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Food Security
https://doi.org/10.1007/s12571-018-0861-9

ORIGINAL PAPER

# Quantitative and quality losses caused by rodents in on-farm stored maize: a case study in the low land tropical zone of Kenya

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Received: 8 May 2018 / Accepted: 29 October 2018
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## Abstract
Rodents are one of the major storage pests in on-farm maize storage in the tropics. However, information on actual magnitude of weight and quality losses caused by rodents in maize stores and species of rodent associated with the losses is scarce and if available would help to improve maize postharvest management. Maize stores of small-scale farmers in the lowland tropical zone of Kenya were monitored for actual weight losses caused by rodents and rodent trapping was conducted to determine species and estimate population of the rodents associated with the losses. Moulds and total aflatoxin contamination and nutritional value of rodent-damaged grain and non-damaged grain samples were also compared to evaluate the impact of rodent infestation on grain quality. In a sample of 20 farmers, we found that cumulative weight losses due to rodents ranged from 2.2 to 6.9% in shelled maize grain and from 5.2 to 18.3% in dehusked cobs after storage for 3 months. *Rattus rattus* was the only rodent species captured over the whole trapping period with a trap success rate of 0.6–10.0%. Total mould count, *Fusarium* spp. incidence and total aflatoxin contamination were significantly higher in rodent-damaged grains than in the non-damaged ones whereas no significant differences were observed for the incidence of *Aspergillus* spp. There were also significant decreases in dry-matter, fat, crude protein and fatty acid content in rodent-damaged grain compared to non-damaged grain. These findings show that rodents are a significant cause of postharvest losses in on-farm maize storage and impact negatively on food nutrition and safety. Mitigation strategies for postharvest losses should therefore include rodent control.

**Keywords** Postharvest losses · Rodent · Fatty acids · Moulds · Aflatoxin

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## 1 Introduction

Maize (*Zea mays* L.) represents the primary staple grain for many households in Sub-Saharan Africa (SSA), accounting for 36% of daily calorie intake (Kumar and Kalita 2017). Hence occurrence of quantitative and quality losses in on-farm or off-farm storage can be a significant contributor to food insecurity in SSA. Postharvest losses not only affect food security but also pose challenges to sustainability of food systems as they compound the pressure on the available land and scarce natural resources (Schuster and Torero 2016). Insects are the main cause of postharvest losses in maize storage (Boxall 2002; Abass et al. 2014). A number of studies across the globe, however, have demonstrated that rodents present a significant challenge in storage and, in some cases, they are the main storage problem (Cao et al. 2002; Brown et al. 2013; Belmain et al. 2015; Edoh Ognakossan et al. 2016; Mwangi et al. 2017).

Published online: 19 November 2018

The roof rat (*Rattus rattus)*, the house mouse (*Mus musculus)* and the natal multimammate mouse (*Mastomys natalensis*) are the rodent species usually associated with postharvest losses in grain stores in East Africa (Makundi et al. 1999). Most current and past research in SSA on postharvest losses in on-farm maize storage due to storage pests focused on insects (Boxall 2002; Affognon et al. 2015), whereas attention to rodents seems to be minimal (Swanepoel et al. 2017). In Kenya, for instance, rodents contribute 30% of the total postharvest losses on maize stored in farmers' stores (Edoh Ognakossan et al. 2016) and 11% of the storage losses in off-farm stores (Mwangi et al. 2017). In the lowland tropical (LLT) zone specifically, rodents are the greatest storage problem in on-farm stores, contributing 63% of their total postharvest losses (Edoh Ognakossan et al. 2016). Moreover, rural storage is usually characterized by poor hygiene and a predominance of non-rodent proof grain storage structures (Edoh Ognakossan et al. 2016). These conditions attract commensal rodents and favour their proliferation (Panti-May et al. 2012). Thus exclusion of rodents from food stores is difficult. Furthermore, poor socio-economic conditions strongly influence rodent infestation in human dwellings (Langton et al. 2001).

Apart from direct weight losses due to physical damage of grains, rodent infestations in grain stores can lead to quality losses, as well as food safety and public health concerns (Meerburg et al. 2009; Belmain et al. 2015). Maize grain includes four distinct parts; the endosperm (80–85%), the germ or embryo (9–10%), the pericarp (5–6%) and the tip cap (Chaudhary et al. 2014). The germ contains most of the nutrients of the grain; it has high concentrations of fat (33%), protein (18–19%), minerals and vitamins (vitamins B complex and E) (Watson 1967). Moreover, the germ is a rich source of unsaturated fatty acids mainly oleic and linoleic acids (Chaudhary et al. 2014). In addition, the proteins with the best amino acid profile are concentrated in the germ (Gupta and Eggum 1998; Shewry 2007). Typically, rodent damage on maize grain is by removal of the germ, and thus may reduce significantly the nutritional value of the grain. Furthermore, grain contaminated by rodents' droppings may harbour pathogens, making them unfit for human consumption (Meerburg et al. 2009; Hodges et al. 2014). Rodents' urine may raise the water activity of the affected area, increase the nitrogen availability and thus encourage development of storage fungi (Stejskal et al. 2005). Furthermore, the feeding activity of rodents itself could aid in disseminating fungal spores (Reichman et al. 1985; Reichman et al. 1988; Vander Wall 1990). Rodents also cause damage to storage materials and equipment (Gwinner et al. 1996) and germination failure of seeds intended for planting.

