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Short Communication

Temperature as a factor in the elaboration of mycotoxins by two fungi in groundnut fodder

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Production of patulin and terreic acid by *Aspergillus terreus* and zearalenone by *Fusarium oxysporum* in groundnut fodder in relation to temperature was investigated. Biodeterioration activity of *F. oxysporum* and *A. terreus* was more at incubation temperature of 20 and 30°C respectively. *F. oxysporum* elaborated maximum amount of zearalenone at 20 to 25°C, while *A. terreus* could produce maximum terreic acid and patulin at 25°C.

Key words: Groundnut fodder, temperature, *Aspergillus terreus*, *Fusarium oxysporum*, terreic acid, zearalenone and patulin.

INTRODUCTION

The nutritive value of groundnut fodder is comparable with most of the pulse straws like cowpea, green gram, etc. Storing of groundnut fodder in open atmosphere is an unusual practice at least in Andhra Pradesh, India and practically no attention is given to the storage of fodder. Under fluctuating environmental conditions, the fodder is exposed to variety of stress conditions such as moisture and temperature creating congenial conditions for proliferation of storage moulds (Lacey, 1991; Visconti and Girolamo, 2002). Of these factors, temperature is of major concern and responsible for changing the metabolic activity of the mycotoxigenic fungi. Though the influence of temperature on biodeterioration and elaboration of mycotoxins in food grains by some fungi has been studied (Girisham et al., 1987; Schneweis et al., 2000; Cairns-Fuller et al., 2005; Hope et al., 2005), practically there is limited information is available for feeds and fodder (Dos et al., 2003; El-Shanawany et al., 2005). The present investigations were aimed to study the effect of temperature on biodeterioration and mycotoxin production in groundnut fodder by two mycotoxigenic fungi.

MATERIALS AND METHODS

The freshly harvested groundnut fodder from fields of Warangal was collected and brought to the laboratory. It is air dried at laboratory conditions. From this bulk sample thirty gram of fodder is randomly collected and chopped into pieces and inoculated separately with 7 day old fungal mat of *Aspergillus terreus* and *Fusarium oxysporum* which were grown on cellophane implanted malt agar medium. The groundnut fodder thus prepared was incubated at different temperatures (15, 20, 25, 30, 35, and 40°C) for 30 days. Moisture and other parameters were kept constant in all the experiments. Uninoculated groundnut fodder treated in a similar manner was served as control. At the end of incubation period, protein (Lowry et al., 1951), crude fibre, lignin, cellulose, total nitrogen and starch (Chopra and Kanwar, 1982), ash content (AOAC, 1990) and loss of weight were determined by using standard methods. The experiments were conducted in triplicate and repeated atleast twice. The results obtained are statistically analysed using SPSS software (Version 12.0). Extraction and estimation of patulin (Subramanian, 1982), terreic acid (Subramanian et al., 1978) and zearalenone (Kamimura et al., 1981) were carried out by employing standard methods.

RESULTS AND DISCUSSION

Groundnut fodder inoculated with both the fungi suffered maximum weight loss at incubation temperature of 25°C (Table 1). The deteriorating activity of *F. oxysporum* was comparatively high at low incubation temperature (15°C), while *A. terreus* was more active at 25°C and above. Biodeteriorating activity of both fungi under investigation was only marginal at incubation temperature of 40°C.

The protein and ash content increased due to fungal inoculation. The increase in protein and ash content was more significant at 25°C which decreased both under low

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Table 1. Statistical analysis of effect of temperature on biodeterioration and toxin production in groundnut fodder by two fungi.

