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Research Article
Identification of Maize Insects and Fungi Affecting Sanitary and Physiological Quality of Stored maize Grains in Central Cote d'Ivoire

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Abstract
Background and Objective: Maize is one of the most important food cereal crops. Unfortunately, in Cote d'Ivoire, maize storage is mainly impaired by biotic factors that are not well documented and controlled. Therefore, this study aimed to identify occurring insects and fungi and their respective impacts on the physiological and sanitary qualities of harvested maize grains. **Materials and Methods:** Six major producing zones in central Cote d'Ivoire were sampled during harvest. Sampled grains were stored in polypropylene bags for six months in laboratory conditions. The 18 out of36 bags, randomly selected, were treated with the insecticide PROTECT DP to control insects. Taxa of insects and fungi present in stored samples were morphologically identified using standard keys. Then, occurrence and relative abundance were recorded monthly for insects, while fungal occurrences and infection rates were assessed during a 7-days paper blotting germination test at the beginning and the end of the storage. Furthermore, moisture content and germination rate were recorded at the beginning and the end of the experiment. **Results:** Five insect species, *Sitophilus zeamais*, *Tribolium castaneum*, *Oryzaephilus surinamensis*, *Cillaeus* sp. and *Ephestia cautella* were observed. *Sitophilus zeamais* was found with the highest occurrence and relative abundance (respectively 57.1 and 98.02%). As for fungi, microscopic observations revealed occurrences of eight fungal species. Then six species (*Aspergillus versicolor*, *A. flavus*, *A. terreus*, *Rhizopus* sp., *Fusarium* sp. and one unidentified species) were constant (50% $\leq$Ci <100%),while one *A. niger* was common species (38.27$\pm$25.55) and *Penicillium* sp. was rarely encountered (3.7$\pm$4.9). The moisture contents at the end of the storage of the treated grains were close to those of grains before storage but significantly lower than those of the untreated grains. Besides, the germination rates at the end of the storage of the treated grains were similar to those of grains dried before storage but significantly higher than those of the untreated grains regardless of the sampled zones. **Conclusion:** Insects and fungi are the main biotic agents which deteriorate the quality of stored grains.

Key words: Maize, storage, insects, fungi, biotic agents

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Maize (*Zea mays*) is an important staple food crop and a source of income for most smallholder farmers[1]. However, the achievements in grain maize production have not been matched by improvements in agricultural marketing services, particularly in storage and transportation[2].Post-harvest losses of grain maize caused by insect pests during storage are a major constraint to household food security[1]. The most economically threatening post-harvest pests of grain maize in Africa are the maize weevil (*Sitophilus zeamais*), the larger grain borer (*Prostephanus truncatus*), the lesser grain weevil (*Sitophilus oryzae*) and the Angoumois grain moth(*Sitotroga cerealella*)[3]. Similarly, pathogenic fungi spoil food grains by producing mycotoxins during storage, thus reducing their nutritional quality[4]. The main pathogenic fungal genera commonly seen in grain maize are *Fusarium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Ustilago* and *Rhizopus*[5], of which the first three are the most predominant and cause reduced seed germination[6,7]. These fungi produce agriculturally important mycotoxins and carcinogenic substances for humans and animals[8,9]. The major mycotoxins frequently present in cereals are aflatoxins, fumonisins, ochratoxins, trichothecenes and zearalenone, although the first two are most common on maize in tropical and subtropical regions[8]. In West Africa, Ayeni *et al*.[9] and Adja *et al*.[10] had, respectively, reported fungi and insects on stored food products. Besides, the authors noted that insects and fungi reduced grains quantity (weight loss) and grains quality (viability, germination and moisture content). Given the damages caused by these biotic agents, it is crucial and cost-effective to protect grains from spoilage. To protect the grains, it is necessary to know exactly which pests are present. This study aimed to inventory the insects and fungi pests affecting maize grains at harvest time and during storage and to determine their impact on the sanitary and physiological parameters of the grains (infection, moisture content and germination rate).

MATERIALS AND METHODS

Grain maize sampling and storage: The study duration continues from October, 2017 to December, 2021. Eighteen (18) grain maize samples were collected from six major maize-producing areas located in Central Côte d'Ivoire: Yamoussoukro, Attiégouakro, Toumodi, Djékanou, Tiébissou and Didiévi. Three farms per zone were sampled during harvest and placed in polypropylene bags. Each bag contains 500 g of grain. The grains in 18 bags out of 36 were treated with 0.25 g of the insecticide PROTECT DP (0.1% Deltamethrin and 1.5% Pyrimiphos-Methyl) while 18 other bags remained untreated before being stored for 6 months at the Department of Agriculture and Animal Resources of the National Polytechnic Institute in Yamoussoukro (6°47' N and 5°15' W). The daily temperature and relative humidity in the store were 28.5±2.3°C and 83.5±5.8%, respectively.

Insect identification, occurrence and relative abundance: Using a completely randomized experimental design with three repetitions established in the store, observations were made monthly on the occurrence and relative abundance of insect species. Maize grains were sieved (mesh Ø 1.5-2.5 mm) and all visible insects were collected. All collected insects were identified under a light microscope (×50) using identification keys[11].

