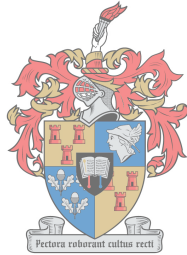**Mycotoxin contamination of maize and groundnut produced by subsistence farmers in northern KwaZulu-Natal**

by

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DECLARATION

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SUMMARY

Subsistence farmers in South Africa face many production challenges including infection of their grain crops with mycotoxigenic fungi and concomitant mycotoxin contamination. *Fusarium* spp. and *Aspergillus* spp. are the most common fungal species infecting maize and groundnuts while plant-parasitic nematodes are also associated with groundnuts in South Africa. Maize and groundnut questionnaires regarding production practices were presented to subsistence farmers in Pongola, Vryheid, Jozini, Manguzi and Mbazwana districts of northern KwaZulu-Natal (KZN), South Africa. Maize and groundnut grain samples were also collected at harvest and after three months of storage during the 2012/13 and 2013/14 seasons. Groundnuts, roots and soil samples were collected before harvest during the 2013/14 season, only. *Fusarium graminearum*, *F. verticillioides* and *A. flavus* target DNA levels were quantified in maize using quantitative polymerase chain reaction and the presence of multi-mycotoxins were determined using the liquid chromatography tandem mass spectrometry. Nematodes were extracted using sieving method and identified microscopically. Questionnaires revealed that over 90% of farmers were not aware of mycotoxins or their implications on human and livestock health. Visually diseased grain was often fed to livestock sensitive to mycotoxicosis such as chickens. Production practices amongst some farmers including crop rotation and the well-ventilated storage of grain may contribute to reduced mycotoxin contamination. In maize grain the *Fusarium graminearum* levels were significantly higher than *F. verticillioides* and *A. flavus* levels in both seasons. Contrary to expectations, zearalenone, produced by *F. graminearum*, was very low ($\leq$0.02 µg/g) at harvest and storage during both seasons while deoxynivalenol and nivalenol was not detected. There were significant differences between districts (localities) and collection periods (harvest and storage) and localities per seasons ($P \leq 0.05$) for all mycotoxigenic fungi and mycotoxins evaluated. Maize sampled in Jozini district was the most contaminated with mycotoxigenic fungi and mycotoxins while Mbazwana and Manguzi districts were the least contaminated. Four plant-parasitic nematodes, namely *D. africanus*, *Pratylenchus* spp., *Meloidogyne* spp. and *Helicotylenchus* spp., were identified from groundnut samples obtained in Jozini, Manguzi and Mbazwana during the 2012/13 and 2013/14 seasons. Furthermore, *Tylenchus* spp. was identified for the first time in groundnuts, pegs and soil collected before harvest during the 2013/14 season. Results from this study showed that there is a need for mycotoxin awareness campaigns and additional surveillance to continuously monitor mycotoxin contamination and potential exposure. More in-depth analyses of all the potential factors contributing to mycotoxin contamination and exposure, particularly in the subsistence production are of northern KZN, is warranted.

OPSOMMING

Bestaansboere in Suid-Afrika het talle produksie uitdagings, insluitende die infeksie van hul graangewasse met mikotoksigeniese swamme en gepaardgaande mikotoksien besmetting. *Fusarium* spp. en *Aspergillus* spp. is die algemeenste swam spesies wat mielies en grondbone besmet, terwyl plant-parasitiese aalwurms ook met grondbone in Suid-Afrika geassosieer word. Vraelyste aangaande mielie- en grondboon verbouingspraktyke is vir bestaansboere in die Pongola, Vryheid, Jozini, Manguzi en Mbazwana areas van noord KwaZulu-Natal (KZN), Suid-Afrika, weergegee. Mielies- en grondboon- graanmonsters is teen oestyd en na `n drie maande opbergingsperiode, gedurende die 2012/13 en 2013/14 seisoene, ingesamel. Grondbone, wortels en grondmonsters is net voor oestyd gedurende die 2013/14-seisoen versamel. *Fusarium graminearum*, *F. verticillioides* en *A. flavus* geteikende DNA vlakke is in mielies deur die gebruik van kwantitatiewe polimerase ketting reaksie (kPKR) gekwantifiseer en die teenwoordigheid van multi-mikotoksiene is met behulp van die vloeistof kromatografie massaspektrometrie bepaal. Aalwurms is deur middel van `nsiftings metode vanuit die grond geïsoleer en mikroskopies geïdentifiseer. Vraelyste het aan die lig gebring dat meer as 90% van die boere nie bewus is van mikotoksiene asook die gesondheidsrisiko's wat dit vir mens- en dier inhou nie. Grane wat visueel siek vertoon, word dikwels aan diere wat sensitief is vir mikotoksikose, soos hoenders, gevoer. Sommige verbouingspraktyke wat deur sommige boere toegepas word, insluitend wisselbou en die gebruik van goed geventileerde opberging kondisies vir graan, kan tot verminderde mikotoksien besmetting bydra. *F. graminearum* vlakke in mielies was aansienlik hoër as die van *F. verticillioides* en *A. flavus* vir beide seisoene. In teenstelling was zearalenone, wat deur *F. graminearum* geproduseer word, baie laag (<0,02 µg/g) gedurende oes en opberging vir albei seisoene, terwyl dexoynivalenol en nivalenol nie opgetel is nie. Daar was betekenisvolle verskille tussen areas (lokaliteite) en versamelingsperiodes (oes en opberging) en lokaliteite per seisoen (P $\leq$ 0.05) vir alle mikotoksigeniese swamme en mikotoksiene geëvalueer. Mielies vanaf Jozini distrik was die meeste besmet met swamme en mikotoksiene terwyl mieliegraan vanaf Mbazwana and Manguzi distrikte die minste besmet was. Vier plant-parasitiese aalwurms, naamlik *D. africanus, Pratylenchus* spp., *Meloidogyne* spp. en *Helicotylenchus* spp., is uit grondboon-monsters wat gedurende die 2012/13 en 2013/14 seisoene in Jozini, Manguzi en Mbazwana verkry is, geïdentifiseer. Alhoewel, *Tylenchus* spp. is gedurende die 2013/14 seisoen vir die eerste keer in grondbone, penne en grond wat voor oes versamel is, geïdentifiseer. Resultate van hierdie studie toon dat daar 'n behoefte aan bewusmakingsveldtogte vir mikotoksien besmetting ontstaan en dat bykomende hulp nodig is om mikotoksien kontaminasie asook potensiële blootstellings te monitor. Meer in-diepte ontledings van al die moontlike faktore wat bydra tot die besoedeling en blootstelling van mikotoksien, veral in die bestaansproduksie area van noord KZN, is geregverdig.

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CONTENTS

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CHAPTER 1

Impact of mycotoxigenic fungi and plant-parasitic nematodes on maize and groundnut production within subsistence farming systems in South Africa

INTRODUCTION

Maize (*Zea mays L.)* is a globally important food and feed crop with the majority being consumed by people living in the sub-Saharan Africa (Fandohan *et al.*, 2003; Alakonya *et al.*, 2008). The South African Grain Information Service reported South Africa as the largest maize producer for four consecutive seasons in the Southern African Development Community (SADC) (Anonymous, 2017). In South Africa, the increase in food prices, especially maize, affects many poor families resulting in the increase of food insecure households. Low income households may experience severe chronic food insecurity as a result of food price shocks (Altman *et al.*, 2009). The food price inflation increased the number of food insecure people worldwide from 900 million to more than 1 billion during 2007-2008 (FAO, 2009).

Various factors affect maize production in small holder farming systems in Southern Africa such as decline in soil fertility, variable climate, inappropriate and insufficient fertilizer application, labour constraints and lack of improved cultivars hence leading to food insecurity (Thierfelder *et al.*, 2015). Ozone (O_3) is known to damage maize crops (Van Tienhoven *et al.*, 2006), in many regions of South Africa and; there is a potential for high concentrations of O_3 due to air pollution from human and natural resources (Laban *et al.*, 2015). However, the major climatic factors affecting maize production include wind, hours of sunshine, temperature, humidity and rainfall which can affect both the quantity and quality of the maize crop (Geyser, 2004).

Changing rainfall patterns and increasing temperatures are already threatening crop production in Southern Africa, whereby the period of crop growth is shortened and plant water demand is increased (Rurinda *et al.*, 2015). Relative humidity and moisture content determine the period at which maize is harvested and the resulting yield, thereby affecting maize production. Other environmental factors affecting maize production include soil nutrients and water availability (Evans and Fischer, 1999; Ono *et al.*, 2002; Mahboubi *et al.*, 2007).

Serious pests such as the European corn borer, *Ostrinia nubilalis* (Hübner) and African maize stalk borer, *Busseola fusca*, affect maize production worldwide; this pest favours fungal infection and subsequent mycotoxin contamination through wounds on the maize kernels (Saladini *et al.*, 2008; Mazzoni *et al.*, 2011). Fungal development leads to symptomatic and asymptomatic infection on maize kernels whereby ambient temperatures and moisture content

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are key factors for subsequent mycotoxin production. The hazard, actual and exposure levels determine the importance of mycotoxins (Naicker *et al.*, 2007; Russel *et al.*, 2010).

The aim of this study is to provide a comprehensive background on the constraints of maize and groundnut production by subsistence farming in South Africa, with particular focus on mycotoxigenic fungi and their associated mycotoxins. South African small holder farmers suffer from economic losses due to pre-harvest and post-harvest contamination of maize due to fungal species and insect pests. Poor storage facilities, ecological and environmental factors further contribute to mycotoxin contamination. This study will also highlight pre and post-harvest management practices to control fungal infection and mitigate grain contamination in subsistence farming.

MAIZE PRODUCTION AND ITS CHALLENGES IN SOUTH AFRICA

During 2014, the average maize production in South Africa was estimated to be 11 million tonnes, which increased with about 18 percent from the previous year (FAOSTAT, 2014). The main production regions of maize in South Africa are the Free State, Mpumalanga and North West as reported on the maize quality report for the years 2010/2011 by the Southern African Grain Laboratory. The Free State, Mpumalanga and North West produced 39%, 21% and 23% of commercial maize grown in South Africa, respectively (www.sagl.co.za). The two main producers of maize in South Africa are resource-poor subsistence and intensive commercial farmers (Ncube *et al.*, 2011; Dawlal *et al.*, 2012). Home-grown crops are major food-sources for subsistence farmers, and in other African countries maize is the main source of income for these farmers (Probst *et al.*, 2010; Thembo *et al.*, 2010). Many subsistence farmers sort their grain after harvest into visually healthy and mouldy grain. The mouldy grain is not discarded but used for traditional maize beer. In areas like the Eastern Cape and the Limpopo province of South Africa, hence posing a risk to human health due to mycotoxin contamination (Phoku *et al.*, 2013; Shephard *et al.*, 2013).

All the factors associated with a decline in maize quality subsequently promote food insecurity, where people do not have access to sufficient and safe food (Bashir *et al.*, 2013). To reduce food insecurity in rural households, South Africa has adopted small-scale agriculture for the economic development of rural farmers (Musvoto *et al.*, 2014). The access to more different dietary substances due to the increased income may, therefore, result in improved nutrition and health. KwaZulu-Natal, Eastern Cape and Northern Cape Provinces were found to suffer most in term of malnutrition (Hendriks, 2003).

Maize can be infected by various fungal pathogens with infection possible both in the field and during storage (Kaaya and Kyamuhangire, 2006; Ncube *et al.*, 2011). Different fungal species infecting maize cause diseases such as root, ear and stalk rot as well as seedling blight (Rahjoo *et al.*, 2008). In addition to fungal pathogens, maize crops in Sub-Saharan Africa

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are affected by pests such as post-harvest weevils, larger grain borer and lepidopterous ear and stem borers (Ognakossan *et al.*, 2013). Certain characteristics of a maize plant can make it difficult for the fungal pathogen to enter and develop within the plant; these characteristics include rigidity of the husks, thickness of the pericarp and humidity of the kernels (Cao *et al.*, 2014). Also, certain maize hybrids have been found to be more resistant to mycotoxin contamination (Lauren *et al.*, 2007).

Maize forms part of the diet of many people because of the ease of cultivation, adaptation to various agro-ecological zones and high yields per hectare (Fandohan *et al.*, 2003). It is ranked as the third most important cereal grain worldwide and it is beneficial to animal and human nutrition due to the antioxidant compounds it contains (Lee *et al.*, 2010). The chemical and physical properties of the maize grain affect the quality and its general acceptability (Zilic *et al.*, 2010).

Small holder farmers in South Africa are threatened by poor soil fertility as it reduces maize grain yield. The reduction in soil fertility is associated with decreasing levels of soil nutrients and organic matter (Mkhabela, 2002; Dube *et al.*, 2012). Lack of resources due to limited cash income is also a major constraint to small holder farmers in southern Africa (Kassie *et al.*, 2013). Both the commercial and smallholder farmers are threatened by environmental factors such moisture and temperature which can favour fungal infection of maize; maize samples from 29 localities of South African commercial farmers were naturally infected with *Fusarium* spp. over a three year period as reported by Janse van Rensburg et al (2015).

GROUNDNUT PRODUCTION AND ITS CHALLENGES IN SOUTH ARICA

One of the main oil-seed crops in South Africa is the *Arachis Hypogaea L.* crop, commonly known as the groundnut. This crop is composed of 48 % oil, 26 % protein, 3 % fiber and other important elements like calcium (Sarvamangala *et al.*, 2011). South Africa is one of the countries known to produce groundnut products with high oleic acid content (Barkely *et al.*, 2013). Growing the groundnut grain crop boosts the economy of South African small-holder and commercial farmers, however small holder farmers constitute the majority of the people growing the groundnut crop (Steenkamp *et al.*, 2010).

Groundnut is produced from sea level to above 1500 m. Main areas of groundnut production are between 900 to 1200 m altitude in the Southern African Development Community (SADC) region; with South Africa being one of these regions (Subrahmanyam *et al.*, 1997). In these SADC regions, the food demand is always high due to lower crop yields caused by diseases and pests (Sharma *et al.*, 1990). Yield loss also affects the export potential of the groundnut crop (Diome *et al.*, 2013). Fungal infection and insect infestations on groundnut crop significantly impacts on food security due to the reduced yields. The groundnut

serves as a protein and fat source for both livestock and humans; providing the necessary nutrients (Sarvamangala *et al.*, 2011).

Changes in nutritional value and physical properties of the groundnut are caused by the storage fungi; which are *A. flavus, A. parasiticus* and *A. nomius*. These include weight loss of the peanut, kernel discoloration and germination capability decrease (Bulaong & Dharmaputra 2002). Frequent consumption of groundnut impacts positively on human health as cholesterol levels are maintained, blood glucose levels reduced and atherosclerosis slowed down in the body (Ros *et al.*, 2012). Groundnut is consumed as soup or snacks, either when roasted or boiled (Kayode *et al.*, 2013).

Fungi are common pathogens of maize and groundnuts (Palencia *et al.*, 2010). Fungal genera and species producing mycotoxins are *Aspergillus, Claviceps, Fusarium, Penicillium* and *Alternaria* (Mostafa *et al.*, 2012). This study will focus on *Fusarium* and *Aspergillus* species. *Aspergillus* species produces Aflatoxins (AF) B_1, B_2, G_1 and G_2, Fusarium species produces fumonisins (FB) and trichothecenes, the latter is a collective name for deoxynivalenol (DON) and zearalenone (ZEA) mycotoxins (Bayman *et al.*, 2002; Bulaong & Dharmaputa 2002; Fandohan *et al.*, 2003; Malbran *et al.*, 2012; Kosawang *et al.*, 2014) (Fig. 1).

TOXIGENIC FUNGAL SPECIES AND ASSOCIATED MYCOTOXINS

Maize and groundnut crops are usually contaminated by mycotoxins prior to and after harvesting (Alakonya *et al.*, 2008). Mycotoxins affect 25 % of crops annually throughout the world and also affect the quality of marketable food products in South Africa (Lezar and Barros, 2010; Iqbal *et al.*, 2013). Sub-Saharan Africa constitutes the largest region with prevalence of maize and groundnut contamination due to mycotoxins (Ilesanmi and Ilesanmi, 2011). Mycotoxins are defined by van Egmond *et al.* (2007) as "metabolites of fungi capable of having acute toxic, carcinogenic, mutagenic, teratogenic, immunotoxic, and oestrogenic effects in man and animals. There are more than 300 known mycotoxins originating from fungal pathogens (Zain *et al.*, 2011).

***Fusarium verticillioides* and fumonisin production**

Fusarium verticillioides (Sacc.) Nirenberg was first described as *Fusarium moniliforme* Sheldon. *Fusarium moniliforme* culture (MRC 826) was first isolated from maize found in the Transkei region of South Africa (Bezuidenhout *et al.*, 1988). Colonization of maize by *F. verticillioides* can lead to either symptomatic or asymptomatic infection (Adejumo, 2012; Brown *et al.*, 2012). On maize, *F. verticillioides* infects stalks, cobs and seedlings and it mainly contaminates maize kernels with fumonisins (Brown *et al.*, 2007; Cao *et al.*, 2014). *Fusarium*

verticillioides is most commonly associated with maize as compared to other grain crops (Brown *et al.*, 2012).

During 1988, fumonisins were first isolated from *F. verticillioides* cultures (Gelderblom *et al.*, 1988; Rheeder *et al.*, 2002). The *F. verticillioides* strain that the fumonisins were first isolated from was MRC 826 (Mogensen *et al.*, 2009; Small *et al.*, 2012; Waskiewicz *et al.*, 2012). The three most predominant fumonisin analogues produced by *F. verticillioides*, which are fumonisins B_1, B_2 and B_3. Fumonisin B_1 (FB_1) constitutes between 70-80 % of the total contents of fumonisins in naturally contaminated food and *F. verticillioides* cultures (Shephard *et al.*, 2011; Waskiewicz *et al.*, 2012). The chemical structure of fumonisins has methyl, hydroxyl and tricarboxylic acid groups that substitute a linear carbon backbone (Mostafa *et al.*, 2012).

Fumonisins are polyketides produced from the expression of a fumonisin biosynthetic (*FUM*) gene cluster (Brown *et al.*, 2007; Lanubile *et al.*, 2013). Seventeen genes are located in the *FUM* gene cluster. *Fusarium verticillioides* genome consists of four other biosynthetic gene clusters in addition to the *FUM* gene cluster; these genes encode fusarin, perithecial pigment, bikaverin and fusaric acid (Butchko *et al.*, 2012). The *FUM* gene cluster is under the influence of the global regulatory *velvet gene* (*FvVE1*), morphogenesis in *F. verticillioides* is also regulated by this gene (Myung *et al.*, 2012). Fumonisins cause programmed cell death when they inhibit a key enzyme in sphingolipid metabolism, known as a synthase gene (ceramide synthase) (Myung *et al.*, 2012; Lanubile *et al.*, 2013).

The average fumonisin contamination rate from good-quality and mouldy-grain collected from subsistence farmers at the Transkei region was 71% (Mogensen *et al.*, 2011). Other maize production regions in rural South Africa were found to be highly infected with *Fusarium* species which had a positive correlation with fumonisin contamination (Mohale *et al.*, 2013). Commercial maize samples in South Africa were also highly contaminated with fumonisins (Chilaka *et al.*, 2012). Among other mycotoxins known, the fumonisins are best studied in South Africa (Lezar and Barros, 2010). A study by Rubert *et al.* (2013) reported high fumonisin levels in organic cereal-based products from Spain, France and Germany. Amongst the South African products, the 'Braaipap' meals had the highest mean level of fumonisins. Total fumonisin levels in the products ranged from 0-3605 ng/g (Schlechter *et al.*, 1998). Other *Fusarium* species producing fumonisins are *Fusarium proliferatum* (Matsushima, Nirenberg) and *F. nygamai* (Burgess, Trimboli) (Mukanga *et al.*, 2010).

