FUMONISIN LEVELS AND *FUSARIUM* SPECIES IN MAIZE IN ISABELA PROVINCE, PHILIPPINES

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ABSTRACT

Fumonisins are toxic contaminants associated with various human and animal diseases. The extensive and intensive cultivation of maize in Isabela Province, Philippines, may increase the risk of fumonisin contamination in maize kernels. The study aimed determine the occurrence of fumonisin and identify toxigenic *Fusarium* species in maize varieties. Maize samples were collected from six municipalities during the 2019 dry season harvest. Fumonisin levels were quantified using enzyme-linked immunosorbent assays (ELISA), while *Fusarium* species identified were through cultural and molecular methods. Fumonisin was detected in 27.72% of the hybrid (*Bt*) maize samples; 72.27% samples were less than the limit of detection (LOD), 12.87% with 200-500 µg/kg, 8.91% with 501-1000 µg/kg, 2.97% with 1000-1500 µg/kg, and 0.99% with 1500-2000 µg/kg. The highest fumonisin concentration, ranging from 2120 to 2410 µg/kg, was detected in 1.98% of samples. All samples were within the acceptable limits based on the Philippine National Standard Code of Practice for cereals. The identified *Fusarium* species consisted of *Fusarium verticillioides (*68%), *Fusarium proliferatum (*28%), *Fusarium oxysporum* (2%), and *Fusarium kyushuense* (2%). *F. kyushuense* is the first report in maize grain in the Philippines. The presence of *F. verticillioides and F. proliferatum* may indicate the occurrence of fumonisin in maize. Continued monitoring of fumonisin levels is recommended to manage fumonisin levels in maize kernels and ensure food safety.

Key words: hybrid, OPV, kernels, fungal spp., ELISA

INTRODUCTION

Fungi are present under certain conditions and contaminate maize grains through the release of mycotoxins, the secondary metabolites that cause serious health hazards to both humans and animals through consumption of contaminated food and feed (Schmale III and Munkvold 2009). Fumonisins are one of the mycotoxins commonly found in maize. It is mainly produced by *Fusarium verticilliodes* and *Fusarium proliferatum* (Cumagun et al. 2009; Yamashita et al. 1995). Fumonisin, particularly fumonisin B_1 and B_2 are the most potent carcinogens with FB_1 mostly observed because of high frequency of occurrence and high toxicity (Li et al. 2024; Oguz 2017). In humans, fumonisins are involved in neural tube defects in babies by reducing the uptake of folates which leads to nerve damage,

spinal bifida, leg paralysis, and stillbirth. Also, it causes carcinogenesis primarily esophageal cancer, and acute mycotoxicosis which are characterized by abdominal pain, diarrhea, and borborygmi. In animals, it causes leukoencephalomalacia in horses, pulmonary oedema in swine, and feed refusal, hepatocellular apoptosis, and renal tubular necrosis in ruminants (Anumudu et al. 2025; Edrington et al. 1995; Imran et al. 2020). In poultry, it increases severity and susceptibility to other microbes that increase significantly mortality in chicken embryos and chicks (Anumudu et al. 2025; Javed et al. 1993). It can cause acute and chronic symptoms in animals infecting the liver and kidney, depending on the strains and species (Kamle et al. 2019; Voss et al. 2007). When metabolized by animals, fumonisin as a substance may still be found in meat, eggs, and milk because of its chemical and thermal stability, posing a risk to human health (Council for Agricultural Science 2003; Gazzotti et al. 2009; Wang et al. 2021). The International Agency for Research on Cancer classified FB_1 in group 2B as a possible carcinogen in humans (IARC 1993; IARC 2002). In the Philippines, clinical studies directly associated with fumonisin as the cause of adverse health effects are limited. Even information on local monitoring of fumonisin levels in maize and maize products is scarce and unavailable (Rustia et al. 2022). Due to a serious threat to humans and animals, the Philippine National Standard/Bureau of Agricultural and Fisheries Product Standards (2014) released the guidelines for the maximum level of fumonisin for raw maize grains, flour, and meal, which are 4,000 µg/kg and 2,000 µg/kg, respectively.

Maize is considered the most widely grown cereal crop in the world. It is used as food, feed for livestock, and raw materials for industries, thereby making it a source of livelihood for many Filipinos (Gerpacio et al. 2004; Reyes et al. 2009). In terms of production, Isabela province in Region II, Philippines, is the number one producer with an area harvested of 59.9% compared to other provinces like Cagayan, Quirino, and Nueva Vizcaya. The maize type produced in the region was composed of 98.6% yellow maize and 1.4% white maize. The type of maize seed planted was about 97.3% hybrid (97.1% is hybrid yellow and 0.2% is hybrid white maize) and 2.7% open-pollinated varieties (OPV), consisting of 2.4% modern OPV and 0.3% native OPV. White maize is a staple food harvested as green maize for human consumption. The yellow maize is planted for feed purposes, and it is used as a main ingredient in the manufacture of feeds for livestock and poultry, thus the existence of feed mills and buying stations in the region (PSA 2021). Post-harvest facilities such as Mindanao Grains presently located in the town of Reina Mercedes considered as the largest post-harvest maize processing facility in Asia, converting maize into grains and supplied to the food-milling and food-processing industries. On the other hand, a breeding station like the Cagayan Valley Research Center (CVRC) is a known institution in growing and developing OPV maize. The Corn World Breeding System Corporation, BMD Farms, located in Aurora, Isabela, Philippines is another breeding company that develops hybrid varieties. There is apparent evidence that maize is cultivated intensively and extensively in the province, in particular agrogeographic conditions of rainfed lowlands, upland plains, and rolling and hilly terrains. These types of cultivation practices may encourage *Fusarium* growth and increase the incidence of fumonisin contamination in maize. Further, this would give maize farmers and workers a high risk of fumonisin exposure, and the consumption of high level fumonisin in the processed products for food and feeds if climatic conditions are met for fungal growth and fumonisin production. It was projected that in changing climate conditions, fumonisin poses a higher risk in the future than aflatoxin in areas where maize is cultivated (Salvacion et al. 2015).

As a major producer of maize in the country, Isabela province is limited in studies of fumonisin and reports of *Fusarium* species. The identified fumonisin level was determined in Cauayan City municipality only in comparison to other places and other countries (Campa et al. 2005). The fumonisin produced by identified *Fusarium* species was quantified *in-vitro* using maize grain culture (Cumagun et al. 2009). The identification of *Fusarium* species was conducted but focused on only one species, and maize specimens were collected from one to two municipalities. Earlier studies focused on the isolation of *Fusarium verticillioides* (Cumagun et al. 2009; Magculia and Cumagun 2011. *Fusarium* species were isolated from different locations in the country, including part of Isabela, but the specific location(s) in Isabela province were not mentioned (Pascua et al. 2016). Records of fumonisin levels

from the other municipalities and/or top-producing maize municipalities, including identification of *Fusarium* species are lacking. The City of Ilagan, Isabela known as the corn capital of the Philippines, due to its large production area of maize, has an information gap on mycotoxin, particularly fumonisin levels. Moreover, the tropical climatic condition in the province and the increasing of heat index makes the plant susceptible to insect attack, in danger to drought stress, and favor the growth of *Fusarium* species.

Thus, the study determined the incidence of fumonisin contamination and *Fusarium* species in maize grains in Isabela province. Specifically, the study sought to determine the level of fumonisin from the six municipalities, representing the different agroecological locations where maize crops are planted in lowland rainfed, upland plains, and rolling to hilly areas. Also, it aimed at determining the fumonisin levels in hybrid (*Bt*) and open-pollinated maize varieties present in the province. Lastly, the study determined the diversity of *Fusarium* species found in maize kernels collected from sampling areas.

MATERIALS AND METHODS

Sampling areas and sample collection. Maize samples were collected from the six municipalities of Isabela (Fig. 1). Ilagan, Cauayan, Echague, and San Mariano were selected from the topmost producing districts, while San Pablo and Aurora were the two low-producing municipalities. The collection of samples was done during the warm season production from the month of March to April 2019. Data on temperature, relative humidity, and rainfall during dry season production in 2019 were obtained from NASA POWER, Data Access Viewer (https://power.larc.nasa.gov/data-access-viewer). The agroecological information, whether the crop was planted in lowland, upland plains or hilly locations, were also recorded.

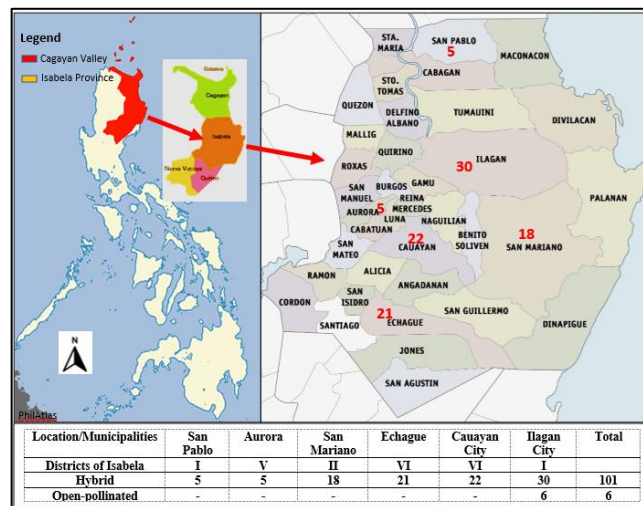| Location/Municipalities	San Pablo	Aurora	San Mariano	Echague	Cauayan City	Ilagan City	Total
Districts of Isabela	I	V	II	VI	VI	I	
Hybrid	5	5	18	21	22	30	101
Open-pollinated	-	-	-	-	-	6	6

Figure 1. Map of Isabela province showing the location of municipalities where the maize grain samples were collected.

The sample size was determined using the formula for determining sample size in a finite population (Cochran 1977). The distribution of samples per area was determined by dividing the total number of maize farmers per area over the population size, then multiplied by 100. This resulted in the distribution of samples for the following: 30 samples from Ilagan, 22 samples from Cauayan, 21 samples from Echague, 18 samples from San Mariano, 5 samples from San Pablo, and 5 samples from Aurora. These

101 samples were all hybrids (*Bt*) and were collected from the six areas, while the open-pollinated varieties (OPV) consisted of six samples from the Ilagan area.

One kilogram of maize kernels for each sample was collected at harvest maturity, R6 stage of the maize crop. Twenty subsamples were randomly collected to have a composite one-kilogram sample from a field or bulk lot of harvested maize. In preparation for mycotoxin analysis, the grain samples were stored immediately below zero temperature, packed in a brown envelope and sealed with plastic, indicating the varieties and location of the sample areas.

One week after the collection, five hundred grams were taken (per sample) and ground into powder using a grinder to increase the uniformity and allow greater precision and accuracy in detecting contaminated particles. After the sample was comminuted, it was thoroughly mixed to homogenize the sample. After homogenization, 100 grams were taken as a representative of one sample. The prepared 107 analytical samples were packed and labeled and later sent for analysis at Romer Labs Singapore Ptre Ltd for the detection of the total fumonisin using the ELISA test.

