Aflatoxin Contamination of Chilli Powder in Canteens of King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

Dusanee Thanaboripat[1]*, Ounruan Petcharawan[2] and Sujitra Sukonthamut[3]

[1]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand
[2]Royal Park Ville, Nonchok, Bangkok, Thailand
[3]Department of Statistics, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

Abstract

Chilli powder from 8 canteens serving various faculties of King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand was surveyed for aflatoxin contamination. Seventy-two samples of chilli powder from 24 food stalls in 8 canteens were collected. These samples including 9 control chilli samples belonging to the same commercial brand were analysed for aflatoxin using DOA-Aflatoxin Test Kits. The results showed that all chilli powder samples collected from all food stalls were contaminated with aflatoxin B1. Chilli powder samples collected from food stalls at 5 canteens (Prathep building, Engineering Faculty, Industrial Education Faculty, KMITL building and Agricultural Technology Faculty) contained the highest average amount of aflatoxin B1 (8.94±1.10, 8.43±1.32, 8.37±1.13, 8.21±0.65 and 8.12±0.96 ppb, respectively). Chilli powder samples collected from all food stalls contained significantly higher average amounts of aflatoxin when compared to commercial chilli powder samples (5.44±0.15 ppb) used as control ($p<0.05$). However, the aflatoxin levels of all chilli powder samples did not exceed the legal limit (20 ppb) prescribed by Thai Ministry of Public Health.

Keywords: Chilli powder, aflatoxin B1, canteen

1. Introduction

Aflatoxins, a worldwide health hazard to humans and animals, are among the most potent mutagenic and carcinogenic compounds known to be produced in nature. Various agricultural commodities have been found to be contaminated with either aflatoxin producing fungi or aflatoxins. Although the presence of *Aspergillus* mould does not necessarily indicate aflatoxin contamination, there is certainly an increased risk [1]. Aflatoxin poisoning have been reported from all parts of the world in almost all domestic and non domestic animals like cattle, horses, rabbits, and other non human primates [2]. Foods at the highest risk of aflatoxin contamination are corn, peanut and cotton seed [3]. Ingestion of aflatoxins in contaminated food or feed can cause

*Corresponding author: Tel: 662-3298415 Fax: 662-3298412
E-mail: ktdusane@hotmail.com

aflatoxicosis. Diet is the major way through which humans and animals are exposed to aflatoxins. In at least three parts of the world, East Africa, the Philippines and Thailand, good epidemiological evidence has been collected showing a correlation between the incidence of liver cancer and exposure to aflatoxins [3] and at least two studies have reported the association between exposure to aflatoxin B1 and the development of gallbladder cancer (GBC) [4,5]. These findings suggest that aflatoxin-contaminated red chili pepper consumed by Bolivian and Peruvian people may be associated with the development of GBC [6]. Athipanyakul *et al.* [7] reported that 3.8 persons in 100,000 persons would be at risk of liver cancer due to consumption of peanut contaminated with aflatoxin.

Aflatoxin B1 has been detected in 80% of maize samples obtained from different locations in Southeast Nigeria [8] and similarly 92 % of animal feed samples taken from commercial sources in Thailand were contaminated with aflatoxin B1 [9]. Fifty-one percent of noodle dishes sold in three areas of Bangkok were found to be contaminated with aflatoxins ranging from 0.01-17.3 µg/kg [10]. Five herbal medicinal products out of 28 products were also found to be contaminated with detectable amount of aflatoxins ranging from 1.7-14.3 ng/g [11]. Furthermore, aflatoxin producing fungi were found in fermented foods and beverages such as fermented rice, fermented soybean paste, peanut butter, soy sauce, Thai red and white wine and rice sugar wine [12].

Aflatoxin contamination in chilli powder has been reported in many countries. Low-grade chillies and chilli powder sold in supermarkets in India and maize and groundnut samples employed in preparing poultry feed were shown to be contaminated with aflatoxin [13]. Chilli (*Capsicum annuum*) has been widely consumed as condiment in Thailand in the forms of fresh or dried chilli, chilli powder and chilli paste. Ooraikul *et al.* [14] reported that the average chilli intake rate of people in Hua Rua sub-district, Ubonratchathani province was 7.6 g/day higher than the average of general Thais (5 g/day) surveyed by the Ministry of Public Health. Thus, the objective of this study is to survey the risk of aflatoxin contamination in chilli powder consumed in 8 canteens of King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

2. Materials and Methods

2.1 Sampling of chilli powder

Seventy-two samples of chilli powder (20-50 g) served as condiment for noodle dishes from 24 food stalls at 8 canteens of KMITL, i.e. Science Faculty, Industrial Education Faculty, Agricultural Technology Faculty, Engineering Faculty, Architecture Faculty, Information Technology Faculty, Prathep Building and KMITL canteen, were collected. Nine control samples belonging to the same commercial brand bought from three local markets were used as control.

