# 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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## 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 while rodent trapping conducted to determine species and estimate population of the rodents associated with the losses. Moulds and total aflatoxin contaminations and nutritional value of rodent-damaged grains and non-damaged grains samples were also compared to evaluate the impact of rodent infestation on grains quality. In a sample of 20 farmers, we found that cumulative weight losses due to rodent ranged from 2.2 to 6.9% in shelled maize grain and, from 5.2 to 18.3% in dehusked cobs over 3 months of storage. *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 *Aspergillus* spp. incidence. There was also significant decrease in dry-matter, fat, crude protein and all fatty acids contents in rodent-damaged grains compared to the non-damaged grains. 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. Hence, postharvest losses mitigation strategies should include rodent control mechanisms.



*Keywords*: Postharvest losses; Rodent; Fatty acids; Moulds; Aflatoxin

## 1. Introduction

Maize (*Zea mays* L.) represents the primary staple grain for many households in Sub-Saharan Africa (SSA), accounting for 36% of daily calories intake (Kumar and Kalita2017). 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 the food system sustainability 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 indeed 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).

The roof rat (*Rattus rattus)*, the house mouse (*Mus musculus)* and the natal multimammate mouse (*Mastomys natalensis*) are the rodent species usually associated to postharvest losses in grain stores in East Africa (Makundi et al. 1999). Most current and past researches 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) and

65 therefore can make the exclusion of rodents in food stores difficult. Furthermore, poor socio-
66 economic conditions have been shown to strongly influence rodent infestation in human
67 dwellings (Langton et al. 2001).

68 Apart from direct weight losses due to physical damage on grains, rodent infestations in grain
69 stores can lead to quality losses, as well as food safety and public health concerns (Meerburg et al.
70 2009; Belmain et al. 2015). Maize grain includes four distinct parts; the endosperm (80–85%),
71 the germ or embryo (9–10%), the pericarp (5–6%) and the tip cap (Chaudhary et al. 2014).The
72 germ contains most of the nutrients of the grain; it has high concentrations of fat (33%), protein
73 (18–19%), minerals and vitamins (vitamins B complex and E) (Watson 1967). Moreover, the
74 germ is a rich source of unsaturated fatty acids mainly oleic and linoleic acids (Chaudhary et al.
75 2014). In addition, the proteins with best amino acid profile are concentrated in the germ (Gupta
76 and Eggum 1998; Shewry 2007). Typically, rodent damage on maize grain is by removal of the
77 germ, and thus may reduce significantly the nutritional value of the grains. Furthermore, grains
78 contaminated by rodents' droppings may harbour pathogens, making them unfit for human
79 consumption (Meerburg et al. 2009; Hodges et al. 2014). Rodents' urine may raise the water
80 activity of the affected area, increase the nitrogen availability and thus encourage development of
81 storage fungi (Stejskal et al. 2005). Furthermore, the feeding activity of rodents itself could aid in
82 disseminating fungal spores (Reichman, et al. 1985; Reichman et al. 1988; Vander Wall 1990).
83 Rodents also cause damage to storage materials and equipments (Gwinner et al. 1996), and
84 germination failure of seeds intended for planting.

85 Given the negative impact rodents may have on food security in maize storage, there is a need
86 to assess the magnitude of the actual weight loss and grain quality issues associated with them, as

a basis to address postharvest losses and assure better grain quality for consumers. Although farmers' perception on weight losses caused by rodent 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 deeper evidence for improving rodent management in on-farm storage. Indeed according to Gwinner et al. (1996), successful management of rodents in stores prior implementation, should answer questions related to (i) the species of rodent causing damage to the produce, (ii) the approximate degree of infestation (loss estimation) and (iii) the extent of the infestation, among others. Furthermore, to our knowledge, there are no reports on how rodent damage affects grains nutritional value. Thus the objectives of this study were to quantify actual magnitude of weight losses due to rodent infestation in maize stores, determine rodent species associated with the losses, and evaluate the quality of grains damaged by rodents with respect to nutritional value diminution, moulds infection, and aflatoxin contamination by following rodent activity in on-farm stores in a rodent-prone zone.