F. verticillioides epidemiology

The life cycle of *F. verticillioides* is affected by the presence of insects, which also facilitate the infection process as they damage maize cobs thereby allowing fungal entry (Richard, 2007). The European Corn Borer (ECB) (*Ostrinia nubilalis* Hübner) is the main insect

damaging maize ears in the United States of America (USA), high insect attack on maize is facilitated by drought stress (Miller, 2008). There are no reports of ECB in South Africa, the insect has only been found in north parts of Africa (www.cabi.org). These insects facilitate fungal invasion by first feeding on maize whorl tissue and penetrate the stalk to disrupt vascular transport (Dafoe *et al.*, 2013).

Fungal micro and macroconidia are dispersed by rain-splash or wind from the tassels (male part) to the silks (female part) (Munkvold, 2003); it was estimated by Ooka and Kommedahl (1977) that viable spores of *F. verticillioides* can travel a distance of between 300-400 km. *Fusarium verticillioides* enters the ear of the maize mostly during silking. Prior to silking, basal organs of the plant for example; the roots and stalks serves as a pathway for *F. verticillioides* infection (Venturini *et al.*, 2011).

Fusarium verticillioides infection through the stalks, roots and seeds occurs systematically in the maize plant (Oren *et al.*, 2003). Infected plant residues left in the field contaminates the soil, hence infecting new seed. Severity of *F. verticillioides* infection is mainly through insect damage and silk route as compared to through contaminated seeds, with effective growth happening at temperatures above 28°C (Miller, 2008; Murillo-Williams and Munkvold, 2008). Fumonisin production occurs immediately after *F. verticillioides* entry as shown in Figure 2 (Maiorano *et al.*, 2009). Fungal infection of maize plants can occur without causing any apparent symptoms. The infection process is facilitated by conidia which are necessary for reproduction, dispersal and survival of *F. verticillioides* (Glenn *et al.*, 2004). A characteristic visual symptom caused by *F. verticillioides* on the maize ear is a light pink or white mycelium (Venturini *et al.*, 2011).

Fusarium verticillioides causes Fusarium ear and kernel rot disease which is a huge problem to maize quality in Southern Africa, rendering maize undesirable for consumption. Complete resistance to the ear rot disease has not been found (Chandra Nayaka *et al.*, 2009; Small *et al.*, 2012). Ear rot infection of maize happens when *Fusarium* species invade maize ears, entrance is through ear wounds caused by insects or bird or through the maize silks (Presello *et al.*, 2007). *Fusarium verticillioides* can still cause maize ear rot in the presence or absence of fumonisins (Lanubile *et al.*, 2013).

Physical injury of the kernels leads to the development of Fusarium ear rot (Munkvold, 2003). The severity of Fusarium ear rot is associated with increased pre-harvest rainfall and elevated temperatures during maturity of the maize kernels (Cao *et al.*, 2014). Once in the ear, *F. verticillioides* infection spreads to husks and glume tissues and finally colonizing the unwounded maize kernels (Cao *et al.*, 2013). Significant losses in the maize ears can also be caused by *Mussidia nigrivenella* Ragonot (Pyralidae), also known as the maize cob borer (Cardwell *et al.*, 1999) and *B. fusca*. The feeding insects damages the maize tissues, entrance of *F. verticillioides* through damaged tissues is passed to offspring through seed-borne

infection. Infection of the maize kernels can also happen when fungal spores are inoculated at the maize silks (Duncan and Howard, 2010), hence causing kernel rot.

Aspergillus species and aflatoxin production in maize and groundnuts

Aflatoxins are naturally occurring mycotoxins produced by five fungal species; which are *Aspergillus flavus* Link ex Fries *Aspergillus parasiticus* Speare, *A. bomycis, A. nomius* Kurtzman, Horn & Hesseltine and *A. tamari* Kita, the latter two *Aspergillus* species rarely produce aflatoxins (Mehan, 1989; Bulaong & Dharmaputa 2002; Liang *et al*., 2006; Iqbal *et al*., 2013). These five fungal species are members of the *Aspergillus* section Flavi group (Sultan and Magan, 2011). *Aspergillus flavus* is most often associated with aflatoxins, contamination of developing crop plants with aflatoxins occuring when there is plant stress and also physical damage (Whitlow and Hagler, 2001). Aflatoxins were first discovered during the outbreak of the Turkey X disease on poultry and were found to be both carcinogenic and toxigenic (Amare and Keller, 2014). Environmental conditions favouring *A. flavus* also lead to aflatoxin contamination of grain crops, for example during unventilated, unhygienic, hot and humid conditions during storage (Egal *et al*., 2005; Probst and Cotty, 2012).

Common habitats of *A. flavus* are decaying organic matter, soil, air and dust (Heinemann *et al*., 2004). Some species of *Aspergillus* cause disease in plants; however most of these species are soil borne (Wiatrak *et al*., 2006; Chaytor *et al*., 2011). Twenty aflatoxins have been identified and only four of them contaminate different foods and feeds. These are aflatoxins B_1, B_2, G_1 and G_2 (Sherif *et al*., 2009). *Aspergillus parasiticus* can produce all the four aflatoxins whereas *A. flavus* can primarily produce aflatoxin B_1 and B_2 (Abbas *et al*., 2006). The most toxic aflatoxin that occurs naturally is the aflatoxin B_1 and it has been classified by the International Agency for Research on Cancer (IARC) as a Group 1 human carcinogen (IARC, 2002). In the Eastern Cape Province of South Africa, levels of about 30 times higher than the international legalized levels (10 parts per billion (ppb) of aflatoxins were found in peanut butter (Wagacha and Muthomi, 2008). In the year 2004, there was an aflatoxicosis outbreak in Kenya due to high aflatoxin contamination of maize (Lewis *et al*., 2005).

Aspergillus flavus epidemiology

Sclerotia and asexual spores are two survival modes of *A. flavus* in the soil. During favourable environmental and nutritional conditions, the asexual spores germinate and grow on plant tissue, the same can happen both on animal and human tissues as hosts. At this stage, the mycelia will form and develop into conidiophores. Sclerotia contain the sexual ascospores of *A. flavus* (Amare and Keller, 2014). In plant tissues, *A. flavus* exists as mycelia. During harsh environmental conditions in the soil such as high temperatures and drought, the sclerotia produce conidia. Before pollination, the *A. flavus* spores colonize the silk, germinate and enter

the maize cob. Entrance pathways could also be through stress cracks in the pericarp, silk scars, the pedicel, bird and insect damage. Nitidulid beetles (*Carpophilus hemipterus* L.) and cornstalk borer (*Elasmopalpus lingosellus* Zeller) are insects that facilitate *A. flavus* infection on maize. The silks of young maize ears are more prone to *A. flavus* colonisation than silks of mature maize crops (Cardwell *et al.*, 1999; Amaike and Keller, 2011).

Aspergillus flavus causes Aspergillus ear rot (AER) of maize. Also, insects invading the maize cob may promote the development of AER and subsequent aflatoxin contamination (Woloshuk and Wise, 2011). Placinta *et al.* (1999) reported a significant interrelations between AER incidence and temperature, relative humidity and rainfall. Futhermore, AER is a non-continous and infrequent disease, however, when it occurs it has a significant impact on maize grain yield and quality (Smart *et al.*, 1990).

A. flavus infection and aflatoxin contamination on groundnut

Whether processed or raw, groundnuts can be contaminated by mycotoxin-producing fungi, which obtain nutrients from the crop (Kayode *et al.*, 2013). The groundnut is highly susceptible to invasion by *Aspergillus flavus* and the production of aflatoxins which contaminate the groundnuts before harvest (production stage), during transportation and after harvest (storage stage) (Liang *et al.*, 2006; Hepsag *et al.*, 2014). Aflatoxin contamination of groundnuts before harvest is also favoured by insects that are present in the soil; contamination can be reduced by the late season irrigation however this does not apply to arid and semi-arid areas (Wang *et al.*, 2010). Pre-harvest contamination of groundnut happen during crop maturity under heat and drought stress (Sultan and Magan, 2011). Water activity (A_w) around 0.82 and temperatures between 25°C and 30°C are best known to favour aflatoxin contamination on groundnuts (Toregeani-mendes *et al.*, 2011). Shackleton *et al.* (2011) stated that by the year 2025, 65 % of people living in South Africa could experience drought stress which also means that the crops will suffer from water shortages. In addition to drought stress, different disease patterns and floods are expected.

Timper *et al.* (2004) stated that the combinations of drought stress and high soil temperatures before optimal groundnut maturity are needed for aflatoxin contamination in groundnuts. High aflatoxin contamination of groundnuts results from higher toxigenic *A. flavus* frequencies (Horn, 2005; Wiatrak *et al.*, 2006). Antimicrobial compounds known as phytoalexins are inhibited during drought, favouring the growth of *A. flavus* (Hamidou *et al.*, 2014). Apart from *A. flavus* infection, there are other factors influencing the production of aflatoxins (Hepsag *et al.*, 2014). Damaged pods are more prone to aflatoxin contamination than undamaged pods. *Aspergillus flavus* penetrates through cracked pod walls due to the decrease in water activity under drought stress. However, upper parts of the plant including leaves, fruits and flowers are more subject to *A. flavus* infection (Diener, 1989).

Previous work by Ncube *et al*. (2010) reported that *Aspergillus flavus* contaminated groundnuts at some northern Kwa-Zulu Natal (KZN) localities in 2006 and at all northern KZN localities in 2007 with levels being higher in 2007 than in 2006. In the two sampled localities of the northern KZN, aflatoxin levels were above the level of allowed for human consumption as set by the Department of Health in South Africa. Linear regression analysis showed that there was no notably correlation between *Aspergillus* spp. and aflatoxin contamination.

Fusarium graminearum

Fusarium graminearum (*Gibberella zeae*) is one of the crucial fungal pathogens infecting maize (Desjardins, 2006) and causes Giberrella ear rot (GER) or red ear rot (Boutigny *et al*., 2011; Martin *et al*., 2012). Yield losses due to *F. graminearum* leads to unmarketable maize grain loses which results in small-holder farmers facing major economic constraints (Geng *et al*., 2014). Cool and moist conditions favour the growth of *F. graminearum* and it is also able to spread rapidly (Sikhakolli *et al*., 2012; Minenko *et al*., 2014). *Fusarium graminearum* produces the mycotoxins zearalenone (ZEA*),* deoxynivalenol (DON*)* and nivalenol (NIV), which is the derivative of DON (Malbran *et al*., 2012)*.* High temperatures do not aid in the decomposition of ZEA and its stability is retained during milling/storage and food processing (Atoui *et al*., 2012). Larsen *et al*. (2004) reported that trichothecenes are relatively heat stable with DON being stable at 120°C.

A positive correlation was found between *F. graminearum and F. culmorum* aggressiveness and the production of DON (Malbran *et al*., 2012). DON and NIV are known as trichothecene mycotoxins commonly found in maize (Velluti *et al*., 2000). The distribution of *F. graminearum* in maize is facilitated by these trichothecene mycotoxins (Mehan, 1989; Taylor *et al*., 2008). DON is the most detected trichothecene in food compounds with maximum allowed limits of between 500-1000 μg/kg (Van Egmond and Joker, 2004), it causes vomiting hence known as vomitoxin (MacDonald *et al*., 2004; Berthiller *et al*., 2005; Numanoğlu *et al*., 2011).

Fusarium graminearum epidemiology

The sexual stage is the most important central part of the life cycle of *F. graminearum*, which is dispersed by ascospores, the perithecia encloses these spores. Asexual spores are also known as conidia, produced in the life cycle of *F. graminearum*. Both ascospores and conidia are found during maturity of the infected plant. During transmission to host plants, the spores are able to survive harsh environmental conditions due to their resistant characteristic. Sources of *F. graminearum* inoculum include maize roots, stems, ears and stalks; however the main source is infected plant debris. Dissemination measures include the rain-splash, wind and insect vectors (Sikhakolli *et al*., 2012; Geng *et al*., 2014).

In maize seedling, *F. graminearum* visual symptoms after infection include wilting, stunting, chlorosis and yellowing which happen when a root system is weak. *Fusarium graminearum* transmission to seedling from seeds occur during favourable environmental conditions, seeds contaminated with this fungal pathogen exhibit a pink to reddish brown colour (Galli *et al.*, 2005). The Gibberella ear rot covers a large part of the ear, initiating from the tip of the ear; and prevails in areas that are cool (Munkvold, 2003).

Fusarium graminearum is known as a broad host range pathogen; of which maize is one amongst other crops it infects. However the molecular basis of infections is not extensively known. The virulence is enhanced by secreted extracellular enzymes called the lipases (Voigt *et al*, 2005). Infections by *F. graminearum* reduce maize quality and yield (Harris *et al.* 1999).

Gibberella ear rot (GER) caused by *F. graminearum* generally commences from the tip of the ear and causes reddish or pinkish coloured mold on maize kernels (Harris *et al.*, 1999; Munkvold, 2003). The GER is prevalent in cooler or higher precitation areas during the growing season and favoured by high moisture levels around silking (Munkvold, 2003).

MYCOTOXIN EFFECTS ON PUBLIC HEALTH AND ECONOMY

The scientific study of mycotoxins began in 1960 after the death of a large number of turkey poults due to consumption of contaminated groundnut meal in England (Bankole and Adejumo, 2003). Humans are exposed to mycotoxins through ingestion of mycotoxin contaminated food and also through inhalation of contaminated air, while animals are exposed through consumption of mycotoxin-contaminated feed. Mycotoxins are released into the atmosphere during colonization and sporulation of the mycotoxigenic fungi (Rao *et al.*, 1997; Iha *et al.*, 2013). Fungal spores contaminated hospital environments during the study period from 1995 to 1998 and caused aspergillosis in patients, a disease normally caused by *Aspergillus fumigatus* but which can also involve *A. flavus* (Alberti *et al.*, 2001; Heinemann *et al.*, 2004).

From both the inhalation and ingestion of mycotoxin-contaminated food and feed, a pathological abnormality known as mycotoxicosis develops (Bankole and Adebanjo, 2003). Human and animal mycotoxicosis syndrome emerges from the ingestion of fumonisins, aflatoxins and trichothecenes for example (Peraica *et al.*, 1999). In the Eastern Cape Province of South Africa, a human oesophangeal risk due to the consumption of fumonisin-contaminated home-grown maize was detected (Ghiasian *et al.*, 2005; Leslie *et al.*, 2005). Daily intake levels of 4.4-8.7 µg kg^{-1} body weight in average were reported from people living in the province (Van der Westhuizen *et al.*, 2011; Adejumo, 2012). The interaction between the ingestion of fumonisins and cancer development in humans is not clear (Nikiema *et al.*, 2004). Equine leukoencephalomalacia, rat liver cancer and pulmonary edema in swine are also caused by the exposure to fumonisins (Pietri *et al.*, 2004; Samprieto *et al.*, 2013).

Aflatoxins cause liver cancer and reduces immunity in humans (IARC, 2002; Kamika *et al.*, 2014). Also, decreased levels of serum immunoglobulins A and B including human hepatocellular and gastrointestinal carcinomas are caused by ingestion of food contaminated with aflatoxins; aflatoxin B_1 induced these neoplasms in humans in China, Philippines and Africa (Ilesanmi and Ilesanmi, 2011; Williams *et al.*, 2011). Impaired growth has been associated with exposure to aflatoxins (Egal *et al.*, 2005; Gong *et al.*, 2004). Aflatoxins impact extensively on the socio-economic status of Africa at large, prevailing in toxicity and carcinogenicity among other mycotoxins. Thus the impact is higher on health costs than on trade costs (Wu and Khlangwiset, 2010). Production of aflatoxin-contaminated groundnuts pose a serious threat. This leads to problems with commercialization of groundnut-derived products (Mehl and Cotty, 2013; Hamidou *et al.*, 2014).

There are certain limits of aflatoxins allowed in food. For instance, in Europe, permitted aflatoxin levels in human food are 4 parts per million (ppm) and 2ppm for total aflatoxin and aflatoxin B1 respectively (Larou *et al.*, 2013).   However, the implementation of aflatoxin regulation in food has not been effective in other nations. Application of regulations is not practical in developing countries because people either consume home-grown food or food from informal markets, some countries like Haiti totally lack food regulations. It is often in countries like these where people are affected by high aflatoxin levels. Tian *et al.*, 2012 stated that aflatoxins affect about 4.5 billion of the people in developing countries. This is where products are being manufactured from grain that was not controlled for aflatoxin contamination, as in the situation in Haiti. The implementation of management methods that reduces the risk of contamination could work best for people under these circumstances; these include dietary, clinical and agricultural interventions (Wu and Khlangwiset, 2010; Filbert and Brown, 2012).

Aflatoxins, fumonisins, trichothecenes, ochratoxins, zearalenone, ergot alkaloids and tremorgenic toxins are significant mycotoxins that affect the agro-economy and public health (Zain *et al.*, 2011). Animal food products such as milk, meat and eggs that contain mycotoxins have a negative impact also on international trade (Bryden, 2012). Also very important for international trade are groundnut and groundnut derived-products, contamination by aflatoxin-producing species remarkably leads to economic loss (Yong and Cousin, 2001). Mycotoxins have an economic impact on health costs and international trade estimated at hundreds of million dollars annualy (Brown *et al.*, 2012).

Mwaza *et al.* (2013) stated that anorexia, weakness and depression develop in dogs (Canis lupus familiaris) due to aflatoxin contaminated feed, during the development of these symptoms the dogs suffer from intravascular clotting, extended livers and internal haemorrhage. Death in South African dogs was due to aflatoxin contaminated feed, aflatoxicosis outbreak resulted in the death of an estimated number of 100 dogs. The dogs

died in the Gauteng Province of South Africa, from April to July 2011 (Arnot *et al.*, 2012). The products and derived products for animal feed can be prepared from contaminated groundnuts, maize, sorghum, cotton seed, oilseed and millet. Other domestic animals like cattle do consume contaminated maize silage, which has high moisture content that favours fungal growth. A great harm is posed to the public health as dairy cattle that consume aflatoxin contaminated feed produce milk containing aflatoxin metabolite of aflatoxin B_1 (aflatoxin M_1). The AFM_1 is has been evaluated as a human carcinogen of the class 2B (Cavallarin *et al.*, 2010).

Other mycotoxins like ZEA and DON cause estrogenic syndrome and decreased weight gain in swine respectively, DON is also associated with oesophageal cancer and liver disease (Reid *et al.*, 1999; Velluti *et al.*, 2000). ZEA complicates reproduction in mammals as it alters the internal and external genitals by binding to oestrogen receptors (Kosawang *et al.*, 2014). Male infertility and swine estrogenic syndrome have been associated with the factors that lead to economic losses due to mycotoxins and mycotoxigenic fungi. These factors are seed contamination, yield reduction and poor seedling germination (Dyer *et al.*, 2006).

PLANT-PARASITIC NEMATODES

Plant-parasitic nematodes are usual parasites of plant roots, although they are also found in other organs of the plant. These plant parasitic nematodes are classified into endo and ectoparasitic nematodes, the former survive within the host plant whereas the latter survive on plant roots (Haegeman *et al.*, 2012). Plant-parasitic nematodes are also known as obligate parasites because their reproduction and development is dependent on viable plants (Oka et al., 2000). Most plant-parasitic nematodes are confined to the roots; these are usually migratory parasites and endoparasites such as the root-knot nematode. The penetration and migration of these nematodes into the plant roots is facilitated by molecules known as effectors, which also manipulate the structure and function of the host cell (Torto-Alalibo *et al.*, 2009). The nematode effectors also play roles in initiating or maintaining the development of feeding sites and in preventing the plant defence response (Gheysen and Mitchum, 2011). Plant-parasitic nematodes are fewer than free-living nematodes; however the damage these plant feeders causes in plants is significant. Water and wind are means of dissemination for nematodes even at long distances (Cadet *et al.*, 2002).

