Benguet State University Research Journal (BRJ) April-August 2016, 76: 83-92

Pre-Harvest Aflatoxin Contamination of Peanuts Collected from Regions I, II and Cordillera Administrative Region

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ABSTRACT

Pre-harvest aflatoxin contamination is a priority concern considering the effects of combined pre-and post-harvest aflatoxin contaminations in peanut products. This can cause liver cancer and weakened immune system in humans, and high mortality and reduced productivity in livestock. It can be carried by cow's milk to processed milk, cheese, and other dairy products (CAST, 2003). The study was undertaken to determine the presence of pre-harvest aflatoxin contamination in peanuts grown in Regions I and II, and the Cordillera Administrative Region. There were 165 peanut samples collected from standing plants ready for harvest in the farm (78), fresh in-shell harvests sold in the market (34), and just dried in-shell brought to the seed store (46) then deshelled ready for processing (7) to ensure that whatever aflatoxin detected is from pre-harvest contamination. Immunochromatographic test strips with 20 parts per billion (ppb) as cutoff limit showed that 7.88% had pre-harvest aflatoxin contamination. These were collected from farms and storage in Region I (4.24%) and Region II (3.64%). There could be some aflatoxin contamination below 20ppb in the samples considering the limitation of the test strip. Results therefore imply the need for appropriate management of pre-harvest aflatoxin.

Keywords: Peanut, pre-harvest aflatoxin contamination, immunochromatographic test strips

INTRODUCTION

Peanut (*Arachis hypogaea L.*) is an economically important legume and staple crop worldwide. It is often consumed as an important dietary component in the form of nutritious snacks and as feed or feed additive due to its high protein, unsaturated fats, carbohydrates, vitamins, and mineral contents (APC, 2013; Hill, 2002; ICRISAT, 2000). In the Philippines, peanut is an excellent source of cash to both small and big farmers accounting for 29,088.93 tons in 25,599.82 hectares in 2013 (PSA, 2015). Peanut could contribute greatly to the current trend of organic farming and good agricultural practices that can be attributed to its ability to fix nitrogen. Peanut is normally grown either in shortened maturity period of about 70 days after rice/corn in the lowlands, grown with corn in Cagayan Valley, north of the Philippines, or in the uplands as rainfed crop.

Due to its high protein and oil content, peanut is susceptible to *Aspergillus flavus* and *A. parasiticus* infection that produce aflatoxin. The low soluble sugars when dry and the high oil content make peanut susceptible to aflatoxin contamination (Pitt *et al.*, 2012). The aspergilli are favored by postharvest moisture content of kernels above 10.5 – 11% coupled with 20-350C and >83% relative humidity (CAST, 2003). When improperly handled, processed peanut products can harbor aflatoxin beyond the acceptable limit of 20 ppb that can happen in home-made products that do not undergo aflatoxin detection. In principle, the Philippines just like the United States of America (USA) and Southeast Asian countries complies with the 20 ppb aflatoxin contamination threshold which has long leeway over the 4 ppb limit set by the European Union. On the other hand, pre-harvest aflatoxin contamination of peanut is enhanced by the interrelation of prolonged drought and high soil temperature of 27-300C during the last 3-6 weeks before harvest (*Abbas et al*., 2009).

Eating aflatoxin-contaminated peanuts, corn, grains, cassava, and tree nuts results to liver cancer

and weakened immune system in humans as well as high mortality and reduced productivity in livestock. Aflatoxin can be carried by cow's milk to processed milk, cheese and other dairy products (CAST, 2003). These all call for the appropriate management of aflatoxin contamination in peanut which can be targeted via pre-harvest aflatoxin contamination management.

Losses due to aflatoxin were estimated at USD900 M annually. Losses in the USA were reported at over USD25.8 M annually in 1993-1996. Most of the cost of losses were shouldered by the shelling industry while grower losses were at USD2.6 M annually (Schmale and Munkvold, 2013).

In practice, aflatoxin contamination of peanut is regularly monitored in the industry through the sampling of lots or stocks. Accurate and convenient estimation of aflatoxin contamination is very essential for effective monitoring and management of aflatoxin contamination (Wilson *et al.*, 1995). In the USA, peanut samples are examined visually for characteristic green or yellow-green *Aspergillus* colonies. Detection of fungal colonies on any pod/kernel causes the entire lot to be designated as Segregation III which is recommended not to be used for direct human or animal consumption. Nevertheless, aflatoxin can be present without visible fungal growth which can be detected by Fuorier transform near-infrared spectroscopy, high performance liquid chromatography (HPLC), liquid chromatography – tandem mass spectrometry, enzyme-linked immunosorbent assay (ELISA) and ion mobility spectrometry. The ELISA and HPLC are most commonly used.