## 2. Materials and methods

### *2.1. Study area*

The study was conducted in Mwarakaya ward (03°49.17'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 from April and ends in July whereas the

short rain cropping season begins from 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 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 account of encountering rodent problems during storage. The farmers were divided in two groups of 5 based on maize storage form (cobs storage or shelled maize grain storage). An individual farmer in each group of maize storage form constituted a replicate in the trial.

One hundred and ten (110) kg of freshly harvested clean shelled maize grain or dehusked maize cobs were purchased locally from farmers. The shelled maize grain and cobs were later treated with Actellic Super dust (pirimiphos-methyl 1.6% w/w + permethrin 0.3% w/w) two weeks before the set-up of the trial. The insecticide was applied to minimize insect infestation during the course of the experiment. For the maize stored on cobs, only cobs which did not present any visible insects or rodent damages were selected during the purchase. 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 ($Wgt_0$). The shelled maize was filled in 50

kg polypropylene bags whose open ends were then twisted and tied shut using sisal twine. The bags were placed on a clean mat in order to collect the spilled grains when the rodent attacked. For the maize stored as cobs, cobs were counted and total weight recorded 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 in the living houses, in the kitchen, or in a traditional granary (*lutsaga*) as the maize storage places differed from one farmer to another. The traditional granary was a wooden platform plastered with mud constructed above the fire place in the kitchen. This type of granary was the most predominant 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 interval. 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 separately shelled. 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 whereas the other was used for determination of live insect counts and insects damaged grains. The spilt shelled grains and loose grains from cobs were also collected as sample. 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.

152 *2.4. Determination of dry matter content*

153 Moisture content of grains was determined by the oven drying method (ISO 1980). About10
154 g of maize grains was ground using a laboratory mill (Knife Mill Cup KM-400 MRC Lab, MRC
155 International, Westminster, UK). The sample was transferred into an aluminium dish and
156 weighed ($W_i$), and then dried in an air-oven maintained at 130°C for 2 h after which it was cooled
157 in a desiccator containing silica gel for 2 h and the new weight of the dish and dry sample ($W_d$)
158 determined. The moisture content (m.c) was determined using the expression: m.c (%) =
159 $100[(W_i-W_d)/W_i]$, and dry matter content obtained by subtracting the moisture content from 100.

160 *2.5. Determination of live adult insect counts and insect damaged grains*

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

165 *2.6. Determination of cumulative weight losses caused by rodents*

166 Actual weight losses, on dry matter basis, were estimated every month from each of the stores
167 where rodent attack was evident; losses in the stores that were not attacked by rodents were
168 assumed to be zero (Hodges et al. 2014). The grains spilled out from damaged bags or loose
169 grains from the maize cobs on the mat were carefully separated and weighed, and their weight
170 added to weight of the shelled maize or cobs remaining in the bags or mats to obtain the weight
171 $Wgt_i$. Cumulative weight loss ($CWgtL_i(\%)$) at each month ($i$), where $i$ is one, two or three storage
172 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 percent fraction 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 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 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 living house 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 the 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 grains 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 12:12h light - darkness regime for 4 days. Mould colonies developing on plates were counted and recorded as colony forming units per gram ($cfug^{-1}$).

215 *2.8.2. Determination of moulds incidence*

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

228 *2.8.3. Aflatoxin analysis*

229 For each sample (rodent-damaged grains and the non-damaged grains), 9 sub-samples of 50 g
230 were each milled using a laboratory mill (Knife Mill Cup KM-400 MRC Lab, MRC International,
231 Westminster, UK). A portion of each of the milled samples (5 g) was mixed with 25 mL of 70:30
232 v/v methanol: distilled water solution, and vigorously homogenized for 3 minutes using a vortex
233 mixer at room temperature (20 - 25°C). The extracts were filtered through a Whatman #1 filter
234 and the filtrates were collected for analysis. Extracts were assayed for total aflatoxin using
235 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 obtained and transferred to antibody-coated wells, and incubated at room temperature for 2 minutes. 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 minutes 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 muffle furnace at 550°C overnight. The dry matter (DM) was determined by subtracting moisture content from 100. 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).

