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PREVENTION OF AFLATOXIN CONTAMINATION IN FENUGREEK (*Trigonella foenum graceum*) SEEDS BY SOME ORGANIC ACIDS

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ABSTRACT

A highly toxigenic aflatoxin producing strain of *Aspergillus flavus* was isolated from fenugreek seeds. Some organic acids viz., propionic acid, benjoic acid, sorbic acid, lauric acid and sodium benzoate have been screened to inhibit mould count and contamination of aflatoxin in fenugreek seeds. The maximum inhibition in mould count and aflatoxin contamination was noted at 0.4% concentration of propionic acid and the minimum inhibition in both parameters was recorded in case of sodium benzoate. The next most effective organic acid was benzoic acid. It completely inhibited aflatoxin B_2 and G_1 at the concentration of 0.4% but aflatoxin B_1 could be inhibited only up to the extent of 95.70% even at the concentration of 0.5%. However, mould count was totally suppressed at this concentration. More or less similar findings have been recorded for sorbic and lauric acids. These acids completely inhibited aflatoxin B_2 and G_1 at 0.4% concentration but aflatoxin B_1 was inhibited to the extent of 94.53 and 93.75 percent respectively.

KEYWORDS: Aflatoxin, Fenugreek seeds, *Aspergillus flavus*, organic acids

Fenugreek (*Trigonella foenum graceum*) is one of the oldest cultivated spice crops of the world and is much used in herbal medicines in India. It has a wide range of medicinal applications. Fenugreek seeds are commonly contaminated with moulds particularly species of *Aspergillus* (Prasad et al., 1984). The mould contamination of seeds not only decreases the nutritive value but also causes harmful effects on consumers health, due to production of highly toxic metabolites called mycotoxins.

Aflatoxins are highly toxic metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* and aflatoxin B_1 is reported to be carcinogenic, mutagenic, teratogenic and cytotoxic to plants and animals (Dickens and Jones, 1965). Consumption of aflatoxin contaminated food by human beings and animals lead to many pathological disorders and even death (Krishnamachari et al., 1975). Recently, Koul and Sumbali (2008) noted the presence of aflatoxin B_1 and B_2 in medicinally important dried rhizome of *Acorus calamus*. Mariella et al., (1998) reported the bovine hepatic metabolism of aflatoxin B_1. In view of the growing concern about health hazards of aflatoxins, present investigation was carried out to find the effect of some organic acids on mould count and aflatoxin production by *Aspergillus flavus* on fenugreek seeds.

MATERIALS AND METHODS

In the present investigation seed samples from freshly harvested fenugreek crop were obtained and treated with 5 organic acids viz., propionic acid, sorbic acid, benzoic acid, lauric acid and sodium benzoate at the rate of 0.1, 0.2, 0.3, 0.4, and 0.5% by weight following Rani and Singh (1992). After adding the chemicals, the seeds were shaken thoroughly to disperse organic acids and then stored at room temperature for 6 months. Untreated seeds served as control. After the stipulated storage period both treated and untreated seeds were analysed for the association of moulds and aflatoxin contamination.

The moulds associated with seeds were isolated by seed plating method following rules of ISTA (1966) using PDA medium. For each seed sample, 200 seeds were taken and plated at the rate of 10 seeds per plate on PDA medium and incubated at 28±2°C for 6 days in an alternating cycle of 12 hours fluorescent light and 12 hours darkness. After incubation period the seeds were examined under stereo-binocular microscope for the presence of moulds and finally fungal population was determined. Further, seed samples were analysed for presence of aflatoxins by thin layer chromatography following method outlined by Jones(1972).

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RESULTS AND DISCUSSION

Among all the organic acids, propionic acid was found to be most effective organic acid in checking mould infestation and aflatoxin production up to 100% at 0.4% concentration. This acid inhibited the production of aflatoxin B_1, G_1 and B_2 completely at 0.4% concentration. However, at 0.5% concentration, this acid completely inhibited mould incidence and aflatoxin B_1, B_2 and G_1. It is interesting to note that aflatoxin G_1 was completely inhibited at 0.1% concentration of propionic acid. Further, aflatoxin B_2 and G_1 could not be detected beyond 0.1% concentration but only aflatoxin B_1 was recorded upto 0.3% concentration of propionic acid. The next effective organic acid was benzoic acid. It completely inhibited aflatoxin B_2 and G_1 at the concentration of 0.4% but aflatoxin B_1 could be inhibited only upto the extent of 95.70% even at the concentration of 0.5%.