On the other hand, immunochromatographic test strips are low-cost, easy to handle, and usable on-site qualitative tests developed and integrated into routine quality monitoring procedures (Zhang *et al*., 2011). Luis (2014) demonstrated the utility of the strips in 12 months even without refrigeration. While limited to qualitative detection only at 20ppb aflatoxin content and beyond, the study was done to determine the presence of pre-harvest aflatoxin contamination of peanut that can be used as a basis in planning for its eventual management in the field right on the standing plants.

MATERIALS AND METHODS

Collection of Peanut Samples

Peanut samples were collected from farms, processing centers, storage facilities and public markets in Regions I and II, and the Cordillera Administrative Region (CAR) (Plate 1). The visual assessment of *Aspergillus* infection in the samples was noted, indicated by the presence of brownish to greenish moldy growth in either the pods or kernels. Farm samples were either harvested from standing plants ready for harvest or from fresh harvests. To be sure of pre-harvest aflatoxin contamination in samples collected from processing centers, small and deformed kernels indicative of pre-harvest contamination were selected. Samples collected from storage facilities purposely for seeds are assured of freedom from post-harvest aflatoxin contamination. Only the relatively quality pods that were properly handled and maintained at 11% moisture content were brought to the seed store and were kept inside cold temperature storage areas. Samples collected from public markets were both fresh harvest still in-shell and adequately dried kernels free from visual symptoms of postharvest aflatoxin contamination considering that the kernels were sold to consumers for roasting.

Visually infected samples were packed in separate paper bags to avoid contaminating other samples. These were brought to the Plant Disease Clinic, Benguet State University (BSU) for processing. Fresh peanut samples were sun/air - dried to prevent molding and rotting. Samples in plastic bags were transferred to net bags or brown paper bags to avoid accumulation of moisture. The samples were segregated as to province where samples were collected and stored in the refrigerator for further processing.

Detection of pre-harvest aflatoxin contamination was done using the qualitative aflatoxin detection kit. Thirty grams peanut kernels were randomly picked from each sample. The kernels were ground using a blender and were passed through a 20-mesh screen so that 75% of the samples were obtained (Plate 2). A 10g ground sample was weighed and placed in a 100 ml Whirl-Pak Stand-Up bag and was added with 20ml of 70% methanol. The sample was vigorously shaken for 1 minute and allowed to

settle before proceeding with the test. The samples were replicated twice.

The immunochromatographic test strips from AgraStrip ® (Romer Labs) were used following the manufacturer's instructions (Plate 3). The production of one visible line is a positive reaction that indicates the detection of aflatoxin level at ≤ 20pbb; two visible lines as a negative reaction indicative of 0-19 ppb aflatoxin contamination; and no line indicative of invalid result. As reported by Luis (2014), strips showed positive results for aflatoxin contamination beyond 20 ppb but not at exactly 20 ppb. All aflatoxin contamination below 20 ppb gave negative results. This means that an undetermined fraction of the samples giving negative results may be harboring aflatoxin below 19 ppb.

Plate 1. Preparation of peanut samples for aflatoxin detection: a) peel and weigh, b) blend, c) weigh ground peanut, d) measure 70% methanol, e) add 70% methanol, f) thoroughly mix ground peanut and methanol, and g) sample extract ready for aflatoxin detection