Plant-parasitic nematode infestations on groundnut

Groundnut yield loss of 10 % has occurred 3 decades back in the South African groundnut industry due to plant-parasitic nematodes and devastating yield losses have been incurred worldwide due to nematode infestations (Venter *et al.*, 1992; Tirumalaraju *et al.*, 2011). Sharma *et al.* (1990) reported that 12 % groundnut yield has been lost globally as a result of

plant parasitic nematodes, with about 20.6 % groundnut yield losses reported in India (Sharma *et al.*, 1992; Rizvi *et al.*, 2012). *Ditylenchus africanus* Wendt, Swart, Vrain, and Webster (1995) is known as a plant-parasitic nematode in groundnuts and is ubiquitous in South Africa where groundnut is produced (Wendt *et al.*, 1995). This nematode normally affects the quality of the peanut and it has high reproductive and damage potential. In South Africa, severe groundnut losses are caused by *D. africanus*, thereby limiting groundnut production and hence income losses for farmers (Steenkamp *et al.*, 2010). There is a need for the implementation of highly effective control measures that will lead to better control of *D. africanus* (Steenkamp *et al.*, 2011). It was first isolated in South African groundnut, in the hulls and kernels and was first identified as *Ditylenchus destructor* (De Waele *et al.*, 1991). The groundnut cultivar Sellie, was found to be highly susceptible to *D. africanus,* however no resistant groundnut cultivar has been recently identified (Steenkamp *et al.*, 2011). *Ditylenchus africanus* can also feed and reproduce on *Botrytis cinerea* Pers. ex Fr, *Rhizoctonia solani* Kuhn*, F. oxysoprum* f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hansen and *A. parasiticus* hyphae (Pedras *et al.*, 2005; Chen *et al.*, 2009; Haegeman *et al.*, 2009; Steenkamp *et al.*, 2011).

Another nematode that is widespread in grape-cultivated areas of South Africa is the *Pratylenchus* spp. These species are known as root-lesion nematodes. In South Africa, four root-lesion nematode species have been identified; namely the *Pratylenchus crenatus* Loof, *Pratylenchus vulnus Allen and Jensen, Pratylenchus penetrans* Filipjev and Schuurmans Stekhoven and *Pratylenchus minyus* Sher and Allen (Loubser and Hoppener, 1986; Frederick and Tarjan, 1989). *Pratylenchus penetrans*, is one of the prevailing plant-parasitic nematodes in the roots and the soil (Kimpinski *et al.*, 1998).

Nematode species known to be destructive are the *Meloidogyne* spp., described as the root-knot nematodes (RKNs). As the name implies, the life cycle of RKNs is completed within the roots. The formation and maintenance of *Meloidogyne* spp. feeding sites helps in continuous supply of nutrients for this species, thereby enhancing development (Ozalvo *et al.*, 2014). In South Africa, the RKNs are known to affect the yield and quality of crops; some of these nematodes have limited distributions like the *M. kikuyensis* de Grisse, *M. vandervegtei* Kleynhans, which are all only found in the KwaZulu-Natal province. Like other pathogens, nematodes differ in terms of prevalence and distribution. Four economically important RKN species are *M. incognita* Kofoid and White, *M. javanica* Treub & Chitwood, *M. arenaria* Neal and *M. hapla* Chitwood (Fourie *et al.*, 2001; Hunt and Handoo, 2009; Eisenback and Dogde, 2012). The former three were described as the closely related group (Trudgill *et al.*, 2000).

Root-knot nematodes decompose and release endospores which are tolerant to desiccation and heat. This allows the survival of endospores in the soil for a prolonged period (Timper, 2009). Two *Meloidogyne* spp. that were found associated with groundnuts are *M. hapl*a and *M. arenaria* race 1(R1). Groundnut cultivars are susceptible whereas others are

resistant to different species of RKNs, like the Chalimbana groundnut cultivar which was found to be susceptible to *M. arenaria* R1 but resistant to *M. hapl*a. The study that was done in Malawi revealed that *M. incognita* and *M. javanica* as the two most damaging species of RKN in crops (Saka, 1990). In groundnut production regions worldwide, remarkable economic losses have occurred due to the root-knot nematode *Meloidogyne arenaria* R1. Symptoms exhibited by plant infected with nematodes include wilting, hindered growth and they display increased susceptibility to other pathogens (Liao and Holbrook, 2007).

Nematodes can supress diseases in plants, caused by different fungal pathogens; these are called fungivorous nematodes. The *Ditylenchus* genera are one of the nematodes known to have an effect on pathogenic fungi, whereby they use the stylet mouthpart to damage the fungal mycelium (Lagerlöf *et al.*, 2011).

Fungal and nematode interaction on groundnut

High *Aspergillus flavus* infestations on groundnuts have been associated with lesion and root-knot nematodes (Motsinger *et al.*, 1976). Nematode infection has a variable effect on kernel colonization by *A. flavus*. This may be due to insect damage and kernel immaturity. Aflatoxin contamination of groundnut kernels during drought stress may also be increased due to nematode damage, however the mechanism is unknown (Timper *et al.*, 2004). The *Fusarium* spp. has also been tested in relation to the *Ditylenchus* genera. However, the authors did not elaborate on the findings of this relationship (Friberg *et al.*, 2005; Lagerlöf *et al.*, 2011).

MANAGEMENT OF MYCOTOXIGENIC FUNGI AND MYCOTOXINS

Control of *F. verticillioides*

Due to its endophytic characteristics, *F. verticillioides* is difficult to control. Biotic and abiotic factors together with genetic and morphological characteristics of maize then changes *F. verticillioides* from an endophyte to a pathogen (Rocha *et al.*, 2014).

It was stated by Van der Westhuizen *et al.* (2011) that physical measures such as sorting and washing significantly reduce fumonisin contamination in maize kernels than is the cooking process. Fungicides have been used as seed treatments; however they are not cost effective and have not been efficient as they leave residues within the grain crop or seeds (Chandra Nayaka *et al.*, 2009). Chemical fungicides to control Fusarium ear rot pose harm to the environment. Other fungicides used are synthetic however, information about the effects the synthetic fungicides have on Fusarium ear rot and fumonisin contamination is very limited (Formenti *et al.*, 2012). Also, fungal resistance develops, hence limiting the use of fungicides as a control measure (Garcia *et al.*, 2012).

Biological control using fungal or bacterial isolates was proposed as an alternative measure to fungicides. The aim of this kind of biological control was to increase maize seed quality (Chandra Nayaka *et al.*, 2009). The proposed method is environmentally friendly and not a hazard to human health (Pereira *et al.*, 2009). Also, the use of synthetic fungicides is replaced with the use of natural compounds, known as polyphenols. These are secondary metabolites which inhibit fungal enzymes and could inhibit growth and fumonisin production (Ferrochio *et al.*, 2013).

Thembo *et al.* (2010) investigated the activity of aqueous and organic extracts of four weedy plant species against isolates of *F. verticillioides* amongst other *Fusarium* and *Aspergillus* species. The weedy plant species collected were mainly used as insect repellents and fumigants in different provinces of South Africa. Methanol extract of one plant species was found to inhibit the growth of two *F. verticillioides* strains (MRC 8559 and MRC 8267) but not MRC 826. Cultural methods investigated to control *F. verticillioides* on maize include the use of less susceptible maize cultivars and also insect and weed control (Torres *et al.*, 2003).

Control of *A. flavus* on maize

Plant stress reduction and plant health maintenance throughout the growing season are current recommendations for *A. flavus* management; however these measures can be affected by environmental conditions (Dolezal *et al.*, 2013). Aflatoxin contamination in the field due to the presence of *A. flavus* can be reduced by planting early and employing good agronomic practises. Drying also prevents development of moulds by reducing water activity. Other methods to control aflatoxins directly in food and feed by destroying or removing the aflotoxin in food include physical, biological and chemical methods (Abrar *et al.*, 2013).

A promising control method is the use of atoxigenic *A. flavus* strain that is competitive when applied. When a fungal strain does not produce any aflatoxins, it is known to be atoxigenic/non-toxigenic. An atoxigenic *A. flavus* strain completely eliminates toxigenic strains when in contact. In the West Africa, over 90% reductions in contamination were achieved from the investigated atoxigenic isolates (Atehnkeng *et al.*, 2008). These isolates belong to the vegetative compatibility groups (VCGs) with do not carry toxigenic genes. One of the advantages of using this method is that is it non-labour intensive as it can be applied directly on soil, this method was successful on groundnuts (Lyn *et al.*, 2009).

Control of *A. flavus* in groundnuts

Hell *et al.* (2010) stated that in Africa, there have been other technologies to reduce the risk of aflatoxin contamination which include; fertilizer application, insect and weed, timing of planting and the use of resistant varieties. However, these interventions have largely been ineffective in reducing the risk of contamination. The use of atoxigenic *A. flavus* strain to

control the toxigenic fungi at timely harvest has been suggested as a biological control method (Hell *et al*., 2010). This intervention has been successfully used in Africa on maize and groundnuts with 77 to 98 % reduction in aflatoxin contamination being recorded. After the development of the groundnut canopy, a grain substrate with fungal conidia is applied directly to the soil surface remaining in close contact with the groundnut pods. The toxigenic strains are competitively eliminated by the atoxigenic fungal strains (Horn and Donner, 2009).

In addition to fungal pathogens, groundnuts can also be infected by nematodes, viral and bacterial pathogens and have been tested for resistance to *Aspergillus flavus*, aphid vectors, rust, drought and nematodes (Subrahmanyam *et al*., 1997; Sarvamangala *et al*., 2011). However the focus here will be on nematode infestations on groundnut.

Control of *F. graminearum*

Fungicides were commonly used to control *F. graminearum* on maize but have been replaced with microbial agents that are antagonist to *F. graminearum* (Chan *et al*., 2009). *Bacillus* spp. have been applied to control different maize diseases caused by *F. graminearum*. This method of biological control is considered due to its cost-effectiveness and posing no harm to the environment (Pal *et al*., 2001). However, Raupach and Kloepper (1998) stated that the use of adversary micro-ogranisms as one of the biological technologies to control plant pathogens could offer a disadvantage due to the narrow-range activity of the micro-ogranisms. Another alternative to reduce yield loss due to mycotoxin contamination were to breed and grow resistant maize cultivars (Loffler *et al*., 2010).

Control of plant-parasitic nematodes

Nematicides have been used to control nematodes; however the disadvantages are that they are costly, hazardous and the effectiveness is short-lived (McElderry *et al*., 2005), in other words these chemicals are a threat to the environment, animals and humans (Ann, 2013). Use of nematicides is also being limited by environmental effects and government regulations (Dong *et al*., 2007; Tirumalarajua *et al*., 2011). Chemical nematicides have been used before for the control of the *Meloidogyne* spp.. Some nematicides were effective like the methyl bromide, ethylene dibromide (EDB) and dibromochloropropane (DBCP). However they were banned due to the consideration of environmental and human safety (Mostafanezhad *et al*., 2014).

There has been wide use of resistant cultivars and crop rotation, adding to the advantage that harm is not posed to the environment when using these two methods (Saka, 1990). However, crop rotation has not been an effective method due to the broad host range of the RKNs (Trudgill *et al*., 2000). Nematode development was inhibited in plants in which the proteinase-inhibitor gene was inserted; these genes that confer resistance in plants are

being widely developed as effective means for nematode control (Oka *et al.*, 2000). Plant-derived chemicals have also been tested for nematode control. These chemicals are effective against other types of nematodes and are environmentally-safe (Akthar and Mahmood, 1994). The *Tagetes* spp. was found to be effective for control of *Pratylenchus* and *Meloidogyne* genera (Oka *et al.*, 2001). Nematode suppression methods such as soil biofumigation have also been used; during this process toxic compounds are produced from the decomposition of either an animal-by product or a plant material (Ploeg and Stapleton, 2001).

Other methods that have been generally employed to control nematodes are biological control, induced resistance, organic and inorganic soil amendments and interruption of host recognition (Collange *et al.*, 2011). Current studies focus on the implementation of nematode-control methods that lead to the production of good-quality, safe and healthy food and food products, for example the Integrated Pest Management (IPM) system. Target-specific methods were proposed by agricultural institutions, these methods are to focus on diseases and nematodes on different crops (Kruger *et al.*, 2013). The other proposed method was to use antagonistic microorganisms that will act against the RKNs, affecting their mobility and viability (Meyer *et al.*, 2000).

Preharvest management strategies

Chemical: Research conducted in South Africa by Thembo *et al.* (2010) has found *Tagetes minuta* chemical extract to inhibit the growth of *F. verticillioides* MRC 8559 and MRC 8267, except for MRC 826. Fungicides and post-emergence herbicides are also used in the field to protect maize against disease infection whereas chemical insecticides are mainly used to control European corn borer (ECB) (Blandino *et al.*, 2012). Essential oils derived from plants provide anti-toxigenic and anti-fungal properties hence are used to inactivate microbes (Tian *et al.*, 2012). The mint (*Mentha viridis*) essential oil tested against *A. flavus* growth on stored maize and it was found to be fungicidal and anti-aflatoxigenic at different concentrations per 100g of maize from 7 days storage of maize up to the end of storage at 21 days (Gibriel *et al.*, 2011).

Cultural practices: Planting date, irrigation, tillage practices and crop rotation are some of the cultural practices employed by farmers to reduce mycotoxin contamination of crops (Munkvold, 2003). Tillage is applied because crop residues harbour most mycotoxigenic fungi, hence the need to also rotate crops to reduce mycotoxin contamination (Munkvold, 2003). Contrast to tillage farming, no-tillage influences root growth and crop productivity through reduced soil cultivation (Himmelbauer *et al.*, 2012). Verhulst *et al.* (2011) reported that zero tillage with residue retention prompted the highest soil water content particularly in extended,

variable drought periods. No-tillage was also found to improve soil moisture content and infiltration (TerAvest *et al.*, 2015).

Crop rotation: Crop rotation was listed by Wambacq *et al.* (2016) as one of the appropriate field management practises to prevent the occurrence of mycotoxins. Pests and disease cycles are broken down due to crop rotations (TerAvest *et al.*, 2015). In certain regions of South Africa, the maize yield was increased due to crop rotation (Nel, 2005). Maize yields were also increased in smallholder farms of Malawi after diverse crop rotations with bean, cassava, cowpea, soybean and sweetpotato regardless of residue retention or tillage (TerAvest *et al.*, 2015). Intercropping is also responsible for reducing contamination of maize crops with mycotoxins (Van Asselt *et al.*, 2012). Destruction or removal of infected crop residues from the field reduces fungal inoculums (Wambacq *et al.*, 2016). Report by Jaime-Garcia and Cotty (2010) suggested that the average aflatoxin-producing potential of *A. flavus* in soil may be reduced by crop rotations.

Harvesting: Mycotoxin contamination of maize planted by subsistence farmers in South Africa was found to be due to their improper farming practices (Ncube *et al.*, 2011; Shephard *et al.*, 2013; van der Westhuizen *et al.*, 2011). These farmers practise maize monoculture, late harvesting and leaving crop residues in the field (Ncube *et al.*, 2011). Timely planting, irrigation and use of transgenic hybrids were found to be effective in lowering the incidence of mycotoxin contamination, these include harvesting techniques and methods (Bruns, 2003). Poor harvesting practices were reported to contribute to fungal growth, farmers are adviced to harvest their crops early to reduce fungal infection in the field before harvest (Wagacha and Muthomi, 2008). Early harvesting and threshing of groundnuts resulted in consistently lower aflatoxin levels and higher gross returns of up to 27% as compared to delayed harvesting (Rachaputi *et al.*, 2002). Many factors can affect early harvesting such as unpredictable weather, labour constraits, need for cash, threat of thieves and animals compelling farmers to harvest at inappropriate time (Amyot, 1983).

Postharvest management strategies

Storage: The quality and safety of grain products is normally protected by controlling the temperature in storage systems. Grain crops are often stored for a long period in different storages systems for example at the silos and storehouses, further infection by fungal pathogens happen at storage. Three recurrent fungal species of partially overlapping ecological niches isolated from stored maize are *Aspergillus*, *Penicillium* and *Fusarium* species (Gregori *et al.*, 2013). However, the *Penicillium* and the *Aspergillus* species are the

two prevalent species at storage; these two species do not require high moisture levels for growth (Boudra and Morgravi, 2008; Njobeh *et al.*, 2009).

Unfavourable storage conditions leads to further mycotoxin contamination of grain thereby causing critical losses of the resulting grain products. Fungal growth is considerably affected by moisture and temperature, with the former factor being greatly increased at storage (Leung *et al.*, 2006). Contamination at storage can occur when humidity or moisture content is above 14 % and 20°C temperatures (Richard, 2007). Storage of improperly dried grain results in rapid proliferation of mycotoxigenic mould and spoilage at storage producing grain products with reduced quality, nutrition and dry matter (Mylona *et al.*, 2012).

Changes at storage occur due to interactions of biological, physical and chemical parameters. Water activity of 0.70 (a_w= 0.70) is required for safe storage of grain crops, this corresponds to moisture content just below 14 %. Some insects occur at storage, damaging maize and thereby allowing fungal entry, mycotoxin contamination and moisture accumulation. *Sitophilus zeamais* Mots, is an example of one these storage insects (Chulze, 2010). Mboya *et al.* 2011 stated that moisture content of stored maize can be increased due to insect activity. Also, the odour caused by fungal decay on maize increases risks by insect pests (Mboya *et al.*, 2011). One control method that has been carried out to hinder fungal growth and mycotoxin production at storage is the application of synthetic mould inhibitors e.g. Antitox Plus (AP) (Elsamra *et al.*, 2012).

Traditional storage practices employed by subsistence farmers in northern KwaZulu-Natal were reported as having an effect on the vigour and germination of maize. This is due to the fact that moisture levels of 14 % or higher may promote fungal infection at storage leading to the reduction in the quality of maize seeds (Govender *et al.*, 2008). Subsistence farmers can be advised on the use of silo bags, which are also known as hermetic bags as new storage systems. These bags are flexible, less costly and have an internal environment that is slightly anaerobic thus not favouring fungal growth. Insect mortality was also observed due to the hermetic bags (Gregori *et al.*, 2013). Stored grain is affected by fungal contamination also in food industries, especially contamination due to *A. Flavus* (Tian *et al.*, 2012). Hence use of hermetic bags can be extended to such industries.

Storage facilities can be improved, leading to reduced levels of contamination. Intervention measures that were introduced in Guinea villages successfully led to the improvement of their storage facilities and a 60 % reduction in aflatoxin contamination of groundnut was accomplished (Turner, 2005). Intervention measures that led to decrease in aflatoxin contamination at storage included hand-sorting before storage, drying on mats, sun-drying, storage in natural-fibre bags, use of wooden pallets and insecticide sprinkling at storage (Turner, 2005). There has not been much attention given on problems associated with storage. Normally what is happening in the field cannot be controlled, however post-harvest

control technologies can minimise mycotoxin contamination in the food chain (Magan and Aldred, 2007). Storage facilities are some of the control points that have to be re-evaluated in the production chain; these will lead to good marketable agricultural products.

Subsistence farmers consume high quantities of home-grown maize and their exposure to mycotoxins is higher than consumers in cities and towns. Therefore, there is a need to determine the extent of multi-mycotoxin contamination. Previous surveys have been carried out in South Africa (Marais and Swart, 2003; Mkhabela, 2002; Ncube *et al.,* 2008, van der Westhuizen *et al.*, 2011), only the survey by Ncube *et al.* (2008) focused on mycotoxins and mycotoxigenic fungi. However, there is still a huge gap on control methods to be employed for the promotion of food security, thereby reducing health risks associated with mycotoxin contaminated food.

Detoxification products: Chemical modification and compartmentation are two major plant detoxification mechanisms, a plant can metabolise mycotoxins to defend against a pathogen (Berthiller *et al.*, 2013). Aflatoxins can be treated with chemical agents like formaldehyde, ammonia and hydrogen peroxide. Natural botanicals, ozone and roasting are postharvest methods which have been adapted specifically for the treatment of aflatoxin B1 (Bhat *et al.*, 2010).

CONCLUSION

Mycotoxin contamination is a worldwide problem, surveillance of grain crops and animal feed still stand as a priority. During the year 2004, 125 deaths were reported in Kenya due to acute aflatoxicosis (Williams *et al.*, 2004). The majority of small-holder farmers in the SADC regions are not aware of mycotoxins and the diseases that the mycotoxins cause (Mboya and Kolanisi, 2014). Awareness campaigns aimed at involving personal communications with nematologists, health professions and researchers dealing with mycotoxins are planned, the end goal is to educate small-holder farmers about potential threats in their fields and storage facilities and how to improve their storage systems. Currently, small-holder farmers are only advised to plant and harvest early (Ncube *et al.*, 2011). These are amongst others, methods that were proposed to reduce infection caused by *F. verticillioides* in the field; however storage problems involving other different fungal pathogens also need to be taken into consideration. The awareness will extend to the public at large; through creation of pamphlets on "mycotoxins and their solutions" and these will be translated to different South African languages. Distribution of these phamplets will cover clinics, hospitals and local schools.