The occurrence of insect species (C) and the relative abundance was calculated according to the formulas used by Adja *et al*.[10]:

$$\text{Occurrence (C\%)} = \frac{\text{Oi}}{\text{O}} \times 100 \quad (1)$$

Where:

Oi = Occurrence of a species

O = Total number of observations

Five classes of occurrence were set up as follows:

- Ubiquitous species (C = 100%)
- Constant species (50% $\leq$ Ci <100%)
- Common species (25% $\leq$ C <50%)
- Moderated common species (5% $\leq$ C <25%)
- Rare species (C<5%)

$$\text{Relative abundance Ar} = \text{ni x } \frac{100}{\text{N}} \quad (2)$$

Where:

ni = Number of individuals of a given species

N = Total number of individuals of all species

Four classes of relative abundance were set up as follows:

- Highly abundant species (Ar $\geq$10%)
- Abundant species (5% $\leq$ Ar<10%)
- Moderately abundant species (1% $\leq$ Ar<5%)
- Scarce species (Ar<1%)

Then, grain maize moisture content was assessed at the beginning and the end of the storage on three batches of 100 g of grains per insecticide treatment, incubated in the oven at 60°C for 72 hrs. The moisture content and the germination rates were calculated according to the formulas used by Monira *et al.*[12]:

$$\text{Moisture content (MC)} = (\text{IW} - \text{FW}) \times \frac{100}{\text{IW}}$$

Where:

IW = Initial Weight
FW = Final Weight

The germination rate was determined as follows:

$$\text{Germination rate (GR)} = \text{G} \times \frac{100}{\text{N}}$$

Where:

G = Germinated grains
N = Total number of grains

Four replicates of 25 randomly selected grains per insecticide treatment were subjected to a paper blotting germination test in four Petri dishes previously sterilized in an oven (100°C, 24 hrs). The Petri dishes were then placed inside a dark and humid chamber tightly closed for seven days. The observations took place 2, 3, 4 and 7 days later. All analyses were performed before and at the end of the storage. The fungal infection rate was according to the formula used by Kouadia[13]:

$$\text{Infection rate (IR)} = \text{I} \times \frac{100}{\text{N}}$$

Where:

I = Number of fungi-infected grains
N = Total number of grains

After 7 days in the dark chamber, macroscopic observations were performed regarding the mycelia growth and colour. Subsequently, PDA (*Potato Dextrose Agar*) plates were used to isolate and purify during 3-7 days, growing fungi on the grains[4,13]. The species of the fungi were determined under a light microscope with AM SCOPE camera per insecticide and storage treatments based on taxonomic features such as conidia and hyphae found on identification key[14].

Data analysis: The insect, moisture content, fungal infection and germination rates data were subjected to an analysis of variance (ANOVA) using STATISTICA 7.1 follows contingently by the Fisher post hoc test at a 5% significance level afterwards checked data normality by Levene test. Principal Component Analysis (PCA) was carried out on all quantitative parameters to reveal correlations between them and sampled zones features.

RESULTS

Impact of the storage on insect diversity and their distribution: At the beginning of the experiment, insects were absent in the samples collected in the localities. Insects appear during the storage. The individuals were collected, classified into five species, five families and two orders (Coleoptera and Lepidoptera) in Table 1. One primary insect (*Sitophilus zeamais*) and four secondary pests (*Tribolium castaneum*, *Oryzaephilus surinamensis*, *Ephestia cautella*, *Cillaeus* sp.) have been recorded. *S. zeamais* was "constant" (C = 57.1%) and "highly abundant" (Ar = 98.02%), while *T. castaneum* and *O. surinamensis* were "moderated common" (C = 9.1 and = 5.4%, respectively) and "scarce" (Ar = 0.87 and 0.53%,respectively). The other species (*Cillaeus* sp. and *E. cautella*) were "rares" (C = 3.09 and 3.24%) and "scare" (Ar = 0.34 and 0.24%) (Table 1). During the storage, insect populations fluctuated from the first month to the sixth. *S. zeamais* population was more important than those of the other insects. *S. zeamais* population mean per sample each month varied from 0.51±0.84-3.2±4.57 on treaded samples and from 5.41±6.76-83.48±40.85 on untreated samples in Fig. 1a. For the other insects, population means ranged from 0±0-1±0.71 on treaded samples and from 0±0-2.94±0.83 on untreated samples in Fig. 1b.

For the whole insects collected during 6 months, populations were more important on untreated samples (171.77±144.42-413.33±56.76), significantly (df = {1, 107}, F = 300.79, p = 0.0001) higher than on treated samples (1.88±1.45-23.77±10.87). Moreover, those populations were significantly higher in the untreated samples from Tiébissou (413.33±56.76) and Yamoussoukro (313.88±98.7) (df = {11, 107}, F = 31.19, p = 0.0001) compared to the other localities(171.77±144.42-274.55±107.95) in Supplementary data 1.

Impact of storage on grain maize moisture content: Before storage, the moisture content of the grain maize ranged from 9.27±0.79-11.38±1.17% in Supplementary data 2. Analysis of variances showed significant differences (df = {5, 53}, F = 12.46, p = 0.0001) between the samples. The moisture content of Didiévi samples (11.38±1.17%) was significantly

