

Entomofauna, mycotoxin accumulation and storage loss dynamics in cereals in the Tshopo province, Democratic Republic of Congo

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ABSTRACT

Post-harvest losses of cereals remain a major challenge for food security in tropical regions, yet data from Central Africa are scarce. This study investigated the entomofauna, mycotoxin contamination, and storage loss dynamics of rice (*Oryza sativa* L.) and maize (*Zea mays* L.) stored under traditional conditions in the Tshopo province, Democratic Republic of Congo. Cereals were sampled at nine storage sites across three zones at 0, 30, and 60 days of storage. Insect pests were identified using morphological and molecular approaches, mycotoxins were quantified using immunochromatographic assays validated by UHPLC–MS/MS, and grain quality losses were assessed through weight, moisture, integrity, and germination measurements.

Stored cereals were dominated by three insect pests: *Sitophilus zeamais*, *Sitotroga cerealella*, and *Tribolium castaneum*, accounting for more than 98% of all collected insects. *S. zeamais* was ubiquitous and reached high densities in both crops, while *S. cerealella* showed site-specific infestations, particularly in rice. Aflatoxin contamination was detected in both crops and increased significantly during maize storage, frequently exceeding the European regulatory limit after 60 days in some zones. Fumonisin were detected exclusively in maize at low but consistent concentrations. Grain moisture readings point to a high risk of pre-storage mycotoxin accumulation, with additional in-storage aflatoxin production occurring specifically in stores with the highest moisture levels. Grain quality deteriorated during storage, with significant losses in dry weight, integrity, and germination capacity, particularly in sites with high insect pressure and mycotoxin levels. Correlation analyses revealed strong associations between insect abundance, mycotoxin accumulation, grain moisture content, and grain deterioration. Findings demonstrate that post-harvest losses in stored rice and maize in Tshopo province are driven by synergistic interactions between storage pests and fungal contamination. The study provides the first comprehensive baseline data for the region and highlights the urgent need for improved storage management to reduce food safety risks and cereal losses.

1. Introduction

Cereals such as rice (*Oryza sativa* L.) and maize (*Zea mays* L.) are staple foods in the Democratic Republic of Congo (DRC) (Marivoet et al., 2019). These crops, alongside cassava, provide the bulk of the caloric intake for millions of inhabitants and are a crucial source of income for the Congolese population, which is primarily engaged in agriculture (FAOSTAT, 2022; Marivoet et al., 2019). Post-harvest preservation of

those crops, however, remains a significant challenge, as losses occurring during storage reduce the quantity of edible grain available and compromise their quality (Flores-Rojas et al., 2024). Post-harvest losses are a persistent problem across Sub-Saharan Africa (SSA), with annual average losses in 2022 of 9.7 and 2.8 million tonnes per year, respectively, for maize and rice, which are the first and third most degraded cereals in all of SSA (APHILIS, 2022). In the DRC's Tshopo province (Fig. 1), maize and rice are pillars of rural livelihoods and food security

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in the entire socio-environmental landscape. Still, they are also the two crops most severely damaged. Regionally, the high post-harvest losses of these staple crops contribute to the chronic malnutrition striking the local population, underscoring the urge for research and action on the matter (IPC, 2023; Stathers et al., 2020).

These losses are driven by the combination of biotic and abiotic factors, among which insect infestation and fungal infections are paramount (Nath et al., 2024). Storage insects, particularly those of the genus *Sitophilus* Schönherr 1838, and *Tribolium* MacLeay 1825, are recognized as major pests of stored rice and maize (Naseri and Majd-Marani, 2020; Peschiutta et al., 2022). They cause direct damage by feeding on the grains, leading to weight loss, reduced germination, and diminished market value (Stathas et al., 2023). Furthermore, their activity creates entry points and favourable microenvironments for secondary insect infestation (Shah et al., 2021) and fungal colonization, amplifying the risk of mycotoxin contamination (Lamboni and Hell, 2009). However, the actual diversity of species present in stored cereals in the Tshopo province is yet to be determined.

Mycotoxins are secondary metabolites produced by fungi, notably *Aspergillus* and *Fusarium* species, and are a growing concern in stored cereals (Misihairabgwi et al., 2019). Among these molecules, aflatoxins produced by *Aspergillus* and fumonisins produced by *Fusarium* are bioactive compounds with well-documented health risks (Omotayo et al., 2019). Observed effects range from acute food poisoning causing death to chronic effects such as immunosuppression and carcinogenicity (Yu and Pedroso, 2023).

In the DRC, regulatory oversight and routine screening of the presence of these pathogens and their associated metabolites are non-existent, especially in rural communities such as those in the Tshopo province (Stathers et al., 2020). Consequently, the population is particularly vulnerable to the effects of mycotoxin exposure. Despite this threat, data on mycotoxin prevalence and its correlation with storage practices and insect infestation in the Tshopo province have yet to be determined. The perception of post-harvest losses among cultivators diverges from measurable realities, as many farmers believe that only a small fraction of their stored grain is lost, whereas field measurements reveal substantially higher quantitative and qualitative losses (Midéga et al., 2016). In particular, farmers tend to underestimate both the reduction in grain dry weight and volume and the deterioration of germination capacity and nutritional value that occur during storage. (Kaminski and Christiaensen, 2014). The gap in awareness and perception among these farmers can lead to risky storage management, as traditional practices are rudimentary and ill-suited to the humid tropical climate. Traditional granaries, sacks, and open storage structures indeed offer limited protection against pests (Ndegwa et al., 2016).

Despite the recognized importance of these issues in tropical cereal storage, there is a striking lack of published data in Central Africa, the DRC, or the Tshopo province. To date, no comprehensive surveys have described the entomofauna or fungal communities associated with stored rice and maize in the DRC, particularly in the Tshopo province, nor their combined impact on post-harvest losses (Stathers et al., 2020). This absence of baseline information represents a critical knowledge gap, hindering the development of effective and locally adapted storage management strategies.

This study aims to fill these knowledge gaps by documenting the entomofauna and mycotoxin-producing fungi present in stored rice and maize, quantifying mycotoxin accumulation, and assessing the extent of storage losses under typical local conditions in Tshopo province. The findings will provide a crucial scientific foundation for designing interventions to mitigate post-harvest losses and enhance food security in the region.

2. Material and methods

2.1. Study area

2.2. Sampling of stored cereals

Sampling was conducted across nine representative storage sites distributed within three principal zones of the Tshopo province (cf. Fig. 1): Yangambi, Yabaondo, and Yanonge, each zone comprising three distinct storage locations. Sampling of the cereals took place at three time points: immediately after storage initiation (day 0), as well as after 30 and 60 days of grain storage. For each sampling, six subsamples of 0.4 kg to 0.5 kg, depending on grain density, were collected from different points within each storage unit to account for spatial heterogeneity. All sampled storage units were located inside traditional dwellings with mud walls and roofs made of dried plant material or corrugated iron sheets. At all sampled sites, both paddy rice and maize were already threshed and stored as loose grain in sacks, rather than remaining on the cob or panicle. These subsamples were thoroughly mixed to form a single 2.5 kg composite sample for each site and time point. Immediately after collection at each storage site, grain samples were placed in rigid plastic boxes whose walls and lids were perforated to allow air exchange and prevent hypoxia-related mortality of live insects during transport. The time between collection in the field and consolidation of samples at the laboratory never exceeded 3 days, so that all samples experienced comparable holding times and remained representative of their initial condition. Upon reception at the laboratory, each composite sample was homogenized and partitioned; one portion was immediately stored at -18°C to arrest fungal development and mycotoxin biosynthesis, while the remaining fractions were kept under controlled conditions for entomological analysis and post-harvest loss evaluation. These handling procedures were designed to minimize artefactual changes during transport.