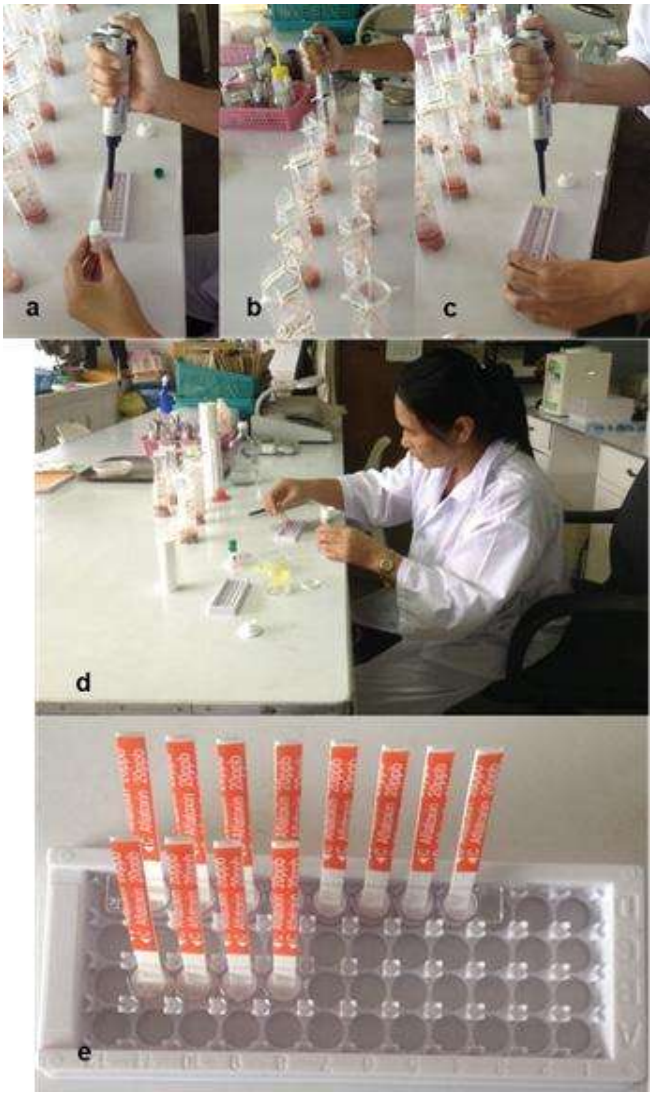

Plate 2. Aflatoxin detection using immunochromatographic test strips from AgraStrip ® (Romer Labs)

RESULTS AND DISCUSSIONS

Collection Sites

Peanut samples were collected from 12 provinces: Ilocos Norte, Ilocos Sur, La Union, and Pangasinan of Region I; Cagayan, Isabela, Nueva Vizcaya, and Quirino of Region II; Benguet, Ifugao, Kalinga, and Mountain Province of CAR.

Peanut Sample Collection

One hundred sixty five samples were collected from Northern Philippines and brought to BSU. Region II samples account for 42.00%, 38.61% for Region I, and 19.39% for CAR. Region II samples were from Nueva Vizcaya (32%) and Isabela (30%). On the other hand, 34% of the Region I samples

were collected from Ilocos Norte, 32% from Ilocos Sur, 18% from Pangasinan, and 16% from La Union. Samples from CAR were collected from Mt. Province (53%), Benguet (22%), Ifugao (12.5%), and Kalinga (12.5%) (Fig.1).

Considering that sample collection was based mainly on peanut availability during the collection visits as can be considered a 'hit or miss' model, no statistical analysis can be derived from the data. It is sufficient to note that adequate samples were collected for qualitative detection of pre-harvest aflatoxin contamination using the immunochromatographic strips as initial guide for a bigger project, i.e. the search for atoxigenic strains of Aspergillus flavus as biological control agents for pre-harvest aflatoxin contamination management.

Table 1. Distribution of peanut samples collected from farm, public market, storage facility and processing center (N = 165)

Collection Site	SAMPLE TYPE			
	Farm	Market	Storage Facility	Processing Center
Region I				
Ilocos Norte	16	0	4	1
Ilocos Sur	14	3	3	0
La Union	9	0	1	0
Pangasinan	5	0	6	0
Sub-total	**44**	**3**	**14**	**1**
Region II				
Cagayan	5	0	4	1
Isabela	15	1	5	0
Nueva Vizcaya	6	17	0	0
Quirino	4	0	13	0
Sub-total	**30**	**18**	**22**	**1**
CAR				
Benguet	1	4	2	0
Ifugao	2	0	2	0
Kalinga	0	3	1	0
Mountain Province	1	6	5	5
Sub-total	**4**	**13**	**10**	**5**
Grand Total	**78**	**34**	**46**	**7**

Table 1 shows the distribution of peanut samples collected from Northern Philippines based on sample type78 from farms, 34 from public markets, 46 from storage facilities, and 7 from processing centers. Forty and four samples collected from Region I were from farms and 14 from seed stores. Thirty collections from Region II were from farms, 22 from seed stores, and 18 from market stalls. On the other hand, CAR samples were from public markets and seed stores.

