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Research Paper

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MEASURING THE IMPACT OF ADHERING TO GOOD PRACTICES ON AFLATOXIN CONTAMINATION IN THE PEANUT MANUFACTURING CHAIN: A MOROCCAN CASE STUDY

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1. INTRODUCTION

Aflatoxins are toxic substances produced by certain Aspergillus species, particularly Aspergillus flavus and Aspergillus parasiticus (Richard & Payne, 2003; Varga et al., 2011). These fungi thrive in warm climates and are more common between latitudes 16[°] and 35[°], but less prevalent above 45° latitudes (Klich, 2007). Although 18 aflatoxins have been identified, only four (AFB1, AFB2, AFG1, and AFG2) are naturally occurring and significant contaminants of various foods and feeds (Frisvad et al., 2019) . AFB1 is the most potent carcinogen produced by Aspergillus species, classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC, 1993). Several epidemiological studies have linked aflatoxins to increased incidence of human gastrointestinal and hepatic neoplasms in Africa, the Philippines, and China. AFB1 has also been associated with human liver cell carcinoma (Goeger et al., 1999).

Many studies have focused on aflatoxins contamination in peanuts, especially in Asia and Africa (Bumbangi et al., 2016; Ding et al., 2012; Galvez et al., 2003; Liu et al., 2017; Mohd Azaman et al., 2016; Mutegi et al., 2013; Sserumaga et al., 2021). In Morocco, a study by (Zinedine & Mañes, 2009) showed that the Moroccan population might be exposed to the risk of consuming food products contaminated with mycotoxins and sometimes with levels above regulatory limits. Peanuts are a widely consumed food item in Morocco, which are highly prone to aflatoxin contamination. Since the discovery of aflatoxins, numerous countries have established regulations to safeguard consumers against the risks posed by mycotoxin contamination of food (van Egmond et al., 2007). However, ensuring the safety of these products requires the active involvement of stakeholders through an integrated strategy from the field until food processing (Torres et al., 2014). Therefore, this study aims to investigate factors that lead to aflatoxins contamination of peanuts and identify differences in respecting good hygiene practices among stakeholders. It also aims to determine how respecting good hygiene practices can contribute to minimizing AFB1 levels at different stages of peanuts manufacturing chain.

2. MATERIAL AND METHODS

2.1 In-site investigations on aflatoxins contamination

The study surveyed 90 stakeholders in the peanut industry, including 30 farmers, 30 shelling stations, and 30 processing factories. The questionnaire assessed their respect to practices for minimizing aflatoxin contamination. The dependent variables were "good hygieneagricultural practices at farms," "good hygiene practices at shelling stations," and "good hygiene practices at processing factories." The independent variables included factors such as agricultural practices, storage conditions, laboratory analysis, sorting methods and knowledge about aflatoxins contamination. A binary coding system was used, where "1" represented "respect" and "0" represented "no respect."

Descriptive statistics were used to summarize the respondents' characteristics, and mean scores were calculated to determine the respect of good hygiene practices, by setting a threshold as follow: If the mean score is greater than or equal to 0.5, the dependent variable was coded as 1 which refers respect of good hygiene practices. If the mean score is less than 0.5, the dependent variable was coded as 0 which refers to no respect of good hygiene practices. A one-way ANOVA was conducted to identify significant differences among the stakeholders. Where significant differences were found, a Tukey post hoc test was performed to identify which specific groups differed from each other. The study tested the hypothesis that there is no significant difference between stakeholders in respecting good hygiene practices towards minimizing contamination in the peanut manufacturing chain.

2.2 Determination of aflatoxin levels with HPCL analysis

After collecting responses to the questionnaires, eight peanut supply chains were selected based on their compliance with good hygiene practices, using a factorial experimental design. Table 1 presents the supply chains used for sampling. Aflatoxin levels in the final peanutbased products were quantified using HPLC with fluorescent detection. The independent variables were the "Farming Stage", "Shelling Stage", and "Processing factory Stage", which were coded as -1 for no respect and 1 for respect. Regression analysis was performed to compare aflatoxin levels and determine which stage had the greatest impact on contamination. The results were used to identify critical points for aflatoxin contamination along the peanut supply chain.

Table 1 Supply chains used for sampling to determine levels of aflatoxins in peanut-based products.

*Note : For each stage a value of -1 is assigned if the answers to the questionnaire by the chosen respondent indicated no respect of good hygiene practices (a value of 0 to 'good hygiene practices'), and a value of 1 is assigned if the answers indicated respect of good hygiene practices (a value of 1 to 'good hygiene practices').

