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Occurrence of aflatoxins in rice (*Oryza sativa* L.) produced in Colombia

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Abstract

In order to investigate the presence of aflatoxins (AFs), a total of 120 samples of paddy rice cultivated by the 'irrigation' and 'rainfed' systems in the main rice-growing regions of Colombia were taken during 2017 and 2018. The Association of Official Analytical Chemists accredited standard method, based on high-performance liquid chromatography, was used to detect and quantify AFs (AFB₁, AFB₂, AFG₁, and AFG₂). The results showed that, in 2017, the occurrence of AFs in paddy rice from rainfed systems was 43% (range 2.1 to 119.5 µg/kg), while in the irrigation system, it was 16.7% (range 0.1 to 1.83 µg/kg). By 2018, the occurrence of AFs had decreased to 31% for the rainfed system and 2% for the irrigation system. AFs contamination levels were higher in the rainfed system compared to the irrigated system ($p<0.05$). No AFG₁ or AFG₂ was detected, irrespective of the cultivation system used. AFB₁ was the most prevalent AF in paddy rice, with a global occurrence of 22.9% in 2017 and 8.62% in 2018. At the national level, the prevalence of AFs in milled rice was 50%. Of the positive samples, 62.5% exceeded the maximum permitted value, with concentrations ranging from 10.3 to 93.9 µg/kg. These findings underscore the critical importance of mycotoxins in the context of food safety, emphasizing the necessity for effective control measures within the rice industry. This study is the first detailed report on the incidence of AFs in paddy rice cultivated in Colombia.

Introduction

Rice (*Oryza sativa* L.) is a staple food crop that accounts for approximately one-quarter of the world's per capita calorie intake (Savi *et al.*, 2018). As one of the most important cereals globally, it ranks third in terms of production and planting area (Chica *et al.*, 2016). In Colombia, the growing demand has positioned the country as the third-largest rice-producing country in Latin America and the Caribbean, with a total production of 2.5 million tons per year (Loaiza *et al.*, 2024). It is the third most extensively cultivated agricultural product in Colombia, after coffee and maize, and accounts for 13% of the harvested area and 30% of transient crops (Espin *et al.*, 2005; Agronet, 2014).

Like many cereals, rice is prone to contamination by filamentous fungi that can generate secondary metabolites during harvest and storage (Alshannaq and Yu, 2017). The main genera responsible for this contamination are *Aspergillus*, *Penicillium*, and *Fusarium* (Khodaei *et al.*, 2021; Santos *et al.*, 2022), whose presence can cause considerable economic losses in the food supply chain. These fungi can synthesize toxins that are associated with various diseases in humans and animals, such as immune function inhibition and carcinogenic effects (Ali, 2019; Arenas-Huertero *et al.*, 2019).

The most harmful category of these toxins in rice grains is the aflatoxins (AFs), produced by *Aspergillus* species, which present high toxicity with acute and chronic effects. AFs are the products of a multifaceted biosynthetic pathway encompassing at least 27 enzymatic reactions (Caceres *et al.*, 2020). Currently, over 20 types of AFs have been discovered, but the most common and important ones are AFs B₁, B₂, G₁, and G₂, with AFB₁ being the most toxic and prevalent (Naeem *et al.*, 2024). These toxins are known to be teratogenic, mutagenic, and they are responsible for toxic hepatitis, hemorrhages, oedemas, immunosuppression, allergic reactions, reproductive deficiencies, fetal alterations, and hepatic carcinoma (Ding *et al.*, 2015; Benkerroum, 2020). Their global impact is a cause for concern, as they can even trigger tumor formation and lead to death (Zhao *et al.*, 2019).

Rice crop demands significant amounts of water, combined with warm temperatures and suboptimal processing and storage conditions, making it especially susceptible to colonization by aflatoxigenic fungi (Kumar *et al.*, 2016). Despite conventional treatments such as washing, peeling, drying, and other unit operations, these methods are not completely effective in eliminating AFs (Shen and Singh, 2021).