The present work is designed as a follow-up study to the earlier work by Ncube *et al.*, 2011 and 2010 that identified hot spots for fumonisin and aflatoxin contamination. There is a need to employ good farming practices, and proper pre-harvest handling of maize and

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groundnuts together with good storage practices to minimize the risk of fungal contamination. Therefore, the research conducted in **chapter 2** will focus on factors that contribute to fungal and mycotoxin contamination of maize and groundnuts and will be identified through the use of questionnaires. In **chapter 3** grain sampled at harvest and following storage over two seasons, from subsistence farmers participating in the study will be evaluated for the i) extent of mycotoxigenic fungal infection, ii) extent of multi-mycotoxin contamination, iii) incidence of plant-parasitic nematode infestations and iv) establish the relationship between plant-parasitic nematodes and fungal/mycotoxin contamination.

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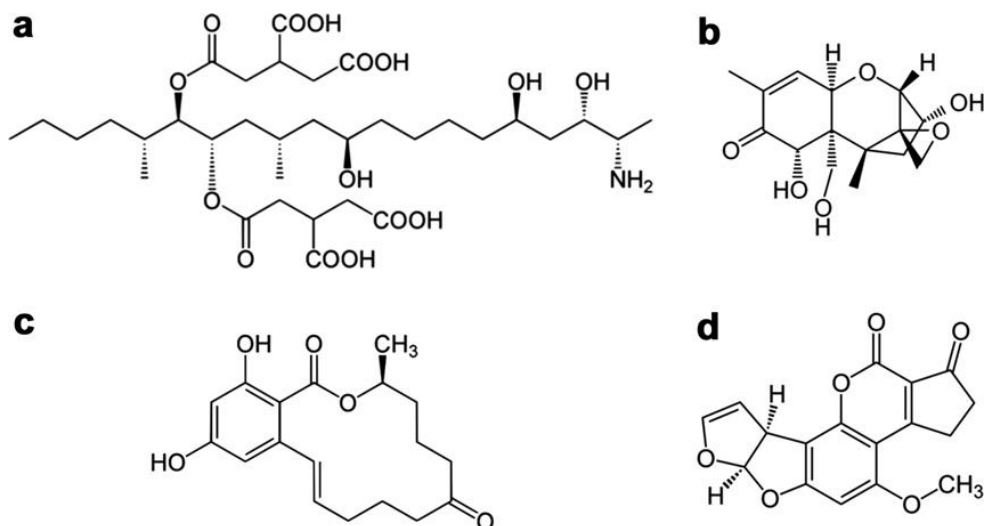
Figure 1. Chemical structures of four prevalent mycotoxins on maize crops; which are fumonisin B1 (a), deoxynivalenol (b), zearalenone (c) and aflatoxin B1 (d) (Covarelli *et al.*, 2011).
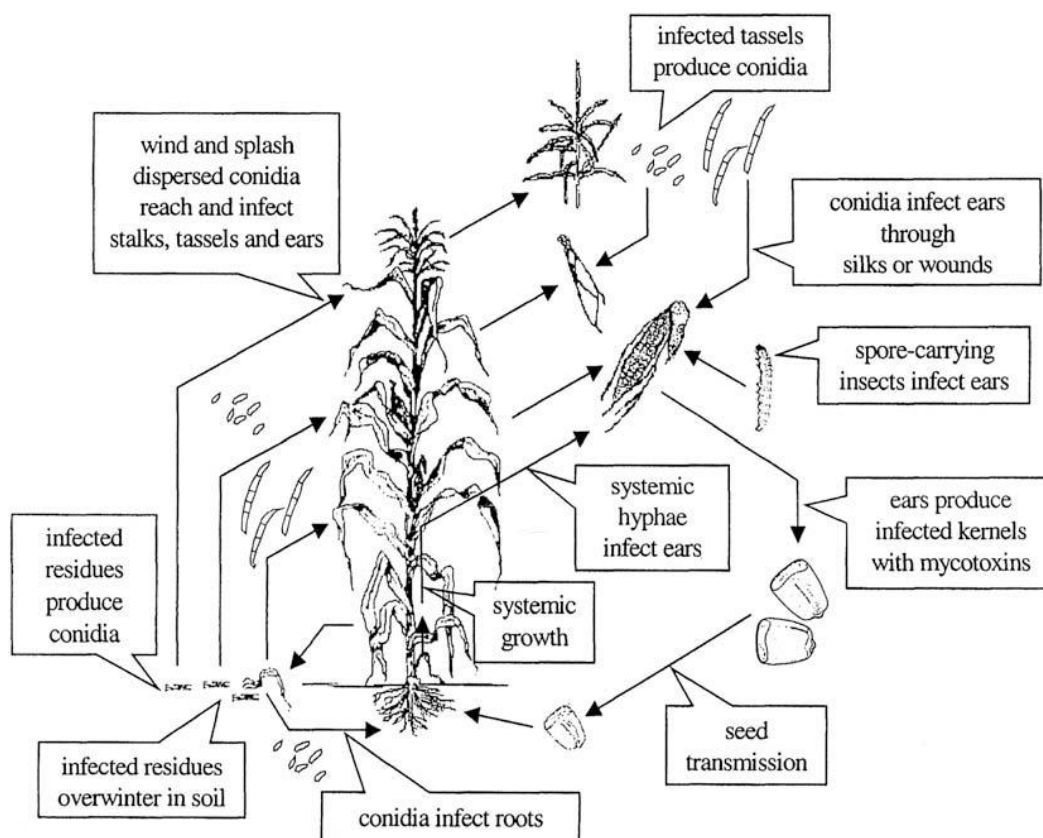
Figure 2. General life cycle of mycotoxigenic fungi which is commonly associated with maize

CHAPTER 2

A survey of agricultural practices in subsistence farming systems and their potential role in mycotoxin contamination of maize and groundnut

ABSTRACT

Maize and groundnuts produced in northern Kwa-Zulu Natal (KZN) can be highly contaminated with mycotoxins such as fumonisins and aflatoxins. Numerous agricultural practices including crop rotation and storage methods have been shown to impact on mycotoxin accumulation. Therefore, the farming and storage practices in maize and groundnut subsistence farming systems in Pongola, Vryheid, Jozini, Manguzi and Mbazwana districts of northern KZN, South Africa were determined. A questionnaire was presented to 52 subsistence farmers of maize; whereas the questionnaire on groundnuts was presented to 30 subsistence groundnut farmers. Fewer farmers grew groundnuts compared to those planting maize as the districts differed in soil types limiting the production of groundnut in certain areas. Storage facilities such as the tank and "*Inqolobane*" (a wooden structure), which differ according to structure, were identified as the most common storage containers with some being ventilated while others were unventilated. The mouldy and damaged grain (groundnut and maize) were largely used as animal feed, hence exposing the animals to increased risk of mycotoxicoses. The questionnaires revealed that at least 90% of the farmers surveyed were not aware of mycotoxins and their consequences to animal and human health. The implementation of mycotoxin awareness campaigns is, therefore, necessary particularly in these districts. Therefore, additional surveillance applying proper sampling and representation strategies should be conducted to obtain unbiased results.

INTRODUCTION

Maize (*Zea mays* L.) and groundnut (*Arachis hypogaea* L.) are important grain crops produced in South Africa. Maize serves as the most important staple food while also being used for animal feed; mostly in rural areas (Ncube *et al.*, 2010; Ncube *et al.*, 2011). Groundnuts are utilised to produce many value-added products such as peanut butter, refined peanut oil and roasted groundnut, the latter representing the most consumed form of groundnut in the northern KwaZulu-Natal. Furthermore, consuming groundnut and groundnut derived products have been shown to protect against many diseases including cardiovascular diseases (Akhtar *et al.*, 2014).

Maize and groundnut are produced commercially as well as by subsistence farming, particularly in the northern KwaZulu-Natal region. The production of these crops is often threatened by numerous microorganisms including fungi. Maize is most commonly infected with *Fusarium verticillioides* (Sacc.) Nirenberg that produces the mycotoxin group, fumonisins (Chulze *et al.*, 1996; Chelule *et al.*, 2001). *Fusarium verticillioides* has been the predominant fungal pathogen isolated from maize collected in northern KwaZulu-Natal (Marasas and Van Rensburg, 1986). These communities were also found to be at higher risk of exposure to fumonisin B_1 (FB_1) than urban communities (Chelule *et al.*, 2001). Groundnut, however, is more readily contaminated with *Aspergillus flavus* (Link ex Fries) which produces the mycotoxins, aflatoxins. Fumonisins and aflatoxins have been associated with oesophageal cancer and liver cancer in humans, respectively (Ghiasian *et al.*, 2005; Kamika *et al.*, 2014). Aflatoxicosis outbreak was reported after the death of 100 South African dogs due to ingestion of aflatoxin-contaminated feed (Arnot *et al.*, 2012). The toxic effects of mycotoxins are as a result of factors such as intake levels, duration of exposure and mechanisms of action (Kabak *et al.*, 2006).

Traders may suffer economic losses when the purchased maize and groundnuts have high moisture content as this may lead to mould growth due to poor handling and improper storage practises (Kutsanedzie *et al.*, 2012). This raises global concerns about food security as the population continues to grow and nutritional demands have to be met (Gustafson *et al.*, 2014). Insufficient storage facilities force farmers to sell their maize at low prices immediately after harvesting to avoid losses due to insect pests and postharvest diseases. This directly impacts on the successes of the smallholder farmers (Tefera *et al.*, 2011). Gueye *et al.* (2013) stated that insects are the main threat for stored maize, feeding and causing losses on both cobs and shelled maize in storage.

Proper management of mycotoxigenic fungi focusses on both pre and post-harvest agricultural practices. The pre and post-harvest period includes planting, harvesting, handling, storage, marketing, transportation and processing of grain (Wagacha and Muthomi, 2008). Physical, chemical and biological management practices are often used during this period

(Munkvold, 2003; Chandra Nayaka *et al.*, 2008; van der Westhuizen *et al.,* 2011; Garcia et al., 2012; Gregori *et al.*, 2013). A study by Kimanya *et al.* (2009) reports good agricultural practices such as intercropping, crop rotation and also sorting of maize before storage. These practices reduce fungal infection and contamination at storage under favourable conditions. Fandohan *et al.* (2005) recommended that farmers use ventilated storage systems to reduce fungal contamination. Significant groundnut yields are favoured on light-textured soils; which range from coarse and fine sands to sandy clay loams. These are usually acidic and highly weathered soils (Murata *et al.*, 2002). Fungal growth on groundnuts is favoured rapidly on these soils especially under dry conditions; heavier soils normally hold water and hence the germinating groundnuts become less prone to fungal contamination due to the prevention of drought stress (Anthony *et al.*, 2012).

The aim of this work was to identify pre and post-harvest factors that contribute to mycotoxin contamination of maize and groundnuts produced in the KwaZulu-Natal province of South Africa. The objective was to interview subsistence farmers on their agricultural farming and storage practices of both maize and groundnut using questionnaires.

MATERIALS AND MEATHODS

Geographic areas surveyed

Sample collection took place in five districts of the northern KwaZulu-Natal (KZN) province of South Africa. These districts were Jozini, Manguzi, Mbazwana, Pongola and Vryheid (Fig. 1). Maize is an important staple food in these districts and groundnut supplements the diet as it provides the fat and protein content needed. Hence hence the risk of mycotoxicosis is high in these districts as the farmers and animals consume the mycotoxin contaminated food and feed, respectively. Agricultural extension officers assisted with the selection of localities and households within the districts which planted maize and groundnuts. Global Positioning System (GPS) was used to detect and mark different localities within the districts. Seven, 17, 13, 17 and 11 subsistence farmers were surveyed for maize and groundnuts in Jozini, Manguzi, Mbazwana, Pongola and Vryheid districts, respectively. All farmers in all five districts planted maize and all farmers in Jozini, Manguzi and Mbazwana planted groundnuts. No farmer in Vryheid planted groundnuts and there was only one identified groundnut farmer in Pongola.

Questionnaires

Questionnaires were produced in English and translated to Zulu. Information requested in the questionnaires (Appendix I) included maize and groundnut intercropping with other crops, storage as maize ears or loose grain, physiological maturity, groundnut harvest size (kilograms), types of storage facilities, cleaning of storage facilities, sorting of damaged and

mouldy grain, means of cleaning storage facilities, problems experienced at storage and mitigating strategies, sources of maize and groundnut seeds, food consumption, trading of home-grown maize and groundnut and awareness of mycotoxins. The questions focused on agricultural farming and storage practices and were asked randomly to ensure adequacy of the questionnaire. Agricultural farming practices and storage facilities were investigated as to which of these could lead to significant mycotoxin contamination of maize and groundnuts.

Interviews

Before the interviews, the farmers were informed about the significance of the survey. Each farmer was interviewed according to questions stated on the questionnaire. Gathering of information was done in collaboration with local extension officers of the KZN, Department of Agriculture and Rural Development. An opportunity was granted for questions after the interviews and appropriate management strategies were discussed with the farmers and local extension officers.

Statistical analyses

The data obtained from the questionnaires was analysed using the Chi-square test for independence. This test was appropriate because the samples were picked randomly from different farmers. One-way analysis of variance (ANOVA) was used to test only the numerical entries. The significance level for both tests were set at a 95% confidence level with $P < 0.05$ and $P > 0.05$ meaning that there are significant differences and non-significant differences between two variables, respectively. For the chi-square test; the tested null hypothesis (H_o) stated that the factor evaluated is independent (no significant relationship) of the different districts surveyed. Conversely, the alternative hypothesis (H_a) stated that the factor is dependent or has a significant association due to the different districts surveyed. The null hypothesis was accepted when $P > 0.05$ and rejected when $P < 0.05$. For the ANOVA, the degrees of freedom (DF) were determined from the formula: $DF = (r - 1) * (c - 1)$, where r is the number of levels for one categorical variable, and c is the number of levels for the other categorical variable (Lombaard *et al.*, 2011).

RESULTS

Crops planted together with both maize and groundnuts

The percentage maize farmers and number of maize districts surveyed were independent of one another ($P = 0.236$) (Fig. 2). However, the percentage groundnut farmers and number of groundnut districts were dependent on each other ($P = 0.004$) (Fig. 3). A variety of crops was intercropped with maize and included beans, groundnuts and pumpkins. Maize was widely

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intercropped with groundnut in the Manguzi (53%) and Mbazwana (92%) districts, respectively. Some farmers in all surveyed maize districts only planted maize (Fig. 4). Intercropping and the districts in which maize farmers were surveyed were, therefore, dependent on each other (P < 0.001) (Fig. 5). Only farmers in the Pongola district did not intercrop groundnuts with other crops while farmers in the other districts intercropped groundnut with crops such as spinach and cowpeas (Fig. 6). Therefore, intercropping was was significantly associated with the groundnut districts surveyed (P = 0.0071) (Fig. 7).

Crop rotation, residue removal and harvest size for groundnuts

The majority of groundnut farmers did not practise crop rotation (Fig. 8) with Jozini (71%), Manguzi (92%) and Mbazwana (100%), respectively, not rotating their groundnut with any another crop. Only farmers in Pongola (100%) practised crop rotation (Fig. 8) and, therefore, a significant relationship between crop rotation and groundnut districts was determined (P = 0.0122) (Fig. 9). Forty-three percent of the farmers in Jozini and Manguzi (54%) removed residues from the soil before planting their groundnuts. All of the farmers in Pongola removed crop residues while none of the farmers in the Mbazwana district removed crop residues before planting groundnuts (Fig. 10). Residue removal and groundnut districts were independent of each other (P = 1.769) (Fig. 11). In Jozini (75%), Manguzi (46%) and Mbazwana (33%) farmers harvested between 100 – 500 kg groundnuts per season and the rest of the farmers in these districts harvested between 10 - 50kg groundnuts per season. All the farmers in Pongola only harvested between 10 - 50kg groundnuts per season (Fig. 12). Harvest size and groundnut districts were thus not associated with each other (P = 0.4996) (Fig. 13).

Physiological maturity and state of storage of maize and groundnuts

Within a district, farmers at different localities planted maize and groundnuts at different times of the year because physiological maturity of maize cobs and groundnut pods was not reached at the same time (Fig. 14 and 15). Maize cobs and groundnut pods produced in Manguzi and Mbazwana reached physiological maturity between December and February (Fig. 14 and15) with the months of physiological maturity and maize districts being dependent variables (P = <0.001) (Fig. 16). Maize was stored as both cobs and shelled grain in all maize districts (Fig. 17) and the storage forms and districts were dependent on one another (P = <0.001) (Fig. 18).

Maize and groundnut storage facilities

A storage facility widely used in all the northern KZN districts surveyed was an "*inqolobane*" which is a Zulu name for a widely ventilated wooden storage facility (Fig. 19). The farmer's houses were prominently used as a storage facility for maize in all the districts. Some farmers

in Jozini, Pongola and Vryheid also stored their maize in metal tanks (Fig. 20). The storage facilities and maize districts surveyed were dependent variables (P = 0.0014) (Fig. 21). Only farmer's homes were used to store the groundnuts in all the groundnut districts (results not shown). Maize farmers either cleaned their storage facilities daily, weekly or only once before harvest. Most maize farmers cleaned their storage facilities daily rather than weekly as it was their residential homes. The highest percentage of farmers that only cleaned once before harvest was those who used an *Inqolobane* which doesn't require much cleaning (Fig. 22). The cleaning period and maize districts surveyed were dependent on one another (P = 0.004) (Fig. 23).

In Manguzi (67%) and Mbazwana (83%) groundnut farmers, respectively, cleaned their groundnut storage facilities (house) daily (Fig. 24) and the cleaning period and groundnut districts surveyed were, therefore, independent of each other (P = 0.15) (Fig. 25). Sweeping was used as a means of cleaning by all groundnut farmers in all districts (results not shown) as well as by most farmers in all maize districts. However, some farmers in Jozini, Pongola and Vryheid also fumigated their maize storage facilities. Additionally, farmers in Pongola and Vryheid removed mouldy and damaged maize at storage (Fig. 26). The cleaning method was not significantly associated with the maize districts surveyed (P =0.1776) (Fig. 27).

Sorting of maize and groundnuts before storage

All the maize farmers in all districts surveyed sorted their maize into apparently healthy, mouldy and damaged maize before storage (results not shown). With the exception of Mbazwana, all the groundnut farmers at Jozini, Manguzi and Mbazwana districts also sorted their groundnuts into apparently healthy, mouldy and damaged groundnuts before storage (Fig. 28) with sorting and groundnut districts surveyed, therefore, being independent variables (P = 0.610) (Fig. 29). All the farmers in Jozini fed the mouldy and damaged maize kernels to chickens only. Some farmers in the other four districts also used the mouldy and damaged maize as chicken feed but also discarded the grain. Additionally, farmers in Pongola (59%) and Vryheid (55%) fed the mouldy and damage grain to other domestic animals such as pigs, cattle and goats. Furthermore, farmers in Manguzi (18%), Mbazwana (8%) and Vryheid (9%), respectively, consumed the mouldy and damaged maize (Fig. 30). The end-user of mouldy and damaged maize kernels and the maize districts surveyed were, therefore, significantly associated (P = 0.0009) (Fig. 31). For groundnuts, all the farmers in Pongola and some farmers in other districts fed the mouldy and damaged groundnuts to chickens only. Less than 30% of farmers in Manguzi and Mbazwana discarded the mouldy and damaged groundnuts. In contrast to maize, more groundnut farmers in Manguzi (50%) consumed the mouldy and damaged groundnuts. Also, 60% of groundnut farmers in Jozini consumed the mouldy and

damaged groundnuts (Fig. 32). The end-user of mouldy and damaged groundnuts and groundnut districts surveyed were also dependent variables (P = 0.0396) (Fig. 33)

Removal of old grain and other crops kept at storage

All the maize farmers across all districts removed the old grain before storing the new harvest (results not shown). However, many farmers indicated that previous season's maize was utilised (consumed or sold) prior to the new harvest. Only 20% of the groundnut farmers in Manguzi did not remove the stored groundnuts while all farmers in Jozini, Mbazwana and Manguzi removed the stored groundnuts before storing the new harvest (Fig. 34). The removal of stored groundnuts and districts were, therefore, independent of each other (P =0.2051) (Fig. 35). Groundnuts and beans were stored together with maize while some maize farmers in all districts stored maize, exclusively (Fig. 36). Other crops at storage and maize districts were dependent variables (P = <0.001) (Fig. 37). Groundnut farmers in Manguzi (40%), Mbazwana (69%) and Pongola (100%), respectively, stored their groundnuts exclusively with maize while none of the Jozini groundnut farmers stored their groundnuts with maize (Fig. 38). The crops stored with groundnuts and groundnut districts surveyed were significantly associated with each other (P = 0.0169) (Fig. 39).