258 *2.8.5. Analysis of fatty acids*

259 A methyl esterification reaction was performed on 5 mg of each the ground sample according
260 to a protocol adapted from Christie (1993). A solution of 15 mg/mL concentration of sodium
261 methoxide in methanol was prepared (Musundire et al. 2016). An aliquot of the solution (500 µL)
262 was added to each ground maize sample, vortexed for 1 min and then sonicated for 5 min. The
263 reaction mixture was incubated at 60°C for 1 h, thereafter quenched by adding 100 µL deionized
264 water followed by vortexing for another 1 min. Methyl esters were extracted using hexane (GC-
265 grade) (Sigma–Aldrich, St. Louis, USA), and then centrifuged (Avanti J-25I, Beckman, CA,
266 USA) at 14,000 rpm for 5 min (Musundire et al. 2016). The supernatant was dried over
267 anhydrous $Na_2SO_4$ and then analyzed using gas chromatography-mass spectrometry (GC/MS).
268 The GC/MS analysis was carried-out on a 7890A gas chromatograph (Agilent Technologies, Inc.,
269 Santa Clara, CA, USA) linked to a 5975C mass selective detector (Agilent Technologies, Inc.,
270 Santa Clara, CA, USA). Injection volume was 1.0 µL in the splitless injection mode using an auto
271 sampler 7683 (Agilent Technologies, Inc., Beijing, China). The following conditions used by
272 Cheseto et al. (2015) and Musundire et al. (2016) were applied: inlet temperature 270°C, transfer
273 line temperature of 280°C, and column oven temperature programmed from 35 to 285°C with the
274 initial temperature maintained for 5 min then 10°C$min^{-1}$ to 280°C, held at this temperature for
275 20.4 min. The GC was equipped with a HP5 MS low bleed capillary column (30 m × 0.25 mm
276 i.d., 0.25 µm) (J&W, Folsom, CA, USA). The carrier gas used was Helium at a flow rate of 1.25
277 mL $min^{-1.}$. The mass selective detector was maintained at ion source temperature of 230°C and a
278 quadrupole temperature of 180°C. Electron impact (EI) mass spectra were recorded at the
279 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 two times.

### *2.9. Statistical analysis*

Data on weight losses (%), insects damaged grain (%) and moulds 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 insects damaged grain data were subjected to repeated-measures ANOVA while total mould count, moulds incidence and total aflatoxin subjected to 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 contents of rodent-damaged and non-damaged grains were compared using t-test. All data were analyzed using SPSS version 20.

## *3. Results*

### *3.1. Dry matter content*

Dry matter contents of the cobs and shelled maize grains storages during the 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

301 matter contents at the baseline and at the end of the trial were significantly lower than the one
302 observed at 1 and 2 months of storage ($F_{3, 18} = 24.55$, $p < 0.001$).

303 *3.2. Live adult insect counts and insect damaged grains*

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

308 *3.3. Weight loss caused by rodents*

309 In cob storage, the average weight loss during the 3 months of storage was 11.37% (range
310 5.2-18.3%) (Table 2). Weight losses increased steadily and significantly with the storage duration
311 ($F_{2.41, 14.47} = 122.661$, $p< 0.001$). Average weight loss was 2.5 times lower (4.6%) in maize stored
312 as shelled grain. Similar to maize stored as cobs, weight losses also increased with storage
313 duration ($F_{1.75, 15.75} = 15.407$, $p< 0.001$) (Table 2).

314 *3.4. Rodents species and population*

315 Over the 4 months trapping period, 65 individual rodents were captured from a total of 1200
316 trap nights (Table 3). Age class structure of the captured rodents over the trapping period was
317 characterized by 63% adults and 18.5% sub-adults and juveniles, respectively. All the rodents
318 captured throughout the trapping period were *R. rattus*. The trap success rate ranged from 0.63 to
319 10%, and overall showed a gradual increase in the last two months of the trapping.