The bigger size of samples collected from Regions I and II from the farms and markets as compared to CAR can be attributed to the sampling time and acreage. Regions I and II have generally wide acreage planted to peanut, i.e. peanut after rice in rice fields and sample collection was done on time with, either as fresh on the farm or in market places where harvest was sold. On the other hand, CAR has only backyard gardens basically for home consumption and being dependent only on rain for irrigation.

The samples collected from farms, markets, storage facilities and processing centers were included for pre-harvest aflatoxin contamination based on the premise that fresh harvests were sold in the market as boiled peanuts. On the other hand, dried kernels which were either sold in the market as roasted or for processing were direct selections from the fresh harvest that were properly maintained in terms of moisture content to avoid post-harvest contamination.

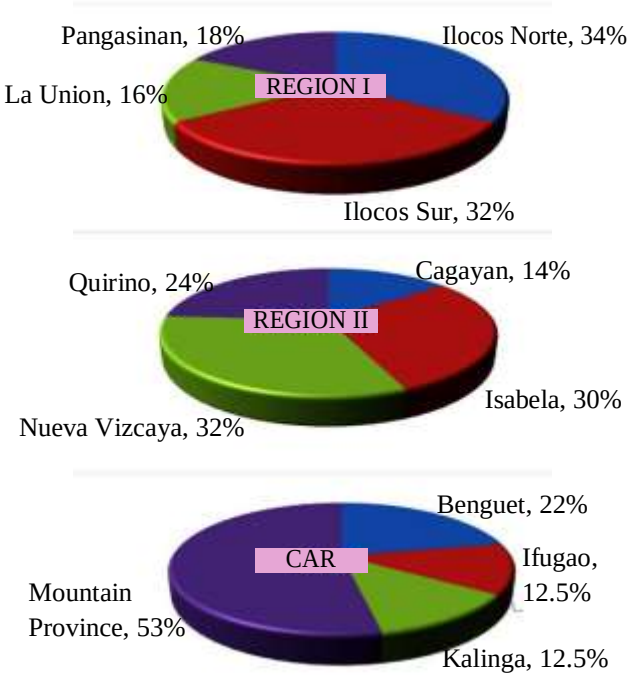

Figure 1. Percentage of peanut samples collected from Regions I and II, and CAR

The qualitative detection of pre-harvest aflatoxin contamination using immunochromatographic test strips showed that 55/62 or 88.7% of the samples from Region I showed negative results, thus, free from pre-harvest aflatoxin contamination (Fig. 2). Seven samples collected from Ilocos Norte, Ilocos Sur and Pangasinan gave positive results meaning 11.29% incidence of pre-harvest aflatoxin contamination (Fig. 3). On the other hand, 65/71 samples from Region II yielded negative results, meaning 91.95% incidence of freedom from pre-harvest aflatoxin contamination while 6 positive results (8.45% incidence of pre-harvest aflatoxin contamination) were obtained from samples in Cagayan, Isabela and Quirino (Fig. 4). All the 32 samples gathered in CAR gave negative results, meaning complete freedom from pre-harvest aflatoxin contamination (Fig. 5).

In the overall population, the negative reactions to qualitative pre-harvest aflatoxin detection shown in Table 2 (92.14%) is encouraging. Nevertheless, the 7.88% positive reaction as shown in Table 3 cannot be ignored considering that this can even be far bigger when actual pre-harvest aflatoxin contamination below 20pbb would have been read. It is reiterated here that the cutoff limit for the qualitative immunochromatographic strips is 20pbb. The provision for pre-harvest aflatoxin contamination management will be an option as initial step in addition to appropriate moisture content management against post-harvest aflatoxin contamination.

The results imply that pre-harvest aflatoxin contamination is favored by the observed relatively warm climate, prolonged drought, and high soil temperature in the peanut – producing areas of Regions I and II. This supports the report of Abbas *et al.* (2009) that pre-harvest aflatoxin contamination of peanut is enhanced by the interrelation of prolonged drought and high soil temperature of 27-30 °C during the last 3-6 weeks before harvest. On the other hand, it is strongly believed that there exists a natural phenomenon protecting the peanuts in CAR and the upland areas of Regions I and II from pre-harvest aflatoxin contamination which can be the case of possible existence of non-aflatoxigenic strains of *A. flavus*. Nevertheless, while no CAR sample yielded any positive result, the limitation of the immunochromatographic strips to detect aflatoxin levels < 20 ppb cannot be underestimated. It could also be possible that pre-harvest aflatoxin was not produced in CAR (possible case of 'escape') as an effect of the production season whereby the rains fall regularly when the peanut pods are towards maturity stage.