Mycotoxin analysis. The ELISA (enzyme-linked immunosorbent assay) analysis was performed with the AgraQuant® Assay, Romer Labs test kit. It is a direct competitive enzyme-linked immunosorbent assay (ELISA) that determines the quantitative level of the presence of total fumonisin (B_1, B_2, B_3). The AgraQuant® Total Fumonisin Assay was validated for maize and other commodities. Fumonisin was extracted from 20-gram representative samples by shaking with 100 mL of 70% methanol for one hour at 180 rpm (rotations per minute) on a GFL shaker. The extracted sample and enzyme-conjugated fumonisin were mixed and added to the antibody-coated microwell. Mycotoxins in samples and control standards were allowed to compete with enzyme-conjugated fumonisin for the antibody binding sites. After a washing step, an enzyme substrate was added, and a blue color was developed. The intensity of the color was inversely proportional to the concentration of mycotoxins in the sample or standard. A stop solution was added, which changed the color from blue to yellow. The microwells were measured optically by a microplate reader with an absorbance filter of 450 nm and a differential filter of 630 nm. The optical densities of the samples were compared to the optical densities of the standards, and an interpretative result was determined.

The optical densities (OD) values are expressed as a percentage of the optical densities (OD) of the zero (0) standard, constructing a dose-response curve using the six standards. The results can be determined using the commercial laboratory spreadsheet, Romer Labs spreadsheets.

The limit of detection (LOD) for total fumonisin was < 200 µg/kg (0.2 ppm). Range of quantification up to 5,000 µg/kg (5.0 ppm). ELISA is reliable, reproducible, cost-effective, sensitive, and applicable for quantitative routine determination of fumonisins in maize (Ono et al. 2000; Sokolovic 2022). Although recovery rate is lower than other methods like high performance liquid chromatography (HPLC), there was no significant differences between the two methods, thus, ELISA an excellent analytical method that reliable, rapid, and inexpensive (Beyene et al. 2019; Minic and Zivkovic 2020; Ono et al. 2000; Rodrigues and Naehrer 2012), the reason why it was used in this study.

Isolation and identification of *Fusarium* species. Blotter tests and agar plate methods (Tsedaley 2015) were used in the isolation of species of *Fusarium*. One hundred seeds were randomly selected per sample and surface disinfected in a 5% sodium hypochlorite solution for one minute, rinsed in sterile water three times, and blotted dry before plating. Fifty seeds were placed into culture dishes with moistened filter paper, and 50 seeds were also plated in potato dextrose agar (PDA). Aseptically, ten seeds were equidistantly placed next to one another in petri plates. The plates were incubated under alternate cycles of 12-hour fluorescent (NUV) light and darkness at room temperature of 25–30°C for 5-7 days (Mew and Misra 1994; Sreenu et al. 2019) for fungal sporulation and growth in culture media (Burgess et al. 1994). After five days of incubation, fungal growths were observed on the top surface

of the seeds and fungal growth on the PDA media under the stereo-binocular microscope. Fungal colonies were picked, isolated, and transferred to test tube slants.

For further purification, a single spore technique developed by Hansen and Smith (1932) was performed per isolate. The transferred conidia from the single spore technique into PDA were incubated for seven days for cultural identification. *Fusarium* species were observed for their macro- and micro-characteristics. Colony growth after 3 and after 5 days of incubation, texture in the PDA medium, and pigment were observed as macroscopic features. Furthermore, the agar block technique was used in the characterization of fungal isolates. *In situ* formation of phialides and conidial chains of the growth of *Fusarium* species in the glass slides in the agar block were observed under the microscope. This is an additional step in the identification to view the undisturbed growth and formation of phialides as one of the major characters in identifying *Fusarium* species. Taxonomic keys of Leslie and Summerell (2006) and Burgess et al. (1994) were used as the basis for morphological identification.

The morphologically identified 50 isolates of *Fusarium* were prepared and sent to Macrogen, South Korea, for molecular sequencing of the ITS rRNA region. For genomic DNA extraction, fungal colonies are picked up with a sterilized toothpick, transferred and suspended in 100µl of sterilized saline in PCR tubes, and centrifuged at 10,000 rpm for 10 minutes. After removal of the supernatant, the pellet is suspended in 50µl of InstaGene Matrix (Bio-Rad, USA), then incubated at 56°C for 30 minutes and heated at 100°C for 10 minutes.

In PCR amplification, the primers used were ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) with PCR mixture of 10X Taq PCR Buffer (2 µl), 2.5 mM dNTP mixture (1.6 µl), primer F, R 10 pmole/µl (1 µl), Template 20 ng/µl (1-2 µl), KOMA-Taq 2.5 U/µl (0.2 µl), and distilled water HPLC grade (up to 20 µl). The PCR cycle conditions contained initial denaturation at 95°C for 5 minutes. The cycles of 30 included the denaturation at 95°C for 0.5 minutes, annealing at 55°C for 2 minutes, extension at 68°C for 1.5 minutes, and the final extension at 68°C for 10 minutes. For purification of PCR products, a Montage PCR clean-up kit (Milipore) was used in removing unincorporated PCR primers and dNTPs. The purified PCR was sequenced by using two primers, ITS5 and ITS4. Sequencing was performed using Big Dye terminator cycle sequencing kit v.3.1 (Applied BioSystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system at the Macrogen, Inc.

The final sequences were generated in FASTA format. The analysis of nucleotide sequences was done using NCBI and Mega 11. Both the morphocultural characteristics and molecular identification of each isolate were used for species identification. The DNA sequences of each isolate were submitted to GenBank, and accession numbers were assigned.

Statistical analysis. Analysis of variance (ANOVA) in SPSS v. 11.5 (IBM) was used to determine significant differences in fumonisin levels among geographical locations and between hybrid and OPV maize varieties. Pearson's correlation analysis was used to determine associations between meteorological variables and fumonisin levels. Average monthly temperature, relative humidity, and rainfall from February 2019 to April 2019 were used with average fumonisin data (log transformed) from each sample location. Correlation coefficients were considered significant at $p<.05$.

Limitation of the study. The limitation of the research study was the shortage of funds to include the determination of fumonisin levels during the wet season harvesting that may have seasonal variation since *Fusarium* species thrive favorably in high moisture. Another, the collection of samples during the post-harvest stage particularly storage period may differ to harvest period collection in terms of fumonisin levels and species of *Fusarium* in grains.

RESULTS AND DISCUSSION

Fumonisin level in maize varieties. Percent positive of total fumonisin was detected in 27.72% hybrid samples (Fig. 2). Samples of 72.28% were below the limit of detection (LOD), 12.87% with 200-500 µg/kg, 8.91% with 500-1000 µg/kg, 2.97% with 1000-1500 µg/kg, and 0.99% with 1500-2000 µg/kg. The highest total fumonisin was detected in 1.98% samples with 2410 µg/kg and 2120 µg/kg, respectively. Furthermore, all samples were within the acceptable level of total fumonisin for raw maize grains. The 95% of samples had total fumonisin ranging from < 200 µg/kg to 950 µg/kg and 5.94% ranging from 1090 µg/kg to 2410 µg/kg, respectively.

For the open-pollinated maize samples (Fig. 3), the lowest was OPV 4 with 1360 µg/kg, followed by OPV 3 and OPV 5 with 1540 µg/kg and OPV 1 and OPV 6 with 2910 µg/kg, respectively. The highest level of total fumonisin was OPV 2 with 3130 µg/kg. All samples were detected above the limit of detection, and within the acceptable level for fumonisin based on the Philippines National Standard for raw maize grains.
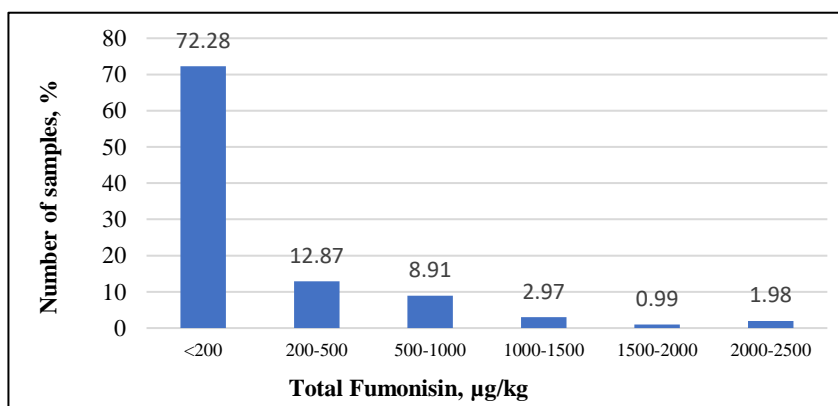

Figure 2. Fumonisin levels of hybrid (*n* =101) maize kernels from six municipalities of Isabela province, the Philippines.
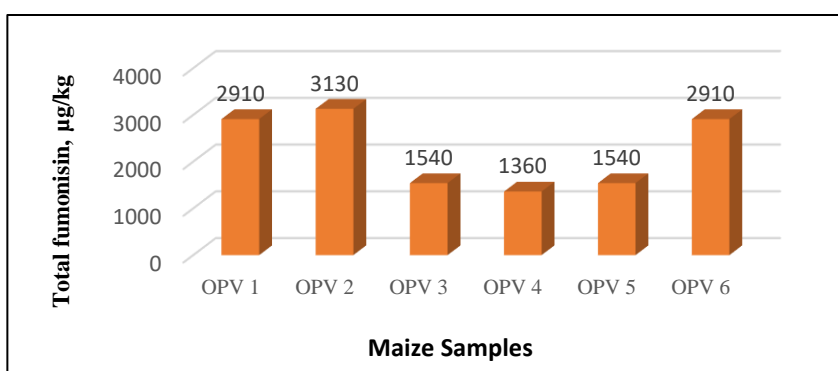

Figure 3. Fumonisin levels of open-pollinated (*n* = 6) maize kernels from Ilagan municipality of Isabela province, the Philippines.

The result of fumonisin in the grains is influenced by *Fusarium* species, climate conditions, insect activity, and water availability before and at harvest. In the present study, the low incidence level of fumonisin in maize grains may be due to low relative humidity during the dry season harvest. The

climatic condition was not suitable for *Fusarium* growth and fumonisin production. *Fusarium verticillioides* and *Fusarium proliferatum* are the primary sources of fumonisin. These *Fusarium* species are field fungi that require high moisture level in the grains for growth. They colonize and produce fumonisin in the field with the maize grains harvested at high moisture content (Milani 2013). *Fusarium verticillioides* grows at 0.87–0.88 water availability with the optimum temperature near 25°C (Garcia et al. 2012; Munkvold 2003). In terms of fumonisin production, 0.97 water availability is optimum and produces high at 0.98 water availability with a temperature of 30°C (Etchevery et al. 2002; Marín et al. 1999a; Marín et al. 1999b). Fumonisin production by the fungi reduces 45–50% at 0.95 (Etchevery et al. 2002) and greatly reduced at 0.92 water availability (Marín et al. 2011).