The impact of AFs on rice is a critical issue in global food security. Various studies have documented the presence of AFs in rice across different continents and countries. The incidence of AFs in rice is a widespread concern in Asia, particularly in high rice-producing countries such as China, Pakistan, Malaysia, and Vietnam. A recent article reported that 60% of rice samples in southern Vietnam were

contaminated with AFs (Phan *et al.*, 2023). Some studies show that semi-polished rice in Pakistan has the highest levels of AFs, while 92% of red rice samples in Malaysia exceed permitted levels (Samsudin and Abdullah, 2013; Akbar *et al.*, 2024). In China, although contamination rates are high, most samples are below the legal limit (Sun *et al.*, 2017). Similar problems are found in Africa and America, with significant AF contamination in Nigeria, Brazil, and Mexico, posing serious risks to public health and food safety in these regions (Suárez-Bonnet *et al.*, 2013; Katsurayama *et al.*, 2018; Ekpakpale *et al.*, 2021). The results of these studies vary according to geographical areas, climatic zones, and agricultural practices, highlighting the importance of addressing fungal and mycotoxin contamination as a critical global issue.

Despite the importance of the presence of AFs in a product as widely consumed in Colombia, studies conducted to determine their incidence are limited. To date, three studies analyzing contamination of rice with AFs have been published. Some years ago, Díaz *et al.* (2001) examined the incidence of AFs in different types of food products purchased in commercial establishments, already raising the alarm about the risks of AFB₁ contamination, mainly in corn and corn products. More recently, the incidence of mycotoxins in the main staple foods (corn, rice, and cassava) of three indigenous groups in the Colombian Amazon was evaluated (Díaz *et al.*, 2015). The latest study was conducted to assess the risk of AFs due to arepa, bread, and rice consumption among the adult population of the Department of Caldas, Colombia (Martínez *et al.*, 2019). The presence of AFs in the rice analyzed was reported in all studies, highlighting their importance to public health. However, the scope was regional in all three cases, and only milled rice was analyzed. It is therefore necessary that studies are conducted on a national scale, taking into account growing conditions to marketing. To address this information gap, the results of the first nationwide study conducted in Colombia are presented here. The objective of this study was to examine the incidence of AFs in paddy rice by comparing cultivation systems and analyzing milled rice marketed in all regions of the country.

Materials and Methods

Sampling

Paddy rice

The study was conducted over two sampling periods: the first in 2017, which included the rice cultivation systems (irrigated and rainfed), collecting 62 samples. The second sampling was carried out in 2018, covering the same systems and obtaining a total of 58 samples. Figure 1 shows the main rice-growing areas of Colombia, highlighting the departments (political divisions) where rice samples were taken according to the cultivation systems. Likewise, the cities where the mills were located, from which milled rice samples were collected, are also highlighted.

A stratified sampling with proportional allocation was applied, classifying the geographical areas into the North zone, Centre zone, Eastern Plains zone, and Northeastern zone, also considering the different cultivation systems. The Northern zone included the departments of Córdoba, Cesar, Bolívar, and Sucre; the Central zone encompassed Huila, Tolima, Valle, and Caquetá; the Eastern Plains zone comprised the departments of Meta and Casanare, while the Northeastern zone focused on the department of Norte de Santander (Table 1).

Milled rice

Additionally, in 2018, a sampling of milled rice was conducted, following the criterion of the number of mills in the different rice-growing areas. Four samples were taken in each of these areas, totaling 16 samples (Figure 1).

Quantification of aflatoxins

The analytical technique employed was the Colombian Technical Standard NTC 1232 (1996-10-23), which was standardized at the Toxicology Laboratory of the Faculty of Veterinary Medicine of the National University of Colombia, based on methods recognized by the AOAC for food analysis.

Twenty-five grams of milled rice were mixed with 100 ml of extraction solvent [CH₃CN (HPLC, Merck, Darmstadt, Germany): water (HPLC, Merck)] (84 + 16), filtered through qualitative filter paper (Whatman No. 4, Merck), and approximately 5 ml of the filtrate was collected in a test tube. A Micotox® M2002 clean-up column (Micotox Ltd., Bogota, Colombia), was then used to obtain approximately 0.5 ml of purified extract, which was quantitatively transferred to a 2 ml conical-bottom vial. After evaporating to dryness under a nitrogen stream, the dry residue was redissolved with 200 µl of acetonitrile (HPLC, Merck). The sample was filtered through a 0.45 µm membrane (Millipore HPLP 1300, Merck) and injected into the liquid chromatograph (Shimadzu Prominence, Canby, OR, USA) with approximate retention times of 7.88 min, 9.47 min, 10.13 min, and 12.49 min, respectively. Total AFs correspond to the sum of the concentrations of AFB₁, AFB₂, AFG₁, and AFG₂.