The composite sample was then subdivided for specialized analyses. Of the composite, 1.5 kg was reserved for insect emergence studies to enable quantification and identification of storage pest populations. A 0.5 kg portion was ground and homogenized for mycotoxin quantification, ensuring a representative assessment of fungal metabolite contamination. The remaining 0.5 kg was allocated for characterization, including grain quality determination, damage assessment, grain moisture determination, and conducting germination tests.

2.3. Entomofauna assessment

From the original sample, 1,5 kg of grains were sieved using a series of analytical sieves from 2×2 cm to 1×1 mm. The procedure enabled the separation of insects from grains. For each sample, the procedure was repeated weekly to monitor the emergence of adults from the grains over a period of three weeks. Insects were stored in a 70% (v/v) ethanol solution.

The insects were identified based on observation of morphometric parameters, using reference identification keys by Delvare and Aberlenc (1989), Delobel and Tran (1993), and Goergen et al. (2005), as well as the Hexapoda Collection of the University of Liège.

For every major morphospecies identified in each site, a molecular validation was performed. DNA was extracted from six different specimens, respectively, for each morphospecies using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the supplier's instructions. PCR amplification was based on the COI region of mitochondrial DNA using the primer pair LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTT-CAGGGTGACCAAAAAATCA-3' in a reaction volume containing 25 μL of Q5 High-Fidelity 2X Master Mix (New England Biolabs, Hitchin, UK), 2.5 μL of each primer (10 μM), and 5 μL genomic DNA (20 ng/ μL).

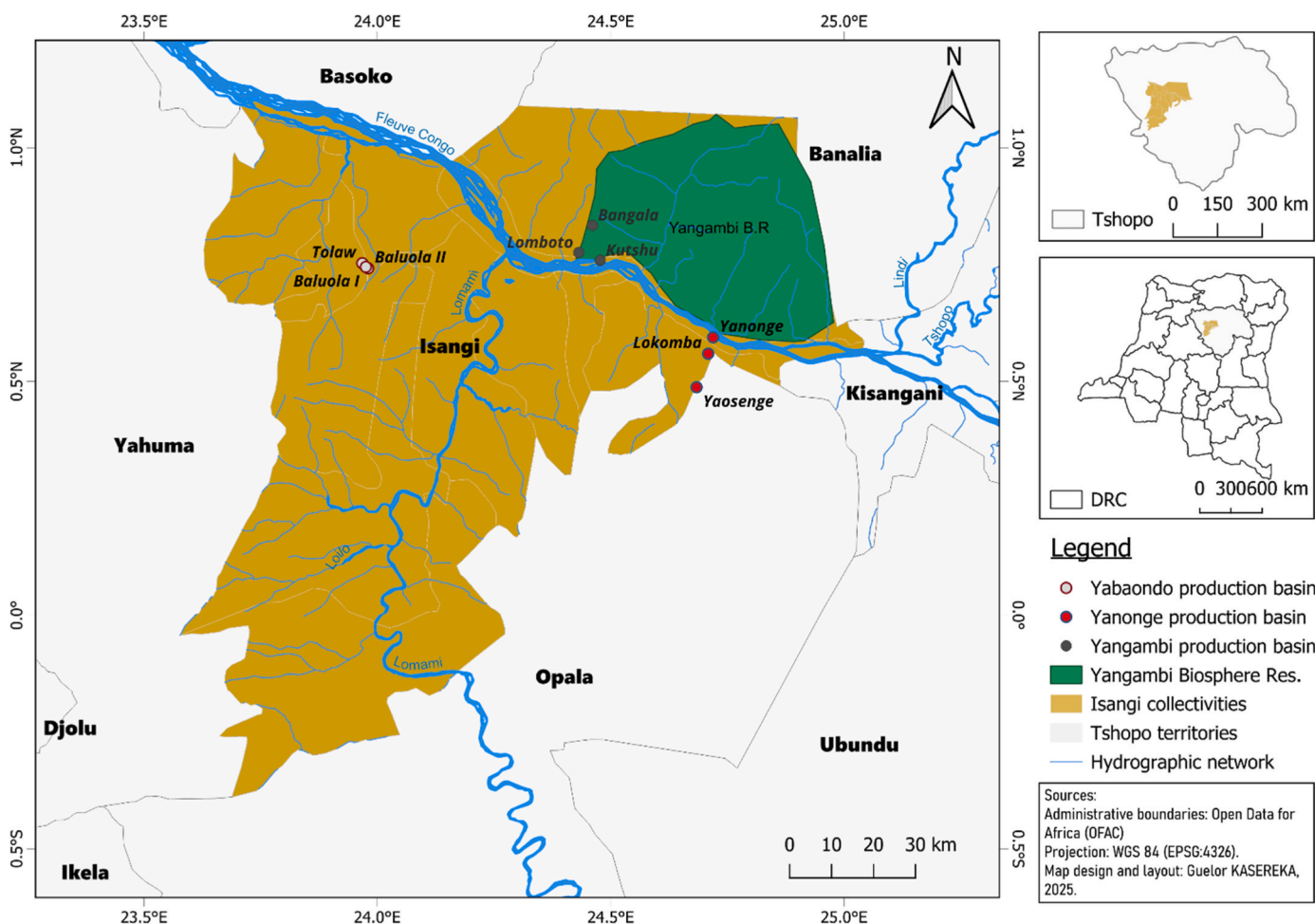


Fig. 1. Geographic distribution of the nine cereal storage sites investigated across the three main zones of the Tshopo province, Democratic Republic of Congo: Yangambi, Yabaondo, and Yanonge. Map created by the authors using ArcGIS software.

Amplification cycle parameters were optimized differently depending on insect taxa. For *Lepidoptera*, PCR cycling parameters were set as follows: an initial denaturation of 30 s at 98 °C, followed by 35 cycles of 10 s at 98 °C, 30 s at 54 °C, and 30 s at 72 °C. Finally, a last extension was done at 72 °C for 2 min. For *Coleoptera*, the PCR cycling parameters were set as follows: an initial denaturation of 30 s at 98 °C, followed by 40 cycles of 10 s denaturation at 98 °C, 30 s of annealing at 48 °C, and 30 s of extension at 72 °C. Finally, a last extension was done at 72 °C for 2 min. Obtained amplicons were run on 1% agarose in TAE buffer by electrophoresis (110 V, 40 min) and visualized using SYBR Safe (Invitrogen, Carlsbad, CA, USA) in a Bio-Rad Universal Hood II Gel Doc Imaging System. Before entering the sequencing procedure, the amplicons were purified using the PCR Clean-Up Kit (Macherey-Nagel, Düren, Germany) according to the supplier's instructions. Eurofins Genomics (Ebersberg, Germany) performed the sequencing using the Sanger method. Finally, using the National Center for Biotechnology Information (NCBI) database and the Basic Local Alignment Search Tool (BLAST), species identities were confirmed.

2.4. Mycotoxin quantification and validation

Before analyte extraction, 500 g of sampled maize and rice were ground and sieved to a granulometry of 0,5 x 0,5 mm. Mycotoxin quantification was performed using the RIDA® QUICK Aflatoxin RQS ECO and RIDA® QUICK Fumonisin RQS ECO (R-Biopharm, Darmstadt, Germany) immunochromatographic tests, following the procedure recommended by the supplier. For each sample, the quantification of both

aflatoxin and fumonisin was done in three repetitions. The scanning of the immunochromatic tests was done using the RIDA® SMART APP. The aflatoxin and fumonisin contents were then respectively calculated using formulas (1) and (2).

$$Aflatoxin\ content\ (ppb) = \frac{W}{10} \times AC_m \times \frac{100}{dW} \tag{1}$$

In (1), *W* is the measured weight of the powdered sample, *AC_m* is the aflatoxin content as measured by the scanner, and *dW* is the dry matter content of the sample.

$$Fumonisin\ content\ (ppm) = \frac{W}{5} \times FC_m \times \frac{100}{dW} \tag{2}$$

In (2), *W* is the measured weight of the powdered sample, *FC_m* is the fumonisin content measured by the scanner, and *dW* is the dry matter content of the sample.