On the other hand, *F. proliferatum* grows at 4°C in 0.994–0.96 water availability but cannot grow at a higher temperature of 40°C to 45°C regardless of water availability level (Marín et al. 1995). In terms of fumonisin production, the optimum temperature for fumonisin production was between 15°C and 25°C at 0.972 water availability (Marín et al. 1999a; Samapundo et al. 2005). It produces a maximum amount of fumonisins at 0.995 water availability at 25°C to 15°C (Cendoya et al. 2018). The growth and fumonisin production of *F. proliferatum* are greatly affected by temperature. At 15°C, fumonisin produces slowly (Samapundo et al. 2005); however, it produces more after 4 weeks (Marín et al. 1999a). The optimum growth rate of *F. proliferatum* is 30°C, however, it produces a small amount of fumonisin (Samapundo et al. 2005).

The climatic conditions for fumonisin production and *Fusarium* growth are specific and independent. In the present study, temperature was suitable for *Fusarium* growth, but water availability in maize grains is low, resulting in low fumonisin levels in samples. In the study of Cao et al. (2014) high fumonisin was observed above acceptable level after physiological maturity of the kernel due to increasing insect damage and stress of *Fusarium* species due to low water activity. It was also observed that drought conditions during ear development increases fumonisin levels (Miller et al. 1995; Rheeder et al. 2005). In the case of the present study, harvest season is done during the dry period while the planting and reproductive stages take place in the last quarter (November to December) and first quarter (January to February) of the year when rainfall is available preventing drought stress to the plant and predispose fungal activity. In addition, the use of *Bt* hybrid varieties can control fumonisin-producing species and reduce fumonisin levels (Alberts et al. 2016; Li et al. 2024) as determined results in the hybrid (*Bt*) maize samples in this study. The low incidence of fumonisin in the study conformed with Tiongson et al. (2005) survey that very low levels of fumonisin were observed across all the production areas in the Philippines.

On the other hand, the high-level incidence of fumonisin in some of the samples may have influenced the post-harvest activities of the farmers and buyers. Maize grains were left in the open area, and the grains were stored temporarily after shelling without drying. This increased the water availability in grains that may resulted in the growth and fumonisin production of *Fusarium* species. This observation conforms to the study of Samuel et al. (2011), that maize grain harvested in warm, tropical temperatures predisposes to microbial attack, and the fluctuation of temperature and relative humidity favor mold growth. Additionally, the intergranular water concentration between the grain and the environment can support different types and rates of mold growth (FAO 1994). Furthermore, when grains are exposed to air, an exchange of moisture between the maize grains and surroundings occurs until equilibrium is reached. In tropical countries like the Philippines, the equilibrium moisture content is above safe storage moisture levels, most especially when grains are stored in open storage systems (IRRI 2013). This caused the rapid propensity of fungal growth and the possible production of fumonisin in the grains. Moreover, the presence of oxygen in the surroundings accelerates respiration, growth, and colonization in grains. This leads to the risk of mycotoxin contamination in maize at high moisture content in storage (FAO 1994; Tubbs et al. 2016).

Correlation between climatic factors such as temperature, relative humidity, and rainfall to the levels of fumonisin in the present study was non-significant as shown in Table 1. Although temperature and relative humidity were not correlated with the fumonisin levels, conditions before and during harvesting may provide suitable conditions for the *Fusarium* growth but not exacerbate fumonisin production. The average temperature ranged from 24.9 to 28.4°C while for the average relative humidity before and during harvest ranged from 67.6% to 77.6%. The temperature, relative humidity, and rainfall before and during harvest shown in Figure 4.

Table 1. Pearson's correlation coefficients between fumonisin level and weather variables.

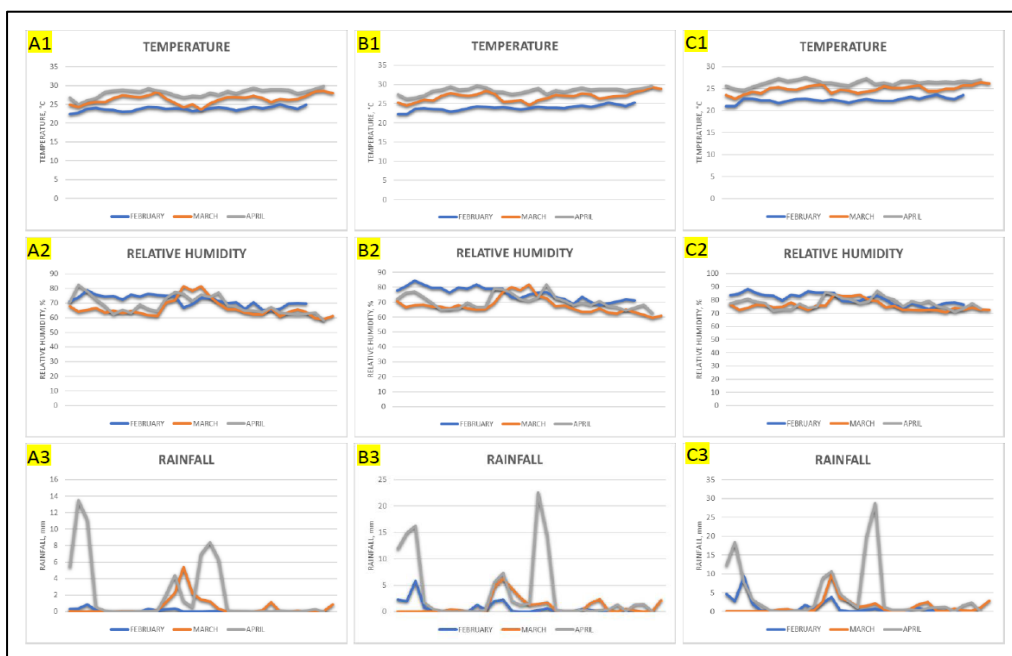| Variable	Months		
	March 2019	**April 2019**	**May 2019**
Temperature	0.16849	0.16033	0.16598
Relative Humidity	-0.15659	-0.17275	-0.17196
Rainfall	-0.13535	-0.10469	-0.12316

Figure 4. Temperature, relative humidity, and rainfall on San Pablo municipality (A1-A3), Aurora, Ilagan, San Mariano, Cauayan municipalities (B1-B3), and Echague municipality (C1-C3).

Fumonisin level by geographic location. The results of total fumonisin by geographic location are found in Table 2. Fumonisin was detected in San Pablo, San Mariano, Echague, Cauayan, and Ilagan but not detected in Aurora above the limit of detection (LOD) of 199.9 µg/kg. The lowest average fumonisin level was Aurora with 199.9 µg/kg followed by Cauayan with 262.65 µg/kg, Echague with 309.92 µg/kg, and San Mariano with 336.03 µg/kg. The highest total fumonisin were in Ilagan and San Pablo with 412.60 µg/kg and 461.94 µg/kg, respectively. However, using ANOVA, the comparison of fumonisin between geographic locations was non-significant (Table 3). This means that the occurrence and level of fumonisin are not affected by agrogeographic locations.

Table 2. Results of total fumonisin in hybrid maize in different agro-geographic locations of Isabela Province, the Philippines.

Location	Fumonisin Level
Aurora	
Number of samples	5
Percent positive (%)	0
Average (µg/kg)	199.9
Average of positive (µg/kg)	0
San Pablo	
Number of samples	5
Percent positive (%)	40
Average (µg/kg)	461.94
Average of positive (µg/kg)	855
San Mariano	
Number of samples	18
Percent positive (%)	22.22
Average (µg/kg)	336.03
Average of positive (µg/kg)	812.5
Echague	
Number of samples	21
Percent positive (%)	23.81
Average (µg/kg)	309.92
Average of positive (µg/kg)	662
Cauayan	
Number of samples	22
Percent positive (%)	27.27
Average (µg/kg)	262.65
Average of positive (µg/kg)	430
Ilagan	
Number of samples	30
Percent positive (%)	36.67
Average (µg/kg)	412.60
Average of positive (µg/kg)	780

Percent positive refers to results above the limit of detection (LOD).
Average (µg/kg) corresponds of the average of all results, including results below
  the limit of detection, which was set as 199.9 µg/kg for fumonisin.
Average of positive (µg/kg) refers to the average that excludes the results below the LOD.

Table 3. Analysis of variance of fumonisin levels on hybrid (*Bt*) maize varieties among six different locations in Isabela province, the Philippines.

Source of variation	Degrees of freedom (df)	Sum of squares (SS)	Mean sum of squares (MSS)	*F*-computed	*F*-test tabulation	
					0.05	0.01
Location	6-1 = 5	480,517.73	96,103.55	0.680[ns]	2.29	3.17
Experimental error	101-6 = 95	13,417,830.17	141,240.32			
Total	101-1 = 100	13,898,347.91				

Abbreviation: ns, not significant at 5% level

Maize was collected from lowland rainfed areas of Ilagan, Cauayan, San Pablo municipalities, upland plains of Echague and part of Cauayan municipalities, rolling to hilly environment of San Mariano, and intercropping lowland rainfed system of Aurora municipality. A strong correlation was determined between fumonisin contamination levels and geographical region due to the influence of climatic conditions, with temperature having the main influence (Lopes et al. 2023). Furthermore, studies showed that regions with higher humidity levels correlated with higher fumonisin concentration (Fandohan et al. 2005; Mupunga 2013). High altitude had significantly high fumonisin content due to higher humidity (Atukwase et al. 2009). The location and environmental factors had a 47% effect on fumonisin levels (Campa et al. 2005). A study on fumonisin levels produced by *Fusarium* isolates from various regions in the Philippines found that isolates from Isabela produced higher levels of fumonisins compared to those from Laguna. The difference in fumonisin production was attributed to the varying climatic conditions between these two provinces (Cumagun et al. 2009). However, a comparison of fumonisin levels within the Isabela Province had a comparable result in the present study despite agro-geographical location, maybe due to the climatic conditions of these municipalities being nearly the same.