Storage-related problems and mitigating strategies

Problems experienced with maize: Mice and weevil damage were experienced in all districts. Mbazwana farmers were mostly affected (77%) by mice damage although least affected by weevil damage (15%) as compared to the other four districts. Some farmers in Jozini (14%), Manguzi (18%) and Pongola (29%) reported mould growth. Less than 15% of farmers in Jozini, Mbazwana and Vryheid did not experience any problems at storage (Fig. 40). The problems associated with grain storage and maize districts surveyed were independent of each other (P = 0.0925) (Fig. 41). Due to mice problems, some farmers in all districts used Rattex® to control the problem while other farmers, with the exception of farmers in Jozini and Pongola, used cats to control the mice. Farmers in the four districts, excluding Jozini, also relied on chemicals such as blue-death, Ptoxin and Alluminium phosphide tablets for storage-related problems. Farmers also kept their maize seeds in bottles to prevent mice entry and weevil damage. Less than 10% of farmers in Manguzi and Mbazwana and 20% of farmers in Jozini and Pongola did not have any means to deal with the problems (Fig. 42). Mitigating strategies and maize districts were independent of each other (P = 0.0792) (Fig. 43). In Jozini (50%), Manguzi (41%) and Pongola (29%), respectively, farmers experienced these problems at the beginning of storage while the other farmers from these three districts experienced these problems after a few months of storage. In addition to experiencing storage problems at the beginning and after few months, some farmers in Mbazwana and Vryheid also experienced these problems

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at other times such as the period from beginning until the storage process was completed (end of storage) (Fig. 44). Time interval of storage problem and maize districts were not significantly associated with each other (P = 0.4652) (Fig. 45).

Problems experienced with groundnuts: Groundnut farmers at Jozini, Manguzi and Mbazwana experienced mice damage, weevil damage and worms at storage. Also, farmers at Jozini (14%) and Mbazwana (39%) experienced both mice and weevil damage at the same time. Similarly the Manguzi district farmers (33%) experienced both mice and worms at the same time. The farmers in Pongola, however, experienced no problems at storage (Fig. 46). The problems experienced at storage were independent of the districts surveyed (P = 0.2392) (Fig. 47). Some farmers in Jozini and Manguzi used cats and some used Rattex® to control the mice. Less than 16% of farmers in Manguzi and Mbazwana did not have mitigating strategies in place and hence discarded the groundnuts. Only 20% of farmers in the Manguzi district used either blue-death, Ptoxin or Alluminium phosphide tablets for control. The farmers in Jozini (43%), Manguzi (13%), Mbazwana (46%) and Pongola (100%), respectively, neither discarded the groundnuts nor used any control measures for the storage-related problems the farmers experienced (Fig. 48). Mitigating strategies did not show a significant relationship with the districts surveyed (P = 0.5341) (Fig. 49).

Sources of maize and groundnut seeds

Farmers at Mbazwana only used home-grown (cultural or traditional) seed for planting, whereas farmers at Jozini (71%), Manguzi (94%), Pongola (6%) and Vryheid (18.0%) districts, respectively, used the home-grown maize as their seed source. The remaining farmers at Manguzi and Jozini used Grovida and Pannar seeds, respectively. Farmers at Vryheid and Pongola used a variety of maize seed sources from AFGRI, Pannar and Monsanto (Fig. 50). The source of the maize seeds and the districts surveyed were dependent on each other (P <0.001) (Fig. 51). Sources of groundnut seeds planted from all four surveyed districts were all traditional/cultural seeds (results not shown-similar entries). The main reason for this could be the lack of groundnut seed companies available in the northern KZN.

Consumption of maize and groundnuts

 In all districts, maize was usually consumed daily and rarely weekly (Fig. 52) with the maize consumption period and districts being dependent of each other (P = 0.0118) (Fig. 53). Farmers who consumed only home-grown maize were those at Pongola and Vryheid, whereas farmers from Jozini, Manguzi and Mbazwana consumed both maize purchased from supermarkets and home grown maize. In Mbazwana, 15% of the farmers only consumed purchased maize (Fig. 54). Maize consumption and maize districts were dependent of each

other (p<0.0001) (Fig. 55). For consumption, maize was either consumed in milled (as maize meal) or unmilled (as roasted cobs) states. All the farmers in Jozini, Manguzi and Pongola prepared and consumed the milled maize as porridge, also known traditionally as "*pap*". Less than 10% of the farmers in Mbazwana and Vryheid also consumed the maize in an unmilled state as roasted cobs (Fig. 56). Differently prepared maize and maize districts were independent of each other (P = 0.4329) (Fig. 57). Groundnuts were consumed in different states than boiled only, farmers often used a combination of both roasted and cooked groundnuts. However, some farmers in Manguzi (47%) and Mbazwana (67%) only roasted and did not boil their groundnuts. Also, other farmers in Jozini (27%), Manguzi (13%) and Mbazwana (33%) only boiled and did not roast their groundnuts (Fig. 58). Differently prepared groundnuts and groundnut districts were dependent on each other (P = 0.021) (Fig. 59).

Trading of home-grown maize and groundnuts

Farmers from all districts either only consumed or both consumed and sold their home-grown maize (Fig. 60). Consumption with trading of home-grown maize and maize districts were independent of each other (P = 0.1766) (Fig. 61). Half of the farmers at Mbazwana only consumed their home-grown groundnuts and the other half both sold and consumed their home-grown groundnuts. Over 60% of farmers at Jozini and Manguzi only consumed their home-grown groundnuts (Fig. 62). Consumption with trading of home-grown groundnuts and groundnut districts were also independent of each other (P = 0.635) (Fig. 63). All the farmers in Jozini and Manguzi only sold their home-grown maize to the local community, farmers in Mbazwana, Pongola and Vryheid also sold their home-grown maize to the nearest markets (Fig. 64). Maize trading areas and maize districts had a significant relationship with one another (P = 0.0046) (Fig. 65).

Maize harvest sizes and household numbers

The Pongola district had highest maize harvest size and Mbazwana had the lowest maize harvest size, there were significant differences between harvest size and maize districts (LSD = 1951.50) (Fig. 66). Also, the Pongola district had the highest percentage of household numbers and Mbazwana also had the lowest percentage of household numbers. There were significant differences between mean household numbers and maize districts (LSD = 4.02) (Fig. 67). Vryheid and Pongola had the highest numbers of children under 12 years within the households, Manguzi and Mbazwana had the lowest numbers of children under 12 years within the households. There were significant differences between mean levels for children under 12 and maize districts (LSD = 1.63) (Fig. 68).

Mycotoxin awareness

None of the farmers in Jozini, Manguzi and Mbazwana were aware of mycotoxins and what produced the mycotoxins. Only farmers at Pongola (6%) and Vryheid (9%), respectively, had an idea of what mycotoxins could be but did not know the cause of these mycotoxins and the implications the mycotoxins have on animal and human health (Fig. 69). Mycotoxin awareness and maize districts were independent of each other (P = 0.1766) (Fig. 70).

DISCUSSION

Improving maize and groundnut subsistence farming is crucial in mitigating mycotoxin contamination within the particular communities surveyed. Good quality maize and groundnut-based products are not only necessary for consumption but also for trade. Hence, it was important to conduct a survey on maize and groundnut subsistence farming in order to determine which factors largely contribute to increased risk of mycotoxin contamination and to determine potential intervention strategies to reduce mycotoxin contamination. This is the first survey which compares agricultural practises used by subsistence farmers within the same province (KZN), hence the specificity of this study. Previous work focused on comparing agricultural practises used by subsistence farmers across Eastern Cape, Limpopo, Mpumalanga and KZN provinces (Ncube, 2008).

More than 90% of farmers were not aware of mycotoxins or their impact on human and livestock health. The small percentage of farmers that were aware of mycotoxins were literate and had interactions with commercial farmers whom might have shared the information on mycotoxins. The lack of mycotoxin awareness in these districts suggests that humans and livestock may be consuming mycotoxin-contaminated maize and groundnuts daily which places them at a high health risk. Incidentally, some agricultural practices used by subsistence farmers (sorting of damaged and mouldy grain from storage, cleaning of storage) may have assisted in limiting mycotoxin exposure.

The South African government implemented new regulations since 2016 for deoxynivalenol and fumonisins B_1 and B_2 limits in maize. Maximum levels of 2 000 ug/kg for deoxynivalenol and 4 000 ug/kg for fumonisins B_1 and B_2 were set (Government Gazette, 2016). Subsistence farmers are not aware of these regulations and this potentially places pressure on subsistence farmers to produce safe and healthy food since a previous survey study by Ncube *et al*. (2011) has reported fumonisin levels in excess of 2 000 ug/kg in maize from subsistence farmers in northern KZN. The monitoring of this regulation in an informal environment is unclear and possibly impractical; however, this supply chain would need to be regulated for quality and safety (Stoev, 2013) considering the potential for trade between subsistence farmers. The majority of subsistence farmers sold their surplus groundnut and maize to local vendors posing great health risk to the consumers. Furthermore, subsistence

farmers have to contend with supermarkets present at local communities and small towns, which sell their good quality products at reduced costs especially maize meal and bread (D'Haese and Van Huylenbroeck, 2005).

During this study, all the farmers sorted their maize into apparently healthy, mouldy and damaged maize before putting in new harvested maize. However, some farmers consumed the sorted mouldy and damaged maize as local brew and also used this sorted maize as animal feed. Hence, increasing the risk of mycotoxicosis. Maize was intercropped with groundnuts by the majority of the subsistence farmers. The study by (Ncube, 2008) supports these findings as it was reported that subsistence farmers in KZN sorted their maize before consumption and over 66% of farmers in KZN practised maize-groundnut rotations. This means that subsistence farmers tend to adopt same agricultural practises over the years hence it is vital for the farmers to be aware of the implications thereof. However, previously none of the farmers were aware of mycotoxin contamination, knowledge of mycotoxin awareness farmers during this survey was due to knowledge imparted during the previous survey (Ncube, 2008).

The ability to intercrop and to rotate maize and groundnuts with other crops may be due to the variation in soil types of the districts surveyed as this directly determines the crops that can be successfully cultivated. For example, the Manguzi and Mbazwana districts had sandy soil types which mostly favours the cultivation of groundnuts over maize. According to Murata *et al.* (2002) light-textured soils; which range from coarse and fine sands to sandy clay loams favour significant groundnut yields. Groundnut harvest size was due to environmental factors such as temperature, rainfall and soil type. Favourable environmental factors are needed for good production of grain crops.

The majority of farmers do not employ crop rotation, possibly due to the lack of knowledge of the advantages of rotating crops. Farmers prefer to grow the same crop throughout, especially when it can be sustainably produced under the prevailing conditions. Rotating crops potentially increases crop yield and the root system health is maintained by the reduced inoculum potential of soilborne pathogens (Nel and Lamprecht, 2011). During 2013, fewer maize and groundnut samples were collected in the Jozini, Manguzi and Mbazwana districts compared to the 2014 season due to a lack of rainfall that severely affected maize and groundnut germination, hence, resulting in significantly reduced yield at physiological maturity.

Farmer preference dictated the use of specific storage facilities in the different districts. The choice of a storage facility may be due to problems experienced at storage relating to the different districts for example the use of tanks and drums to prevent mice damage specifically. The storage facility also ultimately determined the period at which it was cleaned. For example, if the house is used to store the grain it will be cleaned daily. Different cleaning methods were

chosen by farmers due to the effectiveness of the cleaning method based on the storage facility used.

Storage facilities used by farmers in the surveyed districts in northern KwaZulu-Natal are the same as the ones used by other farmers in Sub-Sahara African countries (Fandohan *et al.*, 2005) and some of these storage facilities do not promote proper drying of maize and thus enhance interaction with insects, therefore promoting fungal infection and mycotoxin production (Fandohan *et al.*, 2005). The application of pesticides to control lepidopterous insects is not an effective method and it is also too costly for subsistence use (Khan *et al.*, 2000). Most farmers use wooden granaries for storage, these structures are widely used possibly due to ease of construction and for drying the maize cobs. However, this structure allows invasion by insect pests and rodents as it is not covered on top. Therefore, maize cannot be stored for prolonged periods under such conditions. Farmers could be advised to use a metal silo as described by Tefera *et al.* (2011). This storage facility is airtight and, therefore, prevents any pathogen or pest from invading the stored maize. Subsistence farmers prefer the traditional storage systems as they are cheaper to construct and maintain, although they cause high post-harvest losses (Thamaga-Chitja *et al.*, 2004). The specific storage practices employed were determined by the quantity of maize produced, for instance in high maize production in areas such as Vryheid and Pongola, maize was predominantly stored in tanks.

Storage problems such as weevil and mice damage experienced in northern KwaZulu-Natal were also experienced by subsistence farmers in the Limpopo province, where mice damage was considerably the highest post-harvest problem (Randela, 2003). Similarly, to the northern KwaZulu-Natal farmers, the Limpopo farmers also used ash, ptoxin tablets and synthetic insecticide "blue death powder" to protect their grain crops against insect damage (Randela, 2003). More research is required into developing cost-effective storage facilities suitable for subsistence farming.

The manner in which farmers sorted groundnuts was determined by how much groundnuts were harvested and/or whether this would be kept for household consumption or sold for additional income. Mouldy and damaged maize was used to feed domestic livestock while most farmers across all districts fed the mouldy and damage maize to chickens. Mycotoxin contaminated feed generally affects the growth of chickens (Huwig *et al.*, 2001). Farmers removed maize and groundnuts from storage depending on storage capacity and/or problems experienced during storage. Problems experienced during both maize and groundnut storage may arise due to different factors such as storage temperature and easy access to insect pests, depending on the storage facility used. Therefore, depending on the factors causing the problems, farmers apply different mitigating strategies to solve the problems. A study that was done by Mogensen *et al.* (2011) in the former Transkei region of

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South Africa showed that sorting of damaged and mouldy grain does reduce mycotoxin contamination. This study reported that fumonisin concentration decreased by 71% after removing highly infected maize kernels. Also, washing and sorting of maize kernels was found to reduce fumonisin contamination by 84 % (van der Westhuizen *et al.*, 2010). In the Rombo district of Tanzania, sorting of maize samples from mouldy and damaged ones also led to reduction in fumonisin contamination (Kimanya *et al.*, 2009). Therefore, it is a good practise that the majority of the farmers in the northern KZN sort their maize and groundnut to decrease contamination at storage. The limitation in asking the farmers about the amount of maize consumed is that the answers were dependent on memory and for the groundnuts it was subjective.

The most important factor which contribute to mycotoxin contamination in subsistence farming is the lack of mycotoxin awareness. Therefore, farmers use storage facilities which allow fungal infection of grain crops and subsequent mycotoxin contamination. Farmers and animals then consume mycotoxin-contaminated grain, posing serious health implications. Both pre-harvest and post-harvest technologies are essential for good management of mycotoxins and mycotoxigenic fungi (Jard *et al.*, 2011). When good quality food is produced from South African subsistence farmers, their produce can be incorporated into urban retail markets as suggested by Louw *et al.* (2007). Hence planting of drought tolerant and insect-resistant cultivars of maize, application of atoxigenic *A. flavus* strain (pre-harvest methods) for groundnuts and use of hermetic bags (Chigoverah and Mvumi (2016)) for storage of maize are intervention strategies suggested from this study to help subsistence farmers in South Africa. Sibiya *et al.* (2013) stated that development of maize cultivars with high disease resistance and high abiotic stress tolerance will benefit the smallholder farming sector more. It is also vital that the knowledge of good agricultural practices to minimize mycotoxin contamination be transferred to subsistence farmers together with the agricultural extension officers. This will be part of the mycotoxin awareness campaigns to inform the farmers of the threats and effects of mycotoxins on humans and animals. Additional surveillance is required to continuously monitor and regulate mycotoxin contamination and potential exposure in subsistence farming.

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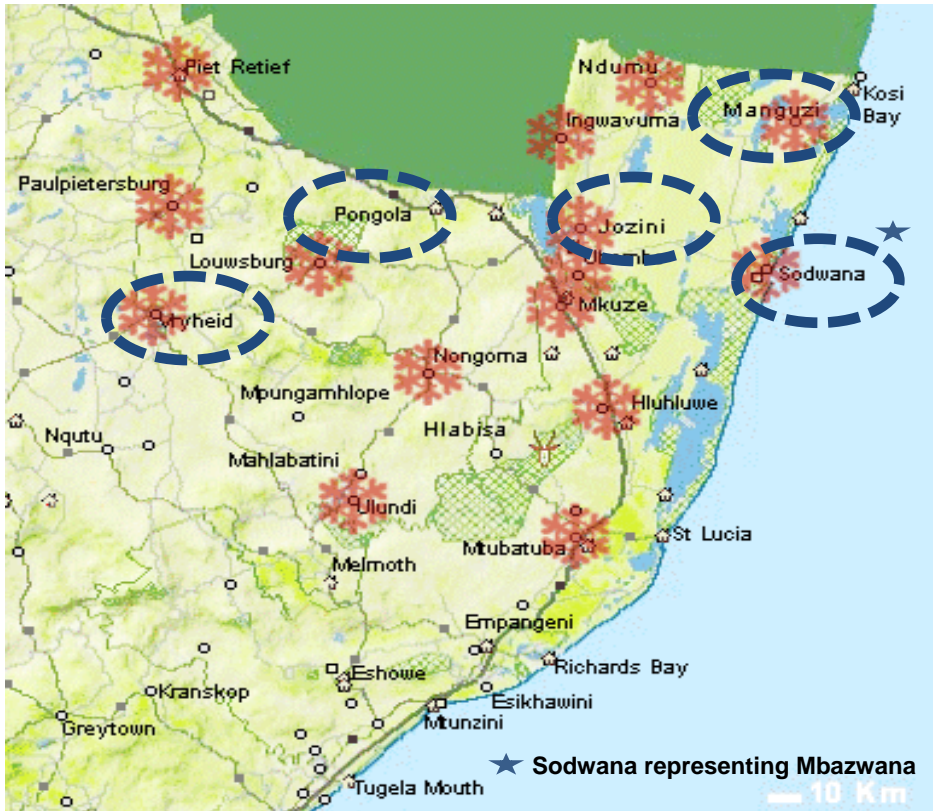
Figure 1. The five districts of the northern KwaZulu-Natal surveyed.
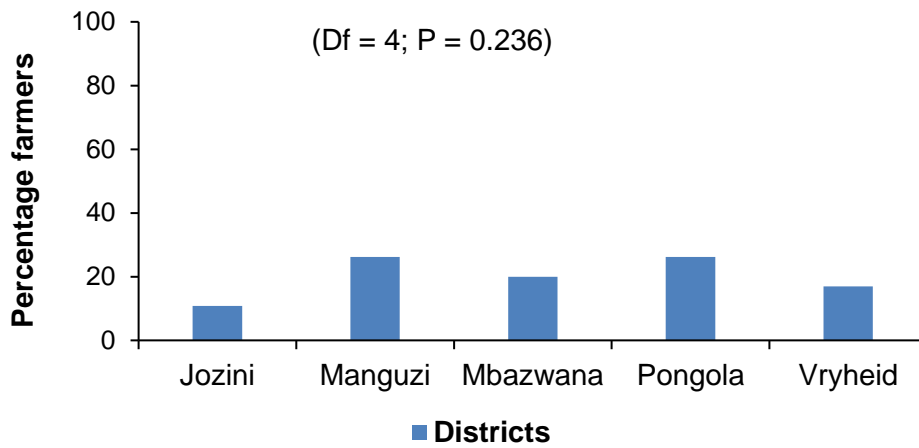
Figure 2. The percentage of maize farmers surveyed across the five districts.