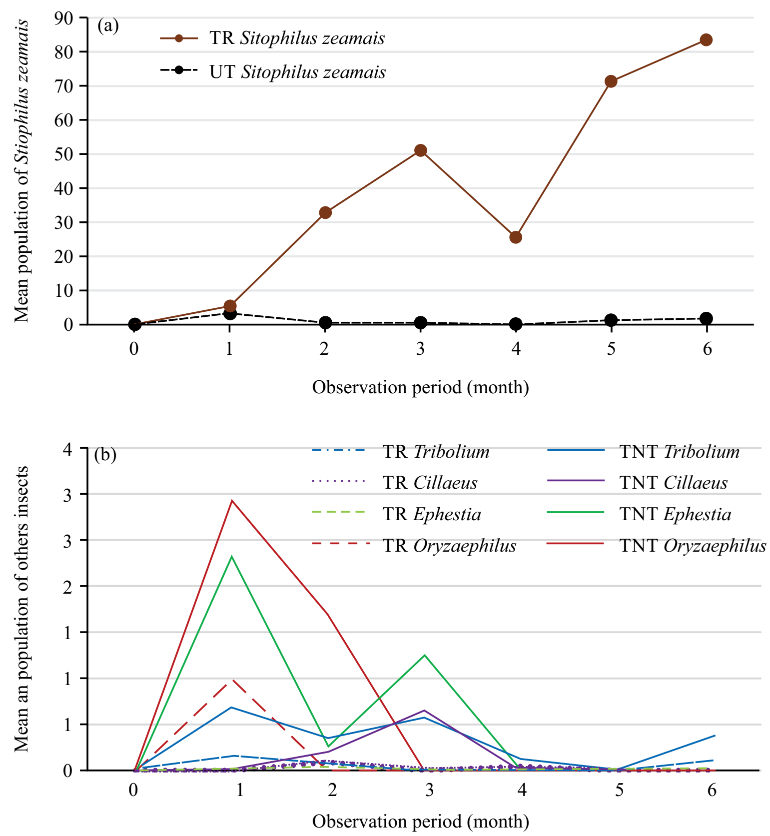

Fig. 1(a-b): Effect of insecticide PROTECT DP on *Sitophilus zeamais* populations of stored grains and (b) The other insect's populations of stored grains

TR: Grain maize samples treated with 0.25 g of the insecticide PROTECT DP (0.1% Deltamethrin and 1.5% Pyrimiphos-methyl), UT: Untreated samples, M: Month

Table 1: Insect identification, occurrence and relative abundance recorded on grain maize

Orders	Families	Species	Status	Occurrence (%)	Relative abundance (%)
Coleoptera	Curculionidae	*Sitophilus zeamais*	Primary pest	57.10	98.02
	Tenebrionidae	*Tribolium castaneum*	Secondary pest	9.10	0.87
	Nitidulidae	*Cillaeus* sp.	Secondary pest	3.09	0.34
	Silvanidae	*Oryzaephilus surinamensis*	Secondary pest	5.40	0.53
Lepidoptera	Pyralidae	*Ephestia cautella*	Secondary pest	3.24	0.24
02	05	05	02		

higher than those of Tiébissou (10.47±0.29%), which was higher than those of Djékanou (9.27±0.79%) and Toumodi (9.42±0.35%). However, the moisture content of the samples of Yamoussoukro (10.01±0.39%) and Attiégouakro (10±0.42%) did not differ from those of Toumodi, Djékanou and Tiébissou (Supplementary data 2). After 6 months of storage, the moisture content was significantly higher (df = {1, 107}, F = 85.47, p= 0.0001)on untreated samples (12.49±1.11) compared to the treated samples (10.92±0.6). It varied from 10.77±0.56-13.28±1.28%. The moisture content of treated samples was statistically similar (10.77±0.56-11.13±0.33%) between the sampled localities.

Regarding the untreated samples, the moisture content of the Tiébissou grains (13.28±1.28%) was significantly higher (df= {11, 107}, F = 8.94, p = 0.0001) than that of Attiégouakro (12.03±1.36%). However, the moisture content in the samples from Yamoussoukro, Toumodi, Djékanou and Didiévi was similar and did not differ from those of Attiégouakro and Tiébissou. The moisture content on treated samples (10.77±0.56-11.13±0.33%) was close to those before storage (9.27±0.79-11.38±1.17%) but lower than those on untreated samples (12.3±1.36-13.28±1.28) regardless of the localities (Supplementary data 2).

Table 2: Fungal Occurrence recorded on maize grains before and after storage

	Before storage	After storage	
Fungi	All grain maize	Treated grains	Untreated grains
Aspergillus versicolor	25.93	98.15	87.04
Aspergillus flavus	00.00	92.59	77.78
Aspergillus niger	9.26	57.41	48.15
Aspergillus terreus	0.00	85.19	87.04
Rhizopus sp.	5.56	100.00	29.63
Penicillium sp.	1.85	0.00	9.26
Fusarium sp.	20.37	75.93	81.48
Unidentified specie	12.96	94.44	94.44
Total species	06	07	08

Treated grains: Samples treated with 0.25 g of the insecticide PROTECT DP (0.1% Deltamethrin and 1.5% Pyrimiphos-methyl)

Impact of storage on grain maize infection by fungi: Before storage, the fungal infection rate varied between 26.67±6.32-50.67±21.17% in Supplementary data 3. Attiégouakro samples (50.67±21.17%) presented the most important infection rate, significantly higher (df = {5, 53}, F = 6.23, p = 0.0001) than those from Toumodi (40±6.93%) and Yamoussoukro (37.33±8.48%), which were higher than those of Tiébissou (29.78±6.04%), Djékanou (26.67±6.32%) and Didiévi (28.44±8.53%)(Supplementary data3). After storage, the treated samples showed infection rates (24.0±10.39-51.33±11.96%) significantly lower (df = {1, 107}, F = 699.31, p = 0.0001) than those of untreated samples (more than 93.33±10.2%). Regarding the treated samples, the infection rates in Attiégouakro, Didiévi and Tiébissou were similar but significantly higher (df = {11, 107}, F = 66.55, p = 0.0001) than that of Djékanou (24.0±10.39%) which remained lower than grain fungal infection from Toumodi samples (51.33±11.96%). Concerning untreated samples, the infection rates were similar (93.33±10.2-100%). Before storage, infection rates (26.67±6.32-50.67±21.17%) were similar to those of treated samples (24.0±10.39-51.33±11.96%) but low than those of the untreated samples (93.33±10.2-100%) regardless of sampled zones (Supplementary data 3).