Fumonisin level between hybrid (Bt) and open pollinated maize varieties. In a comparison of fumonisin level between hybrid (*Bt*) and OPV, the result was highly significant at the five percent level of significance (Table 4). The result shows that hybrid (*Bt*) maize can reduce fumonisin levels and control the growth of *Fusarium* species in maize kernel than OPV. The study of Campa et al. (2005) found that hybrid have a 14% effect on fumonisin accumulation and 11 % with the use of *Bt* hybrids. The significance of total fumonisin between hybrid (*Bt*) and OPV was due to genetic differences between the two varieties, the influence of insect injury, weather, and pre- and post-harvest practices. Hybrid *Bt* maize varieties contain either Cry1Ab or Cry1F insecticidal proteins from *Bacillus thuringiensis* that effectively control insect pests such as maize borer causing kernel damage (Betz et al. 2000; Moscardini et al. 2020). The Cry proteins lessen insect activity, resulting in lesser entry points of fumonisin-producing *Fusarium* species into the kernel, making it more resistant to fumonisin contamination. Open-pollinated varieties do not have these proteins; thus, they are prone to insect attacks. Native unimproved maize varieties such as OPV were more susceptible to mycotoxin-producing fungi and fumonisin contamination compared to improved maize varieties (Cabrera-Meraz et al. 2021). The hybrid (*Bt*) varieties experienced less insect injury and growth of *Fusarium* ear rot, which leads to low fumonisin contamination compared to non-*Bt* hybrids (Bowers et al. 2014). The occurrence of insect damage has a significant correlation to the level of total fumonisins (Clements et al. 2003; Campa et al. 2005; Dowd, 2001) and that hybrid (*Bt*) maize exhibits less fumonisin contamination than non-Bt isolines (Rheeder et al. 2005). This was shown in the result of the present study that hybrid (*Bt*) maize had a significantly lower fumonisin level than OPV maize. In contrast, a study by Esteves et al. (2003) reported no statistically significant difference in fumonisin contamination levels between *Bt* hybrid and non-*Bt* hybrid maize grains across different sampling sites and seasons in the Philippines. Insect damage does not correlate with fumonisin levels and climatic conditions of high

temperature, high relative humidity in the R1 stage, and high rainfall during the R3 to R6 stages of the crop influence high contamination of fumonisin in maize kernels (Sánchez-Zúñiga et al. 2024). However, this is not the condition in the present study since temperature, relative humidity were not high enough for fumonisin production by the fungus during silking, and rainfall was not excessive during the period of physiological maturity as shown on the weather condition of Ilagan municipality (Fig. 4). In addition, preharvest and postharvest agronomic practices may have greatly influence on fumonisin contamination in maize grains. In terms of pesticide application, hybrid (*Bt*) varieties are more tolerant than OPV maize varieties. Therefore, OPV are more vulnerable to weed competition and insect injury and damage. Unhealthy plants are prone to insect attack and infection that may lead to the increase of fungal activity and contamination of fumonisin (Golob, 2007). Furthermore, OPV seeds produced from previous harvest were used as planting materials. Ndemera et al. (2018) observed that FB_1 contamination was higher in seeds from previous harvest compared to early, medium and late maturing hybrid seed varieties. This may explain the high level of fumonisin in OPV maize samples in the present study, although it needs another study to prove the claimed. Moreover, the difference of postharvest practices between hybrid (*Bt*) and OPV can also influence insect activity, fungal growth, and fumonisin production. OPV maize are mostly harvested manually by hand because they are planted in small areas as compared to hybrid (*Bt*). The unshelled maize grains were placed in sacks or dumped in an open area after harvest, covered or uncovered with laminated sheets during the night. Before shelling, harvested OPVs are required to dry for easy detachment of grains from maize cobs and preventing breakage of kernels that may lessen the value of grains for seed purposes and/or for selling. The pilling of unshelled maize directly on the ground with enclosed husk inhibit ventilation, slow drying, develop fungal hotspots, and create opportunity for fungal spores to invade the grain (Golob, 2007; Ndemera et al. 2018; Pitt et al. 2013). Prolonged days of maize ears in the field coupled with climatic variability are more susceptible to the growth of *Fusarium* species and higher FB_1 contamination (Ndemera et al. 2018). In the present study, the exposure of the grains during these postharvest practices may resulted to the high level of fumonisin in OPV maize samples than hybrid (*Bt*) samples.

Table 4. Analysis of variance of fumonisin levels between hybrid (*Bt*) and open pollinated maize samples.

Source of variation	Degrees of freedom (df)	Sum of squares (SS)	Mean sum of squares (MSS)	*F*-computed	*F*-test tabulation	
					0.05	0.01
Location	2-1 = 1	16,544,957.05	16,544,957.05	63.17**	4.17	7.56
Experimental error	36-2 = 34	8,905,078.20	261,914.06			
Total	36-1 = 35	25,450,035.26				

*Note: ** = Highly significant at 1% level of significance*

Identification of *Fusarium* species. In the study, 50 *Fusarium* isolates were identified using both cultural and molecular methods. The isolates comprised 68% *Fusarium verticillioides* (34 isolates), 28% *Fusarium proliferatum* (14 isolates), 2% *Fusarium oxysporum* (1 isolate), and 2% *Fusarium kyushuense* (1 isolate). Table 5 shows the identification and characteristics of the identified species of *Fusarium*.

Table 5. Identification of *Fusarium* species from different maize varieties in Isabela Province, the Philippines.

Isolate	Accession number	Species	Percent Identity (%)	Coverage (%)	Colony Diameter (cm)		Pigment in PDA
					After 3 Days	After 5 Days	
F-01	PP702294	*Fusarium proliferatum*	100	99	3.5	7.25	Pale orange
F-02	PP702295	*Fusarium verticillioides*	99	97-98	3	6.5	Pale orange
F-03	PP702296	*Fusarium verticillioides*	99	99-100	3.4	6	Violet grey
F-04	PP702297	*Fusarium proliferatum*	99	99-100	3.35	6.95	Greyish orange
F-05	PP702298	*Fusarium proliferatum*	100	99-100	3.75	7.2	No pigmentation
F-06	PP702299/ BIOTECH 3456	*Fusarium kyushuense*	98-99	94-96	5.7	8.5	Orange red
F-07	PP702300	*Fusarium verticillioides*	99-100	96-98	2.9	4.6	Violet
F-08	PP702301	*Fusarium verticillioides*	99-100	98-100	2.7	7.3	Light peach
F-09	PP702302	*Fusarium proliferatum*	99	100	3.1	5.3	Peach orange
F-10	PP702303	*Fusarium verticillioides*	100	99	4	8	Pale magenta
F-11	PP702304	*Fusarium proliferatum*	99-100	98-100	3	6.2	Pale magenta
F-12	PP702305	*Fusarium proliferatum*	99	99	3.2	6.4	Orange
F-13	PP702306	*Fusarium oxysporum*	99	99-100	2.5	4.5	Orange peach
F-14	PP702307	*Fusarium verticillioides*	99-100	98-99	3.35	7	Violet
F-15	PP702308	*Fusarium verticillioides*	99-100	95-97	3	6	Magenta
F-16	PP702309	*Fusarium verticillioides*	99	98-99	3.15	6.65	Violet
F-17	PP702310	*Fusarium verticillioides*	100	99	3.3	6.8	Greyish orange
F-18	PP702311	*Fusarium verticillioides*	100	99	2.85	5.95	Pink violet
F-19	PP702312	*Fusarium verticillioides*	99	99	3.3	6.5	Dark violet
F-20	PP702313	*Fusarium verticillioides*	99-100	99	3.3	6.5	Greyish red
F-21	PP702314	*Fusarium proliferatum*	99-100	98-100	4.2	6.1	Dark violet

Isolate	Accession number	Species	Percent Identity (%)	Coverage (%)	Colony Diameter (cm)		Pigment in PDA
					After 3 Days	After 5 Days	
F-22	PP702315	*Fusarium proliferatum*	99	98	3	5.8	Violet
F-23	PP702316	*Fusarium verticillioides*	99	100	2.5	7.05	Greyish rose
F-24	PP702317	*Fusarium verticillioides*	99-100	98-100	3.35	7	Greyish orange
F-25	PP702318	*Fusarium verticillioides*	99	99	3.5	7	Orange
F-26	PP702319	*Fusarium proliferatum*	99-100	99	3.3	6.3	Orange
F-27	PP702320	*Fusarium proliferatum*	100	99	3.5	6.4	Greyish rose
F-28	PP702321	*Fusarium proliferatum*	99	99-100	2.9	6.8	Greyish orange
F-29	PP702322	*Fusarium proliferatum*	99-100	98-99	4.2	7.8	Peach
F-30	PP702323	*Fusarium proliferatum*	100	100	3.3	6.8	Orange
F-31	PP702324	*Fusarium verticillioides*	99-100	98-99	3.2	6.8	Greyish rose
F-32	PP702325	*Fusarium verticillioides*	99	99	3.6	6.8	Greyish orange
F-33	PP702326	*Fusarium verticillioides*	99	100	3	5.8	Dark violet
F-34	PP702327	*Fusarium verticillioides*	99-100	99-100	5.2	7	Orange
F-35	PP702328	*Fusarium verticillioides*	99	99-100	3.5	6.5	Greyish rose
F-36	PP702329	*Fusarium verticillioides*	99	99	3.3	6.6	Magenta
F-37	PP702330	*Fusarium verticillioides*	99-100	98-100	2.6	7	Orange
F-38	PP702331	*Fusarium verticillioides*	99	99	3.5	6.5	Dark orange
F-39	PP702332	*Fusarium verticillioides*	99	98	3.8	7.3	Greyish rose
F-40	PP702333	*Fusarium verticillioides*	100	99	2.75	5.7	Pale magenta
F-41	PP702334	*Fusarium verticillioides*	100	99	2.5	4.6	Dark red
F-42	PP702335	*Fusarium verticillioides*	99	99	3.5	6.4	Orange
F-43	PP702336	*Fusarium verticillioides*	99	99	3.3	6.2	Colorless/ no pigmentat ion
F-44	PP702337	*Fusarium verticillioides*	100	100	3.3	5.5	Orange

Isolate	Accession number	Species	Percent Identity (%)	Coverage (%)	Colony Diameter (cm)		Pigment in PDA
					After 3 Days	After 5 Days	
F-45	PP702338	*Fusarium verticillioides*	99-100	99	3.55	7.5	Greyish rose
F-46	PP702339	*Fusarium verticillioides*	100	100	3.5	7.2	Greyish rose
F-47	PP702340	*Fusarium verticillioides*	99-100	98-99	2.65	5.3	Orange red
F-48	PP702341	*Fusarium verticillioides*	99-100	98-99	3.5	5.7	Pale violet
F-49	PP702342	*Fusarium proliferatum*	99-100	98-99	3.5	6.7	Light pink violet
F-50	PP702343	*Fusarium verticillioides*	99	100	3.9	7.1	Light pink violet