Statistical analysis

A two-way ANOVA was performed to assess the effects of year and cultivation system. Due to the strong skewness data were log10-transformed prior to inference testing. Assumptions of normality and homoscedasticity were evaluated using Shapiro-Wilk and Levene's test, respectively. Additionally, Mann-Whitney U tests were conducted to compare cultivation systems within each year as a complementary non-parametric analysis. Statistical significance was set at $p < 0.05$. Partial eta-squared ($\eta^2 p$) was calculated as a measure of effect size. All analyses were carried out in Python (pandas, scipy, statsmodels and matplotlib libraries).

Results and Discussion

Occurrence of aflatoxins (AFB₁, AFB₂, AFG₁, and AFG₂) in cultivated rice

Table 2 shows the results corresponding to the incidence of AFs (AFB₁, AFB₂, AFG₁, and AFG₂) in rice cultivated during the years 2017 and 2018 in Colombia.

In the samples cultivated through the irrigation system during the year 2017, the presence of AFB₁ and AFB₂ was detected in two of the four rice-growing regions of the country: the Northern and Eastern Plains regions. In samples from the rainfed system, it was observed that two of the three regions (Northern and Central) showed positive results for the presence of both AFB₁ and AFB₂, with particularly high AFB₁ incidence rates of 57% and 67%, respectively (Table 2).

Overall, for the irrigation system in Colombia, the occurrence of AFs during 2017 was 16.7%, with levels ranging from 0.1 µg/kg to 1.83 µg/kg. Of the AFs -positive samples, 57% exceeded the maximum levels permitted by the Resolution of the Ministry of Health and Social Protection of Colombia No. 2671/2014 and Regulation (EU) No. 165/2010 (10 µg/kg). In comparison, the occurrence of AFs in rice cultivated using the rainfed system was higher, registering a 43% (range 2.1 to 119.5 µg/kg), with 50% of positive samples exceeding the permitted maximum limit.

In the rainfed cultivation system, during the year 2018, the data were similar to those obtained in 2017, with the Northern and Central regions showing positive results for AFB₁ and AFB₂. The occurrences in the Central region remained consistent for both mycotoxins, while in the Northern region they were slightly lower (Table 2). Conversely, rice from the irrigation system showed a reduction in AFs occurrence, with only AFB₁ detected in the Northern region, exhibiting a lower occurrence compared to the rainfed system. In summary, in 2018, a higher occurrence of AFs was also observed in the rainfed system than in the irrigation system. The occurrence in rice cultivated using the irrigation system was 2.2%, whereas it was 30.8% for the rainfed system (range 1.6 to 22.4 µg/kg).

A priori, when these results are compared with studies conducted in Asian or African countries, the incidences reported here may seem low; since high occurrences of AFs are normally observed in paddy rice. For example, incidences of 60% in Vietnam, 67.8% in India (with 1200 samples), and even 100% in Iraq have been reported (Reddy *et al.*, 2009; Alhendi *et al.*, 2020; Phan *et al.*, 2023). However, in South and Central American countries, the occurrence of AFs in paddy rice is usually lower (Gruber-Dorninger *et al.*, 2019). In this regard, low rates have been recorded in Brazil, incidences of 6.9% reported in Ecuador, 14.3% in Guyana, and 27.2% in Panama (Ortiz *et al.*, 2013; Katsurayama *et al.*, 2018; Morrison *et al.*, 2019; Troestch *et al.*, 2022). In this context, the results for the irrigation system are consistent with those reported in the extant literature, whereas the incidences recorded for the rainfed cultivation system are comparatively high compared with other countries in the region.