Other organic acids viz., sorbic acid, lauric acid and sodium benzoate completely inhibited aflatoxin B_2 and G_1 at 0.4% concentration but aflatoxin B_1 could not be inhibited completely even at the concentration of 0.5% of these organic acids. The least effective chemical was sodium benzoate which even at 0.5% concentration could inhibit aflatoxins B_1 to the extent of 92.96% only. Thus, it

Table 1: Efficacy of some organic acids against contamination in Fenugreek seeds

Sl. No.	Organic Acid	Conc. of organic acid(%)	No. of Moulds	Amount of Aflatoxin (in ppb)				% inhibition in Aflatoxin Production			
				B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
1	Control	-	39	2590	680	600	-	-	-	-	-
2	Propionic Acid	0.1	22	870	120	-	-	66.01	82.35	100.00	-
		0.2	20	750	-	-	-	70.7	100	100.00	-
		0.3	10	280	-	-	-	89.06	100	100.00	-
		0.4	2	-	-	-	-	100	100	100.00	-
		0.5	-	-	-	-	-	100	100	100.00	-
3	Benzoic Acid	0.1	25	920	150	-	-	64.06	77.94	100.00	-
		0.2	22	850	90	-	-	66.79	87.64	100.00	-
		0.3	14	830	70	-	-	67.57	93.23	100.00	-
		0.4	10	400	-	-	-	84.37	100.0	100.00	-
		0.5	-	110	-	-	-	95.70	100.0	100.00	-
4	Sorbic Acid	0.1	30	1100	340	200	-	57.03	50.00	66.60	-
		0.2	25	950	150	100	-	62.89	77.94	83.30	-
		0.3	17	800	90	-	-	68.75	87.64	100.00	-
		0.4	5	450	-	-	-	82.42	100.00	100.00	-
		0.5	3	140	-	-	-	94.53	100.00	100.00	-
5	Lauric Acid	0.1	24	1120	400	300	-	56.25	46.60	50.00	-
		0.2	20	920	340	150	-	64.06	50.00	75.00	-
		0.3	12	860	100	-	-	66.40	85.29	100.00	-
		0.4	3	500	-	-	-	80.46	100.00	100.00	-
		0.5	-	160	-	-	-	93.75	100.00	100.00	-
6	Sod. Benzoate	0.1	25	1290	444	340	-	50.1	40.00	42.00	-
		0.2	20	1090	340	180	-	57.42	50.00	70.00	-
		0.3	15	900	120	70	-	65.00	82.35	82.00	-
		0.4	5	700	-	-	-	72.65	100.00	100.00	-
		0.5	2	180	-	-		92.96	100.00	100.00	-

can be safely concluded that 0.4% concentration of propionic acid is best for elimination of moulds and aflatoxin contamination in fenugreek seeds. However, this concentration (0.4%) of benzoic acid, sorbic acid and lauric acid can reduce aflatoxin B_1 contamination to the tune of 95.70%, 94.53% and 93.75% respectively, which is also quite satisfactory in table 1.

Lal and Kapoor (1980) suggested that 0.5% concentration of propionic acid can effectively control species of *Aspergillus* in stored maize. Rani and Singh (1992) noted inhibitory effect of lauricidin derivatives including lauric acid on aflatoxin elaboration by *Aspergillus flavus*. Dixit and Singh (2006) reported that among organic acids, benzoic acid was found to be most potent inhibitor of fumonisin elaboration by *Fusarium moniliforme* at 0.4% concentration followed by propionic acid and sorbic acid. Thus, it is quite evident that some organic acids are quite inhibitory to mould infestation and mycotoxin elaboration during storage of seeds. Further, they are cheap, ecofriendly and have no residual toxicity, hence can be safely recommended for stored food grains and edible seeds such as fenugreek seeds and other spices.

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