3. RESULTS AND DISCUSSION

3.1 Results of in-site investigations

3.1.1 Stakeholders profiles

The profiles of respondents are presented in table 2. The majority of farmers and shelling stations were based on the Atlantic coast, while processing factories were distributed across different regions. The study found that none of the farmers had implemented standard operating procedures (SOPs), while 36% of shelling stations and 83.3% of processing factories had implemented SOPs. Employee training was also lacking among farmers and shelling stations, with only one farmer and 11 shelling station employees receiving training. Initial observations of this data showed that more attention must be given to farmers and shelling stations, where the lack of SOP guidelines and employee training was mostly observed.

Note: * N : North , * W : West , *S : South, E* : East.

3.1.2 Investigation results at farms

Table 3 presents the results of 12 statements assessing good hygiene-agricultural practices aimed at reducing aflatoxin contamination on peanut farms. The study found that farmers had low practices, with only 4% of farmers implementing crop rotation, 96.6% using noncertified seeds, and 93.3% misusing fertilizers. Additionally, 96.6% of farmers used fungicides, 50% dried pods on tarpaulins, and 66.6% did not control moisture during drying. The overall mean score of 0.28 indicates that farmers had low practices towards minimizing aflatoxins contamination.

*Overall mean score (n=30): indicates the value of the dependent variable "good hygieneagricultural practices at farms".

3.1.3 Investigation results at shelling stations

Table 4 presents the findings of 12 statements assessing good hygiene practices aimed at reducing aflatoxin contamination at peanut shelling stations. The study found that shelling stations had low practices, with only 36.6% using clean transport vehicles, 50% knowing the origin of the pods, and 3.3% using checklists to accept or refuse batches of pods. Additionally, 96.6% of workers soaked peanuts with water, and 93.3% needed periodic cleaning and ventilation. The overall mean score of 0.19 indicates that respondents had low practices towards minimizing aflatoxins contamination.

	Coding system		Responses				
Explanatory variables	Ω		$\overline{0}$		Mean score		
Independent variables							
1 Use of clean transport vehicle,	Not Applicable	Applicable	19	11	0.36		
2 Use of covered and ventilated vehicle of transport,	Not Applicable	Applicable	19	11	0.36		
3 Known origin of arrived pods	Not Applicable	Applicable	15	15	0.5		
4 Use of checklist to accept or refuse a batch of pods at reception	Not Applicable	Applicable	29		0.03		
5 Check after receipt to remove damaged or moldy pods	Not Applicable	Applicable	29		0.03		

Table 4 Results of the questionnaire on good hygiene practices at shelling stations

* Overall mean score (n=30) : indicates the value of the dependent variable "good hygiene practices at shelling stations".

3.1.4 Investigation results at processing factories

Table 5 presents the findings of 12 statements assessing good hygiene practices aimed at reducing aflatoxin contamination at peanut processing factories. The study found that factories had high practices, with 83.3% controlling transport vehicles, 80% knowing the origin of peanuts batches, and 83.3% performing checklists and laboratory analysis. Additionally, 83.3% of factories controlled moisture, had good ventilation, and used pest control programs. The overall mean score of 0.78 indicates that respondents had high practices towards minimizing aflatoxins contamination.

	Coding system	Responses					
Explanatory variables	$\overline{0}$		θ		Mean score		
Independent variables							
1 Control of transport vehicle	Not Applicable	Applicable	5	25	0.83		
2 Known origin of arrived peanuts batch	Not Applicable	Applicable	6	24	0.8		
3 Use of checklist to accept or refuse a batch of peanuts at reception,	Not Applicable	Applicable	5	25	0.83		
4 Check after receipt to remove damaged or moldy kernels	Not Applicable	Applicable	5	25	0.83		
5 Determination of aflatoxins contamination level with laboratory analysis	Not Applicable	Applicable	5	25	0.83		
6 Add of a sorting step in the manufacturing process	Not Applicable	Applicable	20	10	0.33		
7 Control of temperature and	Not Applicable	Applicable	5	25	0.83		

Table 5 Results of the questionnaire on good hygiene practices at processing factories

* Overall mean score $(n=30)$ – indicates the value of the dependent variable "good hygiene" practices at processing factories".

3.1.5 Differences in good hygiene practices amongst stakeholders:

In this part, mean scores of the dependent variable "good hygiene practices" were compared amongst the different stakeholders. The comparison of the three common independent variables "knowledge about aflatoxins", "moisture control" and "sorting", directly related to aflatoxin contamination was also carried out.