Of the four AFs analyzed, AFB₁ was the most prevalent, which is consistent with what has been reported in different studies worldwide (Ali, 2019; Gruber-Dorninger *et al.*, 2019; Naeem *et al.*, 2024). The overall prevalence of AFB₁ was 22.9% in 2017 and 8.6% in 2018. While the results are concerning, they are consistent with those reported in some South American countries (Ortiz *et al.*, 2013; Gruber-Dorninger *et al.*, 2019; Naeem *et al.*, 2024). AFB₁ contamination levels were higher in the rainfed system compared to the irrigated system. This may be due to specific cultivation system conditions, which, when combined with climatic conditions, favour mycotoxin production. A high incidence of AFB₁ was observed in the Northern region, which had an average air temperature between 27 and 34°C, recorded precipitation between 500 and 4000 mm, with a defined gradient towards the south, and a relative humidity around 70%. In line with the findings of Mousa *et al.* (2016), the environmental conditions in the Northern region of Colombia were conducive to the presence of AFs in rice grain.

Finally, in both seasons and cultivation systems, AFG₁ and AFG₂ were not detected, which is consistent with previous studies (Mazaheri, 2009; Reiter *et al.*, 2010, Korley Kortei *et al.*, 2019).

Occurrence of total aflatoxins in paddy rice

In 2017, samples from the irrigation system showed the presence of AFs in the Northern and Eastern Plains regions, with a high occurrence in the Northern region (Table 3). This finding is significant because 60% of the positive samples exceeded the permitted threshold for AFs according to the regulations. We highlight the detection of levels up to 1834.1 µg/Kg in one of the samples. By 2018, the occurrence had decreased significantly.

In the areas cultivated by the rainfed system, discrepant results were observed. While samples from the Eastern Plains were free of AFs, those from the Central region showed a high occurrence. This tendency was observed in both seasons (Table 3). The above suggests that there are differences, whether at the climatological level or in the agricultural practices, between both regions, which would need to be established in the future. The most notable finding was evidenced by 2018 in the Central region, where the occurrence reached 67%, and 100% of the positive samples exceeded the maximum limit established by the regulations. In 2017, a significant occurrence of 57% was recorded in the Northern region. In this region, a high percentage of positive samples, for both cultivation systems, exceeded the maximum limit established by the regulations (Table 3). As observed for the irrigation system, the occurrence of AFs also decreased in 2018.

The Northern and Central regions are considered ideal areas for rice cultivation due to the climatic conditions, soil characteristics, and flat terrain with slopes less than 3% (Martínez *et al.*, 2016). Since the production of mycotoxins largely depends on the climate for their development, climate change also drives their production. The high temperatures in these two regions can increase the air's capacity to absorb water, generating greater water demand in plants due to decreased soil moisture. This could cause water stress during periods of drought, which could, in turn, give rise to the presence of fungi within the structure of rice plants (Hassan *et al.*, 2023). When considered in conjunction with factors associated

with climate change, this has the potential to encourage the development of mycotoxins (Medina *et al.*, 2017).

The rainfed cultivation system exhibited higher AFs incidence than irrigated system in both years evaluated (Figure 2). In 2017, mean concentrations were 54.4 µg/kg in irrigated rice (n=48) and 20.3 µg/kg in rainfed rice (n=14). In 2018, levels were much lower, with mean values of 0.02 µg/kg (n=45) and 3.55 µg/kg (n=13), respectively. Despite the high frequency of non-detects, the non-parametric Mann-Whitney U test revealed statistically significant differences between cultivation systems in both years (2017: p=0.035; 2018: p=0.001). These findings suggest that rainfed conditions pose a higher risk of AFs presence in paddy rice.

Occurrence of total aflatoxins in milled rice

Simultaneously, milled rice samples were collected from establishments throughout the year 2018, corresponding to rice cultivated in the year 2017. AF contamination in milled rice was observed in all rice-growing regions of the country. The Eastern Plains region had the highest occurrence, at 75%, while the Northern region had the lowest, at 25% (Table 4). The Central and North-eastern regions both exhibited a 50% occurrence of AFs. It is a cause for concern that all positive samples in the Central and Northeastern regions exceeded the maximum limits established by the current legislation. With regard to the findings at the national level, the prevalence of AFs in milled rice marketed in the country, was 50%. Of the positive samples, 62.5% exceeded the maximum value permitted by regulation, with values ranging from 10.3 to 93.9 µg/kg. In contrast to the occurrences observed for paddy rice, higher values were obtained from the analysis of milled rice, which is remarkable.

