenone.

In the present study, *in vitro* zearalenone production potential of different isolates of *F. moniliforme* was tested in Czapek's medium. *Fusarium moniliforme* isolates were inoculated separately in flasks containing 100 ml of Czapek's medium (Yeast extract 10g; $NaNO_3$ 2g; KH_2PO_4 1g; $MgSO_4.7H_2O$ 0.5g; KCl 0.5g and distilled water 1000 mL) and incubated at 26 ± 2°C for 20 days. The culture filtrate was extracted with chloroform for 12h and separated through a separatory funnel. The lower chloroform layer was taken and decanted through a bed of anhydrous sodium-sulphate to remove moisture. The chloroform extract was evaporated to near dryness and the residue was dissolved in 1 ml of chloroform and reserved for thin layer chromatography. The plates were developed in chloroform : methanol (95:5) and toluene : ethyl acetate : formic acid (TEF) (6:3:1) as suggested by Durackowaz *et al.* (1). The bluish-green spot of Zearalenone was observed under short wave UV light (254 nm), which changes to yellowish-brown spot when sprayed with 50 percent methanol in concentrated sulphuric acid and heated at 120°C for 10 min in an oven (2). Blue-green fluorescent spot was enhanced when the plates were sprayed with aluminium chloride solution. The intensity of blue-green colour thus developed was measured at 274 nm. The amount of zearalenone was read from standard curve drawn for zearalenone. Effect of different microbial nutrients on the growth and zearalenone production by *F. moniliforme* was investigated by adding these to Czapek's medium so as to get different concentrations (0.5, 1.0 in percentage) just before the inoculation of the fungus. The flasks thus inoculated were incubated at 25-27°C for 21 days. At the end of incubation period, cultures were harvested on previously dried and weighed Whatman filter paper No. 42 for determination of biomass production. pH of culture filtrate was also recorded with the help of either BDH pH paper or Elico pH meter.

Out of 14 media tried for production of zearalenone by *F. moniliforme*, yeast extract sucrose and Nash and Snyder's media were the best (Table 1). Asthana and Hawkers medium A and nutrient broth were poor substrates for production of zearalenone. Rest of the media tried proved to be intermediate in their nutritive value for induction of zearalenone. *F. moniliforme* could produce maximum zearalenone at pH 7.5, which gradually decreased with increase of alkalinity (Table 2). The toxin production was minimum at pH 10.5. No growth of fungus was recorded at pH 2.5. The

*Corresponding author: profsmereddy@yahoo.com

Table 1. Effect of different synthetic media on growth, pH and zearalenone production by *F. moniliforme*

Media	Dry weight (mg/ml)	Zearalenone (μg/ml)	Final pH
Czapek's	6.51	0.779	4.0
Maize flour	6.30	0.694	6.0
Rice flour	6.27	0.672	5.5
Sorghum flour	6.19	0.689	6.5
Richard's	6.88	0.77	6.5
Nash & Snyders	7.12	0.82	7.0
Smky	4.30	0.472	7.5
YES	7.71	0.882	7.5
Asthana and Hawkers	3.00	0.339	6.0
Malt extract	4.77	0.502	5.5
Singh and Wood	4.68	0.493	7.0
Glucose asparagine	4.85	0.546	6.0
Nutrient agar	1.96	0.283	7.5
S.E.M. ±	0.4660	0.0518	0.3040
C.D. at 5%	0.3266	0.0362	0.2127

Table 2. Influence of pH on growth and zearalenone production by *F. moniliforme*

Media	Dry weight (mg/ml)	Zearalenone (μg/ml)	Final pH
2.5	—	—	2.5
3.5	4.51	0.216	3.0
4.5	4.11	0.352	4.8
5.5	4.16	0.346	5.0
6.5	5.51	0.544	7.0
7.5	7.70	0.991	7.5
8.5	6.00	0.836	8.0
9.5	5.90	0.752	8.5
10.5	1.71	0.187	9.5
S.E.M± 0.913	0.625	0.107	0.826
C.D. at 5% 0.9942	0.7406	0.0144	0.9228

amount of zearalenone produced increased with increasing pH with a maximum at pH 7.5. Similarly the growth of *F. moniliforme* was maximum at pH 7.5 and decreased gradually with further increase of alkalinity or acidity. At pH 10.5 growth of fungus was meagre and toxin produced was also minimum. The final pH recorded varied significantly in relation to initial pH adjusted. The zearalenone production increased marginally with the addition of of microbial nutrients (Table 3). Amount of toxin produced decreased with increase in concentration of nutrients. However, yeast extract stimulated the zearalenone production at higher concentration. The mycelial growth was inhibited in the presence of these nutrients. The pH remained constant through out the observation period.

From the present investigations it is clear that *F. moniliforme* produces zearalenone under varied conditions and potential contaminant of foods and feeds.

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Table 3. Influence of microbial nutrient on growth and zearalenone production by *F. moniliforme*

Nutrient	Concentration (in percentage)	Dry weight (mg/ml)	Zearalenone (in μg/ml)	Final pH
Control	—	9.64	0.882	6.5
Yeast extract	0.5	6.43	0.872	7.5
	1.0	7.72	0.892	7.5
Peptone	0.5	5.91	0.895	7.5
	1.0	6.52	0.881	7.0
Beef extract	0.5	8.43	0.991	7.5
	1.0	7.90	0.989	7.5
Malt extract	0.5	9.45	0.993	7.0
	1.0	7.61	0.871	6.4
S.E.M. ±	0.0945	0.4350	0.0183	0.1510
C.D. at 5%	0.119	0.4736	0.0199	0.1644

REFERENCES

1. **Durackowaz, Betina, V.** and **Nemec, P.** (1976). *J. Chromatograph*. **116**: 141-154.
2. **Ishili, K., Sawanot, M. Ueno, Y.** and **Tsunada, T.** (1974). *Appl. Microbiol*. **27**: 625-628.
3. **Marasas, W.F.O.** (1991). In: *Mycotoxins and Animal Foods,* Eds: J.E. Smith and R.S. Henderson, CRC Press, Boca Raton, FL, pp. 119-139.
4. **Pittet, A.** (1998). *Review Medicine Veterinarie,* **149**: 479-492.
5. **Snijders, C.H.A.** and **Perkowski, J.** (1990). *Phytopathology*. **80**: 566-570.
6. **Stob, J., Baldwin, R.S., Tuite, J., Andrews, F.N.** and **Gillette, K.G.** (1962). *Nature* **196**: 1318.
7. **Sydenham, E.W., Marasas, W.F.O., Thiel, P.G. Shepard, G.S.** and **Nieuwenhusis, J.J.** (1991). *Food Addit. Contam*. **8**: 31-41.
8. **Tanaka, T.** and **Ueno, Y.** (1988). In : *Mycotoxins and Phycotoxins* (eds. S. Natori, K. Hashmoto and Y. Ueno), Elsevier Science Publishers, Amsterdam, pp. 88.

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