Using the taxonomic keys, *F. verticillioides* had a long-chain microconidia mostly in monophialides (Fig. 5A-5D) while *F. proliferatum* had a conidiogenous cells in monophialides and polyphialides (Fig. 5E & 5F). For *F. oxysporum*, the conidiogenous cells consisted of short monophialides in false heads (Fig. 5G) and polyphialides in *F. kyushuense* (Fig. 5H).
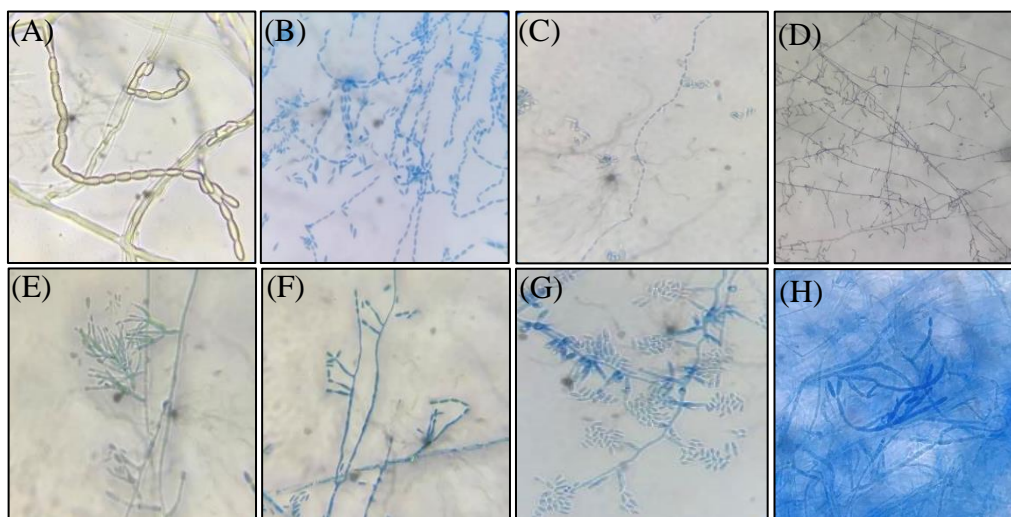

Figure 5. Taxonomic identification of *Fusarium* species: A-C) long chain microconidia of *F. verticillioides*, D) onsite view of *F. verticillioides*, E-F) conidiogenous cells of *F. proliferatum* microconidia in monophialides and polyphialides, G) short monophialides of *F. oxysporum*, and H) production of microconidia in polyphialide.

The isolate with the biggest colony diameter was F-06 (*F. kyushuense*) with 8.5 cm, followed by F-29 (*F. proliferatum*), F-45 (*F. verticillioides*), F-39 (*F. verticillioides*) and F-08 (*F. verticillioides*) with 7.8 cm, 7.5 cm, and 7.3 cm after 5 days observation. The smallest colony diameter was isolate F-13 (*F. oxysporum*) with 4.5 cm, followed by isolates F-07 (*F. verticillioides*) and F-41 (*F. verticillioides*) with 4.6 cm, respectively.

For the shapes of macroconidia and microconidia (Fig. 6), the observed shape for most isolates was blunt apical end and round to barely notched basal end. Some isolates had a hooked apical end and a distinctly notched and foot-shaped basal cells. The isolates F-48 (*F. verticillioides*) and F-07 (*F. verticillioides*) produced needle-like macroconidia while isolates F-19 (*F. verticillioides*), F-37 (*F. verticillioides*), F-12 (*F. proliferatum*) produced sickled or canoe shaped macroconidia. For the isolates that produce only microconidia, F-11 (*F. proliferatum*) had a microconidial shape of pyriform while F-09 (*F. proliferatum*) had obovoid with truncate base microconidia. The microconidial shapes for most *Fusarium* isolates was oval, few obovoid with truncate base and pyriform.
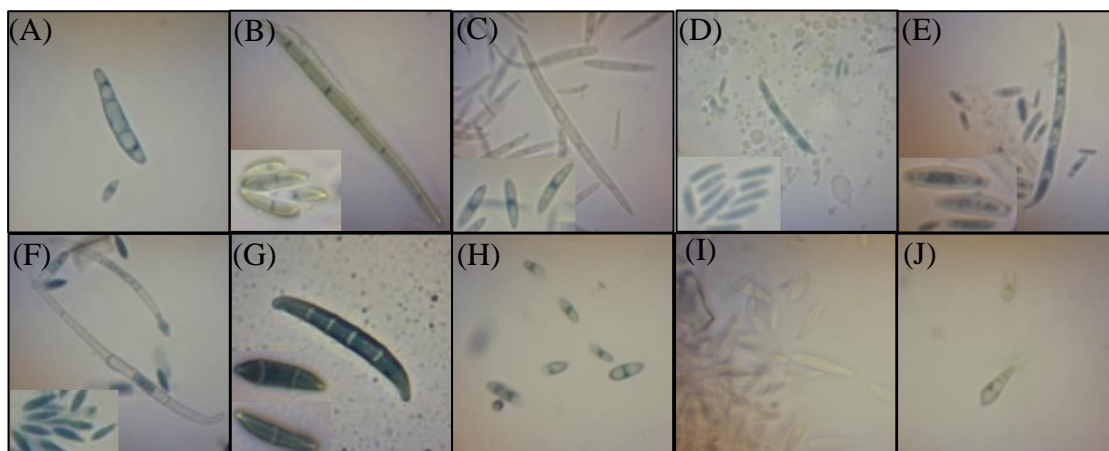

Figure 6. Shapes of macroconidia and microconidia of *Fusarium* species: A) common macroconidia, B) F-48 (*F. verticillioides*) isolate, C) F-07 (*F. verticillioides*) isolate, D) F-19 (*F. verticillioides*) isolate, E) F-37 (*F. verticillioides*) isolate, F) F-12 (*F. proliferatum*) isolate, G) F-06 (*F. kyushuense*) isolate, H) common microconidia, I) F-09 (*F. proliferatum*) isolate, and J) F-11 (*F. proliferatum*) isolate.

For colony pigmentation of *F. verticillioides* isolates varied widely, ranging from no pigmentation to shades of violet, orange, magenta, red, greyish color, and pink-violet. Similarly, *F. proliferatum* exhibited diverse pigmentation. In contrast, *F. oxysporum* colonies displayed an orange-peach color, while *F. kyushuense* formed cottony colonies with white, creamy mycelia and a red-peach pigmentation. Detailed colony characteristics for each isolate are presented in Fig. 7.

The occurrence of *Fusarium* species in maize kernels was also analyzed, revealing variation in isolates across different samples and locations. Isolates F-01 (*F. proliferatum*), F-25 (*F. verticillioides*), F-29 (*F. proliferatum*), F-23 (*F. verticillioides*), F-31 (*F. verticillioides*), F-45 (*F. verticillioides*), F-14 (*F. verticillioides*), F15 (*F. verticillioides*), F-36 (*F. verticillioides*), F-04 (*F. proliferatum*), and F-28 (*F. proliferatum*) were commonly found in both hybrid and open-pollinated maize varieties and were prevalent across nearly all locations. Both *F. verticillioides* and *F. proliferatum* were present in all six sampled areas, though they varied in colony morphology and pigment intensity. Most isolates, however, were unique to specific locations.