Table 6 The study compared the mean scores of good hygiene practices among farmers, shelling stations, and processing factories. The results showed that processing factories had significantly higher mean scores than farms and shelling stations for knowledge about aflatoxins (0.83 vs. 0.03 and 0.06, $p < 0.05$), moisture control (0.83 vs. 0 and 0.06, $p < 0.05$), and overall good hygiene practices (0.78 vs. 0.28 and 0.19, $p < 0.05$). For sorting, farms had higher mean scores than shelling stations (0.33 vs. 0.03, $p < 0.05$), but not significantly different from processing factories (0.33). Many studies showed the importance of sorting to minimize aflatoxins contamination. (Galvez et al., 2003) showed that initially, the raw peanuts had high aflatoxin contents (300 ppb), but after manual sorting, the peanuts were found to be aflatoxin-free. Another study by (Liu et al., 2017), showed that hand sorting resulted in a significant decrease of up to 42.9% of aflatoxins contamination.

In summary, results showed that more attention must be given to upstream stages, especially in terms of knowledge. Therefore, continuous training programs are necessary to improve stakeholders' awareness of aflatoxins contamination.

	Mean scores				
Variables	Farms	Shelling	Processing	F -Value	P - Value
		stations	factories		
Good Practices	0.28 _b	0.19 _b	0.78a	42,61	$0,000*$
Knowledge	0,03 b	0,06 b	0,83a	76,37	$0,000*$
Moisture	0 _b	0,06 b	0,83a	92,77	$0,000*$
control					
Sorting	0,33a	0,03 b	0,33a	5,48	$0,006*$

Table 6 Results of ANOVA and Tukey's honestly significant difference test.

Note: *The mean significance difference at the 5% level of significance. Means followed by the different small capital are significantly different (Tukey's test, $p < 0.05$). a,b: Indicates differences among groups.

3.2 Levels of aflatoxins in peanut-based products samples:

The study used regression analysis to determine the impact of each stage of the peanut manufacturing chain on aflatoxin contamination levels. **Table 7** shows the results of aflatoxin levels in each supply chain with the estimates parameters and statistical significance levels of the regression analysis. Results of aflatoxin levels show that only supply chain 7 and 8 respected Moroccan regulation for peanut based products, which requires a maximum limit in AFB1 of 2 μg/Kg.

From Table 7, the estimate equation **(1)** model is given below:

This model was significant ($p < 0.05$) and explained 99.56% of the variation in aflatoxin levels. The estimated coefficient for the shelling station (-14.914) represents the largest negative coefficient, meaning the shelling stage has the greatest individual effect on reducing aflatoxins. This could be explained by the traditional method used by most shelling stations, soaking pods with water without proper drying. (Waliyar et al., 2015) emphasized the importance of proper drying and storage practices to prevent fungal growth and reduce aflatoxin contamination in groundnut after harvesting.

The estimated coefficient for the farm is also important (-9,436). (Parimi et al., 2018) found good agricultural practices reduced aflatoxin contamination in groundnut by 50%. The interaction between the farming stage and shelling stage has the biggest coefficient (8,169), meaning it has the greatest effect on reducing aflatoxins.

The estimated coefficient for the processing factory (-2.269) presents the smallest negative coefficient. The challenge is that even if aflatoxin reduction recommendations are implemented at the processing factory, they may not be effective if not applied at upstream stages. This is because the peanuts arriving at the factory are already contaminated, making it difficult to reduce high aflatoxin levels at the end of the manufacturing chain.

Results are consistent with previous research showing the importance of respecting hygiene practices at upstream stages in reducing aflatoxin contamination in peanuts. (Xu et al., 2021) showed proper management practices throughout the crop production cycle can significantly reduce aflatoxin contamination.

Supply chain	Aflatoxin B1 levels $(\mu g/kg)$				Estimated	$P -$
	Assay	Assay 2	Mean	Variables	coefficients	values
Supply chain	51.2	55.8	53.5	Constant	17,731	0,000
Supply chain	16	18.2	17.1	Farm	$-9,436$	0,000
Supply chain	5.8	6.8	6.3	Shelling station	$-14,914$	0,000
Supply chain 4	3.8	2.4	3.1	Factory	$-2,269$	0,001
Supply chain	47.69	46.31	47	Farm*shelling station	8,169	0,000

Table 7 Aflatoxin B1 levels in eight supply chains with regression analysis results

4. CONCLUSION

The stakeholders involved in peanut production often lack awareness of the negative effects of aflatoxin contamination, which can lead to serious health and economic consequences. Furthermore, the answers to questionnaires and the analysis of aflatoxin contamination levels in this study highlight the need for more attention to be given to the upstream stages of peanut production in terms of hygiene practices towards minimizing aflatoxins contamination and also in terms of knowledge.

Therefore, continuous training programs are necessary to improve stakeholders' awareness of food safety. Adhering to good hygiene practices, along with the use of simple tools to control factors that contribute to aflatoxin contamination, can help stakeholders decrease aflatoxin levels in peanuts and peanut-based product.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix: Example Chromatogram Results of supply chain 5.

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