Impact of storage on fungal species occurrences: Macroscopic observations of the fungal colonies cultured on PDA plates shown different features including mycelial colour. Microscopic observations of the fungi revealed mainly eight species: *Aspergillus versicolor, A. flavus, A. niger, A. terreus, Fusarium* sp., *Rhizopus* sp. and *Penicillium* sp. and one unidentified species in Table 2. Six species were recorded on grain maize before storage. Then, seven and eight species were respectively recorded after storage on treated and untreated grains. Before storage and according to their occurrence on PDA plates, *A. versicolor* (25.93%) was a "common species" while *Fusarium* (20.37%), *A. niger* (9.26%), *Rhizopus* sp. (5.56%) and one unidentified species (12.96%) were "moderated common" ones. *Penicillium* sp. was"rare" (1.85%). Then, *A. flavus* and *A. terreus* were absent. After storage, we collected, respectively seven and eight species on treated and untreated grains. On treated grains, *Rhizopus* sp. (100%) were "ubiquitous" but *A. versicolor* (98.15%), *A. niger* (57.41%), *A. flavus* (92.59%), *A. terreus* (85.19%), *Fusarium* sp. (75.93%) and the unidentified species (94.44%) were "constants". *Penicillium* sp. was absent on treated grains. On untreated grains, five species are *A. versicolor* (87.04%), *A. flavus* (77,78%), *A. terreus* (85.19%), *Fusarium* sp. (81.48%) and the unidentified species (94.44%) were "constants". In comparison, two species, *A. niger* (48.15%) and *Rhizopus* sp. (29.63%), were "commons" and one species *Penicillium* sp. (9.26%) was "moderated common" (Table 2). Regarding the geographical abundance of the species noted before storage, five species on grain maize from Attiégouakro and Djékanou, four from Toumodi and Tiébissou and three from Didiévi with occurrence ranging from11.11-33.33%. Observations on treated grains revealed seven species from every sampled zone, with occurrence from 44.44-100%. But on untreated grains, eight species were obtained from Didiévi, Djékanou, Yamoussoukro and seven species from Attiégouakro, Toumodi and Tiébissou with occurrence varying between 11.11 and 100%.

Impact of storage on grain germination rate: Before the storage, the germination rate of grain maize varied between 85.33±7.48 and 99.11±1.76% in Supplementary data 4. There was a significant difference between samples from the sampled localities. The germination rates of the samples from Didiévi (99.11±1.76%), Tiébissou (96±2.83), Djékanou (95.11±4.37%) and Yamoussoukro (92.44±8.59) were close but significantly higher (df = {5, 53}, F = 7.74, p = 0.0001) than those of Attiégouakro (85.33±7.48%). However, the germination rate of the Toumodi samples (89.33±3.46%) did not differ from the other localities (Supplementary data 4). After storage, the germination rates of treated samples

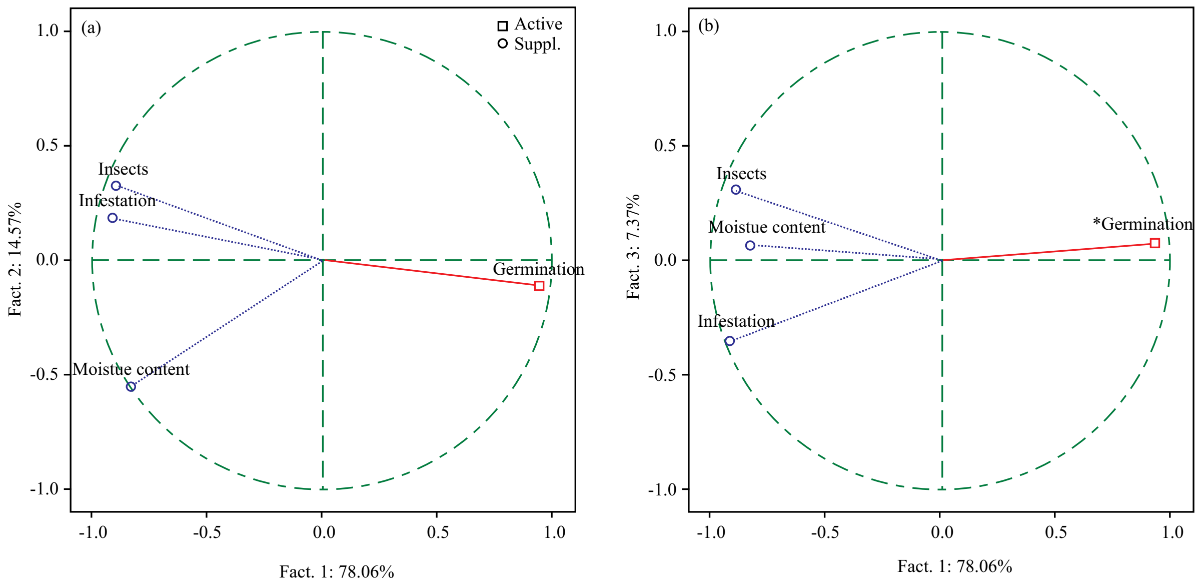

Fig. 2(a-b): Interaction between the storage parameters of maize
Projection of parameters on plan (a) one (factor 1×2) and (b) Two (factor 1×3)

(76.44±16.67-94.66±6.32%), were significantly higher (df = {1, 107}, F= 706.33, p = 0.0001) than those of untreated samples (16.22±5.33-36±15.77%). On the treated grains, the germination rate of the Attiégouakro samples (76.44±16.67%) was significantly lower (df = {11, 107}, F = 67.48, p = 0.0001) than those of the other localities (more than 87.11±7.94%). Regarding the untreated samples, germination rates in Tiébissou (16.22±5.33%) and Yamoussoukro (18.67±22.89) samples were lower than those from other localities (more than 20.44±9.04%). Besides, regardless of the sampled zones, the germination rates on treated samples (76.44±16.67-94.66±6.32%) were similar to the ones before storage (85.33±7.48 to 99.11±1.76%) but higher than those on untreated samples (16.22±5.33-36±15.77%) (Supplementary data 4).