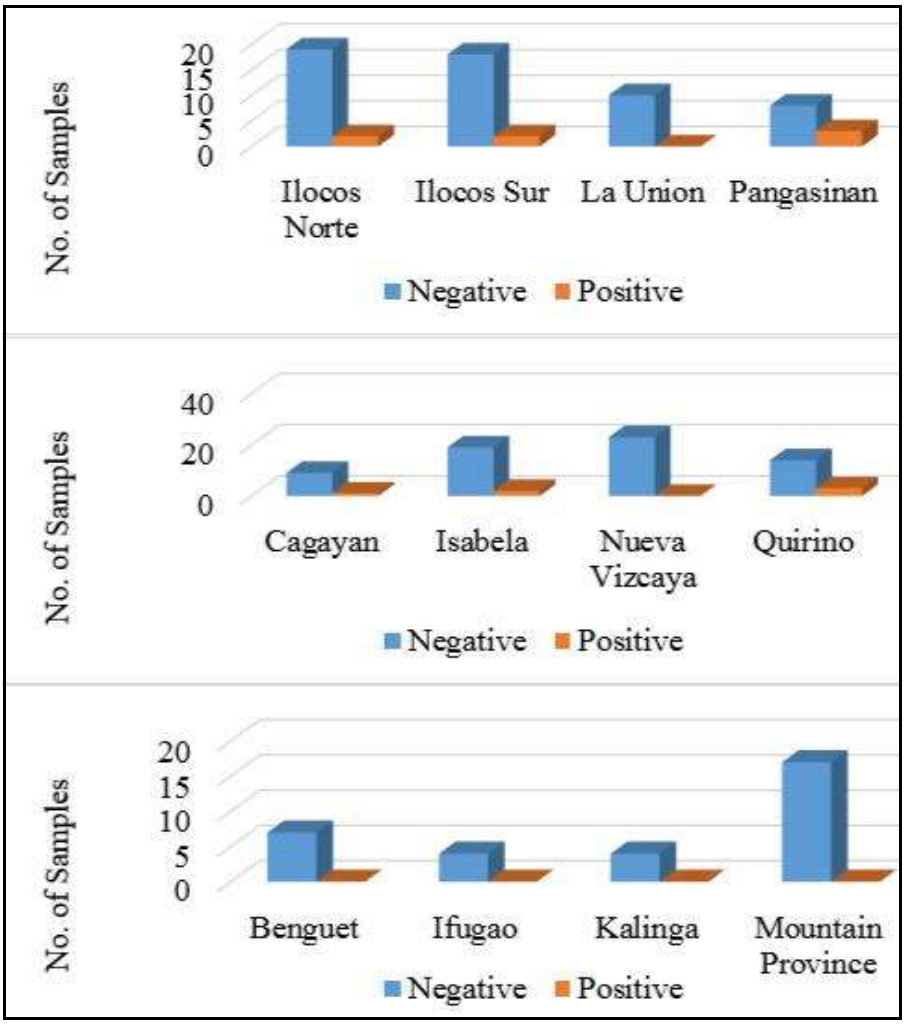

Figure 2. Number of samples collected in Northern Philippines showing negative and positive readings to pre-harvest aflatoxin contamination