(Df = 3; P = 0.004)
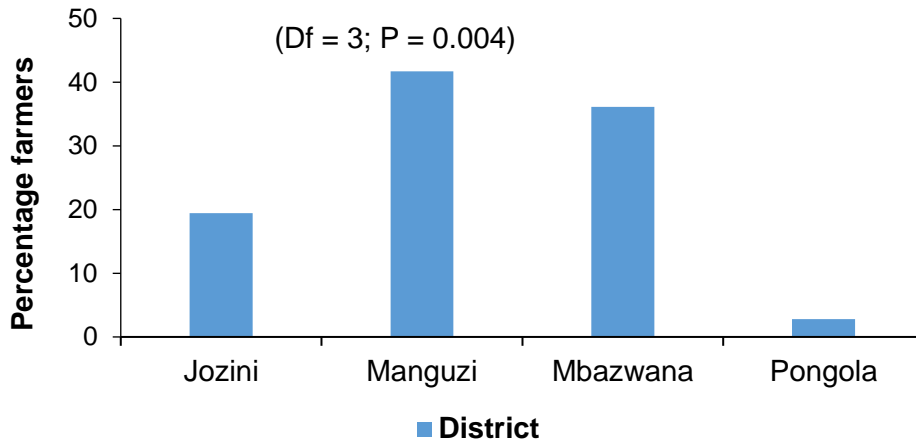
Figure 3. The percentage of groundnut farmers surveyed across the four districts.
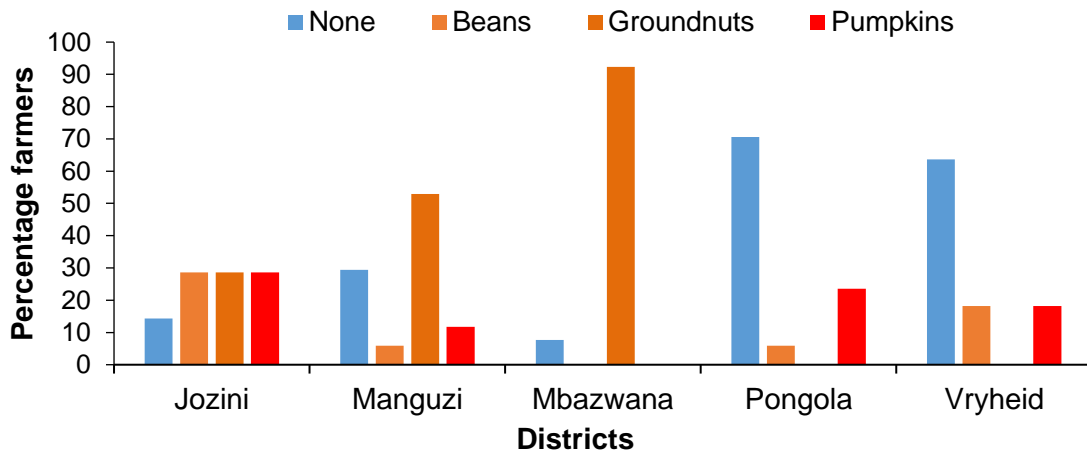
Figure 4. Crops intercropped with maize by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.

(Df=12; P < 0.001)
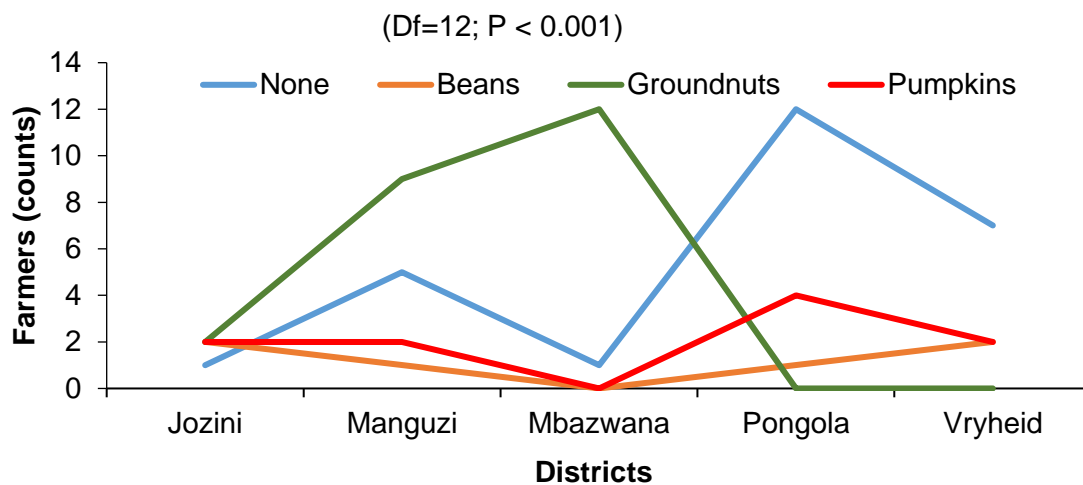
Figure 5. The relationship between intercropping and maize districts.
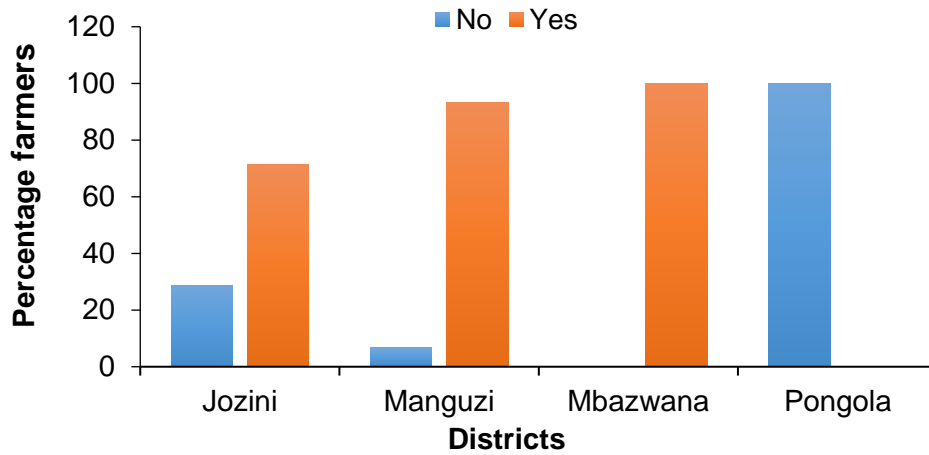
Figure 6. Intercropping practised by groundnut farmers in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
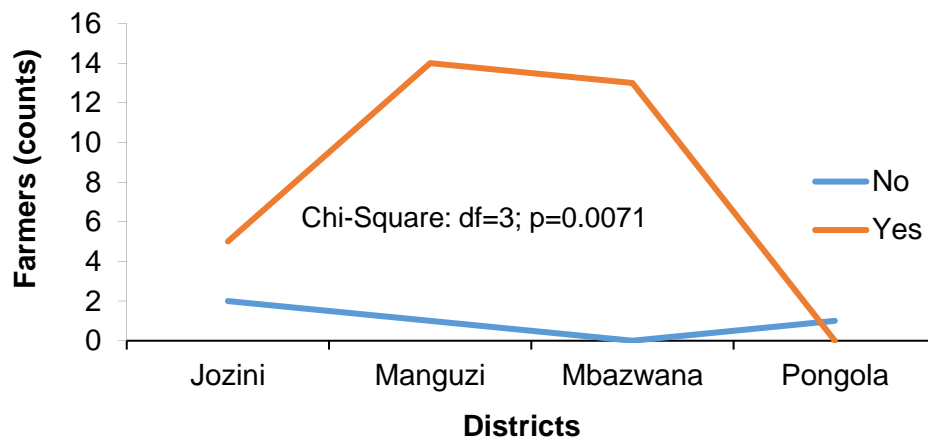
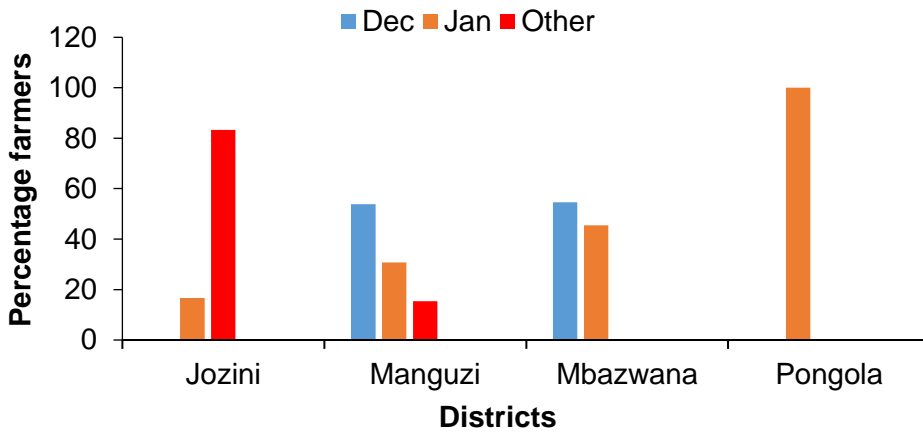**Figure 7.** The relationship between intercropping and groundnut districts.
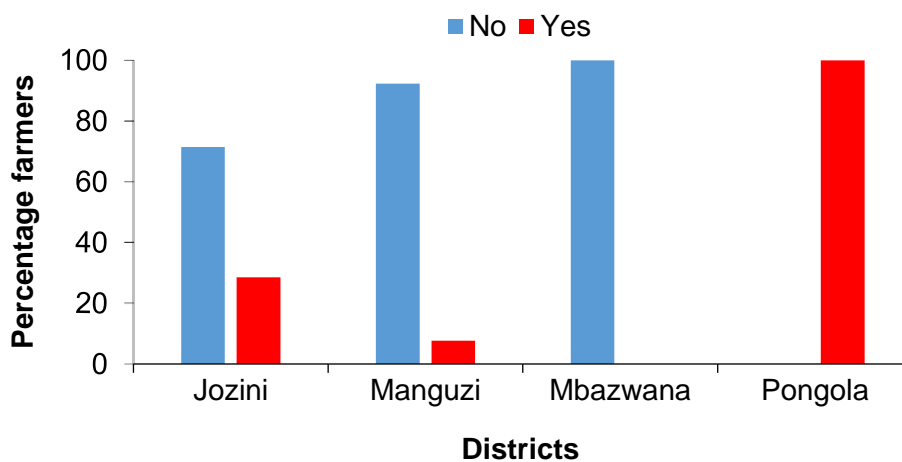
Figure 8. Rotation of groundnuts with other crops practised by subsistence farmers in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.

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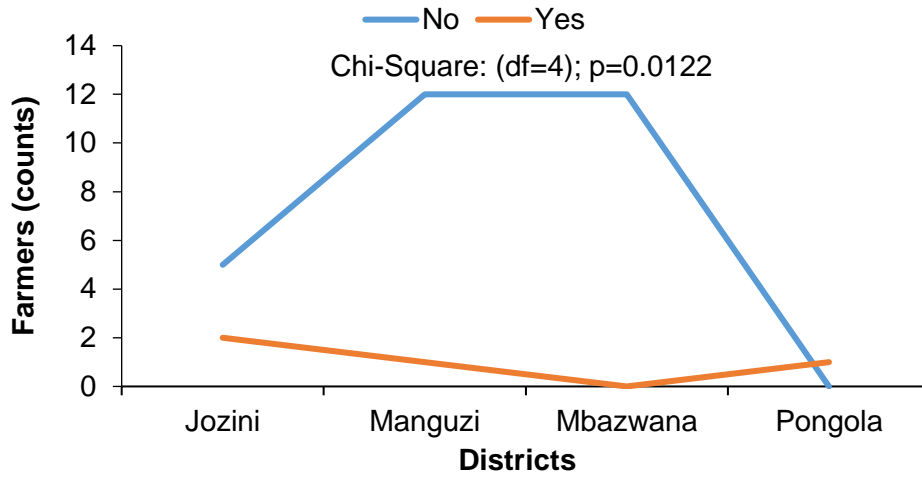
Figure 9. The relationship between crop rotation and groundnut districts.
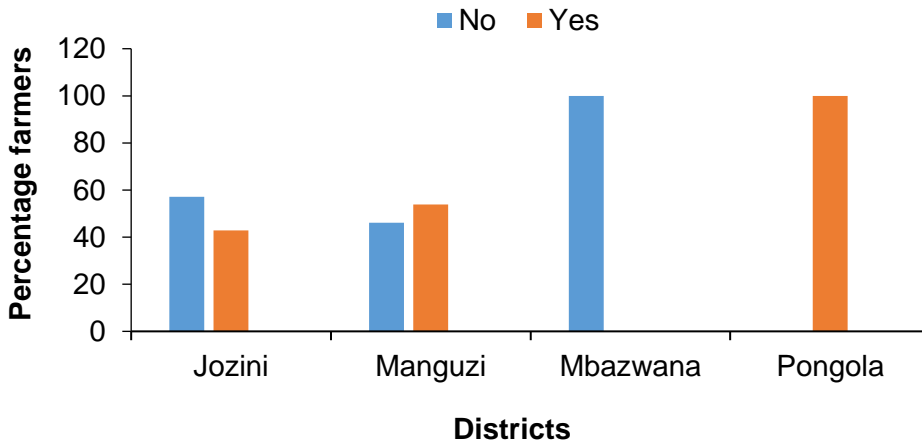
Figure 10. Residue removal before planting groundnuts by subsistence farmers in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
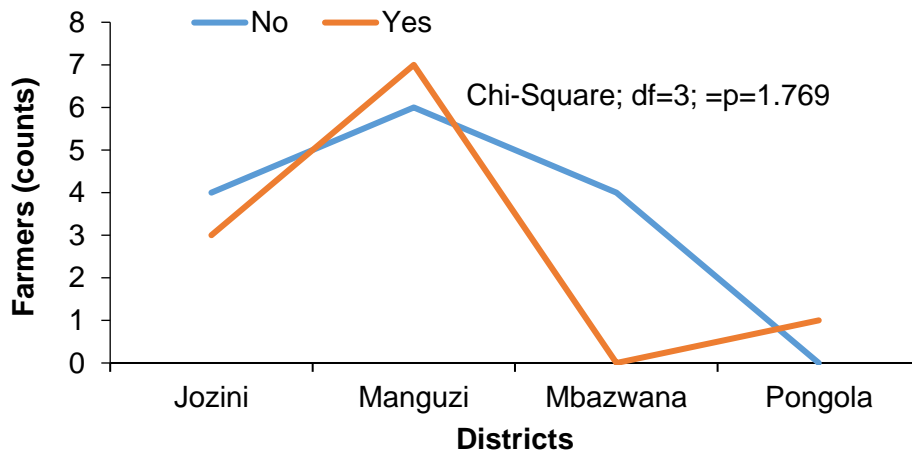
Figure 11. The relationship between residue removal and groundnut districts.
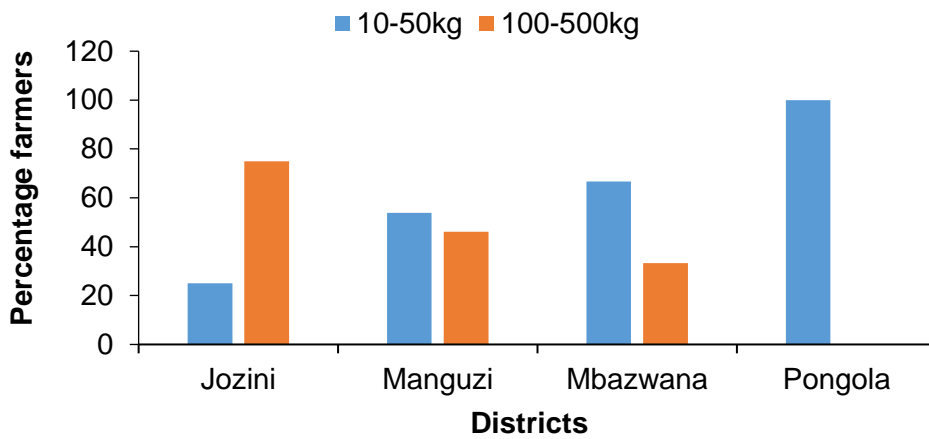
Figure 12. Harvest size for the groundnuts planted by subsistence farmers in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
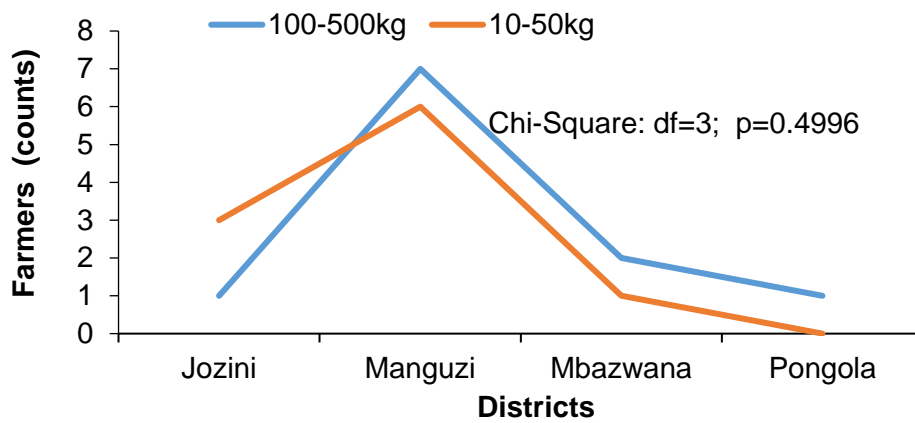
Figure 13. The relationship between harvest size and groundnut districts.
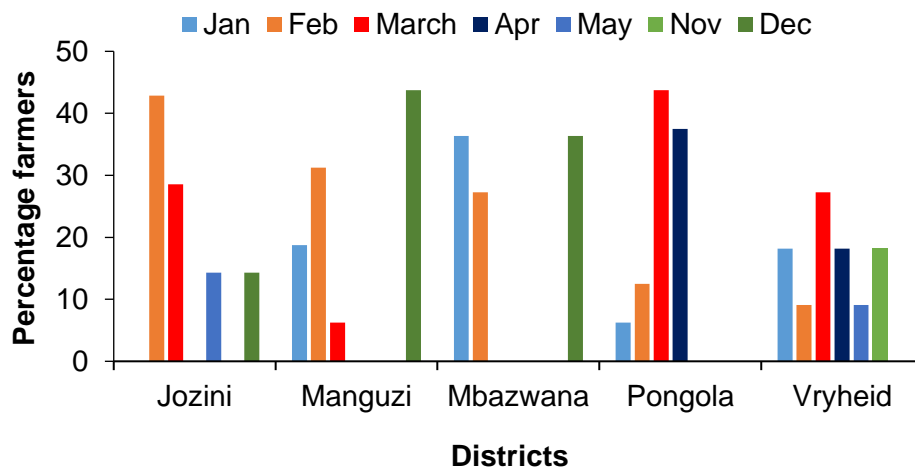
Figure 14. Months at which maize crops of subsistence farmers started drying out in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
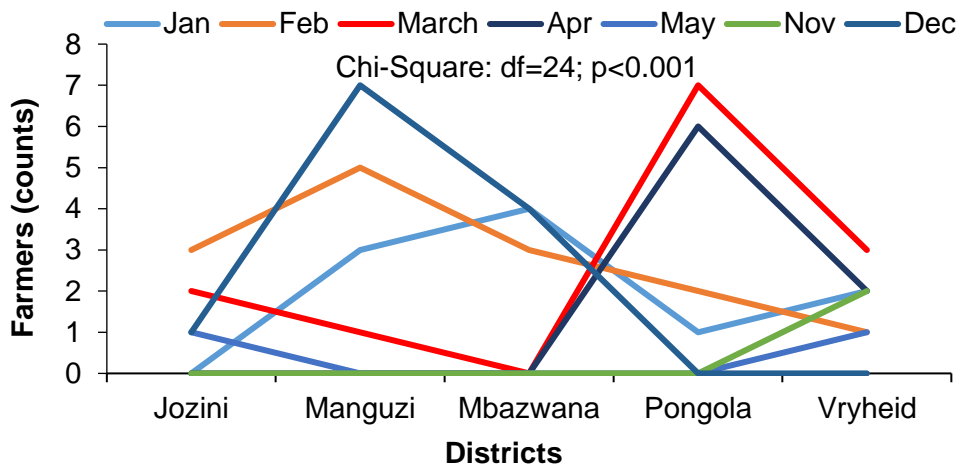
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Figure 15. Months at which groundnut crops of subsistence farmers started drying out in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.