Temperature (ºC)	Name of the fungus	Statistical parameter	Protein (mg/g)	Crude fibre (mg/g)	Cellulose (mg/g)	Lignin (mg/g)	Starch (mg/g)	Total nitrogen (mg/g)	Ash content (mg/g)	Loss of weight (%)	Mycotoxin	Amount
15	*A. terreus*	Mean±St.d	5.40±0.56	274.0±2.05	309.0±1.41	37.2±1.34	104.5±0.70	19.3±0.98	109.4±3.39	9.10±1.83	-	Nil
		S.E	0.40	1.45	1.00	0.95	0.50	0.70	2.40	1.30	TA/Pat	18/Nill
	F. oxysporum	Mean±St.d	5.50±0.70	273.7±2.47	308.2±2.47	37.6±084	104.5±0.70	19.2±1.60	109.5±3.53	10.0±3.18	Zearalenone	-
		S.E	0.5	1.75	1.75	1.75	0.5	0.75	2.5	2.25		
20	*A. terreus*	Mean±St.d	5.75±1.60	272.1±4.73	307.1±4.03	36.2±2.82	102.6±3.32	17.6±3.32	112.3±7.99	12.8±6.43	-	Nil
		S.E	0.75	3.35	2.85	2	2.35	2.35	6.5	3.5	TA/Pat	14
	F. oxysporum	Mean±St.d	5.65±0.91	271.4±5.79	306.9±4.38	34.7±4.94	103.6±1.90	17.6±3.32	113.5±9.19	12.8±6.43	Zearalenone	3
		S.E	0.65	4.1	3.1	3.5	1.35	2.35	6.5	4.55		
25	*A. terreus*	Mean±St.d	6.10±1.55	271.9±5.19	305.2±6.78	35.0±4.52	101.9±4.38	17.0±4.29	114.9±11.1	33.3±21.2	-	Nil
		S.E	1.10	3.60	4.80	3.20	3.10	3.00	7.90	15.0	TA/Pat	2
	F. oxysporum	Mean±St.d	6.15±1.62	270.5±7.00	305.0±7.07	33.4±6.78	101.1±5.51	17.0±4.24	115.8±11.3	33.9±23.2	Zearalenone	3
		S.E	1.15	4.95	5.00	4.80	3.90	3.00	8.00	15.6		
30	*A. terreus*	Mean±St.d	5.65±0.91	272.9±3.67	307.0±4.24	36.2±2.82	102.8±3.11	18.1±2.61	112.2±7.42	27.7±13.5	-	Nil
		S.E	0.65	2.60	3.00	2.00	2.20	1.85	5.25	9.55	TA/Pat	4
	F. oxysporum	Mean±St.d	5.65±0.91	272.6±4.03	307.0±4.17	35.1±4.38	103.9±1.55	17.8±3.11	112.9±8.34	24.5±8.90	Zearalenone	+
		S.E	0.65	2.85	2.95	3.10	1.10	2.20	5.90	6.30		
35	*A. terreus*	Mean±St.d	5.50±0.70	273.6±2.61	308.4±2.26	37.2±1.34	103.9±1.55	18.9±1.55	108.6±2.33	9.45±5.86	-	Nil
		S.E	0.50	1.85	1.60	0.95	1.10	1.10	1.65	4.15	TA/Pat	2
	F. oxysporum	Mean±St.d	5.50±0.70	272.4±4.38	309.3±0.98	37.2±1.34	104.2±1.06	19.4±0.84	108.9±2.66	9.65±6.15	Zearalenone	Nil
		S.E	0.50	3.10	0.70	0.95	0.75	0.60	1.90	4.35		
40	*A. terreus*	Mean±St.d	5.40±0.56	274.4±1.48	309.4±0.84	38.1±0.14	104.8±0.28	19.6±0.56	108.5±2.12	7.30±3.25	-	Nil
		S.E	0.40	1.05	0.60	0.10	0.20	0.40	1.50	2.30	TA/Pat	10/Nil
	F. oxysporum	Mean±St.d	5.05±0.70	274.5±1.34	310.0±0.00	38.1±0.14	104.7±0.35	20.0±0.00	108.5±2.12	6.60±2.26	Zearalenone	Nil
		S.E	0.50	0.95	0.00	0.10	0.25	0.00	0.50	1.60		

S.E= Standard error, St.d= standard deviation. By comparing means, standard deviation and standard error of triplicates of fungi at different temperature we observe that loss of weight is maximum at 25℃ and minimum 40℃. Similarly protein and ash content is maximum at 25℃ and minimum at 40℃ where as in all other cases like crude fibre, cellulose, lignin, starch and total nitrogen is minimum at 40℃.

and higher incubation temperature. Crude fibre, cellulose, lignin, starch and total nitrogen contents of groundnut fodder decreased at 25°C due to inoculation of fungi and the decline of these compounds with increase or decrease in incubation temperature was observed. *A. terreus* elaborated terreic acid at all incubation temperatures tried, and it was maximum at 25°C. On the other hand, patulin could be detected only between incubation temperature of 20 and 30°C. Patulin production was maximum at 25°C.

Zearalenone production by *F. oxysporum* was maximum at an incubation temperature of 20 to 25°C. No mycotoxins could be detected in groundnut fodder when incubated at 30°C and above. Mirocha et al, (1979) reported that low temperature or alternating moderate and low temperature will be favorable for mycotoxins production by species of *Fusarium.* It is reported that the enzymes responsible for the biosynthesis of zearalenone are active at 12-14°C. Similarly Milano and Lopez (1991) have also reported that zearalenone production was inhibited at higher temperature.

From the present investigations it is clear that the temperature plays an important role in infestation of groundnut fodder by *A. terreus* and *F. oxysporum*. Storing of groundnut fodder above 30°C will be more safer and can be made free from fungal infestation by specially *F. oxysporum* and *A. terreus* and mycotoxins contamination. However, more detailed studies dealing with interaction of other environmental factors have also to be investigated before reaching any decesive conclusions.

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