In the Colombian context, Díaz *et al.* (2001) evaluated 40 samples of rice and rice products and found a 10% incidence of AFs. In another study, Díaz *et al.* (2015) reported a 12.5% incidence for 24 samples of milled rice consumed by three indigenous people of the Colombian Amazon. These incidence rates are lower than those reported by us. In contrast, a recent study conducted in the department of Caldas (Colombian coffee axis) found that 75.6% of 90 milled rice samples contained AFs, and 13.5% of the positive samples exceeded the regulatory limit (Martínez-Miranda *et al.*, 2019). These results are more similar to ours. However, our results show a high percentage of samples that exceeded the maximum value permitted by regulation (62.5%), which is different from what was reported by Martínez-Miranda *et al.* (2019).

In terms of incidence, the results obtained are consistent with those reported in various studies around the world (Table 5). In São Paulo markets, incidences of 20% were found for red rice and 66.6% for brown rice (Katsurayama *et al.*, 2018). In Thailand, the prevalence, depending on the region, ranged from 13.3% to 66.6% for colored rice (Panraphee *et al.*, 2016). In China, an incidence of 63.5% was reported for a total of 370 samples (Lai *et al.*, 2015). Recent studies (2021-2025) in countries such as Qatar, Pakistan, and Nigeria also reported high prevalence rates for AFs in milled rice (Alkuwari *et al.*, 2022; Naeem *et al.*, 2024; Ekpakpale *et al.*, 2021) (Table 5). However, with the exception of Nigeria (41.9%), the percentage of samples exceeding the regulatory limits was low in all cases. This observation is consistent with the statistics presented by Gruber-Dorninger *et al.* (2019), in a study analyzing regional trends of mycotoxin occurrence. The percentage of samples exceeding the highest maximum levels is relatively low in most regions of the world, except in regions such as Sub-Saharan Africa or India. Consequently, milled rice marketed in Colombia in 2018 poses a potential health risk due to the high incidence of AFs, as well as the high rate of samples that exceed regulatory limits. The findings presented here will be invaluable in creating awareness among local farmers and regulatory agencies about the risks and health hazards associated with AFs.

Conclusions

A high occurrence of AFs was recorded in rice cultivated in the country during 2017, although this occurrence decreased in 2018. It is hypothesized that this change was associated with climatic variations experienced by the country from one year to the next. The most critical area in terms of AFs presence was the Northern region, affecting both cultivation systems.

In both years, the rainfed system demonstrated a higher occurrence of AFs in comparison to the irrigation system. The growing conditions and agricultural practices associated with the rainfed system may be contributing to this trend in AF contamination.

A high occurrence of AFs was observed, as well as a high rate of samples that exceeded regulatory limits in milled rice marketed in Colombia in 2018. These findings emphasize the presence and relevance of mycotoxins in food safety discussions and the need for rigorous control for their mitigation in the rice value chain.

This study constitutes the first detailed report on the occurrence of AFs in rice cultivated in Colombia, providing an important basis for future research and mitigation strategies.

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Table 1. Number of rice samples in cultivation by rice-growing zone and cultivation system.

Rice-growing zone	Department	System			
		Irrigated		Rainfed	
		2017	2018	2017	2018
North	Córdoba	2	2	2	2
	Cesar	4	4	0	0
	Guajira	2	2	0	0
	Bolívar	0	0	2	1
	Sucre	0	0	3	3
Centre	Huila	6	6	0	0
	Tolima	16	16	0	0
	Valle	3	2	0	0
	Caquetá	0	0	3	3
Eastern Plains	Casanare	4	4	2	2
	Meta	4	3	2	2
North-Eastern	Norte de Santander	7	6	N.A.	N.A.
Total		48	45	14	13

Table 2. Occurrence of aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂) in cultivated rice samples from Colombia.