Interaction between the different parameters: The principal component analysis gave three factors (Fact 1, 2 and 3), with, respectively three eigenvalues (2.34, 0.43 and 0.22), which expressed 100% of the variance. Then, Factor 1 and 2 expressed 92.62% of variances while factor 1 and 3 expressed 85.42% of variances in Fig. 2a, b. Then, axe 1 is significantly correlated (83.47-93.98%) with the different parameters, insect, fungal infection rate, moisture content and germination rate. Axe 2 is moderately correlated with moisture content (54.71%) and Axe 3 is fairly correlated with all the parameters (6.22-35.34%). Thus, the projection of the conservation parameters on the two main planes formed by those three axes revealed various correlations between them. Concerning the correlations between the storage parameters, there were negative correlations between the germination rate and the other storage parameters: Insect and fungal species numbers, fungal infection rate and moisture content. However, the correlations were positive between these last four parameters regardless of the sampled maize-producing localities. When insect populations increased, moisture content, fungal infection rate and the number of fungal species increased but the germination rate decreased.

DISCUSSION

The grain health status varied during the storage, depending on the insecticide treatment. Five insect species belonging to five families and two orders of insect pests were found in the samples during storage. Beetles (four species) dominated this fauna compared to Lepidoptera (one species). The insects generally found in maize grains belong to Coleoptera and Lepidoptera orders[15,16]. The fact that observing these insects on grains could be linked either to their presence in the storage environment or to an infestation from the field. The primary pest (*Sitophilus zeamais*) is more important than the secondary pests (*Tribolium castaneum*, *Cryptolestes ferrugineus, Oryzaephilus surinamensis* and *Ephestia cautella*). The main stored insect pests can be broadly classified into two groups, such as internal feeders (primary pests *Sitophilus, Rhyzopertha* and *Sitotroga*) and external feeders (secondary pests)[16,17]. The primary or major pests could destroy a whole maizegrain[18]. There were other genera found on the stored grains, such as *Tribolium*, *Cryptolestes*, *Prostephanus*, *Trogoderma*, *Oryzaephilus* (Coleoptera), *Plodia* and *Ephestia* (Lepidoptera)[8]. The beetles in which both the larva and adults are responsible for damage (loss) are more diversified and highly destructive compared to moths in which only the caterpillars are the harmful life stage. The species from both orders can complete their life cycles in 30-35 days and lay many eggs, which result in a rapid build-up of populations that consume and contaminate various stored products. They also undergo complete metamorphosis[15]. The third group of insects completes the fauna of the stored grains (mycophagous, necrophagous, detritiphagous, saprophagous, parasite, predator, etc.) appeared when storage conditions are poorly complied[10,17]. Otherwise, the insect population in Tiébissou and Yamoussoukro were important compared to the other zones.

The moisture content of the treated grains was similar between the start and the end of the storage but lower than the untreated grains moisture content. The 12% moisture content in maize grains is the stabilization threshold[17]. Under 12%, the grains contained no free water. Thus, before storage, the moisture content of maize grains (under 12%), below the stabilization threshold recorded inmost zones, was not harmful. Maize grains have been well dried and could therefore be stored for a long time. Current results are similar to those where the moisture content increased from 12.68-13.31% during 50 days of storage[2]. This variation was probably due to the tendency of the grains to come up with hygroscopic balance with the storage environment. Thence, grain moisture in polypropylene bags followed the evolution of the ambient relative humidity due to the permeability of the bags. In hermetic bags, initial moisture content remains largely unchanged during storage[19].

The health status of the samples was influenced by the environmental conditions and the moisture content of the grains. Fungi attacked the samples collected in the different localities. Indeed, the maize grains' fungal infection rate was low in the localities of Djékanou, Didiévi and Tiébissou (under 30%) and average in the localities of Yamoussoukro, Attiégouakro and Toumodi (37-50%). The infection rate recorded in the locality of Attiégouakro, although high (50%), does not match with a high humidity rate (10%). This finding could be explained more likely by the fact that the infection occurred in the field rather than in storage. These fungi hibernated in maize residues in the field or the soil[2]. After 6 months of storage, the untreated samples showed very high infection rates (93-100%), while the treated samples showed low to medium infection rates, close to those obtained before storage. Eight fungi species have been identified on maize grains. Six were "constants" (*Aspergillus versicolor*, *A. flavus*, *A. terreus*, *Rhizopus* sp., *Fusarium* sp. and one unidentified species), one was "common species" (*A. niger*) and one was "rare species" (*Penicillium* sp.). This result is consistent with those of Bressan[5], who reported that the main genera on maize grains were *Fusarium*, *Aspergillus*, *Rhizopus*, *Penicillium* and resulted in a reduced seed germination[20]. The genera *Fusarium* and *Penicillium* infect the grains in the field, while *Aspergillus* and *Rhizopus* a repost-harvest fungi and infected grains during storage[6]. In the current study, the last two fungal species were absent before storage and appeared during the storage while *Penicillium* disappears on stored treated grains. The unequal geographical occurrence of fungal species could be due to interactions between agroecological factors and the fungal mode of fruit colonization[21]. Moreover, the distribution and size of fungal populations on rice seeds are influenced by the harvest period, the provenances and plantvarieties[22]. Besides, five genera of seed-borne fungi (*Aspergillus, Fusarium*, *Penicillium*, *Alternaria* and *Calviceps*) are responsible for the production of agriculturally important mycotoxins and carcinogenic substances for humans and animals[8,9]. The most important mycotoxins that are frequently present in cereal grains are aflatoxins, fumonisins, ochratoxins, trichothecenes and zearalenone[8]. The first two are the most toxic mycotoxins found on maize in tropical and subtropical regions[23].

