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EFFECT OF TEMPERATURE AND MOISTURES ON PRODUCTION OF CITRININ BY *PENICILLIUM CITRINUM* ON AGRICULTURAL COMMODITIES

BY

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Studies on the effect of physical factors like temperature and relative humidities on citrinin production by *P. citrinum* in different agricultural commodities show that 28° to 30°C is the most suitable temperature for citrinin production and maximum citrinin production attained at 70%-80% humiditity.

INTRODUCTION

Mycotoxic fungi are widespread in nature and contaminate foods and decaying vegetable materials. Many agricultural commodities like cereal grains, peanuts etc. appear to be the food and feed substances that may be contaminated with mycotoxins. Mycotoxin production can occur in the field during harvest, processing and storage. Factors governing production of various mycotoxins are not completely understood, although moisture, temperature and nutritional factors are known to play important roles on growth of fungi and toxin production (Ayerst, 1969 ; Christenzen, 1957 ; Snow, 1949 ; Tomkins, 1929).

We investigated the occurrence and distribution of many toxigenic mycoflora on grains and different oil-cakes (Bhattacharya and Majumdar, 1979). Different species of *Aspergillus* and *Penicillium* were isolated and some of them are capable of producing toxic substances. From these we selected two species of *Penicillium* sush as *P. citrinum* and *P. steckii* of producing citrinin. Citrinin, a powerful antibacterial agent was considered as a great threat for sush usage. Citrinin was first isolated from the fungus *P. citrinum* Thom. by Hetherington and Raistrick in 1931 (Hetherington and Raistrick, 1931). It has since been produced by few species of *Aspergillus* and *Penicillium* and is regarded as a potentially important mycotoxin that may be ingested by man and animals, as a contaminant of rice, wheat, barley and oats. The present paper describes the effect of physical factors like temperature and relative humidity on citrinin production by *Penicillium citrinum* on some agricultural commodities.

MATERIALS AND METHODS

The toxin producing strain of *P. citrinum* used in the present study was stored at 4°C on potato-dextrose-agar slant. The toxin produced by the organism in a liquid medium was isolated in the following way. The organism was first grown for 15 days in a medium containing 4% sucrose and 2% yeast extract under stationary condition. The procedure for extraction and isolation of citrinin was essentially the same as described by Hetherington and Raistrick (1931). The mycelium and culture solutions were filtered through Whatman no. 1 filter-paper and the filtrate was acidified to pH 1.5-2.0 with 1N HCl to precipitate the crude toxin. After precipitation was complete, the bright yellow precipitate was collected and dissolved in a minimal amount of chloroform to remove insoluble impurities. The chloroform solution was evaporated to dryness and the residue was taken up in hot ethanol, filtered and crystallised. The toxin was recrystallised thrice by hot alcohol method of Hetherington and Raistrick. The pure material was proved to be citrinin by physico-chemical studies on the compound and its comparison with known citrinin (Hetherington and Raistricks 1931 ; Krogh *et. al.* 1970). For studying the effect of different relative humidities and temperatures the production of citrinin by *P. citrinum* on fermentation was carried out on some agricultural commodities, under stationary condition using 10 gms. solid substrate moistened with 5 ml distilled water in a 100 ml Erlenmeyer flask. The solid medium was inoculated with 0.05 ml of spore suspension (containing 37.5×10^4 spores per ml being collected from 7 days old slant culture). Triplicate flasks were used for each set. The effect of different humidities were studied at $28° \pm 1°C$ in 4 different humid chambers. Citrinin was extracted from different food grains with measured volume of 50% alcohol, three times and extracted broth was assayed by modified cup plate method against the test organism *Bacillus subtilies* var. *mycoides*. Citrinin (sample from USDA, Northern Regional Res. Lab. Peoria, Illinois, U. S. A.) was used as the standard. The results are expressed in terms of micrograms of citrinin per ml of broth being calculated from the standard curve (a standard preparation of citrinin containing 0.05 mg per ml of 50% aq. alcohol produces 20 mm as zone of inhibition).

RESULTS AND DISCUSSION

Effect of temperature on citrinin production by P. citrinum on different agricultural commodities :

The organism was grown on some agricultural commodities at different temperature between 10°C to 37°C. Citrinin production was studied on the 5th, 7th and 9th day. The results are shown in Table 1.