A certified laboratory performed the validation of the quantification, CER Group (Marloie, Belgium, BELAC accreditation number 073-TEST). During validation, UHPLC-MS/MS was employed to quantify aflatoxin B1, B2, G1, and G2, as well as fumonisin B1, B2, and B3, thereby validating the immunochromatographic tests used during the survey.

2.5. Physical parameters

2.5.1. Grain damage

Damaged grain was assessed visually using one hundred grains per repetition (n = 3). The grains were randomly taken from each sample

and inspected under a handheld magnifying glass. Grains showing signs of damage were separated from the undamaged grains. The damaged grains were counted, and the percentage of damaged grains was calculated.

2.5.2. Grain moisture content

Grain moisture (% wet basis) was assessed through gravimetric measurement (Coradi et al., 2020) using one hundred grains per repetition ($n = 3$). Grain moisture and dry grain weight were measured for every composite sample at each sampling date of 0, 30 and 60 days of storage. The grains were randomly selected from each sample and weighed to the nearest 0.01 g before being placed in a drying oven at 105 °C for 24 h. After drying, the grains were weighed again, and the moisture content was calculated using formula (3).

$$\text{Moisture content (\%wb)} = \frac{wW - dW}{wW} \times 100 \quad (3)$$

In (3), wW was the weight of grains before drying, and dW was the dry weight of the grains.

2.5.3. Dry grain weight

Dry grain weight was determined gravimetrically using an adapted version of the thousand grain mass method (Boxall, 1986) with three replicates of 100 grains per sample. For each replicate, one hundred grains were randomly selected and weighed to the nearest 0.01 g. The corresponding dry grain weight was obtained by correcting this value using the measured moisture content on a wet basis (%wb) according to formula (4).

$$\text{Dry grain weight (g)} = GW_{100} \times \left(1 - \frac{\%wb}{100}\right) \quad (4)$$

where GW_{100} is the total mass of 100 grains at the time of weighing and %wb is the moisture content of the grains expressed on a wet basis.

2.6. Seed viability

For the viability assessment, twenty seeds of maize or rice were soaked in distilled water for 30 min, placed in petri dishes containing Whatman grade 1 filter paper (Merck Life Science, Hoeilaart, Belgium) and soaked with 10 mL of distilled water. Germination was observed after 6 days for each repetition ($n = 5$). Germination rate is calculated using formula (5).

$$\text{Germination rate (\%)} = \frac{n_g}{20} \times 100 \quad (5)$$

In (5), n_g was the number of germinated seeds observed.

2.7. Statistical analysis and data visualization

All statistical analyses were performed using RStudio. The packages tidyverse, emmeans, and readr were used for data manipulation and analysis. Group comparisons were conducted using analysis of variance (ANOVA) followed by Tukey's post hoc test to determine statistically significant differences between treatments. Non-parametric tests of Kruskal-Wallis and Dunn's post-hoc tests were done on a population that did not respect ANOVA's necessary conditions. Pearson correlation coefficients were calculated to evaluate the relationships between measured variables of interest.

Data visualization was carried out in RStudio using the ggplot2, ggpattern, patchwork, multcompView, and corrplot packages.

Geographic data analysis and mapping were performed using ArcGIS. Location data were collected in the field using GPS-enabled smartphones during the installation of storage sites. These GPS coordinates were integrated with shapefiles obtained from the OCHA (United Nations Office for the Coordination of Humanitarian Affairs)

and RGD.CD (Référentiel Géographique de la RDC) databases. The processed spatial data were used to generate site maps and visualize the geographic distribution of storage installations.

3. Results

3.1. Entomofauna diversity, abundance, and dispersion in storage sites

To document the entomofauna associated with stored cereals, both molecular and morphological approaches were combined to ensure accurate identification of the insect species present in the stored products. Accurate species identification provided the basis for subsequent analysis of abundance, population dynamics, and geographical distribution within the sampled stored rice and maize. Molecular identification was necessary for two taxa: *Coleoptera* specimens from the *Sitophilus* genus and *Microlepidoptera*.

All *Sitophilus* specimens belonged to *Sitophilus. zeamais*, Motschulsky 1855 and all *Microlepidoptera* insects were *Sitotroga cerealella* (Olivier 1789) *Tribolium castaneum* (Herbst 1797) could be clearly identified by its morphological characteristics and did not require molecular confirmation.

Overall, *S. zeamais*, *S. cerealella*, and *T. castaneum* accounted for more than 98% of the total insect fauna recorded in both crops and are discussed in this study (Fig. 2). Other, less common species were observed only occasionally and are listed in Supplementary Data Table 1.

Overall, *S. zeamais* was detected in all storage sites and in both cereals, frequently reaching values exceeding 100 individuals per kilogram of stored cereals, highlighting its predominance as a primary stored product pest in post-harvest infestations (Fig. 2). Additionally, *S. cerealella* was found in stored rice, particularly in samples from Yangambi, whereas *T. castaneum* was present at most sites but always at significantly lower densities compared to *S. zeamais*.

Regarding rice samples in particular, *S. zeamais* (Fig. 2A) populations remained consistently high from Yangambi throughout the storage period, whereas samples from Yabaondo and Yanonge exhibited lower and more variable infestations. Across all zones and time points, population sizes ranged broadly, from as low as ~20 individuals per kg to over 200. Despite these fluctuations, no significant differences were found between zones and timings. In contrast, *S. cerealella* (Fig. 2B) populations exhibited a distinct spatial trend, with rice from Yangambi showing significantly higher infestations compared to near-absence in samples from Yabaondo and Yanonge throughout the 60-day monitoring period for stored rice infestations. *Tribolium castaneum* was generally rare in rice across all sites and time points (Fig. 2C). It was absent from rice samples from Yangambi, occasionally present in low numbers in rice samples from Yanonge, and significantly more observed in the rice samples from Yabaondo.

Regarding stored maize, *S. zeamais* populations were significantly higher in stored maize from Yangambi (Fig. 2D). Indeed, those populations fluctuated around 200 individuals per kilogram of stored grains during the 60-day monitoring period. Coming in second were *S. zeamais* populations in stored maize of Yabaondo, which reached a maximum of 95 adults per kg after 30 days and then return to their initial count after 60 days. *S. cerealella* populations showed a spatial trend, with maize from Yabaondo having significantly higher infestations compared to near absence in samples from Yangambi and Yanonge throughout the 60 days of monitoring the infestations in stored maize (Fig. 2E). However, in general, *S. cerealella* populations in maize remained very low and rarely exceeded 10 adults per kilogram. *T. castaneum* individuals were very rare in maize across all sites and time points. Even if some individuals were detected in every zone monitored, they were present in small quantities (Fig. 2D).

