Effect of traditional and hermetic bag storage structures on fungus contamination of stored maize Grain (Zea mays L.) in Bako, Western Shoa, Ethiopia

Negasa F.1, Solomon A.2 and Girma D.3

1Ambo Agricultural Research Center, P. O. Box 37, Ambo, Ethiopia.
2Department of food Science and Post-Harvest Technology, Haramaya Institute of Technology, Haramaya University, P. O. Box 138, Dire Dawa, Ethiopia.
3Holleta Agricultural Research Center, Holleta, Ethiopia.

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The experiment was conducted between December 2017 and May 2018 at Bako, Ethiopia to study the effectiveness of traditional (Gombisa, Sack) and Hermetic bag storage structures and storage periods on fungal contamination of stored maize grain on agar plate method. The incidence and frequency of storage fungi was determined at 0, 2, 4 and 6 months of storage periods. The experiment was replicated three times in factorial design. The design was 3×4 in factorial fashion. The treatments were three storage types (Gombisa, sack and Hermetic bag), one variety of maize (Bako hybrid-661) and storage periods (0, 2, 4 and 6) months. The collected data were analyzed statistically using Generalized Linear Model (GLM) procedure of SAS and means that were significantly different were separated using Least Significant Difference (LSD). Fungi were the major causes of deterioration and quality loss on stored maize grains during the storage period. The fungal incidence and frequency significantly different (p<0.05) increased with storage periods. The highest (39.4%) Fusarium species incidence was recorded at the last six months of storage. Fusarium spp. occurred in Gombisa with the highest 29.9% incidence and 23% frequency, respectively. The highest frequency of Aspergillus species 26.7% was recorded in Gombisa whereas the minimum 18% was obtained from Hermetic bag in the six months of storage periods. In this study, Fusarium spp. was the most prevailing storage fungi followed by Aspergillus spp. As a result of this research, the Hermetic bag was determined to be more appropriate for protecting the stored maize grains from fungal attack during the storage periods and the stored grains have low fungal incidence and frequency until initial to four months. Therefore, gombisa and sack storages were inadequate for protecting stored maize from insect pests and fungal attacks. Overall, the hermetic bag storage can protect insect infestation and fungal development and consequently maintains seed viability and nutritional content during storage without use of insecticides.

Key words: Moisture, temperature, humidity, fungi, storage.

INTRODUCTION

Maize (Zea mays L.) is the third most important crop after rice and wheat cultivated in the world and occupying more than 120 million hectares of cropland annually (Marta et al., 2017). In Ethiopia maize is the first most important cereal crop in terms of its production accounting for 26.7% (7.2 million tons) of 87.3% (23.6
millon tons) of the cereal production (Binyam and Girma, 2016). Due to its higher caloric and nutritive values, it is a valuable food for human beings as well as good feed for livestock and poultry (Girma et al., 2006). However, the grain suffers from quantitative and qualitative losses during storage. The losses occur mainly due to improper storage (Ishrat and Shahnaz, 2009) and fungi, bacteria, viruses and insects infecting and infesting stored maize grains and causes combined worldwide annual losses of 9.4% (Verga and Teren, 2005). The main storage fungi associated with stored grains includes Aspergillus and several Penicillium species. Nine fungal species isolated from stored maize were Aspergillus flavus, Aspergillus nidulans, Fusarium moniliforme, Penicillium spp. and the different types of fungi from the stored maize (Bosah and Omorusi, 2014; Chattha et al., 2016). Fungi are the second important cause of deterioration and loss of maize next to insects (Ali et al., 2007).

Fungi could cause about 50 to 80% of damage on farmers’ stored maize grains during storage if conditions are favorable for their development (Ali et al., 2007). Aflatoxin content was increased in the stored maize grain after 12 months of storage. Fungi affect the quality of grains from the results; there were an increase in fatty acid, reduction in germination, increase its mustiness, production of toxins and finally leading to spoilage of grain in many ways. Regardless of the incidence and frequency of these storage fungi which cause losses to the stored grains of maize in Ethiopia, appropriate studies have not been made. Information on these fungi inefficiently exists. In future, many studies to be done on the close-fitting of storage maize grains fungal pathogens are required. The present study was initiated with the objective study of the effectiveness of traditional (Gombisa, Sack) and Hermetic bag storage structures in protecting the stored maize grain from fungal infection in Bako district, Oromia region, Ethiopia (Befikadu et al., 2012).