2.2 Aflatoxin analysis

All chilli powder samples were analysed for aflatoxin B1 by DOA-Aflatoxin ELISA Test Kits provided by Department of Agriculture, Ministry of Agriculture according to the method described by Chinaphuti *et al.* [15]. Twenty gram of ground sample was added with 100 ml of 70% methanol and blended at high speed for 2-3 min before filtering through filter paper. Diluted small portions of filtrate (1:20 or higher) with 0.01 M phosphate buffer saline (PBS). Fifty µl of aflatoxin B1 standards was added into antibody coated wells of micro-titration plate and 50 µl of diluted samples was added into other wells. Fifty µl of AFB1-HRP conjugate was then added into each well, slightly shaken and incubated at room temperature for 30 min. The content in the well was then rinsed 3-5 times with 0.01 M phosphate buffer saline plus 0.5% Tween 20 (PBS-T). One hundred µl of substrate (Tetramethylbenzidine) was then added into the wells, incubated for 10

min. One hundred µl of stopping solution (0.3 Phosphoric acid) was then added into the wells and reacted with substrates. The color of substrates changed from blue to yellow and color intensity was read by Micro ELISA reader at 450nm. Aflatoxin B1 concentration (ppb) was calculated from standard curve.

2.3 Statistical analysis

The experiments were designed by Complete Randomized Design and analysed statistically by Two way analysis of variance and multiple comparison at the 5% level.

3. Results and Discussion

Aflatoxin content contaminated in chilli powder samples collected from 8 canteens of KMITL was shown in Table 1. It was found that all chilli powder samples collected from all food stalls were contaminated with aflatoxin B1. Chilli powder sample collected from food stalls at 5 canteens, i.e. Prathep building, Engineering Faculty, Industrial Education Faculty, KMITL building and Agricultural Technology Faculty, contained the highest average amount of aflatoxin B1 (8.94±1.10, 8.43±1.32, 8.37±1.13, 8.21±0.65 and 8.12±0.96 ppb, respectively). All chilli powder samples collected from all food stalls contained significantly higher amounts of aflatoxin when compared to commercial chilli powder (5.44±0.15 ppb) used as control ($p<0.05$).

Aflatoxins are reported to be the most common mycotoxin found in chilli products around the world [16]. Waiyarattana [17] found that 46 out of 60 (76.67%) samples of ready-to-eat Thai chilli paste, i.e. namprik tadang, namprik pau and namprik narok, frequently contaminated with aflatoxins ranging from 0.39-347.50 ppb. The estimated risk of liver cancer from aflatoxin exposure from daily intake of namprik tadang, namprik pau and namprik narok were 4, 11 and 3 cases per 100,000 persons per year, respectively. Interview data showed the most frequently consumed ready-to-eat Thai chilli paste 1-2 day per week (not everyday). Tansakul *et al.* [18] reported that among 120 samples of Thai commodities analysed, chilli powder had the highest level of aflatoxin contamination followed by chilli pods, peanut and rice, respectively. Iqbal *et al.* [19] detected the amount of aflatoxins in whole chilli and ground chilli from Punjab Pakistan ranging from 0.00-81.5 µg/kg (ppb) and 0.00-84.8 µg/kg (ppb), respectively. The average aflatoxin levels in whole, powdered and crushed red chillies (*Capsicum annuum* L.) in Pakistan were 11.7, 27.8 and 31.2 µg/ kg, respectively [20]. Russell and Paterson [21] reported that high levels of aflatoxin B1 were obtained from ground chilli samples rather than dried whole pod samples.

Many countries have limitation of aflatoxin residue in agricultural products for safety of consumer. Current legislative level of aflatoxins permissible in Thailand is 20 µg/kg or 20 ppb [11] whereas European limit for food is 1-5 µg/kg (1-5 ppb) for aflatoxin B1 and 4-10 µg/kg (4-10 ppb) for total aflatoxins [22] and only 5 µg/kg (5ppb) of aflatoxin B1 and 10 µg/kg (10 ppb) of total aflatoxins are allowed in chillies [21]. The tolerance level for total aflatoxins in red chilli is 20 µg/kg (20 ppb) in United State [23] and 30 µg/kg (30 ppb) in Pakistan [24].