Given the negative impact rodents may have on food security in maize storage, there is a need to assess the magnitude of the actual weight loss and grain quality issues associated with them, as a basis for addressing postharvest losses and assuring better grain quality for consumers. Although farmers' perception on weight losses caused by rodents in storage was recently reported (Edoh Ognakossan et al. 2016), actual measurement of the weight losses with an additional component to determine rodent species and quality decline associated with the losses will give more data which may help to improve rodent management in on-farm storage. Indeed, according to Gwinner et al. (1996), successful management of rodents in stores prior to implementation, should include answers to questions relating to (i) the species of rodent causing damage to the produce, (ii) the approximate degree of infestation and loss estimation and (iii) the extent of the infestation, among others. Furthermore, to our knowledge, there are no reports on how rodent damage affects the nutritional value of grain. Thus the objectives of this study were to follow rodent activity in on-farm maize stores in a rodent-prone zone in order to: quantify the magnitude of weight losses due to rodent infestation; determine rodent species associated with the losses; and evaluate the quality of grain damaged by rodents with respect to nutritional value, infection by moulds and aflatoxin contamination.

## 2 Materials and methods

### 2.1 Study area

The study was conducted in Mwarakaya ward (03°49.17́'S; 039°41.498′E) located in Kilifi-south sub-county, in the low land tropical (LLT) zone of Kenya. This study site was selected based on the findings of an earlier study (Edoh Ognakossan et al. 2016) that rodents were the main storage problem in farmers' stores in this region. The region is characterized by two maize cropping seasons. The long rain cropping season starts in April and ends in July whereas the short rain cropping season begins in September and ends in December. Thus harvesting months are July – August and December – January, respectively. The LLT zone is regarded as one of the lowest potential zones for maize production among the six maize growing agro-ecological zones of Kenya (De Groote 2002) and is characterized by an elevation of <800 m, a daily temperature of 20.0–29.4 °C and an average total seasonal rainfall of <1000 mm (Hassan et al. 1998).

### 2.2 Experimental design

On-farm 3-month storage trials were carried out in two villages (Mbuyuni (03°48.86'S; 039°41.835′E) and Kizingo (03°46.57'S; 039°40.563′E)) from June to September 2015. In each village, ten farmers were selected, based on their own accounts of experiencing rodent problems during storage. The farmers were divided into two groups of five based on maize

storage form (cobs or shelled grain). An individual farmer in each group of maize storage form constituted a replicate in the trial.

Clean, shelled maize grain, freshly harvested or dehusked maize cobs were purchased locally from farmers. The shelled maize grain and cobs were treated with the insecticide Actellic Super dust (pirimiphos-methyl 1.6% *w*/w + permethrin 0.3% *w*/w) 2 weeks before setting up the trial in order to minimize insect infestation during the course of the experiment. For the maize stored on cobs, only cobs which did not present any visible insect or rodent damage were purchased. Each of the 20 farmers involved in the trial was provided with approximately 10 kg of either shelled maize grain or cobs for storage in their ordinary storage structures. The original weight of the maize stored by each farmer was accurately determined and recorded ($Wgt_0$). Polypropylene bags (50 Kg capacity) were filled with shelled maize and the open ends were twisted and tied shut using sisal twine. The bags were placed on a clean mat in order to collect the spilled grains when the rodents attacked. For the maize stored as cobs, cobs were counted weighed and placed on a clean mat. The bagged maize or the cobs were stored in the farmers' usual maize storage places for 3 months. Some farmers stored maize in their homes, in the kitchen, or in a traditional granary (*lutsaga)*. The traditional granary was a wooden platform plastered with mud and constructed above the fireplace in the kitchen. This type of granary was the predominant one in the area. All farmers involved in the study were instructed not to disturb the experiment and also to keep it safe from poultry and domestic animals.

## 2.3 Sampling

Baseline sampling was done during set-up of the trial and subsequent samplings were done at one-month intervals. During each sampling occasion, 200 g of shelled maize grain or 6 cobs were taken randomly from the bags or mat, respectively. The sampled cobs from each store were shelled separately. Only stores showing signs of rodent attack were sampled during subsequent samplings. After sampling from the bags, any sections of the bags damaged by rodents were tied-up with sisal twine and the bags closed again. Each sample was randomly halved into two sub-samples. One sub-sample was analysed for dry matter content and the other was used for determination of live insect counts and insect damaged grain. Spilt shelled grains and loose grains from cobs were also collected as samples. These were separately sorted into rodent damaged and undamaged grains and kept for analysis of quality parameters, including mould infection, aflatoxin contamination, proximate composition and fatty acid profile.

## 2.4 Determination of dry matter content

Moisture content of grain was determined by the oven drying method (ISO 1980). About 10 g of maize grains was ground using a laboratory mill (Knife Mill Cup KM-400 MRC Lab, MRC International, Westminster, UK). The sample was transferred into an aluminium dish and weighed ($W_i$), and then dried in an air-oven maintained at 130 °C for 2 h after which it was cooled in a desiccator containing silica gel for 2 h and the new weight of the dish and dry sample ($W_d$) determined. The moisture content (m.c.) was determined using the expression: m.c. (%) = $100[(W_i\text{-}W_d)/W_i]$, and dry matter content obtained by subtracting the moisture content from 100.

## 2.5 Determination of live adult insect counts and insect damaged grain

Approximately 100 g sub-sample was sieved through a set of 3.35 and 1.4-mm aperture sieves to separate any live adult insects from the grain. Typical insect pests associated with stored maize were identified and counted. The sieved grain was later sorted into insect damaged and undamaged grain.