320 *3.5. Effect of rodent damage on mould and aflatoxin contamination of grains*

321 Total mould count ($log_{10}$ cfu $g^{-1}$) was significantly higher in the rodent-damaged grains (5.3 ±
322 0.2) compared to the non-damaged grains (3.7 ± 0.1) (t (4) = 7.914, $p$ = 0.001). With regard to
323 mould incidence *Aspergillus* and *Fusarium* were the main fungal genera isolated (Fig. 1) in both
324 the damaged and undamaged grains. *Fusarium* incidence was significantly higher in the damaged
325 grains (t (4) = 3.85, $p$ = 0.011), whereas incidence of *Aspergillus* did not differ significantly (t (4)
326 = 1.38, $p$ = 0.239). Irrespective of the fungal genera the percentage of kernels infected with
327 moulds was significantly higher in the rodent-damaged grains (63.5 ± 6.3%) compared to the
328 non-damaged grains (25.4 ± 3.2%) (t (4) = 5.135, $p$= 0.007). Aflatoxin contaminations were
329 significantly higher in rodent-damaged grains (6.1 ± 1.7) than in the non-damaged grains (1.1 ±
330 0.4) (t (8.96) = 2.77, $p$ = 0.022).

331 *3.6. Proximate composition and fatty acid profile*

332 The rodent-damaged grains had significantly lower contents of DM (t (2) = 8.80, $p$= 0.013),
333 CP (t (1.27) = 13.93, $p$= 0.024) and CF (t (1) = 14.95, $p$= 0.043) compared to non-damaged
334 grains (Fig. 2). The DM, CP, and CF in the rodent-damaged grains represented reductions of
335 2.43%, 13.34%, and 87.92%, respectively. However, there was no significant difference in the
336 ash (t (2) = 0.08, $p$= 0.940), NDF (t (1.98) = 2.98, $p$= 0.097) and ADF (t (2) = 8.80, $p$= 0.072)
337 contents between the rodent-damaged grains and the non-damaged grains.

338 Eight fatty acids were identified and quantified (Table 4). The most abundant fatty acids in
339 the non-damaged grains and rodent-damaged grains were oleic acid (C18:1), linoleic acid (C18:2),
340 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 grains. Rodent-damaged grains had significant 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 grains, 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 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 portion (56.18%) of the total fatty acid content in the rodent-damaged grains while oleic acid accounted for the highest portion of the total fatty acid content (43.48%) in the non-damaged grains.

## *4. Discussion*

Filling the gap of actual weight losses and quality decline due to rodents infestation as well as rodents species associated to the losses in storage facilities can help different stakeholders (policy makers, donors, researchers and development agencies) to understand the impact rodents can have on food security, food safety and nutrition and therefore help prioritise extension programs. Lower dry matter content in the rodent-damaged grains results from higher moisture contents 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 *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 of storage, losses may no longer be attributed to rodent infestation alone. The occurrence of insects on the maize at 3-month storage could be explained by a number of

363 factors. Ordinary polypropylene bags are unable to stop insects' proliferation when grains are
364 stored. Moreover, insects are always present in farmers store due to the presence or debris of old
365 stock and lack of storage hygiene. Furthermore, grain treated with insecticides becomes
366 vulnerable to insects infestation with time (usually 3- 4 months) as the potency of the active
367 ingredient gradually decreases. The levels of weight losses associated with rodent infestation
368 during the three months storage period in the present study show that rodents can pose a
369 significant problem for the safe storage of maize. In a similar work in Mozambique with maize
370 cobs, Belmain et al. (2003) reported 3.1-12.8% (average 7%) cumulative weight losses due to
371 rodents within three months. Another study in Tanzania reported an average of less than 0.5%
372 weight losses due to rodents over 7 months of storage on shelled maize grain stored in open cribs
373 and unprotected sacks (Mdangi et al. 2013). However, the difference between losses data in the
374 present study and those reported by Belmain et al. (2003) and Mdangi et al. (2013) could be
375 related to rodent prevalence in the stores which can be linked to the differing habitats and
376 ecologies. Based on the weight loss estimation method used in the present study, no relationship
377 could be established between dry matter and weight loss as the weight of maize available at each
378 sampling date was not constant and highly contingent on rodents' infestation pressure on the
379 stored maize. In the study, although losses were apparently higher on maize stored as cobs than
380 for maize stored as shelled grain, losses in the two cases have to be put into perspective for
381 proper comparison as losses quantified in cobs storage were not corrected for the weight of the
382 cobs without grains. Moreover, it should be noted that the storage of maize as shelled maize grain
383 was not a common practice in the area; farmers predominantly stored their maize as cobs. This
384 situation, that is, presentation of the maize as shelled grain instead of cobs may have influenced
385 the neophobic behaviour of rodents in the stores (Brigham and Sibley 1999).

