

## NATURAL CONTAMINATION OF TOXIGENIC *FUSARIUM* SPECIES AND ZEARALENONE IN SOME OILCAKES

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A survey was undertaken to evaluate the natural occurrence of *Fusarium* species and zearalenone in mustard and linseed cakes. In all, 7 species of *Fusarium* viz., *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. moniliforme*, *F. oxysporum*, *F. semitectum* and *F. sporotrichioides* were encountered with oilcake samples. A total of 119 and 91 isolates of these species of *Fusarium* were obtained from mustard and linseed cakes respectively. In moist-rice medium, 52 (Out of 119), and 43 (out of 91) isolates of *Fusarium* species elaborated zearalenone ranging from 100 to 3680 ppb and 70 to 2920 ppb respectively. Thus, 43.7 and 47.3 per cent of *Fusarium* species isolated from mustard and linseed cake respectively were zearalenone producers. Among the *Fusarium* species, *F. moniliforme* was found to be most potent producer of zearalenone. Natural contamination of zearalenone ranging from 80-3290 ppb were recorded in 64 (out of 157) samples of oilcakes comprising mustard cakes (38, out of 89 samples) and linseed cakes (26, out of 68 samples).

**Key Words :** *Fusarium* species, zearalenone, mustard and linseed cake.

Zearalenone or F-2 toxin is an enone derivative of  $\beta$ -resorcylic acid lactone which possesses potent estrogenic properties. Several species of *Fusarium* are known for the production of zearalenone (Hidy *et al.*, 1977; Bennett and Shotwell 1979, Mirocha and Christensen, 1982; Lee *et al.*, 1986; Chelkowski *et al.*, 1990).

The natural occurrence of zearalenone is reported from different parts of the world including India on various agricultural products (Caldwell and Tuite, 1974; Bottalico, 1977; Reddy *et al.*, 1983k; Bhat *et al.*, 1989; Bresler, 1991; Ansari and Srivastava, 1991, and Bose *et al.*, 1992).

Among the feeds given to dairy cattle, oilcakes constitute a major proportion of their daily diet. In this communication, the incidence of toxigenic *Fusarium* species and level of zearalenone contamination in oilcakes of mustard and linseed have been reported.

### MATERIALS AND METHODS

In the present study, 157 samples of oilcakes comprising 89 of mustard and 68 of linseed were procured from various places of Agra region particularly rural areas. All these samples of oil-cakes were analysed for the presence of moulds by dilution plate method (Graves and Hesseltine, 1966). Out of the various moulds, different species of *Fusarium* were identified following Booth (1971). 210 isolates of *Fusarium* were screened for zearalenone producing potentiality following Scott *et al.* (1970). Each set was

run in triplicate and incubated for 2 weeks at 25°C and then at 10°C for additional one week. After incubation, the contents of each flask were dried at 55±2°C for 24 hours and then extracted chemically for zearalenone following Swanson *et al.* (1984). The ground material was mixed with methanol : water (60 : 40 v/v) and saturated lead acetate solution and blended for 15 minutes. The filtrate of this mixture was defatted with n-hexane and further extracted with Toluene ethyl acetate (9:1 v/v) solution. The extract so obtained was evaporated to near dryness and residue was dissolved in 1 ml benzene : acetonitrile (98:2 v/v) and reserved for TLC.

Subsequently, the samples of mustard and linseed cakes were extracted chemically for the natural occurrence of zearalenone following the method as described above (Swanson *et al.*, 1984). The amount of zearalenone was quantified by visual comparison with varying concentration of standard and also densitometrically. Zearalenone gave bluish green fluorescence under longwave UV light, which was reduced under short wave length.

### RESULTS AND DISCUSSION

In the present investigation, 7 species of *Fusarium* were isolated from the oilcakes of mustard and linseed under natural conditions. Species of *Fusarium* namely *F. equiseti*, *F. graminearum*, *F. moniliforme*, *F. oxysporum*, *F. semitectum* and *F. sporotrichioides* were commonly present in both mustard and linseed

Table 1: *In vitro* production of zearalenone by *Fusarium* species in moist-rice medium.