## 2.6 Determination of cumulative weight losses caused by rodents

Actual weight losses, on a dry matter basis, were estimated every month from each of the stores where rodent attack was evident; losses in the stores that were not attacked by rodents were assumed to be zero (Hodges et al. 2014). The grains spilled out from damaged bags or loose grains from the maize cobs on the mat were carefully separated and weighed and their weight added to the weight of the shelled maize or cobs remaining in the bags or mats to obtain the weight $Wgt_i$. Cumulative weight loss ($CWgtL_i(\%)$) at each month ($_i$), where $_i$ is one, two or three storage months, was calculated as the difference in weight between the originally stored quantity corrected for dry matter content ($Wgt_0 \times DM_0$). The new weight, corrected for dry matter content ($Wgt_i \times DM_i$) was expressed as a percentage of the original weight stored, corrected for dry matter content.

## 2.7 Identification of rodents species and population estimation

A four-month trapping exercise was performed (August–November 2015) on a monthly basis with a group of 10 farmers distributed across two villages: Bokini (03°45.60'S; 039°47.46′E) and Pingilikani (03°47.005'S; 039°46.505′E) located in the Mwarakaya ward. These two villages were different from the villages in which the actual weight loss estimation experiment was conducted in order to avoid interfering with the weight loss estimation. Three types of traps: Snap trap (Wooden Victor® snap traps, Woodstream Corp., Lititz, PA, USA) (kill trap), Sherman live trap (H. B. Sherman's Traps Inc., Tallahassee, FL, USA) (live trap), and the locally-made trap (rectangular box made from wire and small

pieces of metal) (live trap) were used. The Snap traps and Sherman live traps were provided by the National Museums of Kenya while the locally-made traps were purchased from a local vendor. In the two villages, equal numbers of traps were set either in granaries or in the domestic houses where grain was stored. In each room or granary, three snap traps, two Sherman traps and three locally-made traps were set for a total of four consecutive nights. A mixture of peanut butter and white oats were used as bait for the Sherman and snap traps while dried cassava pieces dipped in peanut butter were used as bait for the locally-made traps. Set traps were checked and re-baited every morning. For every individual rodent caught, the age (adult or juvenile), head-body length, tail length, left hind foot length and weight were recorded. Trapped rodent individuals were identified to species level using the Kingdon field guide to African mammals (Kingdon 1997). Further comparative identification of captured specimens was performed at the small mammal collection at the National Museums of Kenya, Nairobi. Animal handling and ethics in the study followed the National Museums of Kenya, Mammalogy section, small mammal capture and handling protocol. Rodent population was estimated based on the relative abundance using trap success rate as described in Aplin et al. (2003). Trap success rate (%) was the number of rodents captured divided by number of night traps multiplied by 100. Trap night is the total number of traps set for four consecutive nights. Adjusted trap night was not used as no case of "null traps" (traps that have been triggered without making a capture) was observed.

## 2.8 Determination of grain quality

### 2.8.1 Determination of total mould count

Total moulds count was performed using the surface plating technique (Pitt and Hocking 2009). Three replicates of 10 g of grain from each of the rodent-damaged and undamaged grain samples were thoroughly homogenised with 90 ml of 0.1% peptone water solution, and serial dilutions of the homogenate were prepared up to $10^{-3}$. Aliquots (0.1 mL) of each dilution ($10^{-1}$, $10^{-2}$, $10^{-3}$) were transferred into Petri dishes containing Sabouraud Dextrose Agar (enzymatic digest of casein 5 g, enzymatic digest of animal tissue 5 g, dextrose 40 g, agar 15 g in 1000 mL distilled water; pH 5.6 ± 0.2 at 25 °C) to which 1 g chloramphenicol per litre had been added. The Petri dishes were incubated at 25 °C under a 12:12 h light - darkness regime for 4 days. Mould colonies developing on plates were counted and recorded as colony forming units per gram (cfug$^{-1}$).

### 2.8.2 Determination of mould incidence

Three replicates of 21 grains of each sample (63 grains per sample) were surface sterilized in 3% sodium hypochlorite solution for 2 min and rinsed twice in distilled water. Seven grains were plated per Petri dish containing Czapek Dox Agar (Sucrose 30 g, Sodium nitrate 2 g, Dipotassium phosphate 1 g, Magnesium sulphate 0.5 g, Potassium chloride 0.5 g, Ferrous sulphate 0.01 g, agar 15 g in 1000 mL distilled water; pH 7.3 ± 0.2 at 25 °C) to which 1 g chloramphenicol per litre had been added. The Petri dishes were incubated at 25 °C under a 12:12-h light and darkness regime for four days. The number of grains infected was recorded and categorized according to colony colour. On the basis of colony colour, pure sub-cultures were prepared and cultivated on Czapek Dox Agar (25 °C; 12:12 h light: darkness regime) for 5 days following which fungal genera were identified using morphological characteristics viewed under a microscope on prepared slides, as described by Pitt and Hocking (2009). The percentage of grains infected by each fungal genus was calculated thereafter to determine their incidence on the grains.