It is evident from the Table 1 that, suitable temperature for citrinin production was in between 28° to 30°C, but yields at 20°C were reduced although citrinin accumulation was only recorded for 20°C, 28°C and 30°C, production flasks were also incubated at 10° and 37°C ; no toxin was observed at the temperature extremes during 9 days of incubation, whereas at 20°C comparatively lower

Table 1. *Effect of temperature on citrinin production by* P. citrinum *on different agricultural products*

Sample	Citrinin (mg/g of substrate)								
	20°C			28°C			30°C		
	5th day	7th day	9th day	5th day	7th day	9th day	5th day	7th day	9th day
Rice	—	0.097	0.11	0.53	0.738	0.84	0.733	0.766	0.623
Wheat	—	0.09	0.132	0.408	0.539	0.551	0.432	0.517	0.559
Corn	0.031	0.053	0.093	0.362	0.506	0.473	0.315	0.532	0.570
Soyabean	—	0.125	0.150	0.336	0.592	0.668	0.382	0.452	0.511
Oat	—	—	0.120	0.120	0.289	0.315	0.210	0.312	0.321
Barley	—	—	0.097	0.315	0.392	0.475	0.305	0.375	0.362
Groundnuts	—	0.092	0.111	0.403	0.439	0.485	0.2	0.302	0.325
Groundnut oil-cakes	—	0.07	0.088	0.205	0.278	0.204	0.235	0.265	0.579
Mustard oil cakes	—	—	0.19	0.237	0.239	0.273	0.15	0.285	0.25

Table 2. *Effect of different humidities on citrinin production by* P. citrinum *on agricuttural commodities*

Sample	Incubation periods (days)	Citrinin (mg/g substrate) at humidities			
		40%	70%	80%	89%
Rice	5th	0.11	0.39	0.6	0.43
	7th	0.21	0.61	0.79	0.65
	9th	0.32	0.69	0.84	0.75
Wheat	5th	0.33	0.53	0.41	0.4
	7th	0.45	0.64	0.59	0.57
	9th	0.47	0 67	0.63	0.61
Corn	5th	0.12	0.36	0.32	0.43
	7th	0.35	0.5	0.57	0.51
	9th	0.38	0.56	0.63	0.57
Oat	5th	0.12	0.11	0.19	0.18
	7th	0.13	0 24	0.31	0.28
	9th	0.16	0.29	0.39	0.3
Barley	5th	0.1	0.22	0.31	0.2
	7th	0.11	0.31	0.36	0.33
	9th	0.18	0.39	0.41	0.38
Groundnut	5th	0.11	0.21	0.3	0.38
	7th	0.23	0.42	0.38	0.41
	9th	0.31	0.41	0.39	0.45
Soyabean	5th	0.19	0.28	0.33	0.29
	7th	0.3	0.37	0.56	0.53
	9th	0.32	0.42	0.61	0.59
Groundnut oil-cakes	5th	0.09	0.2	0.33	0.39
	7th	0.1	0 33	0.42	0.46
	9th	0.25	0.41	0.55	0 5
Mustard oil cakes	5th	0.09	0.1	0.15	0.13
	7th	0.1	0.11	0.18	0.19
	9th	0.16	0.15	0.21	0.23

yield of the toxin was obtained. In general, commodities with a high protein concentration (groundnut and mustard oil cakes support less citrinin synthesis by the fungus at 28°C-30°C whereas substance rich in starch viz. cereal grains provide higher synthesis of the toxin. Even with starchy substrates there was considerable variation with respect to the grain. Rice provided the best substrate for citrinin production.

Effect of different humidities on citrinin production by P. citrinum on different agricultural products

Since humidity is one of the important factor determining the growth of fungi we studied the effect of different humidities for citrinin production. We prepared four humidity chambers in different desiccators. Flasks containing solid sample after inoculation with the organism were incubated in these desiccators at 28°C. Citrinin activity was measured on the 5th, 7th and 9th days. The results are shown in Table 2.

It is evident from the Table 2 that the toxin production increased with increasing humidity and in most of the cases the maximum citrinin value was attained at 70%-80%, humidity, but citrinin production at 40% humidity was reduced dramatically. The optimum time period for toxin synthesis varied with the humidity and the nature of agricultural commodities.

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