MATERIALS AND METHODS

Description of the study area

This study was conducted at Bako Agricultural Research Center located in East Wollega Zone of the Oromia Regional State, Western Ethiopia at an altitude of 1650 m above sea level (m.a.s.l.). Bako lies at 9° 6” north latitude and 37° 9” east longitude in the sub-humid ecology of the country 260 km west of Addis Ababa and 8 km away to the south from the main road to Nekemte. Average annual rainfall at this location is 1237 mm. The rainy season extends from May to October and maximum rain is received in the months of July and August. Agro-ecologically, it has a warm humid climate with mean minimum, maximum and average air temperatures of 15, 30 and 23°C, respectively. The RH minimum, maximum and average of the area is 49, 74.7 and 61.85%, respectively. The major annual and perennial crops of the area include maize, sorghum, teff, nong, hot pepper, haricot bean, sweet potato, mango, banana, and sugar cane in order of importance. The study was conducted for six (6) moths starting from harvesting time in December, 2017 to May, 2018 at Bako National Maize Research Center (Figure 1).

Experimental plan and design

The experiment was arranged in a 3×4 factorial combination with two factors, storage types and storage period in complete randomized design with three (3) replications. Storage types have three levels, that is Gombisa, Sack and Hermetic bag, while storage period have four levels that is 0, 2, 4 and 6 months of storage periods. Data were collected at every two months interval, including at the start of the study making up four levels for the factor storage period.

Experimental materials

The study materials were BH-661 maize of variety harvested in December, 2017 and three types of Gombisa, Sack and Hermetic bag storage structures.

Sampling methods

A total of 90 samples of BH-661 maize variety were collected from each of storage structures periodically starting from the beginning of the storage (0, 2, 4 and 6) months of the storage periods. The samples were taken from the top, middle and bottom of the storage structures. The initial maize samples from each storage structures were taken as a control at the beginning of the storage. Each sample was taken by inserting the spear into the grain mass straight to the maximum depth from the top, middle and the bottom of the storage.

Physical parameters

Moisture content

Grain moisture content was determined by using the AACC (2005) standard procedures of oven dry methods. The grain was dried at a temperature of 105°C for 3 h and after being removed from the oven, it was allowed to cool in a dissector and then weighed. Then, the moisture content was calculated as follows:

\[
MC (\%) = \frac{\text{Weight initial of the sample} - \text{Weight after dry}}{\text{Weight of sample after dry}} \times 100
\]

Storage temperature and relative humidity

The temperature and relative humidity of the internal and external environment of the storage was measured at an interval of every week by using portable digital thermo-hygrometer (Hanna, HI8564) and measurement was done in the afternoon 3.00 p.m. in the day.

*Corresponding author. E-mail: mergafufa@gmail.com.

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(to reduce variations) and at the time three data was taken and its average was recorded. Measurements were taken from the center, side, and top portion of the storage.

**Microbial identification**

**Fungal detection of the stored maize grain**

**Agar plate method:** A sample of stored maize grains with and without surface disinfection was used and 10 grains of each treatment were aseptically placed on potato dextrose agar (PDA) by the method of agar plate according to the procedures used by Binyam and Girma (2016). The laboratory analysis was carried out in the Ambo Plant Protection Research Center mycology laboratory department. Firstly, from each sample, 360 maize grains; in 3 replications of 120 seeds were selected. Initially, freshly harvested seed of BH661 was used and periodically the stored maize grains were used and thoroughly washed with distilled water at each period. From surface disinfected and non-disinfected samples, 10 grains/Petri-dish/plate (9 cm diameter plates) containing potato dextrose agar (PDA) were aseptically placed. The plate that contains fungus was incubated at 26°C for 7 days and after 7 days of incubation, the identification of fungi isolates was done based on: septate, growth rate, color, and morphology of mycelia, conidia and sporulation structures. Then, the isolated fungi were sub-cultured after three days of incubation for purification of the isolate. Finally, incidence of isolation fungi (%) and frequency of isolation of fungi (%) were calculated as follows:

**Incidence of fungi:** Incidence of fungal infection on each samples was calculated by using the following formula:

\[
\text{Incidence} \% = \frac{\text{Number of infected grain}}{\text{total of grain}} \times 100
\]