### 2.8.3 Aflatoxin analysis

For each sample (rodent-damaged grains and the non-damaged grains), 9 sub-samples of 50 g each were milled using a laboratory mill (Knife Mill Cup KM-400 MRC Lab, MRC International, Westminster, UK). A portion of each of the milled samples (5 g) was mixed with 25 mL of 70:30 *v*/v methanol: distilled water solution, and vigorously homogenized for 3 min using a vortex mixer at room temperature (20–25 °C). The extracts were filtered through a Whatman #1 filter and the filtrates were collected for analysis. Extracts were assayed for total aflatoxin using Veratox® Total Aflatoxin ELISA (Enzyme Linked Immunosorbent Assay) kit (Veratox®, Neogen Corporation, Lansing, MI, USA). Enzyme conjugate (100 μL) was added to duplicate mixing wells, then 100 μL of aflatoxin standards (0 ppb, 5 ppb, 15 ppb, and 50 ppb) and extracts in duplicates were added simultaneously using a multichannel pipette. From the mixing well, 100 μL of liquid was transferred to antibody-coated wells and incubated at room temperature for 2 min. Contents were then emptied, and the antibody-coated wells were washed 5 times with sterile distilled water. Excess water was tapped out on to an absorbent paper towel and the wells filled with 100 μL of substrate solution, mixed thoroughly and incubated for 3 min at room temperature before adding 100 μL of the stop solution. Absorbance of liquid in each well was measured at 650 nm using a UT-6100 auto microplate reader (MRC International, UK). Aflatoxin concentrations were determined from a calibration curve prepared from the known standards and multiplied by the dilution factor to obtain the contamination level of the samples in ppb. Detection limit of the assay kit was 1.4 ppb.

### 2.8.4 Proximate analysis

The Association of Analytical Chemists (AOAC 1990) procedures were used. Ash content was determined by incinerating 5 g of the ground sample in a muffle furnace at 550 °C overnight. The dry matter (DM) was determined by subtracting moisture content from 100 (see section 2.4). A VELP® Scientifica solvent extractor (SER 148/6) was used to determine crude fat (CF) content with ethyl ether as extractant. Crude protein (CP) was quantified using the Kjeldahl method. The nitrogen content (%) determined was converted into percentage CP using a factor of 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed with the VELP® Scientifica fibre analyzer (FIWE 6) (VELP Scientifica, Usmate Velate, Italy) using reagents described by Van Soest et al. (1991).

### 2.8.5 Analysis of fatty acids

A methyl esterification reaction was performed on 5 mg of each of the ground samples according to a protocol adapted from Christie (1993). A solution of 15 mg/mL concentration of sodium methoxide in methanol was prepared (Musundire et al. 2016). An aliquot of the solution (500 µL) was added to each ground maize sample, vortexed for 1 min and then sonicated for 5 min. The reaction mixture was incubated at 60 °C for 1 h, thereafter quenched by adding 100 µL deionized water followed by vortexing for another 1 min. Methyl esters were extracted using hexane (GC-grade) (Sigma–Aldrich, St. Louis, USA), and then centrifuged (Avanti J-25I, Beckman, CA, USA) at 14,000 rpm 23,700 *g* for 5 min (Musundire et al. 2016). The supernatant was dried over anhydrous $Na_2SO_4$ and then analyzed using gas chromatography-mass spectrometry (GC/MS). The GC/MS analysis was carried out on a 7890A gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) linked to a 5975C mass selective detector (Agilent Technologies, Inc., Santa Clara, CA, USA). Injection volume was 1.0 µL in the splitless injection mode using an auto sampler 7683 (Agilent Technologies, Inc., Beijing, China). The following conditions used by Cheseto et al. (2015) and Musundire et al. (2016) were applied: inlet temperature 270 °C, transfer line temperature 280 °C, and column oven temperature programmed from 35 to 285 °C with the initial temperature maintained for 5 min then 10°Cmin$^{-1}$ to 280 °C and held at this temperature for 20.4 min. The GC was equipped with an HP5 MS low bleed capillary column (30 m × 0.25 mm i.d., 0.25 µm) (J&W, Folsom, CA, USA). The carrier gas used was Helium at a flow rate of 1.25 mL min$^{-1}$. The mass selective detector was maintained at the ion source temperature of 230 °C and a quadrupole temperature of 180 °C. Electron impact (EI) mass spectra were recorded at an acceleration energy of 70 eV. Fragment ions were analyzed over 40–550 m/z mass range in the full scan mode with the filament delay time set at 3.3 min. Fatty acids were identified by comparison of gas chromatographic retention times and fragmentation patterns with those of authentic standards and reference spectra published by library–MS databases: National Institute of Standards and Technology (NIST) 11. The analysis was replicated twice.

### 2.9 Statistical analysis

Data on weight losses (%), insect damaged grain (%) and mould incidence (%) were arcsine square root (x/100)-transformed while insects count data was log (x + 1)-transformed to normalize them. Total mould count (cfu/g) data was expressed in $\log_{10}$. Transformed weight losses and insect damaged grain data were subjected to repeated-measures ANOVA while total mould count, mould incidence and total aflatoxin were subjected to a t-test. For the repeated-measures ANOVA, degrees of freedom were corrected using Greenhouse-Geisser estimates if the assumption of sphericity was violated (Mauchly's test for sphericity) and the means of the consecutive samplings separated using Bonferroni tests. Data on proximate composition and fatty acid content of rodent-damaged and non-damaged grain were compared using a t-test. All data were analyzed using SPSS version 20.