Rice-growing zone	System	AF	Occurrence (%)		Mean (µg/kg)*		SD	
			2017	2018	2017	2018	2017	2018
North	Irrigation	AFB ₁	63	13	297.50	0.9**	608.50	-
		AFB ₂	25	0	92.59	0	47.52	0
		AFG ₁	0	0	0	0	0	0
		AFG ₂	0	0	0	0	0	0
	Rainfed	AFB ₁	57	33	46.44	1.64	45.98	0.21
		AFB ₂	43	33	2.26	0.14	3.71	0.07
		AFG ₁	0	0	0	0	0	0
		AFG ₂	0	0	0	0	0	0
Centre	Irrigation	AFB ₁	4	0	0	0	0	0
		AFB ₂	0	0	0	0	0	0
		AFG ₁	0	0	0	0	0	0
		AFG ₂	0	0	0	0	0	0
	Rainfed	AFB ₁	67	67	3.75	21.17	3.25	1.55
		AFB ₂	33	33	0.2 **	0.1**	-	-
		AFG ₁	0	0	0	0	0	0
		AFG ₂	0	0	0	0	0	0
Eastern Plains	Irrigation	AFB ₁	25	0	2.53	0	22.56	0
		AFB ₂	13	0	1.40**	0	-	0
		AFG ₁	0	0	0	0	0	0
		AFG ₂	0	0	0	0	0	0
	Rainfed	AFB ₁	0	0	0	0	0	0
		AFB ₂	0	0	0	0	0	0
		AFG ₁	0	0	0	0	0	0
		AFG ₂	0	0	0	0	0	0
North-Eastern	Irrigation	AFB ₁	0	0	0	0	0	0
		AFB ₂	0	0	0	0	0	0
		AFG ₁	0	0	0	0	0	0
		AFG ₂	0	0	0	0	0	0

AF, aflatoxins; SD, standard deviation. *Mean of positive samples for aflatoxins. **Data corresponding to a single sample

Table 3. Occurrence of aflatoxins in paddy rice in Colombia.

Rice-growing Zone	System	Year	Incidence (%)	>LMP (%)	Mean (µg/kg) *	SD	Maximum value (µg/kg)
North	Irrigation	2017	63	60	322.2	657.2	1834.1
		2018	13	0	0.9	-	-
	Rainfed	2017	57	75	48.22	49.44	119.5
		2018	33	0	1.79	0.28	2
Centre	Irrigation	2017	0	0	0	0	0
		2018	0	0	0	0	0
	Rainfed	2017	67	0	3.81	3.39	6.9
		2018	67	100	21.22	1.63	22.4
Eastern Plains	Irrigation	2017	25	50	2.59	23.62	33.6
		2018	0	0	0	0	0
	Rainfed	2017	0	0	0	0	0
		2018	0	0	0	0	0
North-Eastern	Irrigation	2017	0	0	0	0	0
		2018	0	0	0	0	0

LMP, percentage of positive samples exceeding the maximum limit permitted by current regulations; SD, standard deviation.

*Mean of positive samples for mycotoxins. **Data corresponding to a single sample

Table 4. Occurrence of aflatoxins in rice milled in Colombia.

Zone	Company name	AFs incidence (%)	>LMP * (%)	Mean (µg/kg)	SD	Maximum value (µg/kg)
North	1	25	0	0.82	1.65	3.3
	2					
	3					
	4					
Centre	5	50	100	7.77	9.95	20.8
	6					
	7					
	8					
Eastern Plains	9	75	33	35.07	51.05	93.9
	10					
	11					
	12					
North-Eastern	13	50	100	8.85	13.92	30.4
	14					
	15					
	16					

AFs, aflatoxins; LMP, percentage of positive samples exceeding the maximum limit permitted by current regulations; SD, standard deviation. *Resolution No. 2671 of 2014 by the Ministry of Health and Social Protection of Colombia and Regulation (EU) No. 165/2010.

Table 5. Worldwide prevalence of aflatoxins in milled rice.