Chilli pods and chilli powders collected from different areas in India were analysed for aflatoxin B1 content using an indirect competitive ELISA and it was found that there was good agreement between ELISA and HPLC estimations of AFB1 and the results suggested that the ELISA procedure adopted was dependable. Fifty nine percent out of 182 chilli samples tested were contaminated with aflatoxin B1 and 18% contained the toxin at non permissible levels [25]. Forty samples of chilli paste and peanut sauce (satay sauce) marketed in Bangkok, Thailand were examined by TLC method. Thirteen samples were contaminated with aflatoxin B1 and two

samples were found to have the levels of toxin exceeding the tolerance limit (20 µg/kg) regulated by the Ministry of Public Health [26].

One of the most effective ways to control the problems caused by aflatoxins contamination is to prevent the growth of fungi in the substrate, for example by the use of chemical inhibitors to suppress the spore germination of the fungi, as well as the development of the fungal mycelium, in the substrate susceptible to contamination by these toxins[27]. Our previous results also showed that aflatoxin formation was inhibited due to the inhibition of the growth of aflatoxin producing fungi such as *A. flavus* [28, 29]. In addition, Khan *et al* [16] suggested that physical sorting regarding colour, shape and size of chilli could reduce the aflatoxin

Table 1. Aflatoxin contents in chilli powder samples from 24 food stalls in 8 canteens of KMITL, Bangkok, Thailand.

Canteen	Food stall no.	Aflatoxin B1 content (ppb)			Average of aflatoxin B1
		Sample 1	Sample 2	Sample 3	(ppb)*
1. Faculty of Science	1	8.08	7.69	7.48	7.92 ± 0.51^{b}
	2	8.19	7.41	8.01	
	3	8.20	7.30	8.92	
2. Faculty of Industrial Education	4	7.29	7.69	9.96	8.37 ± 1.13^{ab}
	5	9.32	7.04	9.60	
	6	7.79	7.46	9.16	
3. Faculty of Agricultural Technology	7	7.88	7.17	9.28	8.12 ± 0.96^{ab}
	8	7.31	8.20	8.20	
	9	8.08	7.03	9.92	
4. Faculty of Engineering	10	8.53	8.11	11.14	8.43 ± 1.32^{ab}
	11	7.19	8.55	7.41	
	12	7.81	7.24	9.90	
5. Faculty of Architecture	13	6.66	8.15	9.00	7.99 ± 1.06^{b}
	14	7.16	8.55	8.60	
	15	6.15	8.66	9.02	
6. Faculty of Information Technology	16	9.04	8.06	6.08	7.89 ± 1.38^{b}
	17	5.52	8.68	8.15	
	18	7.15	8.67	9.69	

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Canteen	Food stall no.	Aflatoxin B1 content (ppb)			Average of aflatoxin B1 (ppb)*
		Sample 1	Sample 2	Sample 3	
7. Prathep building	19	9.52	8.53	10.92	8.94 ± 1.10^{a}
	20	7.86	8.39	9.37	
	21	8.08	7.68	10.10	
8. KMITL canteen	22	8.76	8.39	8.18	8.21 ± 0.65^{ab}
	23	8.11	7.81	7.60	
	24	7.62	7.82	9.64	
9. Control	25	5.56	5.37	5.69	5.44 ± 0.15^{c}
	26	5.42	5.18	5.40	
	27	5.51	5.32	5.56	

*Means with different superscripts are significantly different ($p<0.05$).

contamination into some extend. However, complete elimination of aflatoxins is not possible. In order to reduce the levels of aflatoxins in chillies and chilli products and to prevent the hazards associated with fungal contamination, number of procedures such as controlling moisture and temperature, proper storage, packing and so on, are required [16].

4. Conclusions

Chilli powder has been regularly consumed by Thai people. The average content of aflatoxin B1 present in all chilli powder at 8 canteens (ranging from 5.32- 11.14 ppb) was lower than the legal limit (20 ppb) in Thailand, however, it is higher than the aflatoxin B1 level allowed in European countries (5ppb). Thus health hazard has to be considered when consuming chilli powder or using as condiment.

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