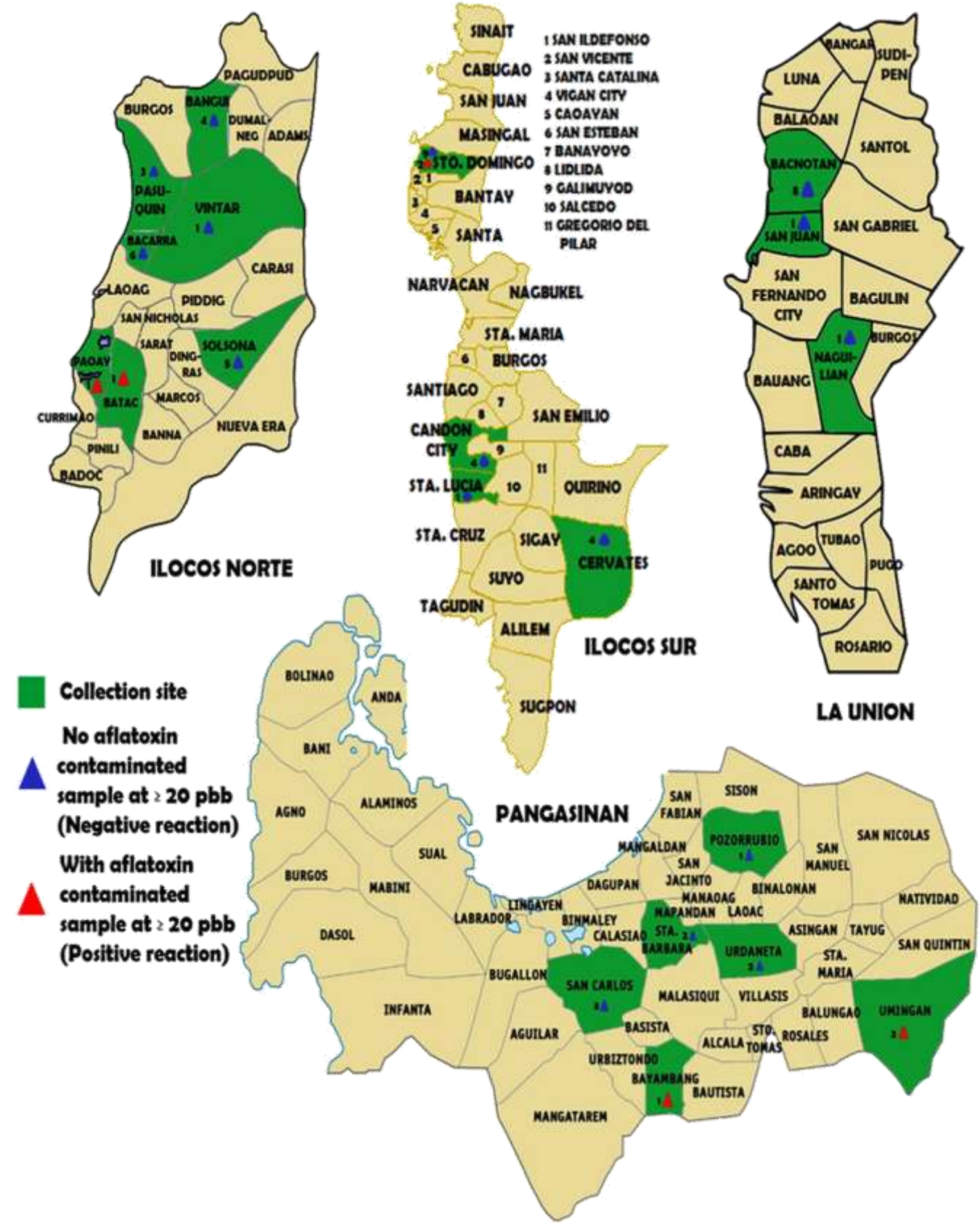

Figure 3. Peanut sample collection sites in Region I showing negative and/or positive reaction to pre-harvest aflatoxin contamination: Ilocos Norte, Ilocos Sur, La Union, and Pangasinan