During storage, maize grains were attacked by various species of insects and fungi, which reduce the germination of the grains. The low germination rate in the locality of Attiégouakro could be explained by the high infection rate of maize grains. This highlights the impact of the presence of fungi and moulds in reducing the germinative capacity of the grains. Consistently with our findings, there were negative correlations between seeds contaminated with pathogenic fungi and germination. Besides, the current study revealed a reduction in the germination rate of the untreated grains compared to the treated grains. Furthermore, before the storage, the germination rates were high (over 85-99%). These rates were close or higher than the norm of stored maize grains (90%)[17]. These high rates may be related to the fact that the samples were collected in the maize field one month after the right period of harvest. The grains, therefore, still have their germinative vigour. Six months after storage, the germination rates of treated grains (more than 76%) are close to those before storage but significantly higher than those on untreated grains (less than 36%). The germination rate of the grains decreases with increasing damage by insects, insect population size and the storage time length[12].

In jute bags, the initial germination rate decreased during storage while the humidity increased. Jute bags are not suitable for long-term packaging, unlike polypropylene bags and airtight jars[12]. Samples from this study were placed in polypropylene bags. However, the samples could face an upturn in grain moisture if the bags were not properly sealed, thus promoting undesirable fungal flora. Moulds develop as soon as the moisture content of the interstitial air is above 65-70%.

The development of fungi during storage is related to the grains' qualities, the moisture content, the storage conditions (heat and light mainly). The stored grains were influenced by environmental parameters, especially the humidity and the temperature. These two factors are closely linked and documented as the most important factors which conditioned astorage[24-26]. During storage, the interactions between abiotic and bio-aggressors lead to the deterioration of grain quality. The two major causes of bio deterioration in stored cereals are insects and fungi[18]. When stored parameters (fungal infection rate, moisture content, fungi and insect populations) increase, the germination rate decreases. These results are similar to those obtained by Adja *et al.*[10] and Likhayo *et al.*[1], respectively, on the cucurbit and maize grain. The negative correlations observed between insects and fungi population sizes, fungal infection rate, moisture content and germination of grains maize were due to these organisms[27]. Furthermore, insects feed on grains and produce waste[10]. The genera *Aspergillus, Penicillium* and *Rhizopus* are known for degrading seeds during storage. The encountered fungi cause fermentation, biochemical alterations and the reduction of the germination capacity of seeds[21]. Positive correlations were obtained between insects and fungi population sizes, fungal infection rate and grain moisture content. Besides, the high insect infestation leads to an increase in moisture content due to insect biological activity which is followed by heat production. Heat associated with moisture content favors the fungal emergence due to the rotting of the seeds and the release of toxins, thus cause the qualitative loss and depreciation of the rains[17]. Hence, grain germination rate decreases with increasing damage from insects and fungi, their high populations and the storage time length[24].

Integrated pest management is essential for the sanitary quality of stored grain. In this study, maize grains were treated with an insecticide and placed in polypropylene bags. For better control of insects and fungi, treatment with an insecticide and fungicide or fumigant is recommended[1]. Nghiep and Gaur[28] showed that a preventive fungicide treatment is necessary to maintain a germination rate above 80% after 6 months of storage. However, this treatment does not apply to edible cereals where post-harvest operations (such as drying, ventilation, cooling, cleaning and separation, sorting, controlled atmospheres, etc.) must be carried out to act on the physicochemical state of the stored grains[27] and then place the grains in hermetically sealed containers[2,12]. In this situation, the factor most responsible for the death of insects in a controlled or modified atmosphere is the lack of oxygen. Traditional methods (pots, bags and earthenware) of storing local varieties of maize grain without fumigation should be prohibited[27]. Grain should be screened and sieved to remove debris and broken kernels to limit the sources and development of insect and fungal pests. Wet grains should be dried to low moisture content (12%) before storage[1]. However, this also means that no further drying is possible in this sealed system, so the grains must be well dried before storage. The moisture content of the grains stored in the airtight storage system remained virtually the same during the storage period, while the levels in the well-sealed polypropylene bags decreased with storage time. Thus, an airtight storage system could be used to store maize, protecting it from insect attack without the need for insecticides[19].

CONCLUSION

The results of these studies reveal five insect species: *Sitophilus zeamais, Tribolium castaneum, Oryzaephilus surinamensis, Cillaeus* sp. (Coleoptera) and *Ephestia cautella* (Lepidoptera). The main pest was *Sitophilus zeamais*. Fungal microscopic observation revealed the presence of eight species: *Aspergillus versicolor, A. niger, A. flavus, A. terreus, Fusarium* sp., *Rhizopus* sp. *Penicillium* sp. and one unidentified species. All these species were constants, except *A. niger* and *Penicillium* sp., which were common and rare. The moisture content of insecticide-treated samples was close to that before storage but significantly lower than that of untreated samples at all locations. Germination rates on insecticide-treated samples were similar to those before storage but significantly higher than those of untreated samples, regardless of sampled localities. There were negative correlations between the germination rate and other storage parameters (insect and fungal population size or occurrences, fungal infection rate and moisture content). However, there were positive correlations between the storage parameters. When insect populations increased, fungal populations, moisture content and fungal infection rates increased but germination rate decreased. Insects promote fungal growth and moisture content during storage.