Fusarium species are major sources of fumonisins with *F. verticillioides* and *F. proliferatum* identified as the primary producers (Milani 2013; Waskiewicz et al. 2010). These mycotoxigenic species have been detected in agricultural crops in the Philippines, particularly in maize and rice grains (Balendres et al. 2019). *Fusarium verticillioides* is frequently found in farm and storage samples across various regions in the Philippines (Esteves et al. 1996). Isolates of *F. verticillioides* have been collected from maize ears, kernels (Hussien et al. 2017; Magculia and Cumagun 2011; Pascual et al. 2016), and
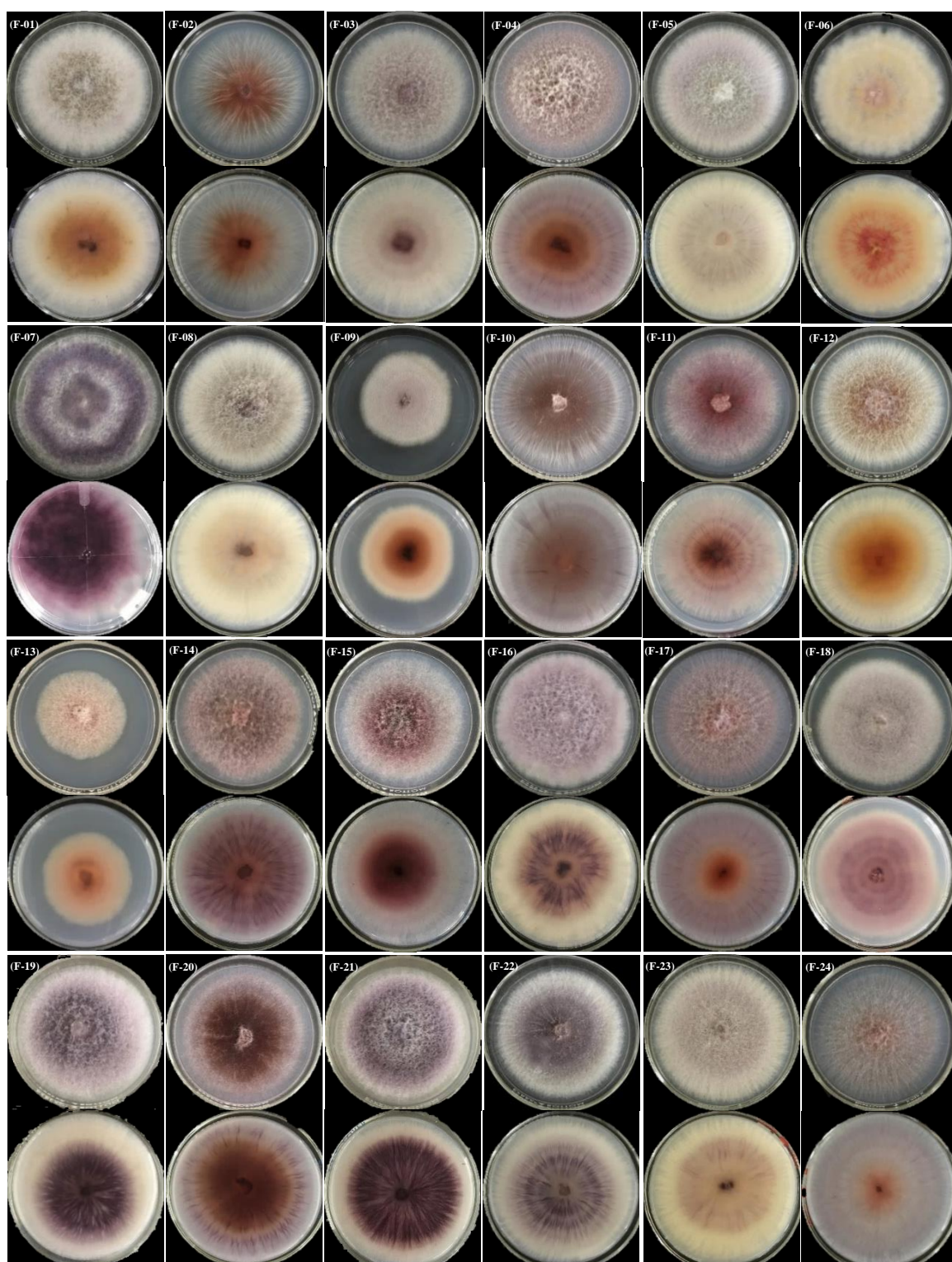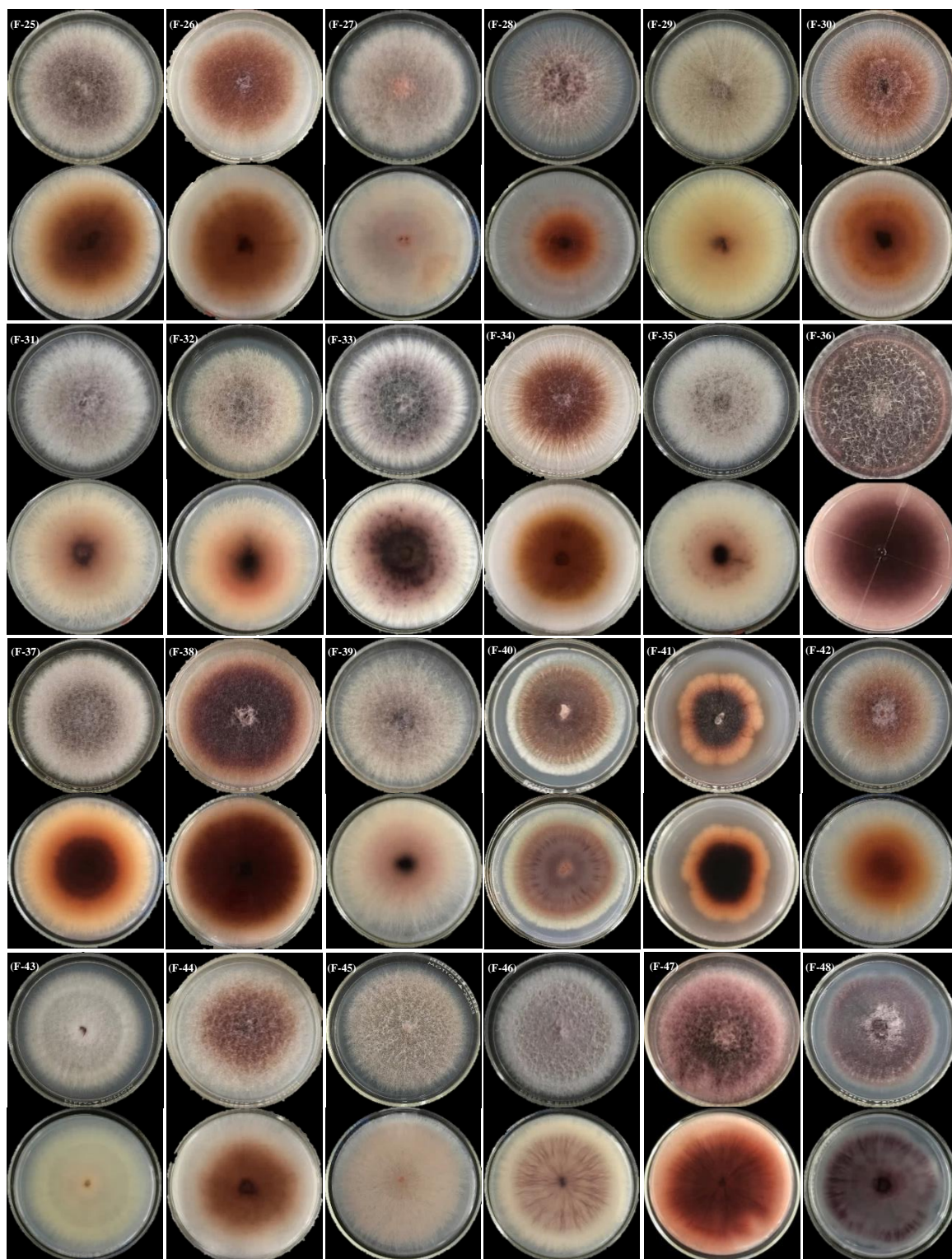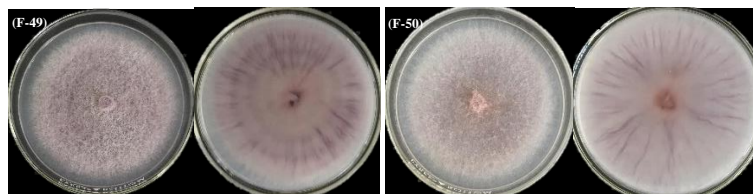
Figure 7. Colony characteristics of *Fusarium* isolates from maize kernels in Isabela Province, the Philippines after 7 days on PDA.

even maize cobs (Cumagun et al. 2009). *Fusarium verticilliodes* is the most dominant and frequent species at harvest and at storage (Carbas et al. 2021). It is predominant, endemic in maize kernels and even in fruit crops in temperate, tropical, and subtropical regions of the world with warmer and drier conditions (Miller 2001; Santiago et al. 2015; Yli-Mantilla and Sundheim 2022; Zakaria 2023). In the Philippines, Esteves et al. (2003) reported that *F. verticillioides* was prevalent in Isabela province during the wet season, which is characterized by warm, humid conditions. This was similarly displayed in the result of the present study with 68% isolated *F. verticillioides* species from maize kernels during the dry season harvest. In addition, the occurrence of *F. verticillioides* has a strong correlation to insect activity (Miller 2001; Stathas et al. 2023). This was shown in the present study with the result of low fumonisin levels in hybrid (*Bt*) maize varieties that can be due to reduce insect activity and further limit by climatic conditions that promote *F. verticillioides* contamintation in kernels.

The cultural characteristics of *F. verticillioides,* as described by Burgess et al. (1994) and Leslie and Summerell (2006), include pigmentation in PDA culture media ranging from no pigmentation to grayish orange to violet and dark violet or dark magenta. In this study, the *F. verticillioides* isolates exhibited similar pigmentation, including shades of grayish rose, red, and pink. A key morphological feature of *F. verticillioides* is the presence of long chains of microconidia produced from monophialides, which was observed and utilized for isolate identification. Additionally, the identity of *F. verticillioides* isolates was confirmed by 99-100% gene sequence similarity with sequences from the National Center for Biotechnology Information (NCBI). However, fumonisin production by each isolate remains unknown.

On the other hand, *F. proliferatum* is widely distributed across various agricultural and non-agricultural substrates globally. However, it is less distributed in lower frequencies in maize ear, and often co-occurs with *F. verticilioides* in warmer regions (Santiago et al. 2015). Biosynthesis of fumonisin reportedly regulated by fumonisin gene clusters in *F. proliferatum* together with *F. verticillioides* (Medina et al. 2013; Sun et al. 2019). The polyketide synthase in the genome of the *F. proliferatum* with PKS11 key enzyme was linked in the biosynthesis of fumonisin (Niehaus et al. 2016).

Key morphological characteristics of *F. proliferatum* as described by Burgess et al. (1994) and Leslie and Summerell (2006) include the production of chains of microconidia from polyphialides, although conidiogenous cells may also form as either monophialides or polyphialides, as observed in this study. These morphological features were instrumental in identifying *F. proliferatum* isolates. Additionally, gene sequencing results from NCBI showing 99%-100% similarity confirm the close genetic relatedness and accurate identification of *F. proliferatum* isolates. Pascual et al. (2016) identified an isolate of *F. proliferatum* from maize ears collected in Bohol.

Another species identified is *F. oxysporum,* commonly recognized as a soil saprophyte and a significant vascular wilt pathogen affecting numerous plant species worldwide. Notably, *F. oxysporum* is also known to produce fumonisin C-series mycotoxins, which exhibit toxicity to that of the B-series

fumonisin (Shephard et al. 2011). The C-series fumonisins have been reported as naturally co-occurring with fumonisin B in moldy maize (Seo and Lee 1999). A distinct characteristic of *F. oxysporum* is the formation of microconidia in false heads on short monophialides, as described by Burgess et al. (1994) and Leslie and Summerell (2006), which was observed in isolate F-13. This identification was further confirmed through gene sequencing, showing a 99% similarity with sequences in the NCBI database. *Fusarium oxysporum* was also cataloged by Santiago et al. 2011 as one of the fungal species isolated from maize grain.

Additionally, *Fusarium kyushuense* is a trichothecene-producing species originally isolated from Kyushu and Shikoku, Japan. Aoki and O'Donnell (1998) describe its macroconidia as arising from sporodochial conidiogenous cells on surface hyphae, falcate to fusiform in shape, straight to slightly curved, with an acutely pointed apical cell and a distinct or indistinct basal foot cell.

Its microconidia are borne from aerial conidiogenous cell, oblong to clavate in shape. The macroconidia and microconidia characteristics of the *F. kyushuense* isolate this study were consistent with these descriptions. *F. kyushuense* was first reported on maize plants causing stalk rot in China (Cao et al. 2021). This study presents *F. kyushuense* as the first report of *Fusarium* species from maize grain and new record for the Philippines. The isolate's identity was confirmed by the Philippine National Collection of Microorganisms (PNCM) and preserved under the accession number BIOTECH 3456. *F. kyushuense* was isolated from a single maize sample collected from San Mariano municipality.

Globally, fumonisin contamination happen primarily in maize and maize products (Bryla et al. 2013; Li et al. 2024). Salvacion et al. 2015 assessed that fumonisin contamination in the Philippines remains at very high risk both present and future conditions. The presence of *F. verticillioides* and *F. proliferatum* in the study suggests the possible contamination of fumonisin in maize grains. However, the fumonisin production capability of each *Fusarium* isolates remain undetermined. These *Fusarium* isolates may or may not produce fumonisins, as Pascual et al. (2016) identify fumonisin-producing and non-producing *Fusarium* within the same species.

CONCLUSION

Fumonisins in maize grains were within the safe limits during the period of this study. It may not pose a threat at the post-harvest stage as the *Fusarium* species responsible for its production generally cannot thrive under low moisture storage conditions unless factors arise that favor fungal growth and fumonisin production. Processing these contaminated grains into flour, meal and other maize by-products may reduce fumonisin level due to the effects of grain treatments and refinements.

Regular monitoring of fumonisin levels in maize kernels is essential to effectively manage and prevent contamination in food and feed. The use of hybrid (*Bt*) maize is recommended to reduce insect injury, which can create entry points for *Fusarium* species and increase the risk of contamination. Optimal planting should coincide with the availability of rainwater to support healthy plant development and avoid drought stress. Immediate shelling and drying after harvest are crucial to minimize the risk of *Fusarium* colonization. Moreover, storing maize in clean, dry and secure facilities is necessary to prevent insect infestation and further fumonisin contamination. Further study is recommended to determine the level of fumonisins during the wet season harvest and to assess the fumonisin producing potential of the *Fusarium* isolates identified in this study

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REFERENCES CITED

Alberts, J.F., W.H. Zyl, and W.C.A. Gelderblom. Biologically based methods for control of fumonisin-producing *Fusarium* species and reduction of the fumonisins. Front Microbiol 7: 548.