3.2. Mycotoxin accumulation

Analyses revealed distinct patterns of aflatoxin and fumonisin

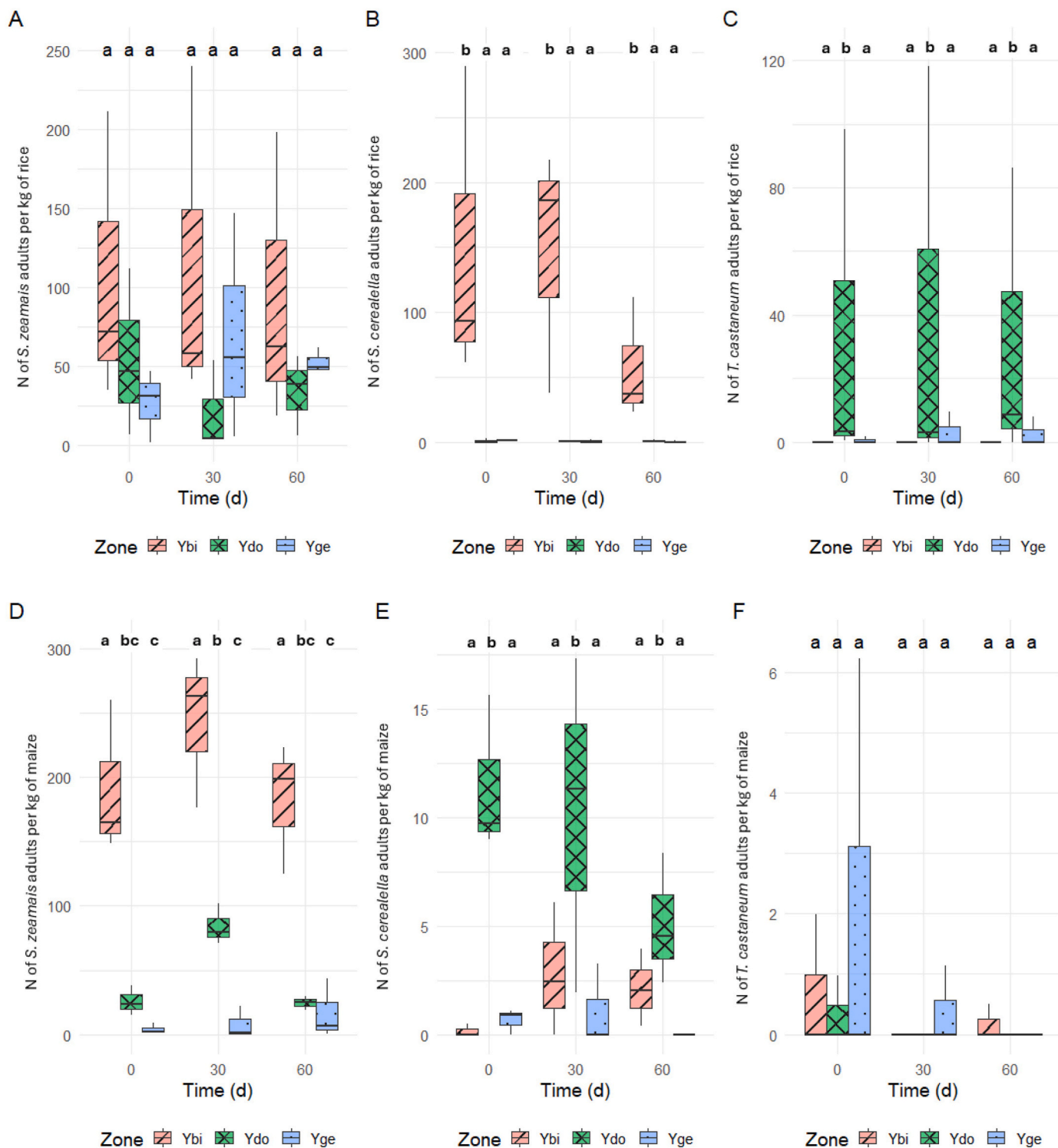


Fig. 2. Boxplots showing the density (number of insects per kg of grain) of *Sitophilus zeamais* (A), *Sitotroga cerealella* (B), and *Tribolium castaneum* (C) in stored rice samples and *Sitophilus zeamais* (D), *Sitotroga cerealella* (E), and *Tribolium castaneum* (F) in stored maize samples across the sampling zones, Yangambi (Ybi), Yabaondo (Ydo), and Yanonge (Yge), and sampling times of 0, 30, and 60 days. Boxes indicate the interquartile range (IQR), horizontal lines represent medians, whiskers show the full range of observed values, and measurements were done in triplicate. A and D were analysed using ANOVA and Tukey's post-hoc test; B, C, E, and F were analysed using Kruskal-Wallis and Dunn's post-hoc test. Different lower-case letters above boxplots indicate statistically significant differences between groups at $\alpha = 0.05$.

contamination dynamics between crops, zones, and sampling times (Fig. 3). Overall, aflatoxins showed substantial temporal and spatial variation in both crops, with Yabaondo being the most contaminated zone at the onset of storage. By contrast, fumonisin occurrence was crop- and zone-specific, with consistently negligible levels in rice and more

pronounced contamination in maize, but always remained under the EU regulatory concentration of 4 ppm.

In rice, aflatoxin levels differed significantly between zones at the start of storage; however, values converged by 60 days to uniformly low concentrations, with no significant differences remaining between sites.

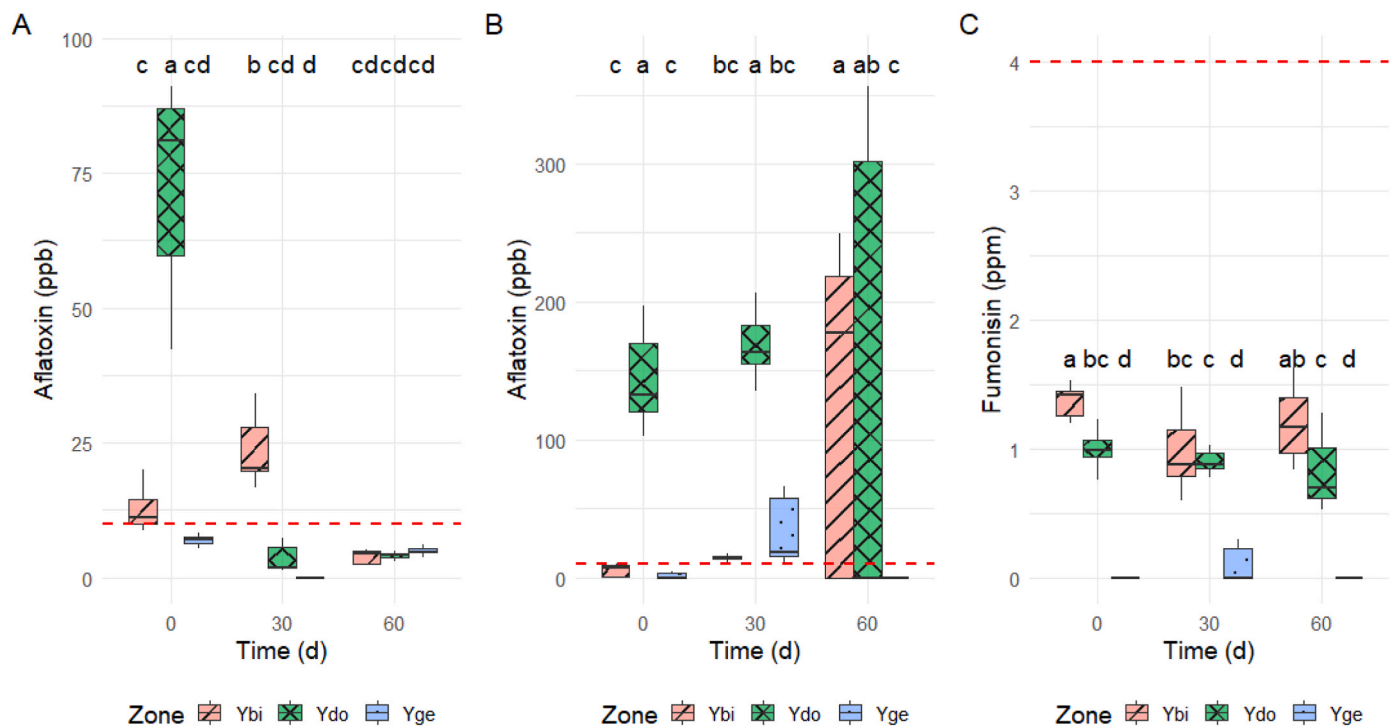


Fig. 3. Boxplots showing the concentrations of aflatoxins in rice (ppb) (A), aflatoxins in maize (ppb) (B), and fumonisins in maize (ppm) (C) across the sampling zones Yangambi (Ybi), Yabaondo (Ydo), and Yanonge (Yge) and sampling times of 0, 30 and 60 days. Boxes indicate the interquartile range (IQR), horizontal lines represent medians, whiskers show the full range of observed values, measurements are done in triplicate. Data were analysed using ANOVA and Tukey's post-hoc test. Red dotted lines indicate EU regulation thresholds of 10 ppb for aflatoxins and 4 ppm for fumonisins. Different lower-case letters above boxplots indicate statistically significant differences between groups at $\alpha = 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