386 Of the three commensal rodent species (*R. rattus*, *M. musculus* and *M. natalensis*) often
387 associated with postharvest crop damage in East Africa (Makundi et al. 1999), only *R. rattus*
388 species was captured in farmers' stores. *Mastomys natalensis* was especially expected to be
389 captured during the last two months of the trapping period which coincided with the end of the
390 harvest period as this rodent moves from the fields into storage structures at the end of the
391 harvest season due to absence of food in fields (Makundi et al. 1999). On the other hand, *M.*
392 *musculus* was expected to be captured during the trapping period as it inhabits houses and storage
393 structures like *R. rattus* (Mdangi et al. 2013). The capture of only *R. rattus* over the 4 months of
394 trapping nevertheless supports the consideration that it is the most abundant rodent species
395 residing inside houses across Africa (Kilonzo 2006), and is consistent with the findings of
396 Belmain et al. (2003) and Mdangi et al. (2013) in Mozambique and Tanzania, respectively.
397 However, three possible reasons could explain the absence of *M. natalensis* and *M. musculus*
398 over the 4 months of trapping in the present study. One reason would be the presence of inter-
399 specific competition. According to Taylor et al. (2012), *M. natalensis* only enters smallholder
400 houses in large numbers when *R. rattus* is completely absent from the regional environment.
401 Several studies (King et al. 1996; Choquenot and Ruscoe 2000; Courchamp et al. 2000; Ruscoe
402 2001) also reported that rats are strong competitors of mice, affecting negatively the rate of
403 change in mouse abundance and even excluding them when resources are scarce. King et al.
404 (1996) for instance found that where mice and *R. rattus* coexisted in New Zealand forests, the
405 mice were scarcer than rats. A second reason for absence of *M. natalensis* is the nesting
406 behaviour difference between *M. natalensis* and *R. rattus*. *Rattus rattus* appears to be
407 predominantly confined to areas of human settlement whereas *M. natalensis* lives in burrows in
408 the fields (Belmain et al. 2003; Mdangi et al. 2013) and therefore trapping inside dwelling places

409 may not result in high capture rates. The absence of *M. natalensis* and *M. musculus* could also be
410 related to the fact that data in this study were limited to 4 months trapping while rodent
411 abundance may vary with a longer trapping periods. Indeed *M. natalensis* population fluctuations
412 vary among seasons, years and localities and are largely influenced by the amount and duration
413 of rainfall (Leirs et al. 1989; Makundi et al. 2005). The increase of the trap success rate during
414 the last two months could be related to the availability of more food resources in the farmers'
415 stores as this period, coinciding with the end of harvesting. According to Krebs (1999), food is
416 clearly one of the dominant ecological factors that influence rodent populations.