**Isolation frequency (IF):** For each fungus, the proportion of samples that yielded its isolates were determined and expressed as percent by using the following formula (Marasas et al., 1988).

\[
\text{IF} \% = \frac{\text{Number of samples of occurrence fungi species}}{\text{total number of sample}} \times 100
\]

**Statistical analysis**

All the data collected were subjected to analysis of variance (ANOVA) by using the PROC GLM procedure (SAS institute, 2004) and difference among means was compared by the Least Significant Difference at 5% level of significance (Steel and Torrie, 1980). The correlation parameters were examined using Pearson’s correlation coefficient using PROC CORR procedure of the SAS software (SAS Institute, 2004).

**RESULTS AND DISCUSSION**

**Relative humidity of the stored maize grain**

Mean relative humidity of stored maize grains over the storage periods is shown in Table 1. The initial loading of data of relative humidity for all storages just before being closed was 23.60% which was the same as that of the ambient relative humidity. In the subsequent months, the relative humidity kept on increasing in each storage as well as the ambient and reached 41.80, 37.15, 36.45 and 35.00%, respectively. Befikadu et al. (2012) reported that the average relative humidity ranged from 30.83 to
54.67% and 29.33 to 65.17% being recorded inside Gombisa and Sack. Abass et al. (2014) reported that the mean relative humidity maintained inside hermetic storage containers was significantly higher from 72.47 to 75.32% in Manyara sites than in Dodoma sites from 60.02 to 61.68%, but the average relative humidity conditions in polypropylene bags without treatment in Manyara (62.28%) is similar to the average humidity in Dodoma (58.52%). Likewise, Chattha et al. (2016) stated that the average maximum ambient temperature at 38.07°C; and the mean relative humidity was 78.0% throughout the study period.

### Temperature of the stored maize grain

Table 2 shows monthly average temperature data of the three storage types and that of the ambient atmosphere. The initial temperature during the loading of the storages was 22.25°C. The temperature readings continued to increase continuously and reached 35.65, 34.15, 33.05 and 31.05°C for Gombisa, Sack, Hermetic bag and the ambient in the six months. Likewise, Befikadu et al. (2014) reported that the average temperature ranged from 21.30 to 35°C for Gombisa and 16.55 to 28.95°C for Sack, while Marek et al. (2018) reported average values of temperature inside of the floored warehouse to be 21.9°C within the timeframe, with the maximum value of 32.6°C and minimal value of 12.6°C.

### Moisture content of the stored maize grain

The average moisture content data of grains stored in the three types of storages for six months are shown in Table 3. The values did not change much after storage periods of one month. As time passed by the moisture contents in
all three storage types decreased. For instance, the moisture content of samples in Gombisa dropped to 7.40% after two months and that of Sack reduced to 8.40% and of the Hermetic bag to 7.80%. The reduction in moisture content of grains could be loss of moisture to the air in the storage through transpiration (Evaporation). In contrast, Niamketchi et al., 2016, reported that with an individual mean of 9.23 and 9.05% at the beginning (0 month), the moisture contents increased significantly (P<0.001) during the storage period. In the third months, the moisture content of grains in Gombisa increased to 8.36% whereas those in Sack and Hermetic bag continued to drop to 8.00 and 7.50%, respectively. The reduction in moisture content of grains could be loss of moisture to the air in the storage through transpiration (Evaporation). The moisture content of the grains at and after the fourth months showed continued increment reach 13.9, 11.7 and 10.70% at the end of six months storage periods for samples in Gombisa, Sack and Hermetic bag, respectively. These increments could be due to the moisture generated during respiration of the grain and other living things in the storages. However, Befikadu et al. (2012) reported that grain stored in the sacks was different in that the moisture content exhibited increment to 11.08 at 120 days and 11.7 at 180 days of storage time.