In maize, contamination was more severe and persistent: storage sites in Yabaondo were heavily affected from the beginning, whereas samples from Yanonge and Yangambi experienced significant increases in contamination during storage after the first 30 days (Fig. 3A and B). After 60 days, maize grains in Yangambi and Yabaondo exhibited similarly high levels above the EU regulatory threshold of 10 ppb. In contrast, no aflatoxins were detected in maize from Yanonge in the final sampling.

In rice, fumonisin was virtually absent, with only rare detections at trace levels in 2 samples. In maize, however, fumonisin contamination was widespread, and it was specifically higher in certain zones (Fig. 3C). While maize from Yanonge remained consistently and significantly unaffected, the concentration of fumonisin measured in samples from Yabaondo was significantly higher and stable at around one ppm throughout the monitoring period. Finally, samples from Yangambi exhibited the highest fumonisin concentrations at the start of storage, with levels stabilizing near 1 ppm over the subsequent 30 to 60 days of monitoring.

3.3. Storage loss dynamics

Seed quality and quantity losses were measured during a 60-day storage period at three sampling times (day 0, 30, and 60) in three zones (Yanonge, Yangambi, and Yabaondo), using germination, dry grain weight, and the proportion of intact grains as indicators for both rice and maize (Fig. 4).

Rice initial germination (Fig. 4A) capacity was overall poor, with averages around 50% in Yangambi's and Yanonge's storage sites. Grains had particularly weak initial germination when originating from the Yabaondo's sites, significantly lower than those from the other storage zones. This disadvantage persisted after 30 days. By 60 days, grains from Yanonge exhibited a marked decline in germination capacity, aligning

with the low levels already observed in Yabaondo, whereas Yangambi samples remained more stable. Dry grain weight remained steady during the first 30 days across all sites (Fig. 4B). After 60 days, however, grains stored in Yangambi exhibited a significant weight loss compared with those from Yabaondo, indicating stronger deterioration under these conditions. Integrity of the rice grains (Fig. 4C) also varied significantly. At the start of storage, grains from Yangambi already showed significantly reduced integrity compared with those from the other sites, and this disadvantage was maintained throughout the experiment. By 30 days, all storage environments exhibited significant declines in the proportion of intact grains, confirming that physical degradation of rice develops rapidly under storage conditions. In rice, grain moisture content remained relatively similar between zones at each sampling time, with only modest differences among sites. Across all zones, a general decrease in grain moisture was observed between the 30 and 60 days of storage period, indicating progressive drying of stored rice. The phenomenon was especially pronounced in samples originating from Yanonge.

Maize grains exhibited high initial germination across all storage environments, averaging above 75% (Fig. 4E). The highest initial values were observed in Yanonge, while Yangambi grains started at slightly lower levels. Germination remained largely stable during the first 30 days for Yanonge and Yabaondo, but grains stored in Yangambi showed an early and significant decline. By 60 days, all storage conditions showed marked reductions in germination capacity. Losses were most severe in Yangambi, where values dropped by more than 60% from the baseline. At the same time, grains from Yanonge and Yabaondo retained comparatively higher viability, though still at significantly reduced levels.

Dry grain weight differed significantly across storage environments and followed a general trend of gradual decline (Fig. 4F). Grains from Yanonge consistently had the highest weights, maintaining near-

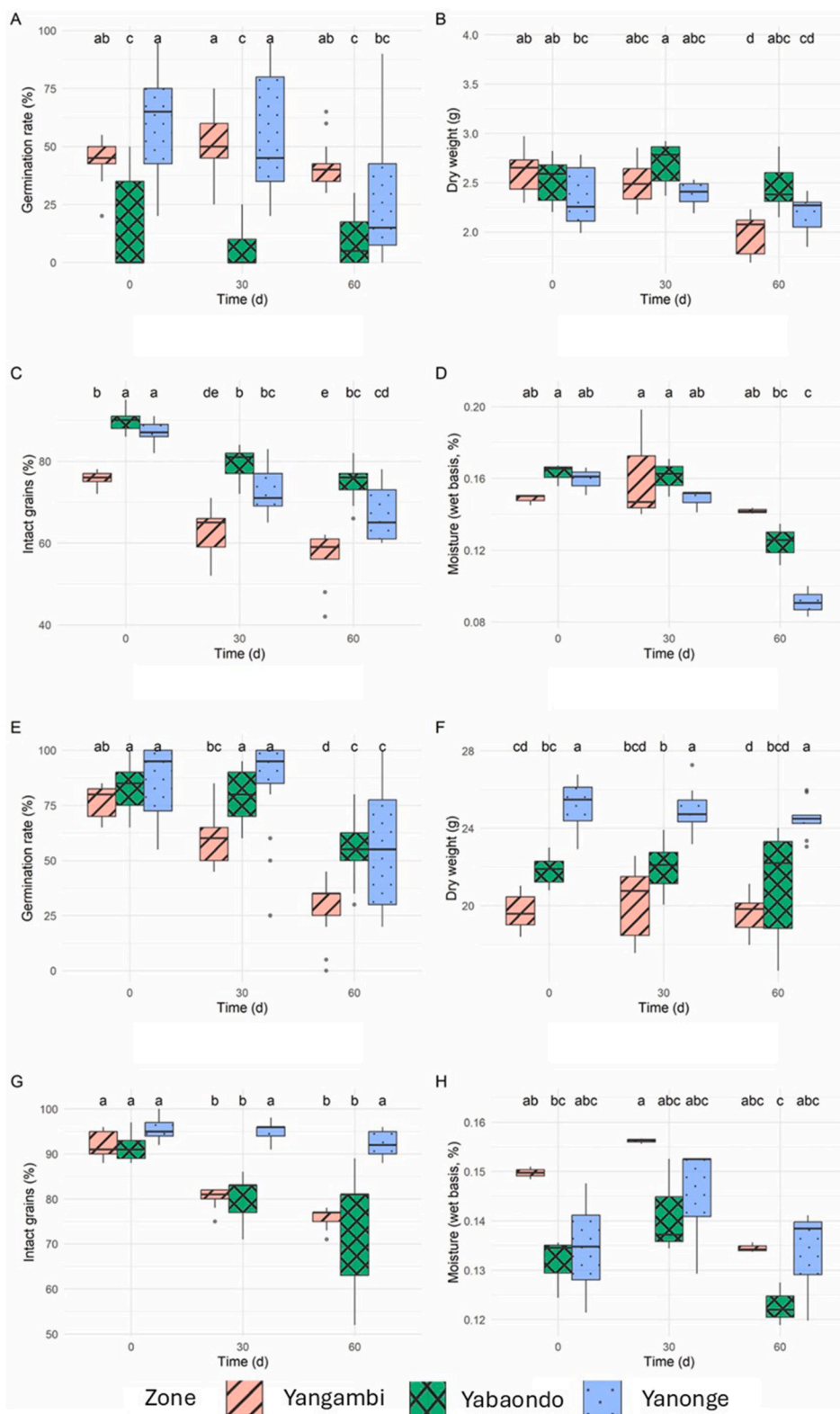


Fig. 4. Boxplots showing the germination rate (%) (A), dry weight of 100 grains (g) (B), and percentage of intact grains (%) (C) in stored rice samples and the germination rate (%) (E), moisture content in rice (D), dry weight of 100 grains (g) (F), and percentage of intact grains (%) (G) and moisture content (H) in stored maize samples across the sampling zones Yangambi (Ybi), Yabaondo (Ydo) and Yanonge (Yge) and sampling times of 0, 30 and 60 days. Boxes indicate the interquartile range (IQR), horizontal lines represent medians, whiskers show the full range of observed values, dots indicate outlier values, measurements are performed in triplicate for B, C, D, F, G and n = 5 technical repetitions for A and E. Different lower-case letters above boxplots indicate statistically significant differences between groups at $\alpha = 0.05$.