SIGNIFICANCE STATEMENT

This study revealed to producers and researchers the main biotic agents that affect the sanitary quality of stored grains. Therefore, it opens the way to develop specific control methods from the field to the storage.

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REFERENCES

1. Likhayo, P., A.Y. Bruce, T. Tefera and J. Mueke, 2018. Maize grain stored in hermetic bags: Effect of moisture and pest infestation on grain quality. J. Food Qual., Vol. 2018. 10.1155/ 2018/ 2515698.
2. Viebrantz, P.C., L.L. Radunz and R.G. Dionello, 2016. Mortality of insects and quality of maize grains in hermetic and non-hermetic storage. Rev. Bras. Engenharia Agrícola Ambiental, 20: 487-492.
3. Tefera, T., S. Mugo and P. Likhayo, 2011. Effects of insect population density and storage time on grain damage and weight loss in maize due to the maize weevil *Sitophilus zeamais* and the larger grain borer *Prostephanus truncatus*. Afr. J. Agric. Res., 6: 2249-2254.
4. Hussain, N., A. Hussain, M. Ishtiaq, S. Azam and T. Hussain, 2013. Pathogenicity of two seed-borne fungi commonly involved in maize seeds of eight districts of Azad Jammu and Kashmir, Pakistan. Afr. J. Biotechnol., 12: 1363-1370.
5. Bressan, W., 2003. Biological control of maize seed pathogenic fungi by use of actinomycetes. BioControl, 48: 233-240.
6. Basak, A.B. and M.W. Lee, 2002. Prevalence and transmission of seed-borne fungi of maize grown in a farm of Korea. Mycobiology, 30: 47-50.
7. Tsedaley, B. and G. Adugna, 2016. Detection of fungi infecting maize (*Zea mays* L.) seeds in different storages around Jimma, southwestern Ethiopia. J. Plant Pathol. Microbiol., Vol. 7. 10. 4172/2157-7471.1000338.
8. Suleiman, M.N. and O.M. Omafe, 2014. Activity of three medicinal plants on fungi isolated from stored maize seeds (*Zea mays* (L.). FUTA J. Res. Sci., 10: 276-281.
9. Ayeni, K.I., O.O. Atanda, R. Krska and C.N. Ezekiel, 2021. Present status and future perspectives of grain drying and storage practices as a means to reduce mycotoxin exposure in Nigeria. Food Control, Vol. 126. 10.1016/j.foodcont. 2021.108074.
10. Adja, N.H., M. Danho, T.A.F. Alabi, J.Y. Zimmer and F. Francis *et al*., 2016. Identification and impact of insects feeding on the stored seeds of *Lagenaria siceraria* molina (Standl., 1930) and *Citrullus lanatus* thumb (Matsum & Nakai, 1916), two oilseed cucurbits of the ivory coast. Am. J. Res. Commun., 4: 104-132.
11. Kumar, R., 2017. Insect Pests of Stored Grain. 1st Edn., Apple Academic Press, Boca Raton, Pages: 412.
12. Monira, U.S., M.H.A. Amin, M.M. Aktar and M.A.A. Mamun, 2012. Effect of containers on seed quality of storage soybean seed. Bangladesh Res. Publ. J., 7: 421-427.
13. Kouadia, A.M.J., K. Abo and K.T. Kouadio, 2019. Evolution des infections naturelles sur les mangues, les avocats et les bananes en Côte d'ivoire et principaux champignons responsables. J. Appl. Biosci., 134: 13710-13721.
14. Kidd, S., C. Halliday, H. Alexiou and D. Ellis, 2016. Descriptions of Medical Fungi. 3rd Edn., Newstyle Printing, Australia, ISBN-13: 9780646951294, Pages: 264.
15. Kra, K.D., K.E. Kwadjo, B.G. Douan, N.N.D. Kouadio and M. Doumbia, 2017. Insect pests of grains stocks in markets of Abidjan's district (Côte d'Ivoire). Int. J. Dev. Res., 07: 15996-16000.
16. Hiruy, B. and E. Degaga, 2018. Insect pests associated to stored maize and their bio rational management options in sub-Sahara Africa. Int. J. Acad. Res. Dev., 3: 741-748.
17. Leblanc, M.P., B. Fuzeau and F. Fleurat-Lessard, 2014. Influence of grain storage practices or kind of structure and pesticide use on insect presence in wheat bulks after a long-term storage: A multi-dimensional analysis. Integr. Prot. Stored Prod., 98: 403-420.
18. Kanyamasoro, M.G., J. Karungi, G. Asea and P. Gibson, 2012. Determination of the heterotic groups of maize inbred lines and the inheritance of their resistance to the maize weevil. Afr. Crop Sci. J., 20: 99-104.
19. Ognakossan, K.E., A.K. Tounou, Y. Lamboni and K. Hell, 2013. Post-harvest insect infestation in maize grain stored in woven polypropylene and in hermetic bags. Int. J. Trop. Insect Sci., 33: 71-81.
20. Tsedaley, B. and G. Adugna, 2016. Detection of fungi infecting maize (*Zea mays* L.) seeds in different storages around Jimma, southwestern Ethiopia. J. Plant Pathol. Microbiol., Vol. 7. 10.4172/2157-7471.1000338.
21. Gnacadja, C., G. Vieira-Dalode, C. Razanaboahirana, P. Azokpota, M.M. Soumanou and M. Sie, 2018. Revue analytique des performances agronomiques, nutritionnelles et perspectives de valorisation du riz Africain (*Oryza glaberrima*). J. Appl. Biosci., 122: 12211-12230.
22. Ibiam, O.F.A., C.I. Umechuruba and A.E. Arinze, 2010. *In vitro* seed-dressing technique for the control of seed-borne fungi of rice variety Faro -29. J. Appl. Sci. Environ. Manage., 12: 39-43.