417 The higher mould infection rates and the high *Fusarium* incidence on the rodent-damaged
418 grains indicate that rodent attack encourages mould contamination. This may be because the
419 injuries inflicted by rodents on grains when feeding, offered entry points to fungal spores.
420 According to Chen et al. (2011), kernel breakage creates an infection court for opportunistic
421 pathogens. It might also be possible that rodents when feeding on the grains transmit fungal
422 spores through their mouth. This hypothesis is supported by the fact that fungi and rodents do not
423 occur independently in natural ecosystem as it is known that their internal organs or shelters of
424 rodents are active sites where fungi proliferate (Otcenášek and Dvorák 1962; Hubálek et al. 1980;
425 Herrera et al. 1997; Hawkins 1999). While *Aspergillus* incidence did not differ significantly
426 between the two samples, total aflatoxin content in the grains was influenced by rodent damages.
427 Observation of higher total aflatoxin content in the damaged grains corroborates with the findings
428 of Mutiga et al. (2014) that maize with the most broken kernels is mostly contaminated with
429 aflatoxins. Payne et al. (2010) for example reported that the susceptibility of maize to infection
430 by *A. flavus* and aflatoxin contamination increases with kernel damage. Other factors such as
431 environmental conditions, moisture content, and cropping history among others play a role in

Aflatoxin contamination. Nonetheless, although many grains were infected by *Aspergillus* genus in the tested samples, total aflatoxin levels were very low in the samples. 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 contents were 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 grains in the literature (Nuss and Tanumihardjo 2010; Chaudhary et al. 2014; Rouf Shah et al. 2016). The decrease in nutrient contents observed in the rodent-damaged grains is attributable to the feeding habit of rodents on the grains, 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 the other grain parts, and therefore damaged-grains are left with lower concentrations of fat and proteins. The 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 justifies the substantial decline in the content of all the fatty acid identified in the rodent-damaged grains. Moreover, lower contents of unsaturated fatty acids in general and particularly for linoleic acid

454 which is an essential fatty acid may deprive consumers the health benefits of these fatty acids
455 when rodent-damaged grains are consumed. Unsaturated fatty acids are generally associated with
456 reduction of cholesterol levels which is often associated with cardiovascular diseases (Lunn and
457 Theobald 2006). Specifically, low linoleic acid levels in diet have been associated with higher
458 risk of cardiovascular disease (Czernichow et al. 2010; Harris et al. 2009; Mozaffarian et al.
459 2010). The Food and Agriculture Organization/World Health Organization recommended that
460 about 2–4% of daily energy should come in the form of essential fatty acids with an additional
461 3% energy for pregnant or breast feeding mothers (Sanjeev et al. 2014).

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

467 **5. Conclusion**

468 The findings of this study demonstrate that rodents are a significant cause of postharvest
469 losses in on-farm stored maize, and have a significant negative impact on grain safety and
470 nutritional value. Thus postharvest losses mitigation strategies should include rodent control
471 mechanisms especially among poor rural communities where living conditions encourage
472 rampant rodent infestations that attack the limited food resources. The findings should enable
473 policy makers to understand the impact rodents may have on national food security, nutrition and
474 health.

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## Conflict of interest statement

The authors declare that they have no conflict of interest

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671 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 |

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

674

675 Table 2. Weight loss due to rodent attack, and level of insect damage of in cobs and shelled
676 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 |

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

679

680 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 |

681 *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
682 and 3 locally made traps) set in the house of each farmer each night, trap nights was calculated as 8 x 3 x 10 = 240 trap nights. For the other
683 months of trapping (September, October and November), traps were set for 4 consecutive nights and therefore monthly trap nights was 320.

684

685 Table 4. Fatty acids profile of the rodent-damaged grains and non-damaged grains. All values are presented as µg/g of sample dry
686 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 |