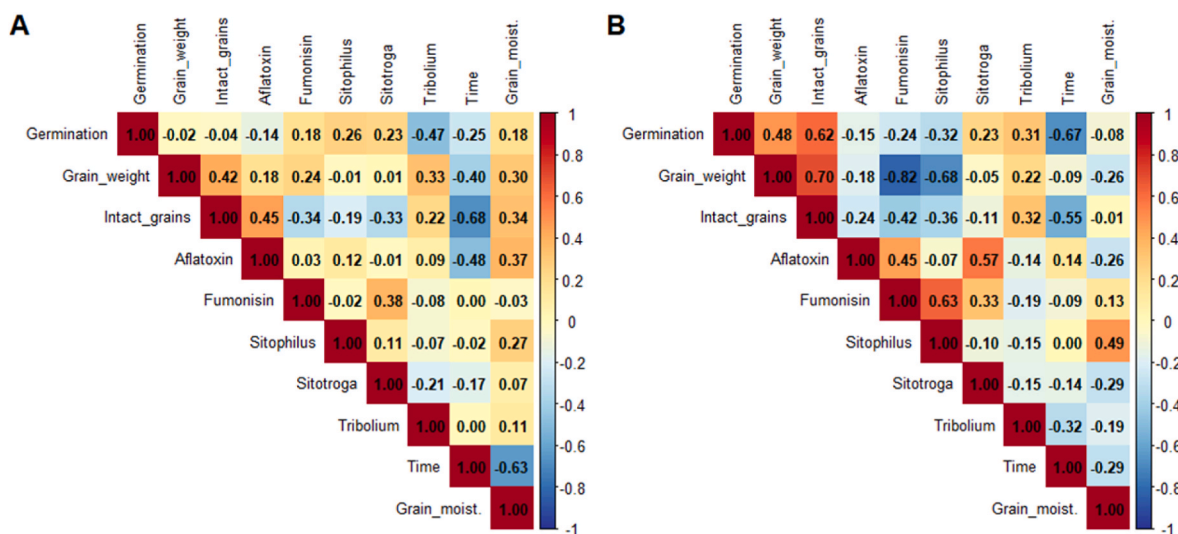


Fig. 5. Pearson's correlation heatmap of variables observed in stored rice (A) and maize (B).

stability over the 60 days. Yabaondo grains showed slight variation during the first 30 days and only modest reductions by day 60. In contrast, Yangambi grains experienced the most significant cumulative weight loss, ultimately reaching the lowest levels despite a temporary stabilization at mid-storage.

The integrity of maize grains was initially high across all sites, with more than 90% intact grains and no significant differences observed at the outset (Fig. 4G). After 30 days, Yanonge grains retained nearly complete integrity, while the Yangambi and Yabaondo samples deteriorated more rapidly, both dropping to around 80%. By 60 days, Yanonge grains still retained more than 90% of their integrity, highlighting their strong resilience during storage. Yangambi and Yabaondo, however, continued to decline, showing the lowest proportions of intact grains at the final sampling point. In maize, grain moisture content (Fig. 4H) also remained relatively similar between zones at each sampling time, with only modest differences among sites apart from Yangambi being systematically higher than samples from other sampling areas. Across all zones, a moderate decrease in grain moisture was observed between 30 and 60 days of storage, indicating progressive but less pronounced drying of stored maize compared with rice.

3.4. Correlation analysis

In rice (Fig. 5A), storage time was negatively correlated with grain integrity ($r = -0.68$), grain moisture content ($r = -0.63$), dry weight ($r = -0.40$), and aflatoxin content ($r = -0.48$), indicating overall deterioration and partial drying with increasing duration. Dry grain weight was moderately associated with the proportion of intact grains ($r = 0.42$), and aflatoxin levels were likewise positively related to intact grain proportion ($r = 0.45$). Insect pressure also played a role, with higher *Tribolium* abundance linked to reduced germination ($r = -0.47$).

In maize (Fig. 5B), dry grain weight was strongly negatively correlated with fumonisin content ($r = -0.82$) and *Sitophilus* abundance ($r = -0.68$) but positively associated with the proportion of intact grains ($r = 0.70$). Germination declined with relatively high correlation with storage time ($r = -0.67$) and was positively related to grain integrity ($r = 0.62$). The proportion of intact grains also decreased over time ($r = -0.55$). In terms of pest-toxin interactions, fumonisin levels increased with *Sitophilus* abundance ($r = 0.63$), while aflatoxin was positively associated with *Sitotroga* presence ($r = 0.57$). Grain moisture content was moderately correlated with *Sitophilus* populations ($r = 0.49$).

4. Discussion

Our study provides the first comprehensive inventory of entomofauna associated with stored rice and maize in the Tshopo province. Insect pest diversity in local storage environments was dominated by *S. zeamais*, *S. cerealella*, and *T. castaneum*, which together account for more than 98% of collected specimens. Molecular and morphological identification techniques have allowed precise species confirmation and clarified the composition of local storage pest communities. This confirms the working hypothesis: storage losses in maize and rice in Tshopo province are primarily associated with a narrow but highly destructive set of insect pests.

Our findings represent a breakthrough in the knowledge of storage pests in Central Africa, specifically in the DRC, where baseline data are scarce. Previous studies across sub-Saharan Africa have established *S. zeamais* as a primary agent of post-harvest loss (CABI, 2021), with its geographical distribution expanding due to regional and international trade, as well as adaptation to climatic changes (Ghareeb et al., 2025). *S. cerealella* is increasingly recognized as a major pest of rice and maize in tropical storage, sometimes overlooked due to its cryptic life cycle and the challenges to identify *microlepidoptera* morphometrically (Salim et al., 2023). The capacity of *T. castaneum* to colonize a broad spectrum of cereal products globally, particularly when stored under inadequate and unhygienic conditions, is well-documented (Campbell et al., 2022). Our results are therefore clearly consistent with continental trends, but extend them by confirming the presence and dominance of these species in a region for which no prior published records existed. By establishing new occurrence records for *S. zeamais*, *S. cerealella*, and *T. castaneum*, this study delivered baseline data urgently needed for national and regional management strategies. These results will have significant implications for food safety policy, extension services, and targeted pest management programs. However, the importance of identifying and reporting these species comes with the caveat that our research was restricted to rice and maize stored in selected rural sites of the Tshopo Province, which limits the broader generalization of our findings. Despite these constraints, the combined use of molecular and morphological identification, together with randomized site selection, ensures robust representation of principal storage pests.