Figure 16. The relationship between month of physiological maturity and maize districts.
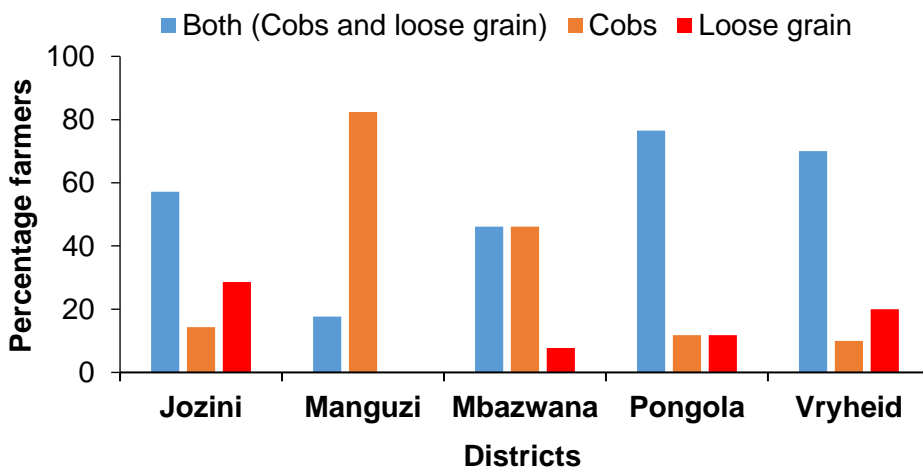

Figure 17. Different forms of maize stored by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
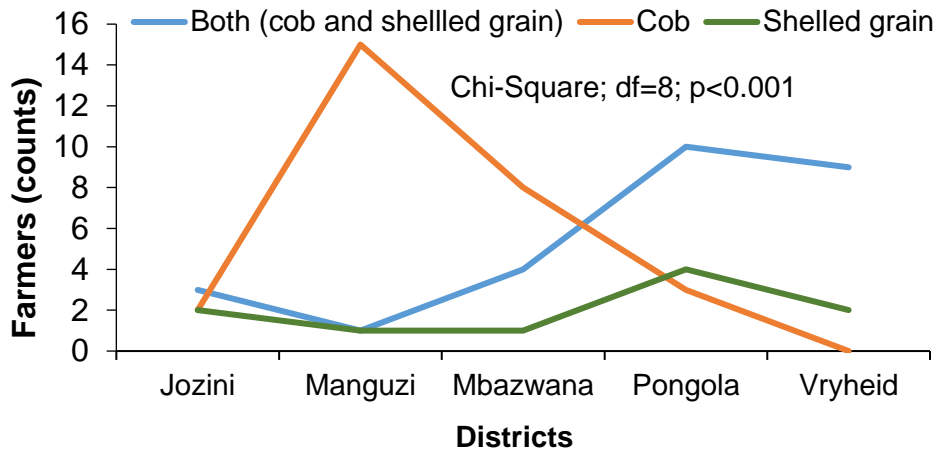
Figure 18. The relationship between forms of stored maize and maize districts.

Figure 19. Wooden storage structure widely used by subsistence farmers in northern KwaZulu-Natal. These farmers refer to this structure as an "*Inqolobane*".
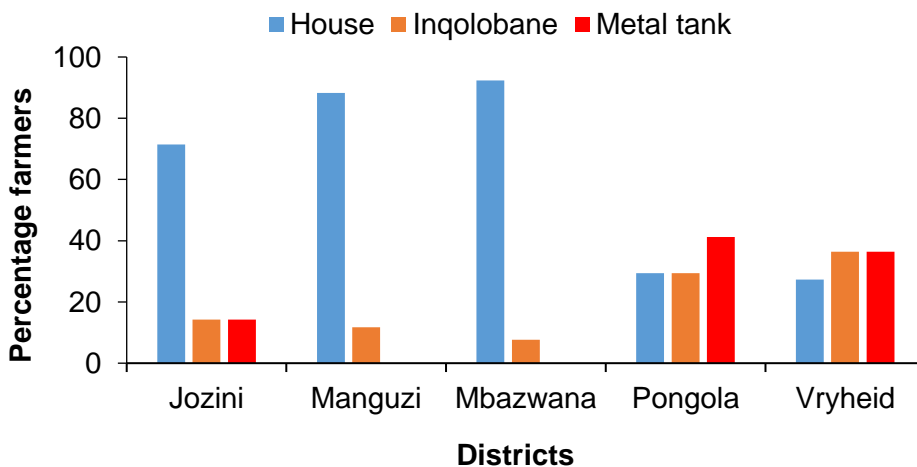

Figure 20. Storage facilities utilised by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
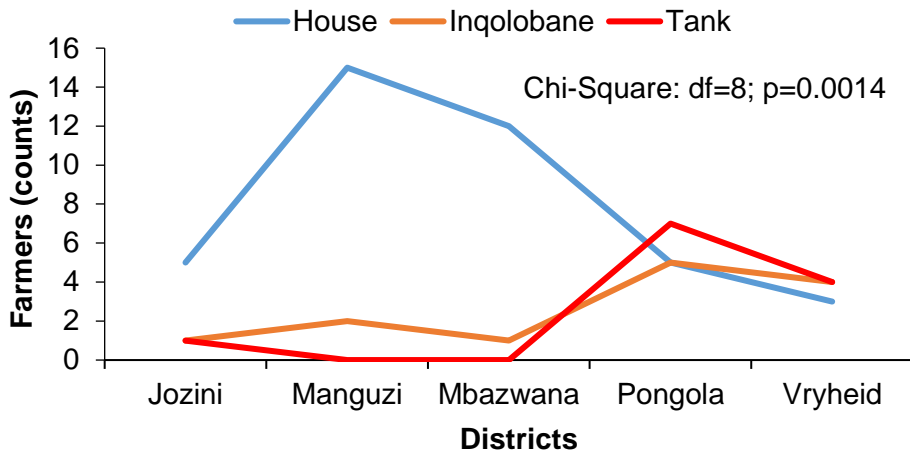
Figure 21. The relationship between storage facility and maize districts.
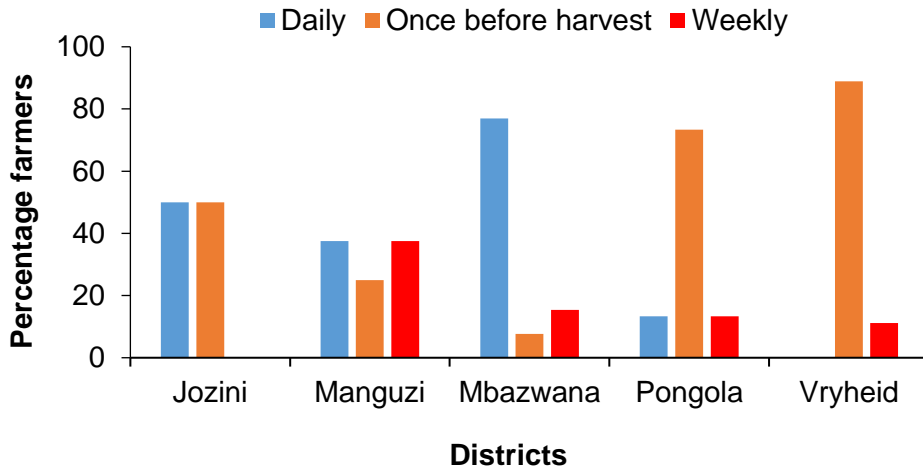

Figure 22. Period at which maize storage facilities were cleaned by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
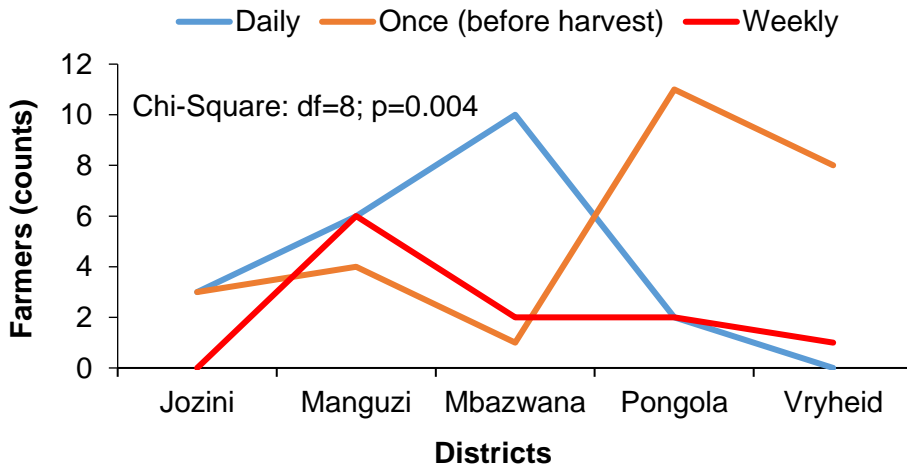

Figure 23. The relationship between cleaning period and maize districts.
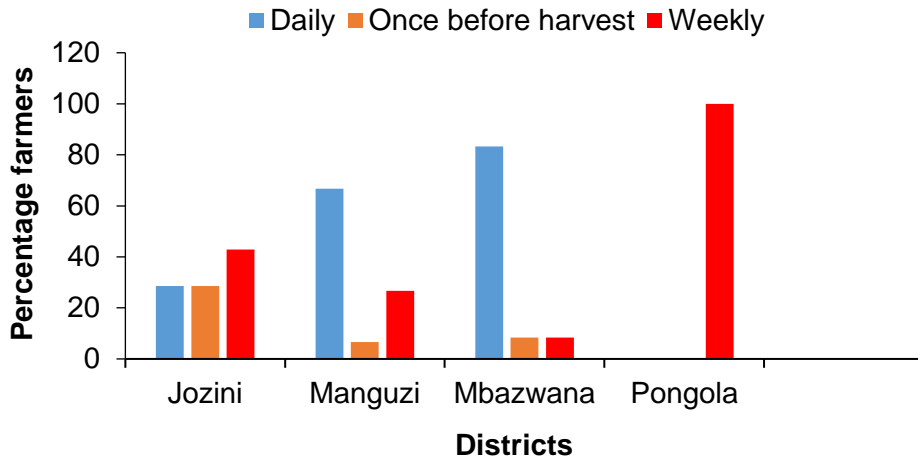
Figure 24. Period at which groundnut storage facilities were cleaned by subsistence farmers in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
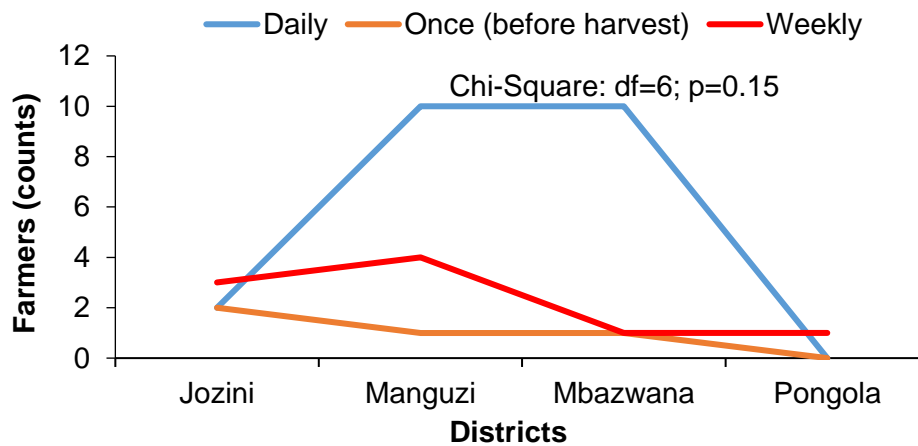

Figure 25. The relationship between cleaning period and groundnut districts.
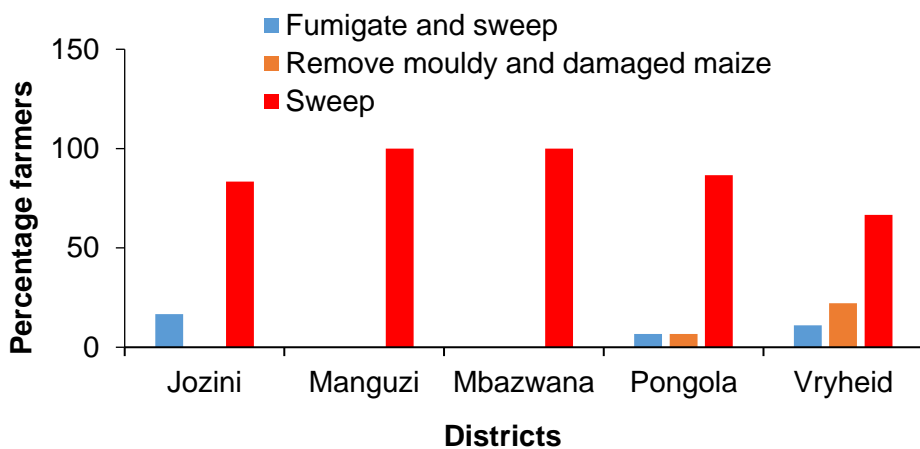
Figure 26. Measures employed by subsistence farmers to clean their maize storage facilities in five districts of the northern KwaZulu-Natal, surveyed during the 2013/2014 season.

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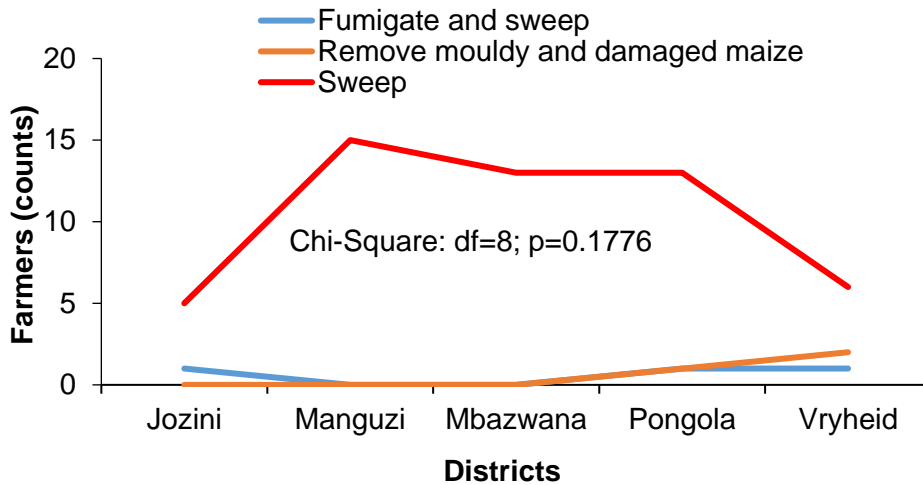
Figure 27. The relationship between cleaning measures and maize districts.

Figure 28. Sorting of damaged and mouldy groundnuts by subsistence farmers in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
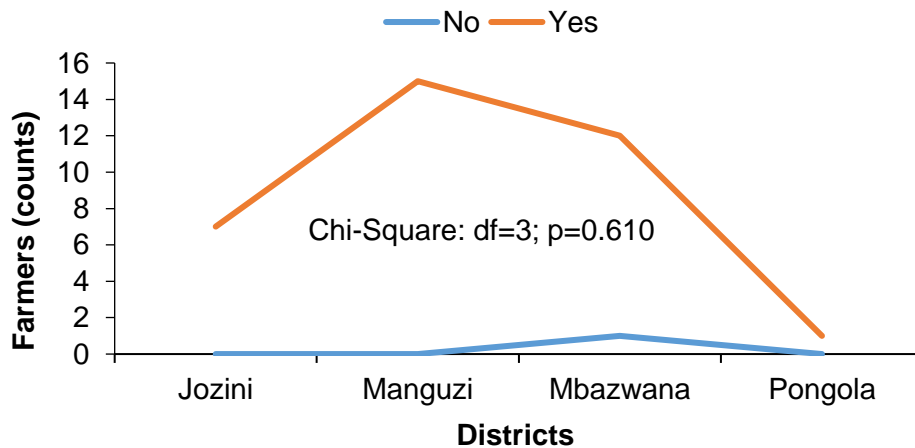
Figure 29. The relationship between sorting and groundnut districts.

Figure 30. End result of damaged and mouldy maize produced by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
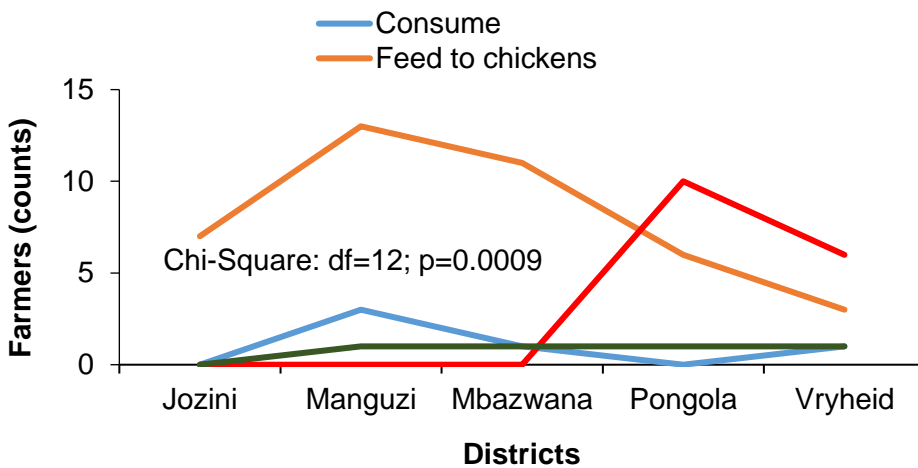
Figure 31. The relationship between end result and maize districts.
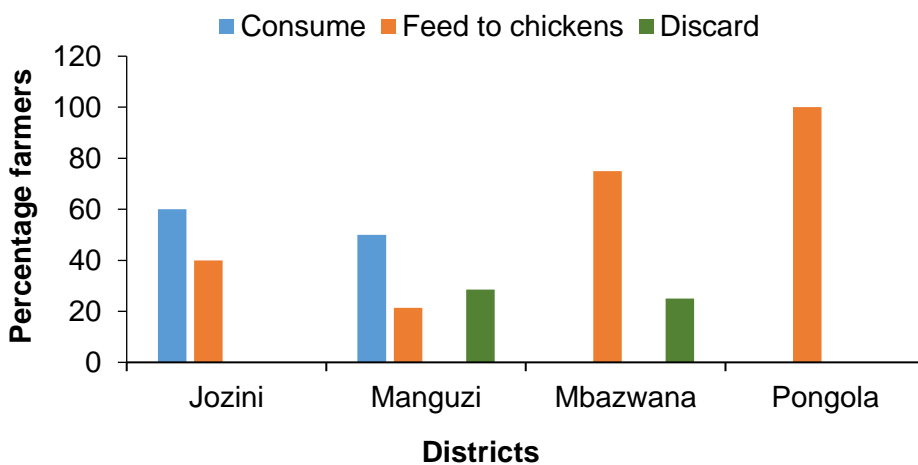
Figure 32. End result of damaged and mouldy groundnuts produced by subsistence farmers in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
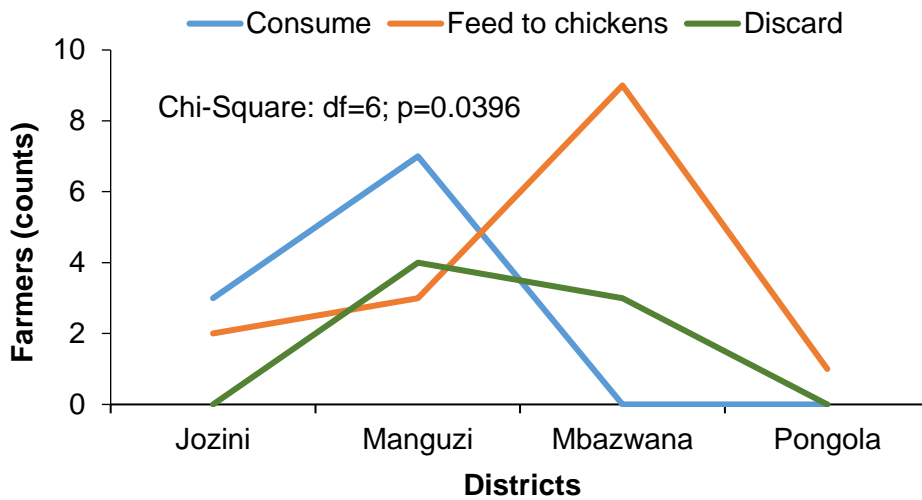
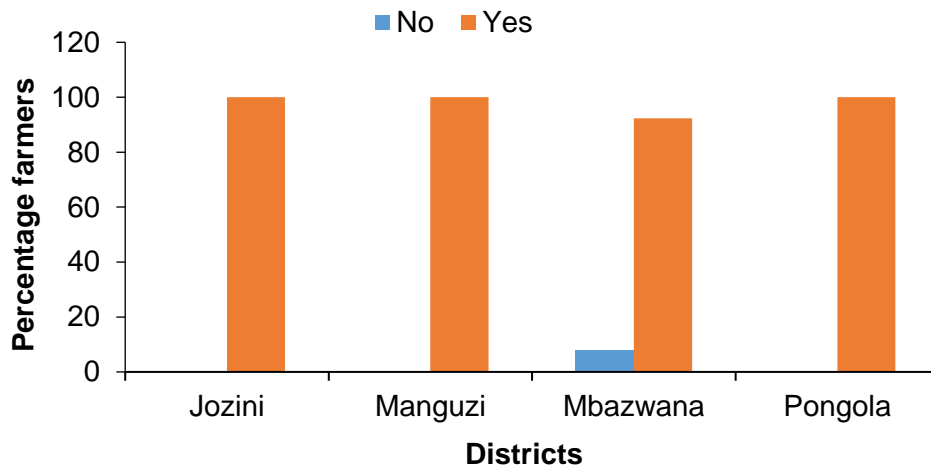**Figure 33.** The relationship between end result and groundnut districts.