## 3 Results

### 3.1 Dry matter content

Dry matter content of the cobs and shelled maize grain stored for 3 months varied between 88.24 ± 0.23 and 89.63 ± 0.18% and between 87.95 ± 0.18 and 89.39 ± 0.11%, respectively (Table 1). Significant decrease of the dry matter content was observed in the shelled maize grains at the end of the storage trial ($F_{3,\ 6} = 24.55$, $p = 0.001$) while on the stored cobs, dry matter contents at the baseline and at the end of the trial were significantly lower than the ones observed at 1 and 2 months of storage ($F_{3,\ 18} = 24.55$, $p < 0.001$).

### 3.2 Live adult insect counts and insect damaged grains

Insect damage levels on cobs and shelled maize grain remained unchanged statistically during the trial compared to baseline. Throughout the trial, insect damage levels were lower than 1%. *Sitophilus zeamais* was the only insect species observed in the trial, and was detected only after 3 months' storage on cobs (Table 2).

**Table 1** Dry matter content of the maize during 3 months storage

| Sampling intervals (month) | Dry matter content (%) |
|---|---|
| Maize stored on cobs | |
| 0 (n = 10) | 88.59 ± 0.23a |
| 1 (*n* = 10) | 89.14 ± 0.14b |
| 2 (*n* = 9) | 89.63 ± 0.18b |
| 3 (n = 7) | 88.24 ± 0.23a |
| Shelled maize grains stored in bags | |
| 0 (n = 10) | 89.33 ± 0.17b |
| 1 (*n* = 4) | 89.39 ± 0.11b |
| 2 (*n* = 7) | 89.13 ± 0.14b |
| 3 (*n* = 6) | 87.95 ± 0.18a |

For each storage form, means (± SE) within a column followed by different letters differ significantly from each other ($p < 0.05$). *n* represents the number of stores sampled

### 3.3 Weight loss caused by rodents

Weight loss of stored cobs increased steadily and significantly over time, ranging from 5.2% after storage for 1 month to 18.3% after storage for 3 months, the maximum storage duration ($F_{2.41,\ 14.47} = 122.661$, $p < 0.001$; Table 2)). Weight loss of shelled grain also increased with storage duration from 2.2% after storage for 1 month to 6.9% after storage for 3 months ($F_{1.75,\ 15.75} = 15.407$, $p < 0.001$; Table 2).

### 3.4 Rodent species and population

Over the 4 months trapping period, 65 individual rodents were captured from a total of 1200 trap nights and consisted of 63% adults and 18.5% sub-adults and juveniles (Table 3). All the rodents captured throughout the trapping period were *R. rattus*. The trap success rate ranged from 0.63 to 10%, and overall showed a gradual increase in the last two months of trapping.

**Table 2** Weight loss due to rodent attack, and level of insect damage of cobs and shelled maize during 3 months storage

| Sampling intervals (months) | Cumulative weight losses (%) | Damage due to insects (%) | Number of live *S. zeamais* adults |
|---|---|---|---|
| Maize stored on cobs | | | |
| 0 (n = 10) | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a |
| 1 (n = 10) | 5.2 ± 0.8b | 0.0 ± 0.0a | 0.0 ± 0.0a |
| 2 (n = 9) | 12.8 ± 3.5c | 0.0 ± 0.0a | 0.0 ± 0.0a |
| 3 (n = 7) | 18.3 ± 1.6d | 0.2 ± 0.1a | 0.9 ± 0.4a |
| Shelled maize grains stored in bags | | | |
| 0 (n = 10) | 0.0 ± 0.0a | 0.4 ± 0.1a | 0.0 ± 0.0a |
| 1 (*n* = 4) | 2.2 ± 1.1a | 0.6 ± 0.3a | 0.0 ± 0.0a |
| 2 (n = 7) | 4.7 ± 1.5b | 0.3 ± 0.1a | 0.0 ± 0.0a |
| 3 (n = 6) | 6.9 ± 2.1b | 0.5 ± 0.2a | 0.0 ± 0.0a |

For each storage form, means (± SE) within a column followed by different letters differ significantly from each other ($p < 0.05$). *n* represents the number of stores sampled

### 3.5 Effect of rodent damage on mould and aflatoxin contamination of grains

Total mould count ($\log_{10}$ cfu $g^{-1}$) was significantly higher in the rodent-damaged grain (5.3 ± 0.2) compared to the non-damaged grain (3.7 ± 0.1) (t (4) = 7.914, $p = 0.001$). With regard to mould incidence, *Aspergillus* and *Fusarium* were the main fungal genera isolated (Fig. 1) in both the damaged and undamaged grain. *Fusarium* incidence was significantly higher in the damaged grain (t (4) = 3.85, $p = 0.011$), whereas incidence of *Aspergillus* did not differ significantly (t (4) = 1.38, $p = 0.239$). Irrespective of the fungal genera the percentage of kernels infected with moulds was significantly higher in the rodent-damaged grains (63.5 ± 6.3%) compared to the non-damaged grains (25.4 ± 3.2%) (t (4) = 5.135, $p = 0.007$). Aflatoxin contamination was significantly higher in rodent-damaged grain (6.1 ± 1.7) than in non-damaged grain (1.1 ± 0.4) (t (8.96) = 2.77, $p = 0.022$).

### 3.6 Proximate composition and fatty acid profile

Rodent-damaged grain had significantly lower dry matter (t (2) = 8.80, $p = 0.013$), crude protein (t (1.27) = 13.93, $p = 0.024$) and crude fat (t (1) = 14.95, $p = 0.043$) compared to non-damaged grains (Fig. 2). The dry matter, crude protein and crude fat in the rodent-damaged grains represented reductions of 2.43%, 13.34%, and 87.92%, respectively. However, there was no significant difference in the ash (t (2) = 0.08, $p = 0.940$), neutral detergent fibre (t (1.98) = 2.98, $p = 0.097$) and acid detergent fibre (t (2) = 8.80, $p = 0.072$) content between the rodent-damaged grain and the non-damaged grain.