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

688

689 Figures caption

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

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

696

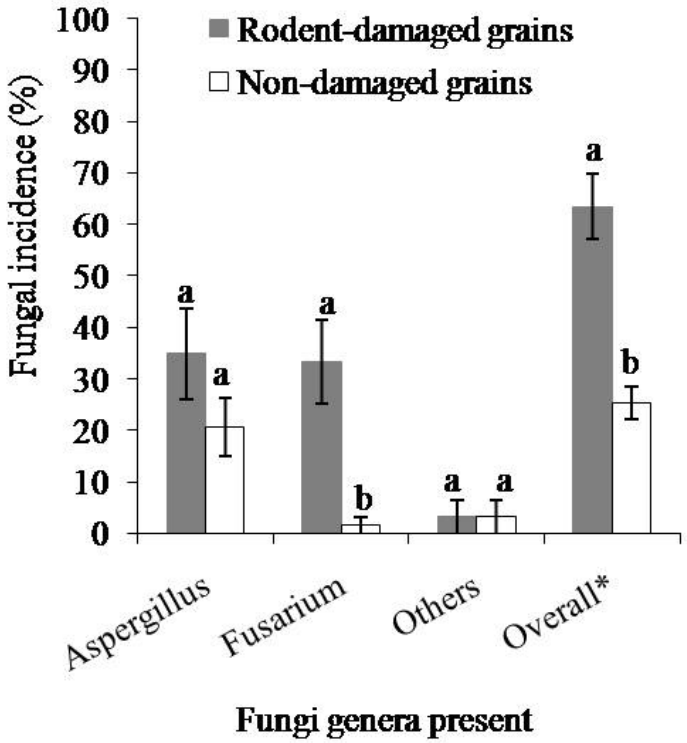
Rodent-damaged grains
Non-damaged grains
Fungal incidence (%)
100
90
80
70
60
50
40
30
20
10
0
a
a
a
b
a
a
a
b
Aspergillus
Fusarium
Others
Overall*
Fungi genera present

Fig. 1

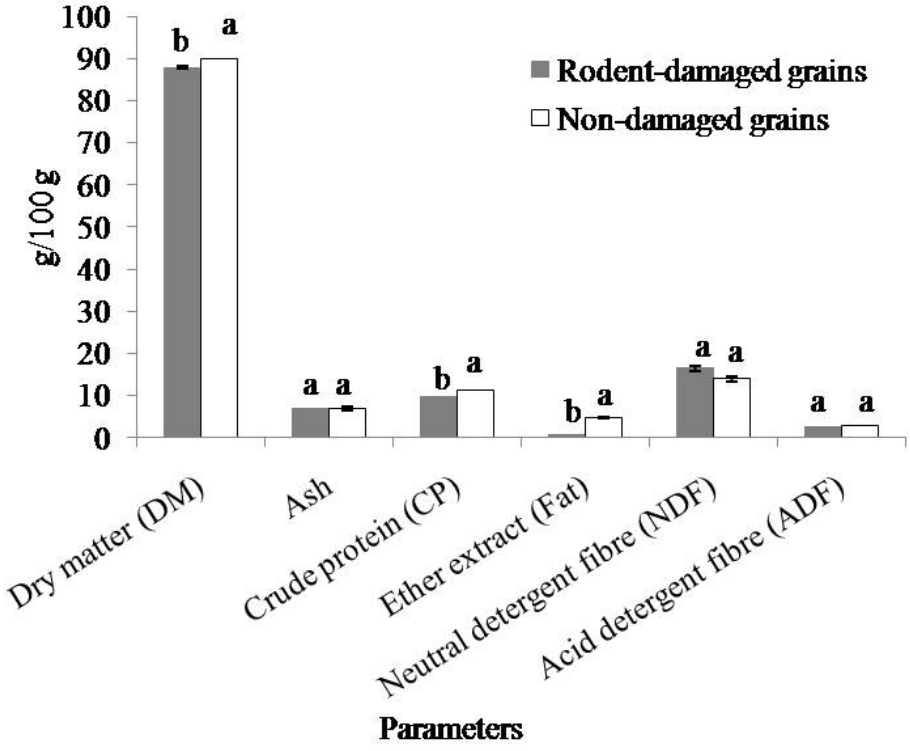
100
90
80
70
60
50
40
30
20
10
0
g/100 g
Rodent-damaged grains
Non-damaged grains
b a
a a
b a
b a
a a
a a
Dry matter (DM)
Ash
Crude protein (CP)
Ether extract (Fat)
Neutral detergent fibre (NDF)
Acid detergent fibre (ADF)
Parameters

Fig. 2