The occurrence of *S. zeamais* in all rice samples, with populations consistently high throughout the three production areas, confirms it as the most ubiquitous storage pest in tropical Africa (Ndiaye and Sembène, 2018). This phenomenon has been well documented, for example, in Senegal (Diop et al., 2021), where *S. zeamais* was found infesting all stored maize varieties, and in Malawi (Kamanula et al.,

2017), where it accounts for a significant portion of post-harvest maize damage, regardless of the storage structure. It was not surprising, therefore, that *S. zeamais* was recovered from every storage location and throughout the storage period. This reflects its capacity for infestation, starting in the field and continuing throughout the entire post-harvest process, leading to eventual storage, as well as its behavioral tendency to move between bags and sites once storage is ongoing (Makundi et al., 2010). Infestations typically begin with a low population count and increase exponentially due to their short life cycle of 25 to 30 days and high reproductive capacity (Makundi et al., 2010). However, this exponential growth was not observed; instead, the population would stabilize on a plateau. This suggests that factors within the stored grains may limit growth and development, such as depletion of the food source or changes in grain quality. More likely, given that traditional storage bags are highly permeable (Edoh Ognakossan et al., 2012), adult weevils may disperse from density infested bags to find new food sources and less competitive environments, thus limiting local population growth. Supporting this hypothesis, *S. zeamais* adults are known to detect both the odours of rice grains and aggregation pheromones emitted by conspecifics (Bandeira et al., 2021; Stuhl and Romero, 2021; Ukeh et al., 2012). This chemical sensing mechanism likely facilitates movement away from overinfested and depleted bags, causing densities to stabilize rather than grow indefinitely until all the available cereals are consumed.

In stored maize, *S. zeamais* populations reached a clear maximum after 30 days of storage before returning to initial levels by 60 days. This dynamic reflects the same fundamental population regulation processes observed in rice storage, but amplified due to the physical, nutritional, and olfactory properties of maize. Indeed, maize grains have larger intergranular spaces compared to rice, providing greater mobility for adult weevils (Murugesan et al., 2024). Moreover, maize is more suitable and preferred by *S. zeamais* as a host due to its nutritional characteristics, which significantly improve reproduction rates (Stuhl and Romero, 2021). Consequently, it is suspected that the population builds up faster and reaches higher than in rice, explaining the observed peaks. The decline after the population peak is unlikely to result from mortality since dead weevils would still be sampled and accounted for. Instead, it also points to the exact dispersal mechanisms as explained above. Indeed, the grains are kept in the same kind of permeable bags, and maize does possess a strong olfactory attractiveness for this species.

Therefore, the observed *S. zeamais* population dynamics reflect a balance between reproductive potential, resource availability, and dispersal. This strongly highlights the importance of controlling within-bag infestations and inter-bag movement to reduce post-harvest losses.

Our results regarding *S. cerealella* show a distinct difference in infestation depending on the grain substrate, but most importantly, the location of the samples. This change was detected throughout the three production zones and during the whole experimentation. *S. cerealella* was never described in the Tshopo province. Still, it is known as a widespread pest of stored cereals with a nearly cosmopolitan distribution, thriving primarily in temperate and tropical climates worldwide (Berhe et al., 2022). As the environmental parameters are very similar throughout the monitored region, it is reasonably expected that all populations would grow simultaneously during the 60-day experimentation period. Nevertheless, a steady adult population was observed in the infested storage sites. Interestingly, all the sites with large *S. cerealella* infestations were storage sites where the cultivators would systematically leave the storage bags open, providing adult moths with surface access for mating and oviposition. Importantly, *S. cerealella* adults are less able to penetrate closed woven polypropylene storage bags commonly used in the region, explaining this distribution pattern (Vigneshwari and Venugopal, 2020). This contrasts with more robust insects, such as *S. zeamais*, which can move through the fabric (Edoh Ognakossan et al., 2012), promoting wider dispersal, precisely as observed in this context. However, this facilitated infestation for *S. cerealella* does not demonstrate why these storage sites do not see a

substantial increase in the measured population during the monitoring process. A critical consideration is the biology and behavior of *S. cerealella* adults. Unlike larvae, which develop within the grain kernels, causing direct feeding damage, adults do not feed and are primarily involved in reproduction (Salim et al., 2023). The larval stages remain concealed within the grain mass (Hossien et al., 2018), making their direct observation during monitoring unlikely given the present sampling method. Consequently, the adults captured on the surfaces of storage bags predominantly reflect mating activity rather than the total reproductive output or larval infestation levels. Therefore, assessments based solely on adult captures can underestimate the actual infestation level within grain masses unless complemented with more invasive sampling targeting larvae inside kernels. This surface mating and subsequent flight for colonizing new sites explain the steady adult populations observed, despite not detecting an increase in internal infestation.

The presence of *T. castaneum* across multiple sampling locations is consistent with its recognized global distribution as a ubiquitous pest of stored products (Campbell et al., 2022). This species is widely established in food storage and processing facilities worldwide, making its detection in even small numbers unsurprising given its dispersion capacity and association with diverse grain environments (Awadalla et al., 2023).

In this study, *T. castaneum* populations were generally low, indicating minor infestations that are unlikely to pose immediate threats to stored grain integrity. However, the relatively extended larval developmental period typical of this species suggests that population increases may manifest later (Duarte et al., 2021), necessitating vigilant monitoring to detect potential surges. The low but persistent presence of *T. castaneum* also signals that hygiene standards within the storage sites are reasonably maintained, as this species often serves as an indicator of compromised sanitation and poor storage hygiene in stored-product ecosystems (Semeao et al., 2012). Therefore, the current infestation levels may reflect effective control hygiene measures limiting extensive population growth, despite the species' capacity for persistence and eventual proliferation under favourable conditions.

Although there are no current legal limits for the number of insects allowed per kilogram of cereals, research shows that infestation levels as low as 100 maize weevils per kilogram can lead to up to 20% loss in grain weight and a 35% decrease in intact stored grains within three months (Saeed and Laing, 2023). Our results regarding mycotoxin quantification demonstrate the presence of aflatoxin and fumonisin contamination in rice and maize samples, respectively, confirming the activity of *Aspergillus* sp. and *Fusarium* sp. (Gao et al., 2023; Kumar et al., 2021). These genera are well documented as primary producers of these mycotoxins worldwide. Although species-level identification was beyond the reach of our analytical approach, the detection itself is a substantial finding given the scarcity of prior comprehensive mycotoxin monitoring in this region. The presence of these toxins signifies a critical food safety threat with far-reaching implications for public health (Gao et al., 2023; Kumar et al., 2021).

Temporal variation of aflatoxin levels in rice reveals a counterintuitive decrease over the storage period, despite established knowledge regarding the chemical stability and thermal resistance of these compounds under normal storage conditions (Mostashari et al., 2021). This observation plausibly reflects fungal colonization dynamics, whereby initial contamination likely originates from pre-harvest stages or immediate post-harvest conditions characterized by high moisture content, which promote fungal proliferation and mycotoxin biosynthesis (Kumar et al., 2021). In addition, the grain moisture levels measured during storage were practically equal or below the threshold of 14 % of wet basis generally considered critical for consequent growth of *Aspergillus* (Wieman et al., 1986) and *Fusarium* (Marín et al., 2004) and their mycotoxin production in cereals. Consequently, much of the aflatoxin and fumonisin contamination detected in both rice and maize likely originates from pre-storage stages, when grain moisture was higher and

drying incomplete, rather than from extensive toxin synthesis during the monitored storage period. The subsequent decline in detectable aflatoxin levels could be attributed to degradation mechanisms (Song et al., 2024). These observations necessitate further investigation to clarify the underlying biochemical or environmental drivers, such as microbial and fungal enzymatic activity or insect consumption, that can effectively decrease the risk of aflatoxin poisoning for consumers.