Anumudu, C.K., C.T. Ekwueme, C.C. Uhegwu, C. Ejileugha, J. Augustine, C.A. Okolo, and H. Onyeaka. 2025. A review of the mycotoxin family of fumonisins, their biosynthesis, metabolism, methods of detection and effects on humans and animals. Int. J. Mol. Sci. 126: 184.

Aoki, T. and K. O'Donnell. 1998. *Fusarium kyushuense* sp. nov. from Japan. Mycoscience 39: 1-6.

Atukwase, A., A. Kaaya, and C. Muyanja. 2009. Factors associated with fumonisin contamination of maize in Uganda. J. Sci. Food Agric.89: 2393–2398

Balendres, M. A. O., P. Karlovsky, and C.J.R. Cumagun. 2019. Mycotoxigenic fungi and mycotoxins in agricultural crop commodities in the Philippines: a review. Food 8: 249.

Betz, F.S., B.G. Hammond, and R.L. Fuchs. 2000. Safety and advantages of *Bacillus thuringiensis* – protected plants to control insect pests. Regul.Toxicol. Pharm. 32: 156–173.

Beyene, A.M., D. Xiangwei, D.E. Schrunk, S. Ensley and W.K. Rumbeiha. 2019. High-performance liquid chromatography and enzyme-linked immunosorbent assay techniques for detection and quantification of aflatoxin B1 in feed samples: a comparative study. BMC Res. Notes 12: 492.

Bowers, E., R. Hellmich, and G. Munkvold. 2014. Comparison of fumonisin contamination using HPLC and ELISA methods in *Bt* and near-isogenic mize hybrids infested with european corn borer or western bean cutworm. J. Agric. Food Chem. 62: 6463-6472.

Bryla, M., M. Roszko, K. Szymczyk, R. Jedrzejczak, M.W. Obiedzinski, J. Sekul. Fumonisins in plant-origin food and fodder - a review. Food Addit. Contam. Part a-Chem. Anal. Control Exposure Risk Assess. 30: 1626-1640.

Burgess, L. W., B.A. Summerell, S. Bullock, K.P. Gott, and D. Backhouse. 1994. Laboratory manual for *Fusarium* research (3rd ed.). Sydney: Dept. of Crop Sciences, University of Sydney / Royal Botanic Gardens.

Cabrera-Meraz, J., L. Maldonado, A. Bianchini, and R. Espinal. 2021. Incidence of aflatoxins and fumonisins in grain, masa and corn tortillas in four municipalities in the department of Lempira, Honduras. Heliyon 7.

Cao, A., R. Santiago, A.J. Ramos, X.C. Souto, O. Aguín, R.A. Malvar, and A. Butrón. 2014. Critical environmental and genotypic factors for *Fusarium verticillioides* infection, fungal growth and fumonisin contamination in maize grown in northwestern Spain. Int. J. Food Microbiol. 177: 63-71.

Cao, Y.Y., J. Zhang, S.B. Han, L.K. Xia, J. Ma, L.F. Wang, H.Y. Li, L.R. Yang, S.L. Sun, Z.D. Zhu, and C.X. Duan. 2021. First report of maize stalk rot caused by *Fusarium kyushuense* in China. Plant Dis. 105: 3759.

Campa, R.D.L., Hooker, D.C., Miller, J.D., Schaafsma, A.W. and Hammond, B.G., 2005. Modeling effects of environment, insect damage, and Bt genotypes on fumonisin accumulation in maize in Argentina and the Philippines. Mycopathologia 159: 539-552.

Carbas, B., D. Simões, A. Soares, A. Freitas, B. Ferreira, A.R.F. Carvalho, A.S. Silva, T. Pinto, E. Diogo, E. Andrade, and C. Brites. 2021. Occurrence of *Fusarium* spp. in maize grain harvested in Portugal and accumulation of related mycotoxins during storage. Foods10: 375.

Cendoya, E., M.D. Monge, S.M. Chiacchiera, M.C. Farnochi, and M.L. Ramirez. 2018. Influence of water activity and temperature on growth and fumonisin production by *Fusarium proliferatum* strains on irradiated wheat grains. Int. J. Food Microbiol. 266: 158-166.

Clements, M.J., K.W. Campbell, C.M. Maragos, C.D. Pilcher, J.M. Headrick, J.K. Pataky, and D.G. White. 2003. Influence of Cry1Ab protein and hybrid genotype on fumonisin contamination and Fusarium ear rot of corn. Crop Science 43: 1283-1293.

Cochran, W. G. 1977. Sampling Techniques. 3rd ed., John Wiley & Sons, Inc., New York, New York. 75 p.

Council for Agricultural Science. 2003. Mycotoxins: risks in plant, animal, and human systems; task force report; Council for Agricultural: Ames, IA, USA. 191 p.

Cumagun, C.J.R., J.S. Ramos, A.O. Dimaano, F. Munaut, and F.V. Hove. 2009. Genetic characteristics of *Fusarium verticilliodes* from maize in the Philippines. The Phytopathological Society of Japan and Springer 75: 405-412.

Dowd, P.F. 2001. Biotic and abiotic factors limiting efficacy of Bt corn in indirectly reducing mycotoxin levels in commercial fields. J. Econ. Entomol. 94: 1067-1074.

Edrington, T., C. Kamps-Holtzapple, R. Harvey, L. Kubena, M. Elissalde, and G. Rottinghaus. 1995. Acute hepatic and renal toxicity in lambs dosed with fumonisin-containing culture material. J. Anim. Sci. 73: 508–515.

Esteves, L., R. Bermundo, B. Talplacido, and M. Dela Cruz. 1996. Survey of mycotoxigenic fungi in Philippines corn. In: Nakase, T., Takeo, K. (eds) Proceedings of Asian International Mycological Congress '96. Chiba University, Chiba. 151 p.

Esteves, L., N. Santiago, E. Regpala, E. Ablaza, and R. Bermundo. 2003. Characterization of fungal microflora and evaluation of mycotoxin contamination level in yieldgard and non-Bt corn grains in The Philippines. Compendium of PhilMech RD&E Abstracts 2001– 2011, 34 p.

Etchevery M., A. Torres, M. Ramirez, S. Chulze, and N. Magan. 2002. *In vitro* control of growth and fumonisin production by *Fusarium verticillioides* and *Fusarium proliferatum* using antioxidants under different water availability and temperature regimes. J. Appl. Microbiol. 92: 624-632.

Fandohan, P., B. Gnonlonfin, K. Hell, W.F. O. Marasas, and M.J. Wingfield. 2005. Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa. Int. J. Food Microbiol. 99: 173-183.

FAO [Food Agriculture Organization]. 1994. Grain Storage Techniques: Evolution and trends in developing countries. FAO Agricultural Services Bulletin No. 109, Rome, Italy.

Garcia, D., G. Barros, S. Chulze, A.J. Ramos, V. Sanchis, and S. Marín. 2012. Impact of cycling temperatures on *Fusarium verticillioides* and *Fusarium graminearum* growth and mycotoxins production in soybean. J. Sci. Food Agric. 92: 2952-2959.

Gazzotti, T., B. Lugoboni, E. Zironi, A. Barbarossa, A. Serraino, and G. Pagliuca. 2009. Determination of fumonisin B1 in bovine milk by LC-MS/MS. Food Control 20: 1171-1174.

Gerpacio R.V., J.D. Labios, R. Labios, and E.I. Diangkinay. 2004. Maize in the Philippines: Production Systems, Contraints, and Research Priorities. Mexico, D.F., CIMMYT.

Golob, P. 2007. On-farm mycotoxin control in food and feed grain. Good practices for animal feed and livestock – training manual. Food and Agriculture Organization of the United Nations, Rome, Italy.

Hansen, H. N. and R.E. Smith. 1932. The mechanisms of variation in imperfect fungi: *Botrytis cinerea*. Phytopathology 37: 369–371.

Hussien, T., A.L. Carlobos-Lopez, C.J.R. Cumagun, and T. Yli-Mattila. 2017. Identification and quantification of fumonisin-producing *Fusarium* species in grain and soil samples from Egypt and the Philippines. Phytopathol. Mediterr. 56: 146-153.

IARC [International Agency for Research on Cancer]. 1993. *Fusarium moniliforme*: fumonisins B1 and B2 and fusarin C, pp 445-466. In IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 56, IARC Working Group, WHO, Lyon, France.

IARC [International Agency for Research on Cancer]. 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Monogr. Eval. Carcinog. Risks Hum. 82: 171-274.

Imran, M., S. Cao, S. Wan, Z. Chen, M.K. Saleemi, N. Wang, M.N. Naseem, and J. Munawar. 2020. Mycotoxins - a global one health concern: a review. Agrobiol. Rec. 2: 1-16.

IRRI [International Rice Research Institute]. 2013. Grain storage and pest management. Postharvest Unit, CESD, International Rice Research Institute.

Javed, T., J. Richard, G. Bennett, M. Dombrink-Kurtzman, R. Bunte, K. Koelkebeck, L. Cote, R. Leeper, and W. Buck. 1993. Embryopathic and embryocidal effects of purified fumonisin B1 or *Fusarium proliferatum* culture material extract on chicken embryos. Mycopathologia 123: 185–193.

Kamle, M., D.K. Mahato, S. Devi, K.E. Lee, S.G. Kang, and P. Kumar. 2019. Fumonisins: impact on agriculture, food, and human health and their management strategies. Toxins 11: 328.

Leslie, J.F. and B.A. Summerell. 2006. The fusarium laboratory manual. Blackwell Publishing, Iowa. USA.

Li, T., J. Li, J. Wang, K.S. Xue, X. Su, H. Qu, X. Duan, and Y. Jiang. 2024. The occurrence and management of fumonisin contamination across the food production and supply chains. J. Adv. Res. 60: 13-26.

Lopes, L.D.S., G.l.P.A. Ramos, V.O. Alvarenga, F.B. Campagnollo, S.B.S. Oliveira, L. Freire, and A.S. Sant'Ana. 2023. Quantitative risk assessment of the presence of fumonisin in corn produced in different regions of Brazil: Influence of climatic conditions. Environ. Res. 237: 116907.

Magculia, N.J.F. and C.J.R. Cumagun. 2011. Genetic diversity and PCR-based identification of potential fumonisin-producing *Fusarium verticillioides* isolates infecting maize in the Philippines. Trop. Plant Pathol. 36: 225-232.

Medina, A., M. Schmidt-Heydt, D.L. Cárdenas-Chávez, R. Parra, R. Geisen, and N. Magan. 2013. Integrating toxin gene expression, growth and fumonisin B1 and B2 production by a strain of *Fusarium verticillioides* under different environmental factors. J. R. Soc. Interface 10: 20130320.

Minic, R. and I. Zivkovic. 2020. Optimization, validation and standardization of ELISA, pp 9-28. In Norovirus Intech Open London, UK.