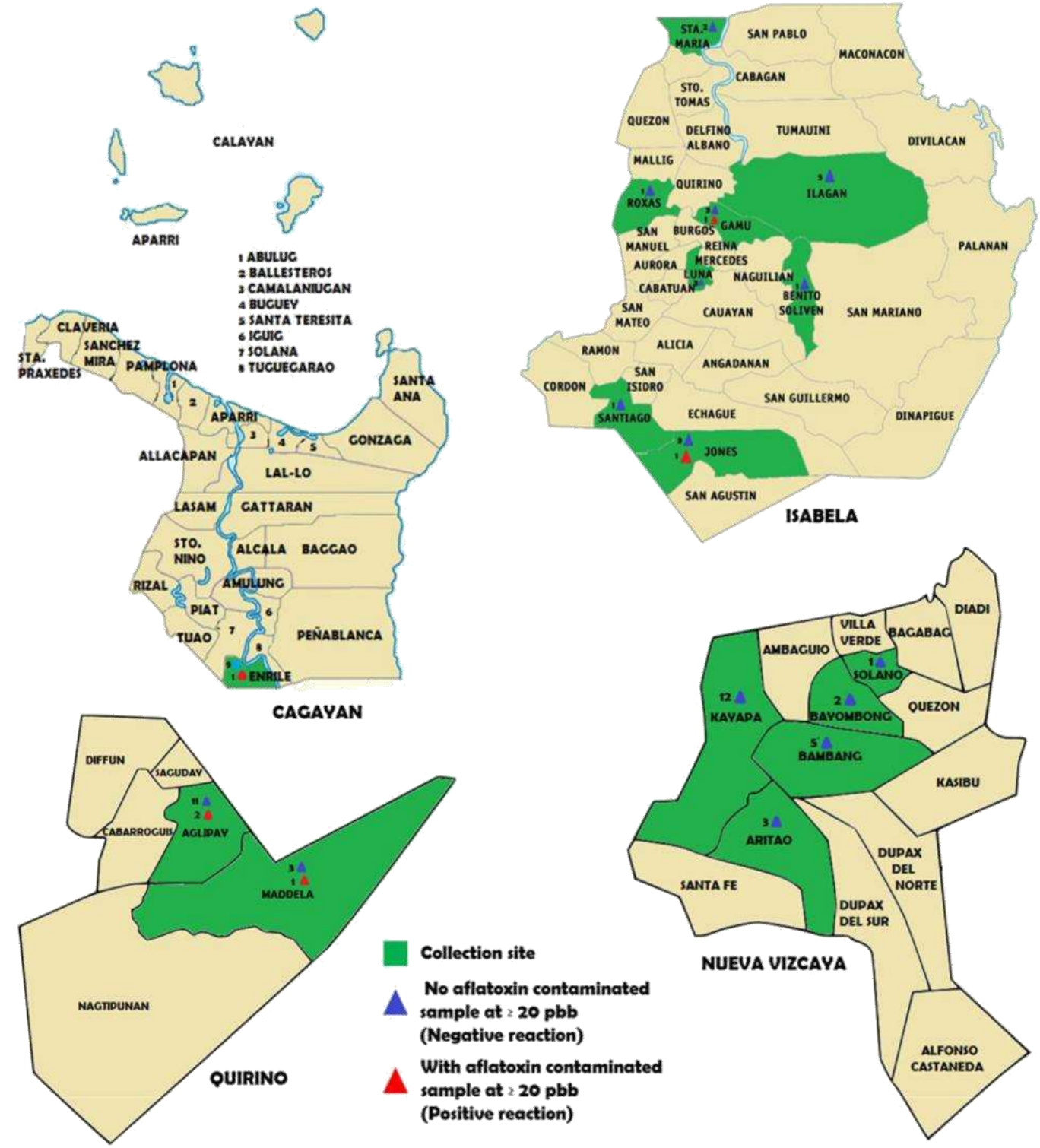

Figure 4. Peanut sample collection sites in Region II showing negative and/or positive reaction to pre-harvest aflatoxin contamination: Cagayan, Isabela, Nueva Vizcaya, and Quirino