Eight fatty acids were identified and quantified (Table 4). The most abundant fatty acids in the non-damaged grain and rodent-damaged grain were oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:0), and stearic acid (C18:0). Other fatty acids were present in minor quantities and were only detected in the non-damaged grain. Rodent-damaged grain had significantly lower levels of oleic acid (t (2) = 77.79, $p < 0.001$), linoleic acid (t (2) = 15.81, $p = 0.004$) and palmitic acid (t (2) = 10.25, $p = 0.009$) compared to the non-damaged grain, corresponding to reductions of 85.71%, 57.90% and 80.40%, respectively. Stearic acid was also lower in the rodent-damaged grains, although the difference was not statistically significant at the 95% confidence level. In both samples, linoleic and oleic acids represented more than 75% of the total fatty acid content. Moreover linoleic acid accounted for the highest proportion (56.18%) of the total fatty acid content in the rodent-damaged grain while oleic acid accounted for the

**Table 3** Rodent species associated with the losses and their population estimation

| Months | Number of captures | | | | | | *Trap nights | Percentage trap success (%) |
|---|---|---|---|---|---|---|---|---|
| | *Rattus rattus* | | | | *Mastomys natalensis* | *Mus musculus* | | |
| | Total | Adult | Sub-adult | Juvenile | | | | |
| Aug-15 | 8 | 8 | 0 | 0 | 0 | 0 | 240 | 3.33 |
| Sept-15 | 2 | 1 | 0 | 1 | 0 | 0 | 320 | 0.62 |
| Oct-15 | 23 | 10 | 5 | 8 | 0 | 0 | 320 | 7.19 |
| Nov-15 | 32 | 22 | 7 | 3 | 0 | 0 | 320 | 10.00 |
| Total | 65 | 41 | 12 | 12 | 0 | 0 | 1200 | 5.41 |

* For the first month of trapping (August), traps were set for 3 consecutive nights. So with 10 farmers and 8 traps (3 snap traps, 2 Sherman live traps and 3 locally made traps) set in the house of each farmer each night, trap nights was calculated as $8 \times 3 \times 10 = 240$ trap nights. For the other months of trapping (September, October and November), traps were set for 4 consecutive nights and therefore monthly trap nights was 320

highest proportion of the total fatty acid content (43.48%) in the non-damaged grain.

## 4 Discussion

Filling the gap of actual weight losses and quality decline due to rodent infestation as well as rodent species associated with the losses in storage facilities could help different stakeholders (policy makers, donors, researchers and development agencies) to understand the impact rodents may have on food security, food safety and nutrition and therefore help prioritise extension programs. Lower dry matter content in the rodent-damaged grain results from higher moisture content associated with them due to hydration of the damaged sites. The unchanged insect damages levels throughout the duration of the trial and the observation of live adults of *S. zeamais* only after three months of storage at an average density of less than 1 insect per 100 g suggest that interference of insects was insignificant, and that cumulative weight losses recorded are mainly attributable to rodent infestation. However, the appearance of live adult insects at three months suggests that beyond three months, losses may no longer be attributed to rodent infestation alone. The occurrence of insects on maize after 3 month's storage could be explained by a number of factors. Ordinary polypropylene bags are unable to stop insects' proliferation when grain is stored. Moreover, insects are always present in farmers stores due to the presence or debris of old stock and lack of storage hygiene. Furthermore, grain treated with insecticides becomes vulnerable to insect infestation with time (usually 3–4 months) as the potency of the active ingredient gradually decreases. The levels of weight losses associated with rodent infestation during the three

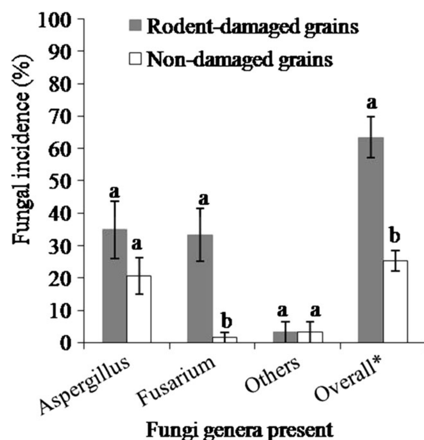


**Fig. 1** Mould incidence in rodent-damaged grains and non-damaged grains. For each parameter, bars marked with same letters, imply that means (± SE) are not significantly different ($p > 0.05$).*Overall: percentage of kernels infected with moulds irrespective of mould genera

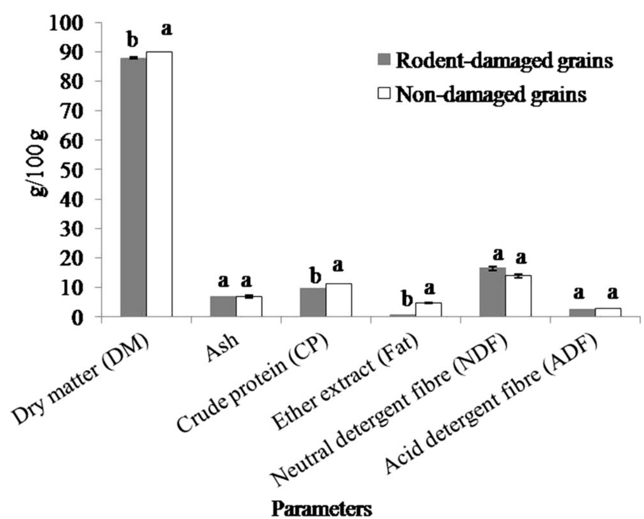


**Fig. 2** Proximate composition of rodent-damaged grains and non-damaged grains. For each parameter, bars marked with same letters, imply that means (± SE) are not significantly different ($p > 0.05$)