Effect of storage type on fungal incidence and frequency of stored maize grain

Interaction effect of storage type with storage periods on fungal incidence and frequency in stored maize grains is shown in Table 6. The values of fungal incidence and frequency are significantly different (p<0.005) to each other with the storage periods (Table 1). No incidence and frequency of Apergillus and Penicillium spp. was recorded during the initial. Maximum incidence 39.4% of Fusarium spp. was recorded in the last six months of storage periods. The highest incidence of Aspergillus spp. 21.3% was recorded in the last six months. This might be due to high relative humidity and temperature that hastened the rewetting of the grains and resulted in fungal infection to the grains. Likewise, Kodwo (2015) reported that Aspergillus flavus was recorded in Ava and Shade storage structures with the highest occurrence rates of 100 and 86.76%, while Sack and Hermetic storage bags record the least rates of 53.33 and 46.67%, respectively. Regarding to fungal incidence and frequency, Fusurium spp. was recorded in more incidentally and frequently in Gombisa 29.9 and 23.9% the least recorded in Hermetic bag with 20.4 and 16.4%. Similarly, Ng’ang’a et al., 2016, reported in the PICS bags, the incidences of Aspergillus (9 - 16%) and Penicillium (3 - 6%), respectively.

Effect of storage type on fungal incidence and frequency of stored maize grain

The effects of storage type on fungal incidence and frequency of stored maize grain are shown in Table 4. The highest 29.9% Fusurium spp. incidence was recorded in Gombisa whereas the lowest 18% was recorded in Hermetic bag. Minimum value 5.2% of Aspergillus spp. incidence was recorded in Hermetic bag. Initially, the frequency of Aspergillus and Penicillium spp. was 0.0% in all the three storage structures and increased significantly to 15.7 and 4.6%. This might be due to the high relative humidity and temperature that hastened the rewetting of the grains and resulted in fungal infection to the grains. Likewise, Kodwo (2015) reported that A. flavus was recorded in Ava and Shade storage structures with the highest occurrence rates of100 and 86.76%, while Sack and Hermetic storage bags scored the least rates of 53.33 and 46.67%, respectively. Regarding of fungal incidence and frequency in storage type, Fusurium spp. was recorded more incidentally and frequently in Gombisa as 29.9 and 23.9%; the least recorded in Hermetic bag with the

### Table 3. Mean moisture content of stored maize grains over the storage periods, 2017/2018.

| Storage period (Months) | Gombisa | Sack | Hermetic |
|------------------------|---------|------|----------|
| ILD                    | 10.00\(^c\) | 10.00\(^c\) | 10.00\(^c\) |
| 1                      | 9.93\(^a\) | 10.00\(^c\) | 9.30\(^c\) |
| 2                      | 7.40\(^d\) | 8.40 \(^d\) | 7.80\(^d\) |
| 3                      | 8.36\(^d\) | 8.00 \(^d\) | 7.50\(^d\) |
| 4                      | 10.50\(^c\) | 10.20\(^c\) | 9.86\(^c\) |
| 5                      | 11.23\(^b\) | 10.46\(^c\) | 10.03\(^c\) |
| 6                      | 13.9\(^a\) | 11.70\(^b\) | 10.70\(^c\) |
| LSD (5%)               | 0.53     | 0.48  | 0.72     |
| CV (%)                 | 3.4      | 2.30  | 2.81     |

Mean values of three replicates within each column sharing similar letters were not significantly different by LSD test at P≤0.05. CV: Coefficient of variation, LSD: least significant different, ILD: initial loading date.
incidence and frequency of 20.4 and 16.4%. Similarly, Ng’ang’a et al., 2016, reported in the PICS bags, the incidences of Aspergillus (9 - 16%) and Penicillium (3 - 6%), respectively.

### Effect of storage periods on fungal incidence and frequency of stored maize grains

Table 5 shows the effects of storage type on fungal incidence and frequency of stored maize grain. The value of the incidence of Aspergillus spp. was 0.0% initially and increased significantly (p<0.05) to 21.3% in the last six months of storage periods. Maximum 39.4% incidence of Fusarium spp. was recorded during six months of storage periods. Fusarium spp. occurred more frequently 33.3% than Aspergillus spp. 15.9% and Penicillium spp. 8.0% in the last six months. As the storage period increased, the incidence and frequency of all fungal species increased significantly (p<0.05). This is due to increase of relative humidity in the storage which favors therewetting of the stored maize grains. The use of Hermetic bag storage structures reduces the fungal infection, since low relative humidity was recorded in the storage and the use of the stored grains before six months of storage periods is better.