S. No.	<i>Fusarium</i> species	Sources	Number of isolates screened	Number of toxigenic isolates	Per cent toxigenic isolates	Concentration of zearalenone elaborated (in ppb)
1.	<i>Fusarium culmorum</i>	Mustard cake	2	-	-	-
		Linseed cake	-	-	-	-
2.	<i>F. equiseti</i>	Mustard cake	20	7	35.0	140-720
		Linseed cake	12	5	41.7	80-560
3.	<i>F. graminearum</i>	Mustard cake	11	3	27.3	210-1170
		Linseed cake	9	3	33.3	140-980
4.	<i>F. moniliforme</i>	Mustard cake	32	21	65.6	230-3680
		Linseed cake	22	14	63.6	110-2920
5.	<i>F. oxysporum</i>	Mustard cake	39	18	46.1	100-1860
		Linseed cake	30	16	53.3	70-1460
6.	<i>F. semitectum</i>	Mustard cake	10	2	20.0	160-380
		Linseed cake	7	2	28.6	90-170
7.	<i>F. sporotrichioides</i>	Mustard cake	5	1	20.0	170
		Linseed cake	11	3	27.3	80-510

cakes but *F. culmorum* was obtained from mustard cakes only. Altogether 210 isolates of different *Fusarium* species were obtained from 157 samples of oilcakes (Table 1) of which 119 isolates were obtained from mustard cakes and the remaining from linseed caked.

Perusal of Table-1 indicates that 52 (out of 119) and 43 (out of 91) isolates of *Fusarium* species associated with mustard and linseed cakes respectively were toxigenic and elaborated zearalenone in the range of 70-3680 ppb. *F. culmorum* was non-toxic; its two isolates obtained from mustard cake were unable to produce zearalenone in moist rice medium. Out of 32 isolates of *F. moniliforme* obtained from mustard cakes, 21 isolates elaborated zearalenone in the range of 230 to 3680 ppb, while 14 (out of 22) isolates of *F. moniliforme* associated with linseed cakes elaborated zearalenone in the range of 110 to 2920 ppb. Thus, the incidence of toxigenic isolates of *Fusarium moniliforme* was 65.6 and 63.6 per cent in mustard and linseed cake respectively. Besides these, 18 (out of 39) isolates and 16 (out of 30) isolates of *F. oxysporum* isolated from mustard and linseed cakes were producing zearalenone in the range of 100 to 1860 ppb and 70 to 1460 ppb respectively.

Isolates of *F. equiseti*, *F. graminearum*, *F. semitectum*, *F. sporotrichioides* were also elaborating zearalenone in different concentrations.

In view of the capacity to produce zearalenone,

Table 2: Natural contamination of zearalenone in mustard and linseed cakes.

Oilcakes	Total number of samples analysed	Number of samples positive to zearalenone	Per cent toxic samples	Amount of zearalenone detected (in ppb)
Mustard cake	89	38	42.7	80-2470
Linseed cake	68	26	38.2	100-3290

*Fusarium moniliforme* was the most potent producer while other species (*F. oxysporum*, *F. graminearum* and *F. equiseti*) were mild producers, while *F. semitectum* and *F. sporotrichioides* were poor producers of zearalenone. It was interestingly noticed that none of the isolate of *Fusarium culmorum* was able to produce zearalenone. From findings incorporated in Table - 1, it was evident that oilcakes of mustard and linseed had high incidence of zearalenone producing strains of *Fusarium*.

Altogether 64 samples of oilcakes were found to be naturally contaminated with zearalenone in the range of 80 to 3290 ppb, thereby indicating 40.8 per cent contamination. The per cent incidence of zearalenone was greater in mustard cake (42.7%) than linseed cake samples (38.2%). Out of 89 samples of mustard cake analysed, 38 samples were found to contain zearalenone ranging from 80 to 2470 ppb. However, in linseed cake, only 26 samples (out of 68) were naturally contaminated with zearalenone ranging

from 100 to 3290 ppb. These findings clearly indicate that samples of oilcakes had an appreciably high quantity of zearalenone under natural conditions.

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