**Table 4** Fatty acids profile of the rodent-damaged grain and non-damaged grain. All values are presented as μg/g of sample dry weight and as percentage of total fatty acid content

| Fatty acids | Non-damaged grains | | Rodent-damaged grains | |
|---|---|---|---|---|
| | (μg $g^{-1}$dw) | % of total | (μg $g^{-1}$dw) | % of total |
| Palmitic acid (C16:0) | 51.00 ± 0.00a | 15.52 | 10.00 ± 4.00b | 11.31 |
| 14-Methylpalmitic acid (a:17) | 0.50 ± 0.50 | 0.19 | nd | – |
| Stearic acid (C18:0) | 12.50 ± 0.50a | 3.79 | 8.50 ± 1.50a | 9.35 |
| Arachidic acid (C20:0) | 2.50 ± 0.50 | 0.71 | nd | – |
| Lignoceric acid (C24:0) | 1.00 ± 0.00 | 0.4 | nd | – |
| Palmitoleic acid (C16:1) | 1.00 ± 0.00 | 0.22 | nd | – |
| Oleic acid (C18:1) | 143.50 ± 0.50a | 43.48 | 20.50 ± 1.50b | 23.14 |
| Linoleic acid (C18:2) | 117.50 ± 2.50a | 35.69 | 49.50 ± 3.50b | 56.18 |

nd, not detected. Values (means ± SE) followed by the same letter, within the same row, are not significantly different ($p > 0.05$)

month storage period in the present study show that rodents can pose a significant problem for the safe storage of maize. In similar work in Mozambique with maize cobs, Belmain et al. (2003) reported 3.1–12.8% (average 7%) cumulative weight losses due to rodents within three months. Another study in Tanzania reported an average of less than 0.5% weight losses due to rodents over a 7 month period of storage of shelled maize grain stored in open cribs and unprotected sacks (Mdangi et al. 2013). However, the difference between loss data in the present study and those reported by Belmain et al. (2003) and Mdangi et al. (2013) could be related to rodent prevalence in the stores, which can be linked to differing habitats and ecologies. Based on the weight loss estimation method used in the present study, no relationship could be established between dry matter and weight loss as the weight of maize available at each sampling date was not constant and highly contingent on the pressure of rodent infestation. In the study, although losses were apparently higher on maize stored as cobs than for maize stored as shelled grain, losses in the two cases have to be put into perspective for proper comparison as losses quantified in storage of cobs were not corrected for the weight of the cobs without grains. Moreover, it should be noted that the storage of maize as shelled maize grain was not a common practice in the area; farmers predominantly stored their maize as cobs. This situation, i.e. maize stored as shelled grain instead of cobs may have influenced the neophobic behaviour of rodents in the stores (Brigham and Sibly 1999).

Of the three commensal rodent species (*R. rattus*, *M. musculus* and *M. natalensis*) often associated with postharvest crop damage in East Africa (Makundi et al. 1999), only *R. rattus* species was captured in farmers' stores. *Mastomys natalensis* was especially expected to be captured during the last two months of the trapping period which coincided with the end of the harvest period as this rodent moves from the fields into storage structures at the end of the harvest season due to the absence of food in the fields (Makundi et al. 1999). On the other hand, *M. musculus* was expected to be captured during the trapping period as, like *R. rattus,* it inhabits houses and storage structures (Mdangi et al. 2013). The capture of only *R. rattus* over the 4 month duration of trapping nevertheless supports the view that it is the most abundant rodent species residing inside houses across Africa (Kilonzo 2006), and it is consistent with the findings of Belmain et al. (2003) and Mdangi et al. (2013) in Mozambique and Tanzania, respectively. However, three possible reasons could explain the absence of *M. natalensis* and *M. musculus* over the fours month when traps were set in the present study. One reason could be the presence of inter-specific competition. According to Taylor et al. (2012), *M. natalensis* only enters smallholder houses in large numbers when *R. rattus* is completely absent from the region. Several studies (King et al. 1996; Choquenot and Ruscoe 2000; Courchamp et al. 2000; Ruscoe 2001) also reported that rats are strong competitors of mice, affecting negatively the rate of change in mouse abundance and even excluding them when resources are scarce. King et al. (1996) for instance found that where mice and *R. rattus* coexisted in New Zealand forests, the mice were scarcer than rats. A second reason for the absence of *M. natalensis* is the difference in nesting behaviour between *M. natalensis* and *R. rattus*. *R. rattus* appears to be predominantly confined to areas of human settlement whereas *M. natalensis* lives in burrows in fields (Belmain et al. 2003; Mdangi et al. 2013) and therefore trapping inside dwelling places may not result in high capture rates. The absence of *M. natalensis* and *M. musculus* could also be related to the fact that data in this study were limited to 4 months trapping while rodent abundance may vary with longer trapping periods. Indeed, *M. natalensis* population fluctuations vary among seasons, years and localities and are largely influenced by the amount and duration of rainfall (Leirs et al. 1989; Makundi et al. 2005). The increase of trap

success rate during the last two months could be related to the availability of more food resources in the farmers' stores as this period coincided with the end of harvesting. According to Krebs (1999), food is clearly one of the dominant ecological factors that influence rodent populations.