Mupunga, I. 2013. A comparative study of natural contamination with aflatoxins and fumonisins in selected food commodities from Botswana and Zimbabwe. Ph.D. Dissertation, University of South Africa, Pretoria.

Marín, S., V. Sanchis, and N. Magan. 1995. Water activity, temperature, and pH effects on growth of *Fusarium moniliforme* and *Fusarium proliferatum* isolates from maize. Can. J. Microbiol. 41: 1063–1070.

Marín, S., N. Magan, N. Belli, A.J. Ramos, R. Canela, and V. Sanchis. 1999a. Two-dimensional profiles of fumonisin B1 production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modelling toxin formation in maize grain. Int. J. Food Microbiol. 51: 159-167.

Marín, S., V. Homedes, V. Sanchis, A.J. Ramos, and N. Magan. 1999b. Impact of *Fusarium moniliforme* and *F. proliferatum* colonisation of maize on calorific losses and fumonisin production under different environmental conditions. J. Stored Prod. Res. 35: 15–26.

Marín, S., V. Sanchis, and N. Magan. 2011. Water activity, temperature, and pH effects on growth of *Fusarium moniliforme* and *Fusarium proliferatum* isolates from maize. Can. J. Microbiol. 41: 1063-1070.

Mew, T.W. and J.K. Misra. 1994. A manual of rice seed health testing. International Rice Research Institute. 27 p.

Miller, J.D. 1995. Fungi and mycotoxins in grain: Implication for stored product research. J. Stored Prod. Res. 31: 1-16.

Miller, J.D. 2001. Factors that affect the occurrence of fumonisin. Environ. Health Perspect. 109: 321-324.

Milani, J.M. 2013. Ecological conditions affecting mycotoxin production in cereals: a review. Veterinarni Medicina 58: 405-411.

Moscardini, F.V., L.H. Marques, A.C. Santos, J. Rossetto, O.N. Silva, P.E. Rampazzo, and B.A. Castro. 2020. Efficacy of *Bacillus thuringiensis* (*Bt*) maize expressing Cry1F, Cry1A.105, Cry2Ab2 and Vip3Aa20 proteins to manage the fall armyworm (Lepidoptera: Noctuidae) in Brazil. 2020. Crop Prot. 137: 105269.

Munkvold, G.P. 2003. Epidemiology of *Fusarium* disease and their mycotoxins in maize. Eur. J. Plant Pathol. 109: 705-713.

Ndemera, M., S. Landschoot, M. De Boevre, L.K. Nyanga, and S. De Saeger. 2018. Effect of agronomic practices and weather conditions on mycotoxins in maize: a case study of subsistence farming households in Zimbabwe. World Mycotoxin J. 11: 421-436.

Niehaus, E.-M., M. Münsterkötter, R.H. Proctor, D.W. Brown, A. Sharon, Y. Idan, L. Oren-Young, C.M. Sieber, O. Novák, A. Pěnčík, D. Tarkowská, K. Hromadová, S. Freeman, M. Maymon, M. Elazar, S.A. Youssef, E.S.M. El-Shabrawy, A.B.A. Shalaby, P. Houterman, N.L. Brock, I. Burkhardt, E.A. Tsavkelova, J.S. Dickschat, P. Galuszka, and U. Güldener. 2016. Comparative "omics" of the *Fusarium fujikuroi* species complex highlights differences in genetic potential and metabolite synthesis. Genome Biol. Evol. 8: 3574–3599.

Oguz, H. 2017. Mikotoksinler ve Önemi. Turkiye Klinihleri (Mycotoxins and their importance). Turkiye Klinikleri Vet. Sci. Pharmacol. Toxicol-Special Topics 3: 113-119.

Ono, E.Y.S., O. Kawamura, M.A. Ono, Y. Ueno and E.Y. Hirooka. 2000. A comparative study of indirect competitive ELISA and HPLC for fumonisin detection in corn of the State of Paraná, Brazil. Food Agric. Immunol 12: 5-14.

Pascual, C.B., A.K.S. Barcos, J.A.L. Mandap and E.T.M. Ocampo. 2016. Fumonisin-producing *Fusarium* species causing ear rot of corn in the Philippines. Philipp J. Crop Sci. 41: 12-21.

Philippine National Standard/Bureau of Agricultural and Fisheries Product Standards (PNS/BAFPS). 2014. Code of practice for the prevention and reduction of mycotoxin contamination in cereals. 13 p.

Pitt, J.I., M.H. Taniwaki, and M.B. Cole. 2013. Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of food safety objectives. Food Control 32: 205-215.

PSA [Philippine Statistical Authority]. 2021. Maize production in Cagayan Valley fell short in 2020. Region II. Retrieved from: rsso02. psa.gov.ph

Reyes, C.M., S.N. Domingo, C.D. Mina, and K.G. Gonzales. 2009. Climate variability, SCF, and corn farming. In Isabela, Philippines: A farm and household level analysis, Discussion paper series No. 2009- 06. Philippine Institute for Development Studies, NEDA.

Rheeder, J., H. Vismer, L. van der Westhuizen, G. Imrie, P.Gatyeni, D. Thomas, G. Shephard, W. Marasas, and B. Flett. 2005. Effect of Bt corn hybrids on insect damage, incidence of fumonisin-producing *Fusarium* species and fumonisin levels in South Africa. Phytopathology 95: S88-S.

Rodrigues, I. and K. Naehrer. 2012. Prevalence of mycotoxins in feedstuffs and feed surveyed worldwide in 2009 and 2010. Phytopathol. Mediterr. 51: 175-192.

Rustia, A.S., D.N.S. Guiriba, K.A.D. Bautista, D. Mahoney, E.B. Barrios, C.B.J. Villarino, and M.V. Capanzana. 2022. Risk profiling of fumonisin in corn (*Zea mays* L.) to the Filipino consuming population. Philipp. J. Sci. 151: 73-93.

Salvacion, A., I. Pangga, and C.J. Cumagun. 2015. Assessment of mycotoxin risk on maize in the Philippines under current and future climate change conditions. Rev. Environ. Health 30: 135-142.

Samapundo, S., F. Devliehgere, B.D. Meulenaer, and J. Debevere. 2005. The effect of water activity and temperature on growth and the relationship between fumonisin production and the radial growth of *Fusarium verticillioides* and *Fusarium proliferatum* on maize. J. Food Prot. 68: 1054-1059.

Samuel, A., A. Saburi, O.E. Usanga, I. Ikotun, and I.U. Isong. 2011. "Post–harvest food losses reduction in maize production in Nigeria". Afr. J. Agric. Res. 6: 4833-4839.

Sánchez-Zúñiga, K., W. Rivera-Méndez, S. Campos-Boza, E. Araya-Valverde, F. Solano-Campos, and M. Vinas. 2024 Factors influencing fumonisin B1 contamination in maize: insights from two production regions in Costa Rica. Mycotoxin Res. 40: 641-649.

Santiago, N.C., B.P. Roderos, L.A. Esteves, F.L.M. Pineda, and A.J.G. Gibe. 2011. Catalogue of fungal cultures and collection. Philippine Center for Postharvest Development and Mechanization (PhilMech), Science City of Muñoz, Nueva Ecija, Philippines.

Santiago, R., A. Cao, and A. Butrón. 2015. Genetic Factors Involved in Fumonisin Accumulation in Maize Kernels and Their Implications in Maize Agronomic Management and Breeding. Toxins 7: 3267-3296.

Schmale III, D.G. and G.P. Munvold. 2009. Mycotoxin in Crops: A threat to human and domestic animal health. The Plant Health Instructor. DOI: 1094/PHI-I-2009-0715-01.

Seo, J.A. and Y.W. Lee. 1999. Natural occurrence of the C series of fumonisins in moldy corn. Appl. Environ. Microbiol. 65: 1331-1334.

Shephard, G.S., L.V.D. Westhuizen, V. Sewram, J.V. Zyl, and J.P. Rheeder. 2011. Occurrence of the C-series fumonisins in maize from the former Transkei region of South Africa. Food Addit. Contam. 28: 1712-1716.

Sokolovic, M. 2022. HPLC and ELISA Methods for Detection and Quantification of Fumonisins in Naturally Contaminated Maize. Research Square. DOI: 10.21203/rs.3.rs-1908427/v1.

Sreenu, B., A.G. Girish, and J.A. Sujeetha. 2019. Identification and detection of maize seed borne pathogens using different seed testing methods. Int. J. Curr. Microbiol. Appl. Sci. 8: 1460–1466.

Stathas, I.G., A.C. Sakellaridis, M. Papadelli, J. Kapolos, K. Papadimitriou, and G.J. Stathas. 2023. The effects of insect infestation on stored agricultural products and the quality of food. Foods 12: 2046.

Sun, L., X. Chen, J. Gao, Y. Zhao, L. Liu, Y. Hou, L. Wang, and S. Huang. 2019. Effects of disruption of five FUM genes on fumonisin biosynthesis and pathogenicity in *Fusarium proliferatum*. Toxins 11: 327.

Tiongson, R., L. Esteves, N. Santiago, E. Regpala, and B. Roderos. 2005. Profiling of aflatoxin B1 and fumonisin B1 contamination in selected corn-growing areas in the Philippines. Compendium of PhilMech RD&E Abstracts 2001– 2011. 34 p.

Tsedaley, B. 2015. Review of seed health tests and detection methods of seedborne diseases. J. Biol. Agric. Health. 5: 176–184.

Tubbs, T., D. Baributsa, and C. Woloshuk. 2016. Impact of opening hermetic storage bags on grain quality, fungal growth and aflatoxin accumulation. J. Stored Prod. Res. 69: 276-281.Voss, K.A., G.W. Smith, and W.M. Haschek. 2007. Fumonisins: oxicokinetics, mechanism of action and toxicity. Anim. Feed Sci. Technol. 137: 299-325.

Wang, Y., H. Quan, X. li, Q. Li, M.A. Hague, Q. Shi, Q. Fu, and C. He. 2021. Contamination with fumonisin B and deoxynivalenol is a threat to egg safety and contributes to gizzard ulcerations of newborn chickens. Front Microbiol. 12: 676671.

Waskiewicz, A., P. Golinski, L. Irzykowska, J. Bocianowski, M. Kostecki, and Z. Weber. 2010. Formation of fumonisins and other secondary metabolites by *Fusarium oxysporum* and *F. proliferatum*: a comparative study. Food Addit. Contam. 27: 608-615.

Yamashita, A., T. Yoshizawa, Y. Aiura, P.C. Sanchez, E.I. Dizon, R.H. Arim, and Sardjono. 1995. *Fusarium* mycotoxins (fumonisins, nivalenol, and zearalenone) and aflatoxins in corn from Southeast Asia. Biosci., Biotechnol., Biochem. 59: 1804-1807.

Yli-Mattila, L. and L. Sundheim. 2022. Fumonisins in African countries. Toxins 14: 419.

Zakaria, L. 2023. *Fusarium* species associated with disease of major tropical fruit crops. Horticulturae 9: 322.