Country	Occurrence	Average level (µg/kg)	Reference
Pakistan	76.7%	4.38	Naeem <i>et al.</i> , 2024
China	63.5%	0.85	Lai <i>et al.</i> , 2015
Qatar	71.0%	2.23±1.07	Alkuwari <i>et al.</i> , 2022
Thailand	35.0%	0-26.6 (range)	Panrapee <i>et al.</i> , 2016
Nigeria	75.0%	6.5±1.6	Ekpakpale <i>et al.</i> , 2021
Colombia	75.6%	0.61	Martínez <i>et al.</i> , 2019

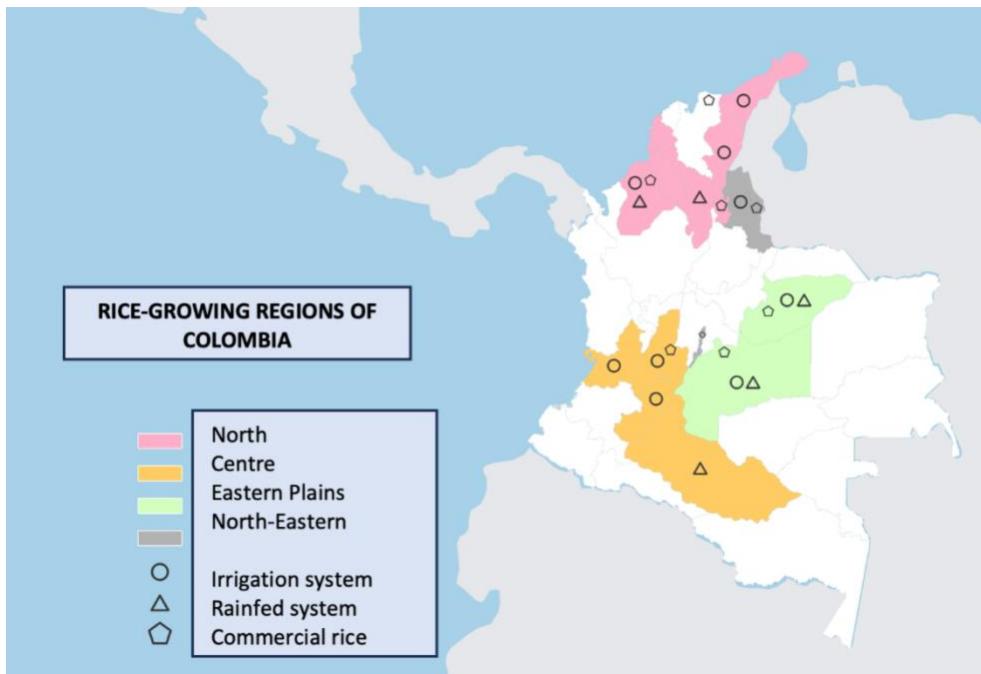


Figure 1. Rice-growing regions of Colombia, indicating the sampling sites for cultivated rice and milled rice.

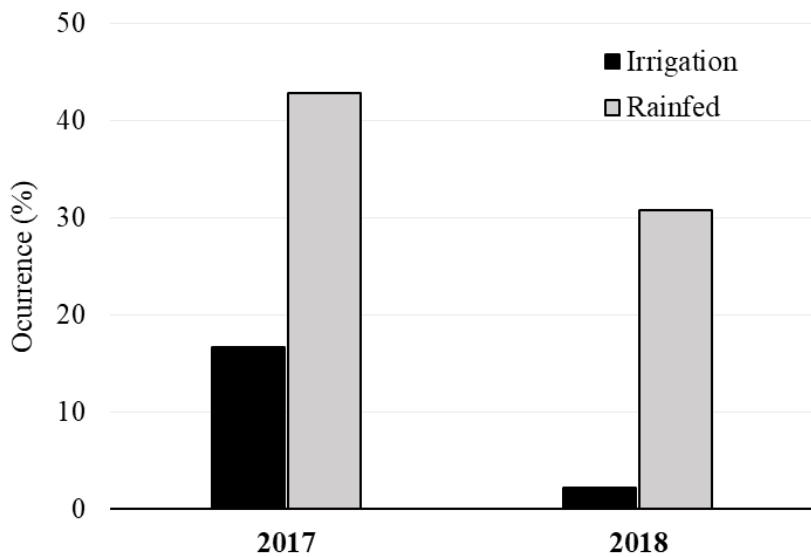


Figure 2. Comparison of aflatoxin occurrence in paddy rice by cultivation systems.