The higher mould infection rates and the high *Fusarium* incidence on the rodent-damaged grains indicate that rodent attack encourages mould contamination. This may be because the injuries inflicted by rodents on grain when feeding, offered entry points for fungal spores. According to Chen et al. (2004), kernel breakage creates an infection court for opportunistic pathogens. Also it might be possible that rodents, when feeding on the grain, transmit fungal spores through their mouths. This hypothesis is supported by the fact that fungi and rodents do not occur independently in natural ecosystem as rodent shelters and their internal organs are active sites of fungal proliferation (Otcenášek and Dvõrák 1962; Hubálek et al. 1980; Herrera et al. 1997; Hawkins 1999). While the incidence of *Aspergillus* did not differ significantly between the two samples, total aflatoxin content in the grain was influenced by rodent damage. Observation of higher total aflatoxin content in the damaged grain corroborates the findings of Mutiga et al. (2014) that maize with the most broken kernels is the most contaminated with aflatoxins. Payne and Yu (2010) for example reported that the susceptibility of maize to infection by *A. flavus* and aflatoxin contamination increases with kernel damage. Other factors such as environmental conditions, moisture content, and cropping history among others play a role in aflatoxin contamination. Nonetheless, although many grains were infected by *Aspergillus* in the tested samples, total aflatoxin levels were very low. The total aflatoxin levels recorded in the two samples were well below 10 ppb which is the allowable limit of aflatoxin contamination for human consumption for many national and international food safety agencies (FDA, WFP, Daniel et al. 2011). Overall, the observation of potentially toxigenic fungi of the genera *Aspergillus* and *Fusarium* on the stored maize grains in the experiment is in agreement with findings from previous investigations on stored maize grains collected from rural households in Kenya (Bii et al. 2012; Wagara et al. 2014). These results suggest rodent infestation can exacerbate the loss of grain quality and safety.

Lower nutrient content was associated with rodent-damaged grains. The nutritional compositions of the non-damaged grains in this study are within the range of nutrient levels known for normal maize grain in the literature (Nuss and Tanumihardjo 2010; Chaudhary et al. 2014; Rouf Shah et al. 2016). The decrease in nutrient content observed in the rodent-damaged grain is attributable to the feeding habit of rodents on the grain, and the distribution of nutrients in the various parts of maize grain. In maize, as well as in other cereal grains, rodent damage is associated with removal of the germ (Bhargava and Kumawat 2010; Mdangi et al. 2013), which has the highest concentrations of fat and crude protein compared to other grain parts, and therefore damaged grain are left with lower concentrations of fat and proteins. Maize germ contains about 33% fat, 18% protein and 8% starch, whereas the endosperm contains ~1% fat, 8.5% protein and 85% starch (Singh et al. 2014). High fat concentration in the germ also explains the substantial decline in the content of all the fatty acids identified in the rodent-damaged grains. Moreover, lower contents of unsaturated fatty acids in general and particularly linoleic acid, which is an essential fatty acid, may deprive consumers the health benefits of these fatty acids when rodent-damaged grain is consumed. Unsaturated fatty acids are generally associated with reduction of cholesterol levels, which is often associated with lower risk of cardiovascular disease (Lunn and Theobald 2006). Specifically, low linoleic acid levels in diets have been associated with higher risk of cardiovascular disease (Czernichow et al. 2010; Harris et al. 2009; Mozaffarian et al. 2010). The Food and Agriculture Organization/World Health Organization recommended that about 2–4% of daily energy should come in the form of essential fatty acids with an additional 3% energy for pregnant or breast feeding mothers (Sanjeev et al. 2014).

Although rodents' consumption of the germ is associated with partial removal of the pericarp around the hilum, fibre contents (NDF (cellulose + lignin + hemicelluloses) and ADF (cellulose + lignin)) of the rodent-damaged and non-damaged grain were similar. The pericarp is the major source of fibre in the grain, consisting principally of hemicellulose, cellulose and lignin (Nuss and Tanumihardjo 2010).

## 5 Conclusion

The findings of this study demonstrate that rodents are a significant cause of postharvest losses in on-farm stored maize, and have a significant negative impact on grain safety and nutritional value. Therefore mitigation strategies for postharvest losses should include rodent control measures, especially among poor rural communities where living conditions encourage rampant rodent infestations that reduce the limited food resources. The findings described in this paper should enable policy makers to understand the impact rodents may have on national food security, nutrition and health and lead to the establishment of methods for their control.

**Acknowledgements** The first author was supported through the Dissertation and Research Internship Program (DRIP) of ICIPE. The present study was conducted with financial support from the German Federal Ministry for Economic Cooperation and Development (BMZ)

(Grant number: 15.7860.8-001.00; contract number: 81202143) through the RELOAD project. We acknowledge the National Museums of Kenya for directly overseeing the rodent trapping activities of the study. We are also grateful to UK Aid of the UK Government, the Swedish International Development Cooperation Agency (Sida), the Swiss Agency for Development and Cooperation (SDC), and the Kenyan Government for their core support to ICIPE that facilitated the present work. The authors sincerely thank all the farmers who were involved for their cooperation. They also acknowledge the support provided by Government Agricultural Officers at Kilifi-south sub-county.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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