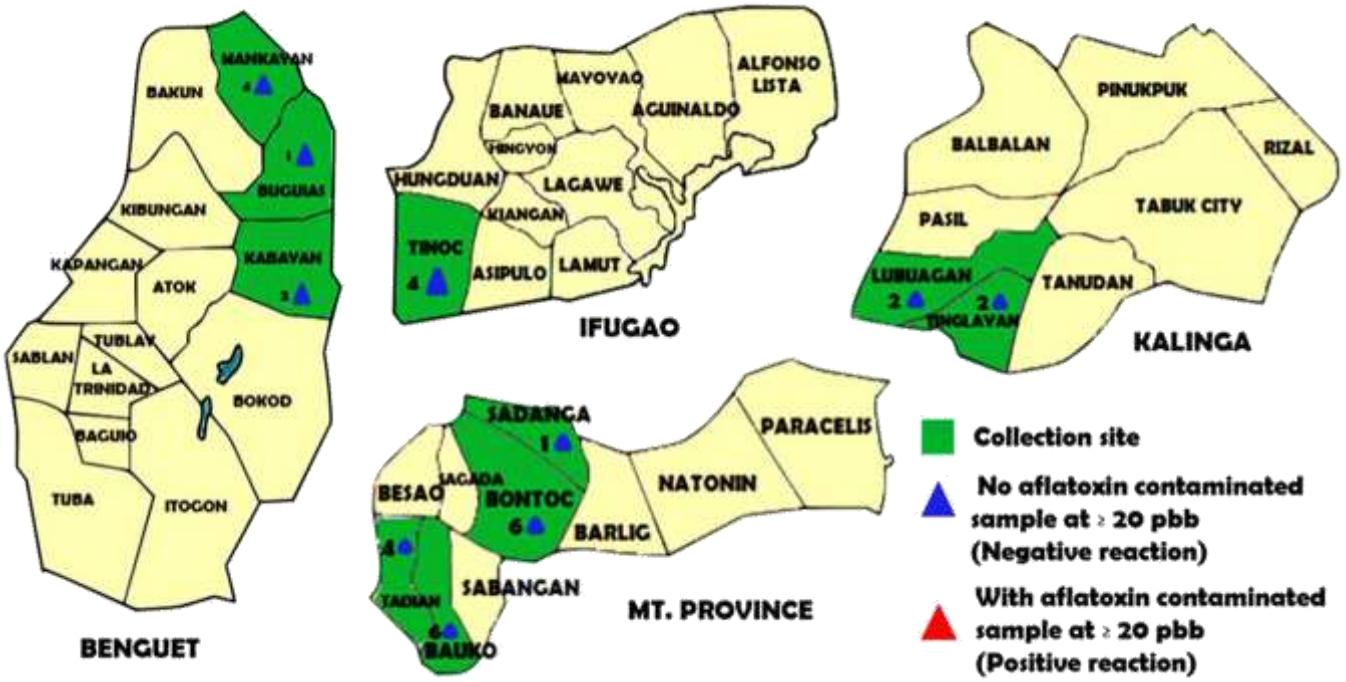

Figure 5. Peanut sample collection sites in CAR showing negative and/or positive reaction to pre-harvest aflatoxin contamination: Benguet, Ifugao, Kalinga and Mt. Province

Table 2. Distribution of peanut samples collected from farm, public market, storage facility and processing center showing negative reaction to pre-harvest aflatoxin contamination (152/165 = 92.13%)

	SAMPLE TYPE			
COLLECTION SITE	Farm	Market	Storage Facility	Processing Center
Region I				
Ilocos Norte	14	0	4	1
Ilocos Sur	12	3	3	0
La Union	9	0	1	0
Pangasinan	5	0	3	0
Sub-total	**40**	**3**	**11**	**1**
Region II				
Cagayan	4	0	4	1
Isabela	13	1	5	0
Nueva Vizcaya	6	17	0	0
Quirino	4	0	10	0
Sub-total	**27**	**18**	**19**	**1**
CAR				
Benguet	1	4	2	0
Ifugao	2	0	2	0
Kalinga	0	3	1	0
Mountain Province	1	6	5	5
Sub-total	**4**	**13**	**10**	**5**
Grand Total	**71**	**34**	**40**	**7**

Table 3. Distribution of peanut samples collected from farm, public market, storage facility and processing center showing positive reaction to pre-harvest aflatoxin contamination (13/165 = 7.87%)

	SAMPLE TYPE			
COLLECTION SITE	Farm	Market	Storage Facility	Processing Center
Region I				
Ilocos Norte	2	0	0	0
Ilocos Sur	2	0	0	0
La Union	0	0	0	0
Pangasinan	0	0	3	0
Sub-total	**4**	**0**	**3**	**0**
Region II				
Cagayan	1	0	0	0
Isabela	2	0	0	0
Nueva Vizcaya	0	0	0	0
Quirino	0	0	3	0
Sub-total	**3**	**0**	**3**	**0**
Grand Total	**7**	**0**	**6**	**0**

CONCLUSIONS

There were 7 (2 farm samples from Ilocos Norte, 2 farm samples from Ilocos Sur, and 3 storage facility samples from Pangasinan) and 6 (1 farm sample from Cagayan, 2 farm samples from Isabela, and 3 storage facility samples from Quirino) samples from Regions I and II, respectively showing positive reaction (13/165 or 7.88% incidence) to pre-harvest aflatoxin contamination qualitative detection. Considering the cutoff limit of immunochromatographic test strips used, there could be more samples with < 20 ppb pre-harvest aflatoxin contamination. The results clearly indicate the need for appropriate and practical pre-harvest aflatoxin contamination management strategy in the warm areas of Regions I and II and other peanut – growing areas in the country with similar environment.

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