### Conclusion

High relative humidity favors the rewetting of the stored grains and made the grains to be mouldy. Moisture content and stored maize grains temperature increased progressively with storage periods. Initially, the incidence and frequency of the fungal species was not recorded except for the Fusarium spp. with the incidence of 10.0%. The values of fungal incidence and frequency showed significant difference (p<0.005) to each other with the storage periods. Maximum incidence 39.4% of Fusarium spp. was recorded in the last six months of storage periods. The highest 29.9% Fusarium spp. incidence was recorded in Gombisa whereas the lowest Fusarium spp. incidence 18% was recorded in Hermetic bag. Fusarium spp. incidence was 21.7% for Gombisa, 15.0% for Sack, 13.0% for Hermetic bag, respectively. Therefore, maize grains should not be stored for more than six months. It is concluded that adoption of improved storage facilities like Hermetic bag storage will reduce maize grain losses, save the resources required for maize grain production, minimizes the maize nutrient quality deteriorations and mycotoxins that causes health risks and ultimately contributes to the improvement of food safety and food security of the region.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of Interests.

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Table 6. Interaction effect of storage type with storage periods on fungal incidence and frequency in stored maize grains, 2017/2018.

| Storage period (months) | Aspergillus incidence (%) | Aspergillus frequency (%) |
|-------------------------|----------------------------|---------------------------|
|     | Gombisa | Sack | Hermetic | Gombisa | Sack | Hermetic |
| ILD | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| 2   | 13.3 ± 1.24 | 7.3 ± 1.63 | 3.6 ± 1.14 | 10.3 ± 0.86 | 5.0 ± 2.12 | 3.7 ± 1.66 |
| 4   | 23.3 ± 2.24 | 15.0 ± 1.46 | 14.3 ± 1.86 | 16.6 ± 2.32 | 11.0 ± 1.96 | 8.5 ± 1.39 |
| 6   | 33.3 ± 3.08 | 28.3 ± 2.99 | 26.7 ± 3.23 | 26.7 ± 2.32 | 23.3 ± 1.47 | 18.0 ± 1.66 |
| LSD (5%) | 2.8 | 3.4 | 16.3 | 17.4 |
| CV (%) | 33.3 | 22.4 | 22.2 | 33.3 | 22.4 | 22.2 |

| Fusarium incidence (%) | Fusarium frequency (%) |
|-------------------------|------------------------|
| ILD | 10.0 ± 0.86 | 10.0 ± 0.86 | 10.0 ± 0.86 | 6.3 ± 1.47 | 6.3 ± 1.47 | 6.3 ± 1.47 |
| 2   | 21.7 ± 1.47 | 15.0 ± 1.91 | 13.0 ± 1.91 | 15.0 ± 1.91 | 11.7 ± 1.96 | 9.0 ± 1.46 |
| 4   | 33.3 ± 2.14 | 26.6 ± 2.50 | 23.3 ± 1.75 | 26.7 ± 0.33 | 21.7 ± 1.47 | 18.0 ± 1.66 |
| 6   | 45.0 ± 5.54 | 40.0 ± 5.75 | 33.0 ± 2.14 | 38.3 ± 3.16 | 33.3 ± 2.14 | 28.0 ± 1.16 |
| LSD (5%) | 2.8 | 2.4 | 10.4 | 15.3 |
| CV (%) | 33.3 | 22.4 | 22.2 | 33.3 | 22.4 | 22.2 |

| Penicillium incidence (%) | Penicillium frequency (%) |
|---------------------------|---------------------------|
| ILD | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2   | 10.0 ± 0.86 | 5.0 ± 1.12 | 2.0 ± 0.14 | 7.0 ± 0.58 | 3.0 ± 1.87 | 1.0 ± 1.01 |
| 4   | 16.7 ± 2.32 | 11.3 ± 1.19 | 9.4 ± 1.10 | 13.0 ± 1.91 | 8.0 ± 1.51 | 7.0 ± 0.58 |
| 6   | 22.4 ± 1.47 | 16.0 ± 3.22 | 12.2 ± 1.17 | 17.3 ± 3.25 | 12.0 ± 1.70 | 9.0 ± 1.46 |
| LSD (5%) | 2.8 | 2.4 | 0.92 | 2.7 |
| CV (%) | 33.3 | 22.4 | 22.2 | 33.3 | 22.4 | 22.2 |

Mean values ± standard deviation of three replicates within each column sharing similar letters were not significantly different by LSD test at P≤0.05. CV: Coefficient of variation, LSD: least significant different, ILD: initial loading date.

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