23. Manu, N., G.P. Opit, E.A. Osekre, F.H. Arthur and G. Mbata *et al*., 2019. Moisture content, insect pest infestation and mycotoxin levels of maize in markets in the northern region of Ghana. J. Stored Prod. Res., 80: 10-20.
24. Haile, A., 2015. Eco-friendly management of chickpea storage pest, *Callosobruchus chinensis* L. (Coleoptera, Bruchidae) under laboratory conditions in Eritrea. J. Stored Prod. Postharvest Res., 6: 66-71.
25. Nasreen, S., B.R. Khan and A.S. Mohmand, 2000. The effect of storage temperature storage period and seed moisture content on seed viability to soybean, Pak. J. Biol. Sci., 3: 2003-2004.
26. Strelec, I., R. Popović, I. Ivanišić, V. Jurković, Z. Jurković Ž. Ugarčić-Hardi and M. Sabo, 2010. Influence of temperature and relative humidity on grain moisture, germination and vigour of three wheat cultivars during one year storage. Poljoprivreda/Agric., 16: 20-24.
27. Olakojo, S.A. and T.A. Akinlosotu, 2004. Comparative study of storage methods of maize grains in South Western Nigeria. Afr. J. Biotechnol., 3: 362-365.
28. Nghiep, H.V. and A. Gaur, 2005. Efficacy of seed treatment in improving seed quality in rice (*Oryza sativa* L.). Omonrice, 13: 42-51.

Supplementary data 1: Insect population means according to the localities

	Yamoussoukro		Attiégouakro		Toumodi		
	TR	UT	TR	UT	TR	UT	p-value
BS	0±0		0±0		0±0		
AS	5±4.35^{a}	313.88±98.70^{d}	2.44±2.24^{a}	274.55±107.95^{cd}	1.88±1.45^{a}	171.77±144.42^{b}	0.00011
	Djékanou		Didiévi		Tiébissou		
	TR	UT	TR	UT	TR	UT	p-value
BS	0±0		0±0		0±0		
AS	3.33±3.27^{a}	235.88±122.16^{bc}	7.55±7.63^{a}	238.11±127.74^{bc}	23.77±10.87^{a}	413.33±56.76^{e}	0.00011

NB: On the same line, the means followed by the same letter are not significantly different, BS: Before storage, AS: After Storage, TR: Treated, UT: Untreated

Supplementary data2: Moisture content means according to the localities

	Yamoussoukro		Attiégouakro		Toumodi		
	TR	UT	TR	UT	TR	UT	p-value
BS	10.01±0.39^{ab}		10±0.42^{ab}		9.42±0.35^{a}		0.0001
AS	11.04±0.76^{ab}	12.30±1.24^{cd}	10.93±0.52^{ab}	12.03±1.36^{bc}	10.9±0.36^{ab}	12.48±0.7^{cd}	0.00011
	Djékanou		Didiévi		Tiébissou		
	TR	UT	TR	UT	TR	UT	p-value
BS	9.27±0.79^{a}		11.38±1.17^{c}		10.47±0.29^{b}		0.0001
AS	12.72±0.99^{cd}	13.28±1.28^{a}	11.13±0.33^{ab}	12.18±0.75^{cd}	10.78±0.95^{a}	13.28±1.28^{d}	0.00011

NB: On the same line, the means followed by the same letter are not significantly different, BS: Before Storage, AS: After Storage

Supplementary data 3: Infection rate means according to the localities

	Yamoussoukro		Attiégouakro		Toumodi		
	TR	UT	TR	UT	TR	UT	p-value
BS	37.33±8.48^{ab}		50.67±21.17^{c}		40±6.93^{b}		0.0001
AS	34.67±4.9^{ab}	99.11±2.67^{d}	46.22±27.35^{bc}	100±0^{d}	51.33±11.96^{c}	97.33±2.64^{d}	0.0001
	Djékanou		Didiévi		Tiébissou		
	TR	UT	TR	UT	TR	UT	p-value
BS	26.67±6.32^{a}		28.44±8.53^{a}		29.78±6,04^{a}		0.0001
AS	24.00±10.39^{a}	100±0^{d}	40.00±19.29^{bc}	93.33±10.2^{d}	40.89±9.33^{bc}	100±0 ^{d}	0.0001

Supplementary data 4: Germination rate means according to the localities

	Yamoussoukro		Attiégouakro		Toumodi		
	TR	UT	TR	UT	TR	UT	p-value
BS	92.44±8.59^{bc}		85.33±7.48^{a}		89.33±3.46 a^{b}		0.00002
AS	87.11±7.94^{de}	18.67±22.89^{a}	76.44±16.67^{d}	20.44±9.04^{ab}	89.33±6.32^{de}	36±15.77^{c}	0.0001
	Djékanou		Didiévi		Tiébissou		
	TR	UT	TR	UT	TR	UT	p-value
BS	95.11±4.37^{bc}		99.11±1.76^{c}		96±2.83^{bc}		0.00002
AS	94.66±6.32^{e}	21.77±6.96^{ab}	88.44±7.33^{de}	26.67±24.24^{bc}	91.55±4.67^{de}	16.22±5.33^{a}	0.0001