The observed pattern of aflatoxin contamination in maize, with severe and persistent levels in Yabaondo from the beginning of storage, was consistent with expectations based on the understanding that pre-harvest and immediate post-harvest conditions critically influence initial fungal contamination (Alaniz Zanon et al., 2022). Such early high contamination likely reflects prior fungal colonization exacerbated by inadequate drying or insect pest damage before storage. In comparison, the delayed but significant increases in aflatoxin levels in Yanonge and Yangambi after 30 days of storage correspond to the well-documented post-harvest fungal dynamics, where temperature and high air humidity inside the storage facilities favour fungal growth and toxin accumulation over time (Kumar et al., 2021). At the end of the monitoring, maize grains from Yangambi and Yabaondo exhibited similarly high aflatoxin levels, exceeding the EU regulatory threshold of 10 ppb. This indicates that prolonged storage under suboptimal conditions facilitates toxin accumulation to levels hazardous for human health. In line with this, the only marked increase in aflatoxin levels was observed in the maize storage area of Yangambi, during the period when grain moisture reached the critical range for aflatoxin B₁ production by *Aspergillus flavus* reported in the literature (Wieman et al., 1986). This supports the idea that moisture near these thresholds can trigger localized mycotoxin production during storage, in addition to pre-storage contamination.

In comparison, the contrasting result from Yanonge, where no aflatoxins were detected at the final sampling, was notable and suggests that local factors, probably similar to the phenomena observed in stored rice, may have inhibited fungal proliferation and toxin biosynthesis while promoting biological degradation (Mostashari et al., 2021; Song et al., 2024). Fumonisin was not detected in rice and remained well below the EU limit of 4 ppm in maize, indicating relatively low contamination risk. However, fumonisin accumulation depends largely on *Fusarium* fungal infection during maize grain development, especially at flowering and maturation stages (Dinolfo et al., 2022). Environmental conditions such as temperature and humidity during these periods strongly influence *Fusarium* growth and mycotoxin production (Gao et al., 2023). Therefore, while current fumonisin levels are low, they may not fully reflect potential contamination risks, as favourable environmental factors during maize maturation can significantly increase fumonisin synthesis.

The observed degradation in both the quality and quantity of stored rice and maize during the 60-day storage period is consistent with expectations, given the known poor storage conditions and prevalent infestations and infections described extensively in this research. The measured indicators: germination capacity, dry grain weight, and proportion of intact grains, demonstrate significant deterioration across all storage zones. This is especially true in Yangambi and Yabaondo, which are precisely the zones with the higher insect pest population and mycotoxin accumulation levels, respectively. Correlations observed in the data further affirm this linkage: dry grain weight and integrity decrease in tandem with higher pest infestations and fungal toxin levels, while germination is negatively impacted by both insects and fungal metabolites. These intricate relationships suggest a synergistic mechanism driving stored grain deterioration, where pest activity enhances fungal colonization and toxin production (Riungu et al., 2024), and both diminish grain quality and quantity simultaneously (de Carvalho Brito et al., 2024). The exceptionally low germination capacity observed in rice from Yabaondo is a concerning anomaly that extends beyond typical storage-related deterioration. While storage pests and fungal infections undoubtedly contribute to seed quality loss, other intrinsic factors may also play a significant role in limiting germination. Poor grain maturity at harvest could result in physiologically immature seeds with

incomplete embryo development, reducing their viability and ability to germinate effectively (Olaoni et al., 2024).

Additionally, environmental stress such as excessive solar radiation during the drying process can damage seed tissues and impair germination potential (Olaoni et al., 2025). Furthermore, genetic deficiencies in the seed stock may reduce inherent germination vigour, mainly if inferior or heterogeneous varieties are used or seed renewal practices are inadequate (Sanghamitra et al., 2021). Such genetic factors manifest as uneven germination and susceptibility to deterioration.

When extrapolating these results to the area, the nearly 25% drop in dry weight of rice grains during storage highlights a substantial quantitative loss that directly diminishes food availability. Such a significant reduction in grain mass across Tshopo province, which covers nearly 200,000 square kilometres, comparable to countries like Senegal or Syria, represents a vast loss that jeopardizes both immediate food supplies and future seed stocks.

Regarding germination, the initial rice seed viability averaged around 50% in Yanonge and Yangambi and was notably lower in Yabaondo. These levels are far below what is typically considered adequate for high-quality seed, where germination rates above 85% are expected to ensure good crop establishment and growth. By 60 days, rice germination declined further, approaching critically low thresholds indicative of poor seed viability. Maize germination also dropped dramatically, by more than 60% in Yangambi, from an initial baseline above 75%, severely impairing its potential for successful replanting.

When viewed across the extensive area of Tshopo, these declines not only represent individual losses but cumulatively threaten regional food security, crop productivity, and livelihoods. Reduced seed quality impairs farmers' ability to regenerate crops effectively, while mass losses mean less grain for consumption and sale. Given the scale, interventions to mitigate these losses are essential to safeguard agricultural sustainability and food security.

5. Conclusions

This study provides the first comprehensive account of the insect pest complex driving post-harvest losses in stored rice and maize in Tshopo province, confirming that predominance of *S. zeamais*, primarily *S. cerealella*, and partially *T. castaneum* are highly destructive under local storage conditions. By combining molecular and morphological identification, the research addresses a critical data gap for Central Africa, offering robust evidence of the species composition and infestation patterns within a region as large as several medium-sized countries. Our results clarify that storage losses in Tshopo are primarily shaped by the synergy between pest infestations and fungal contamination, with measurable consequences for grain mass and seed viability. The pronounced declines in dry weight and germination, especially in high-risk sites, underscore the urgency of improving post-harvest management to protect food availability and agricultural sustainability. This work supported the initial hypothesis by demonstrating that a narrow set of pest species, interacting with fungal pathogens, accounted for most observed losses, which were caused by inadequate post-harvest practices in a tropical climate.

Given the demonstrated critical role of insect pests in driving post-harvest losses, it is essential to implement farmer training programs focused on best practices to minimize pest accumulation during storage. Training should prioritize practical, accessible methods, including proper drying, sanitation, and storage hygiene, to create less favourable environments for pest proliferation. Additionally, the use of locally available ethnobotanicals offers a promising, eco-friendly alternative to synthetic pesticides and is much more accessible in the area, both logistically and financially. Plants such as *Azadirachta indica* A.Juss., *Tetrapleura tetraptera* (Schumacher and Thonn.), and *Scorodophleum zenkeri* Harms, as well as numerous other species, contain natural insecticidal and repellent compounds and have been found effective against key insect storage pests. Empowering smallholder farmers with

knowledge and resources to apply these botanicals can reduce pest pressure sustainably while avoiding the environmental and health risks associated with chemical pesticides. The study also highlights the urgent need for routine surveillance systems at consumption centers to monitor mycotoxin levels entering the human food chain. Regular screening for aflatoxin and fumonisin contamination, combined with systematic tracking of insect pest infestations, would provide near real-time data critical for early warning and rapid response. Without such monitoring, populations remain vulnerable to silent crises of toxin exposure and grain deterioration, as this study demonstrates is currently the case in Tshopo province. Establishing these routine observation frameworks is critical to safeguard public health and food security, enabling targeted interventions before contamination reaches dangerous levels or pest populations spiral out of control. Together, farmer training and robust monitoring protocols form pillars of an integrated pest and mycotoxin management strategy, essential for sustainable post-harvest protection in Tshopo and similar agroecological zones.

CRedit authorship contribution statement

Jeremy Berdy: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Hervé Ambakina:** Writing – original draft, Methodology, Investigation, Formal analysis. **Papy N'Sevolo:** Conceptualization, Methodology, Writing – review & editing. **Manon Genva:** Writing – review & editing, Writing – original draft, Supervision. **Rudy Caparros Megido:** Writing – review & editing, Supervision, Methodology. **Frédéric Francis:** Writing – review & editing, Supervision. **Marie-Laure Fauconnier:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of generative AI use

During the preparation of this work the authors used perplexity.ai in order to improve spelling, grammar and phrasing of the manuscript. After using this service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jspr.2026.103049>.

Data availability

Any data or information used in this study can be requested by contacting the corresponding author.

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