Figure 34. Removal by subsistence farmers of old groundnuts from storage before putting new harvested groundnuts in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.

Chi-Square: df=3; p=0.2051
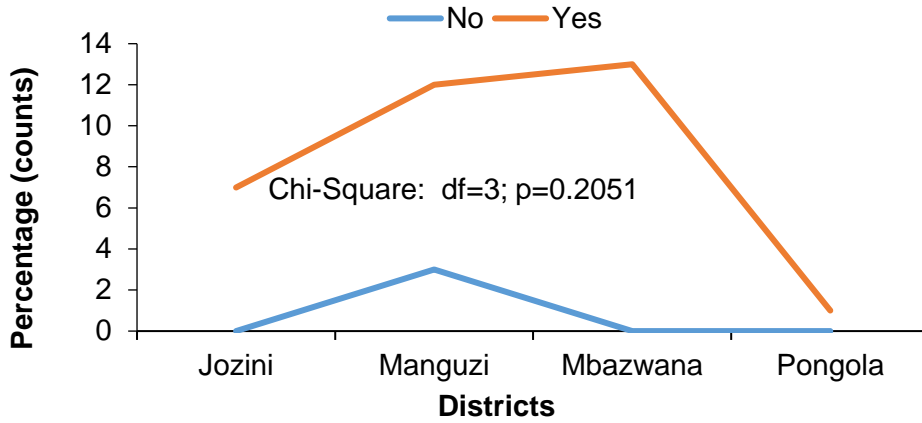
Figure 35. The relationship between removal of old groundnuts and groundnut districts.
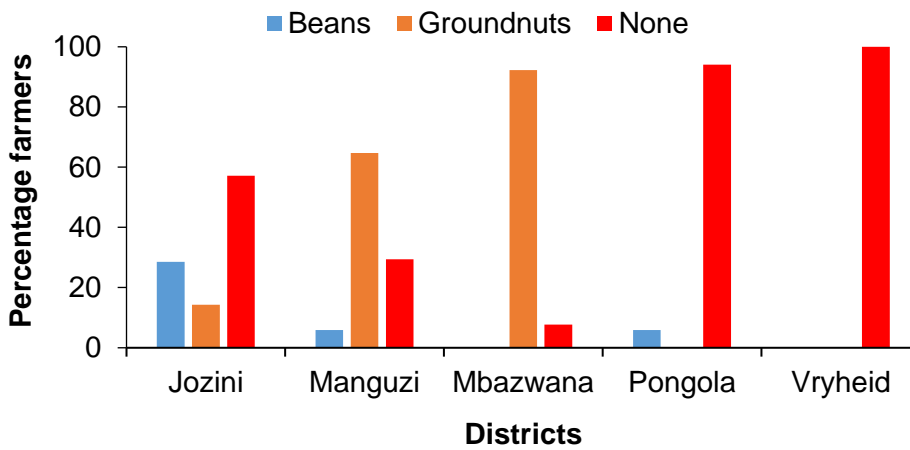
Figure 36. Crops which were kept at storage together with maize in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.

Chi-Square: df=8; p<0.001
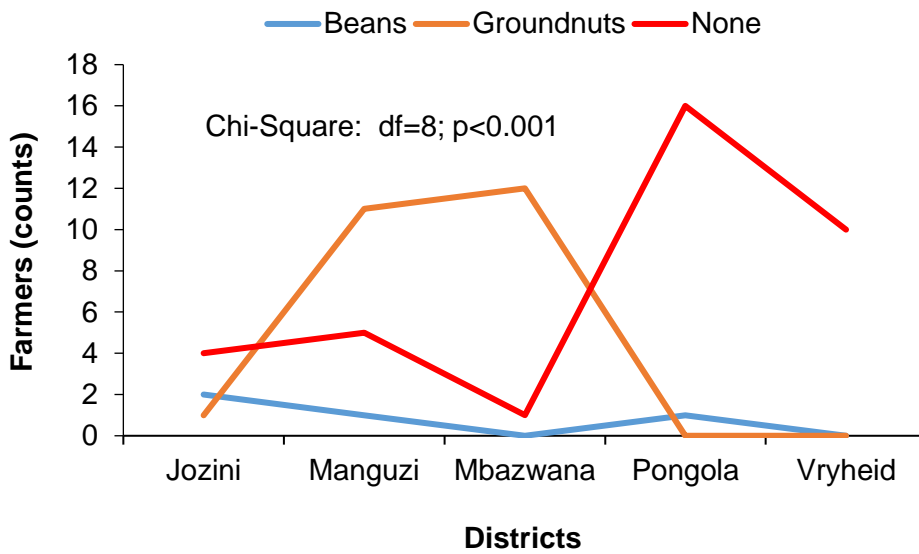
Figure 37. The relationship between crops at storage and maize districts.
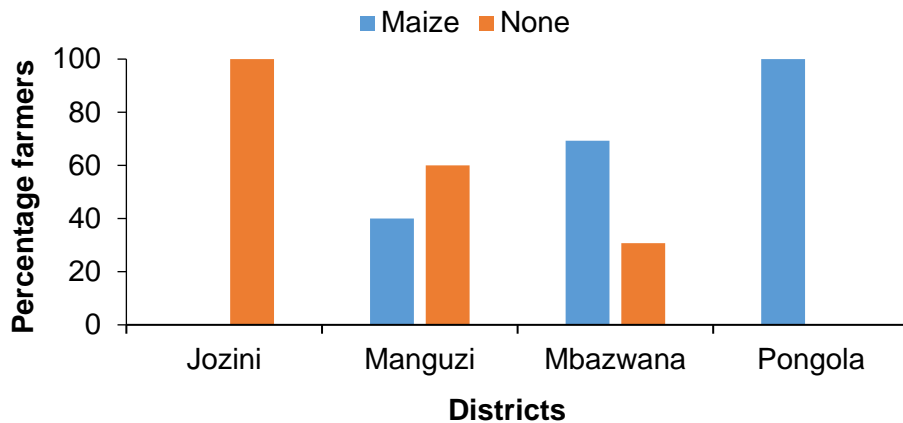
Figure 38. Crops which were kept at storage by groundnut farmers in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
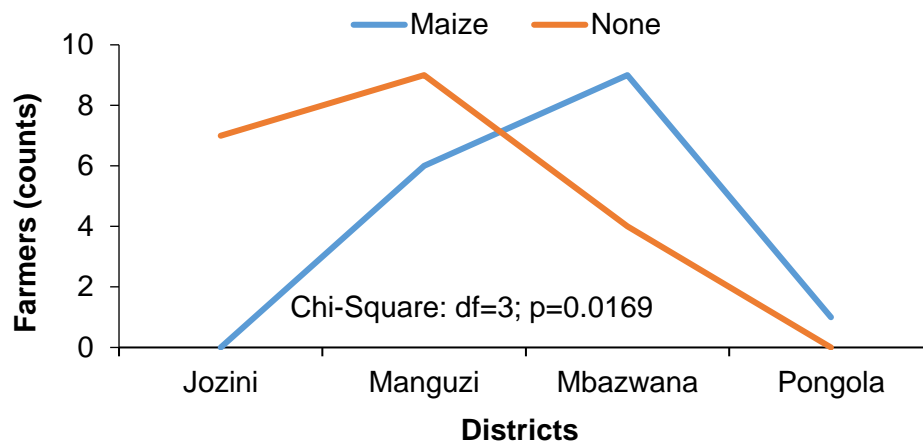
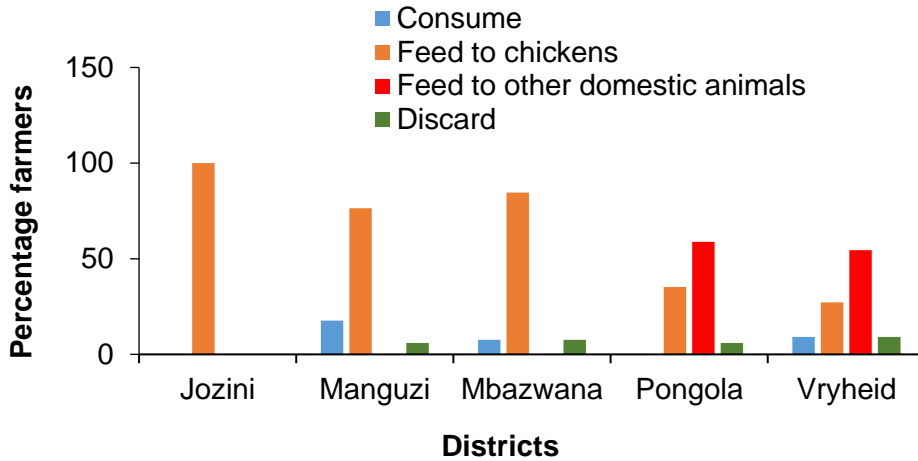**Figure 39.** The relationship between crops at storage and groundnut districts.
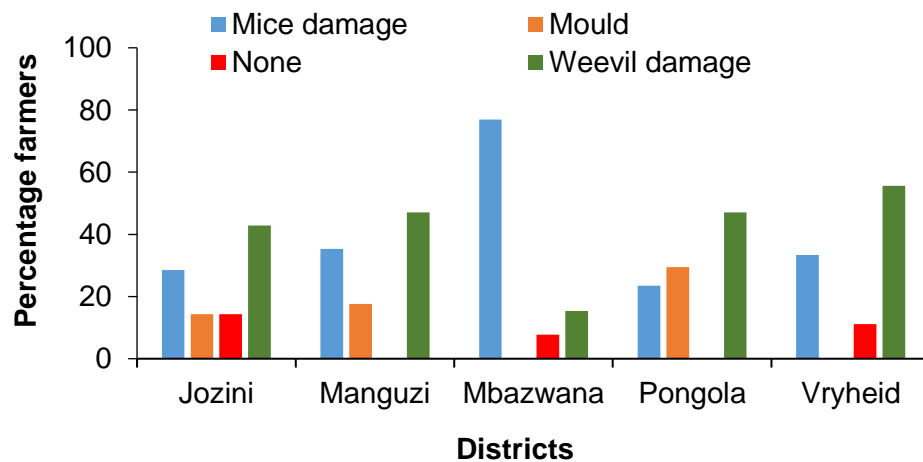
Figure 40. Problems which were experienced by subsistence farmers during maize storage in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
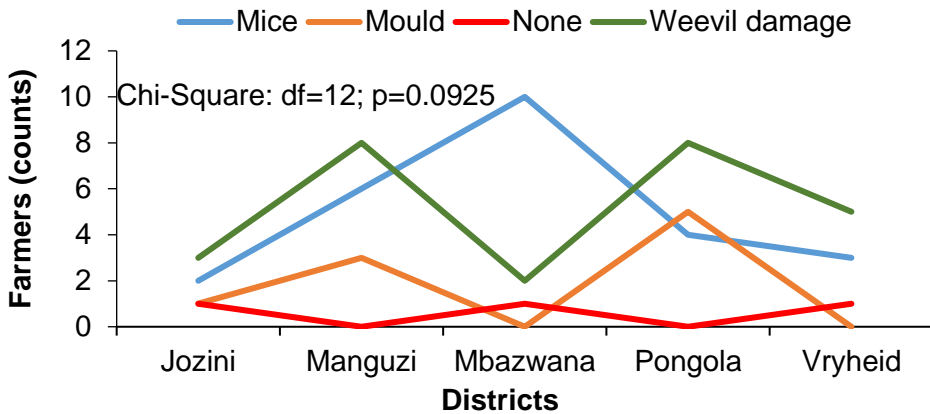
Figure 41. The relationship between problems experienced at storage and maize districts.

Figure 42. Different control measures employed by maize subsistence farmers for storage-related problems in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
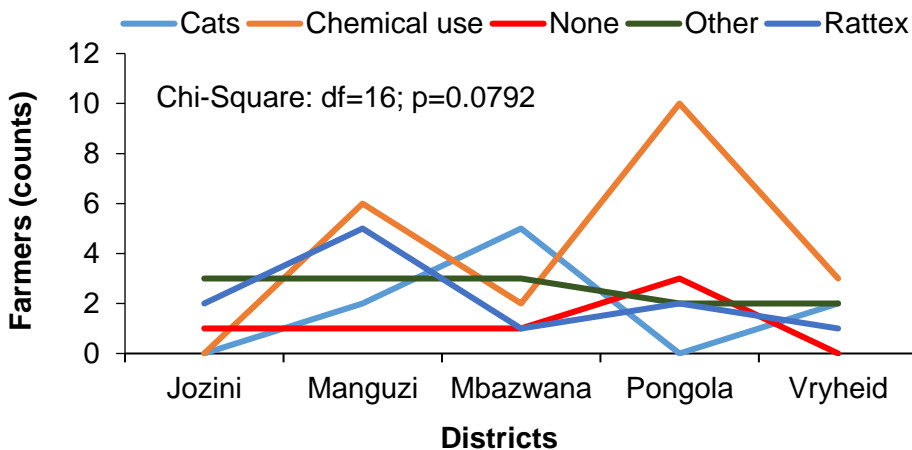
Figure 43. The relationship between mitigating strategies and maize districts

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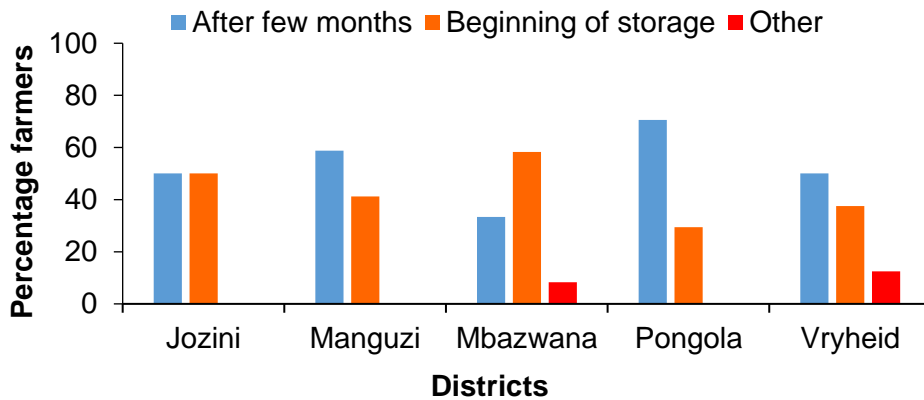
Figure 44. Period at which problems associated with maize storage were experienced at storage by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
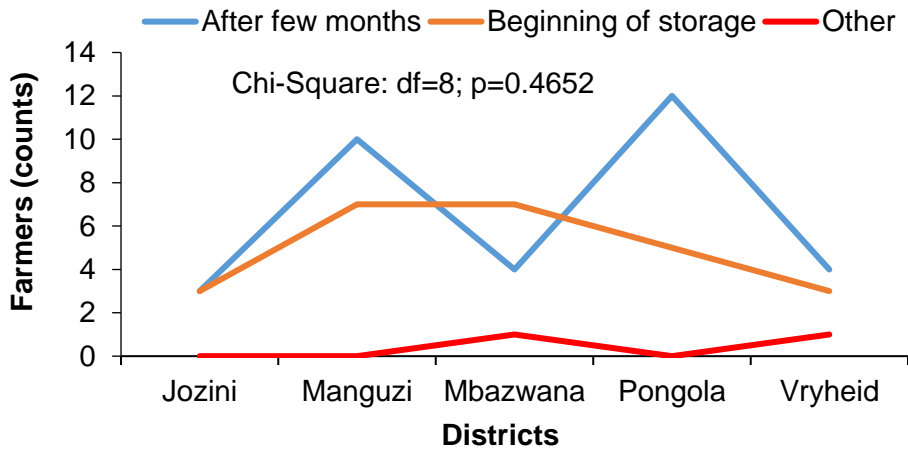
**Figure 45.** The relationship between observed period and maize districts.
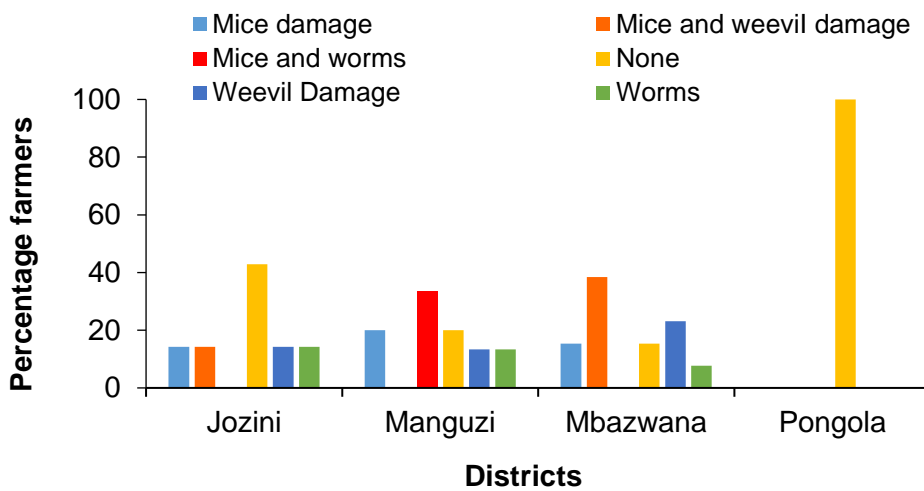
Figure 46. Problems which were experienced by subsistence farmers during groundnut storage in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
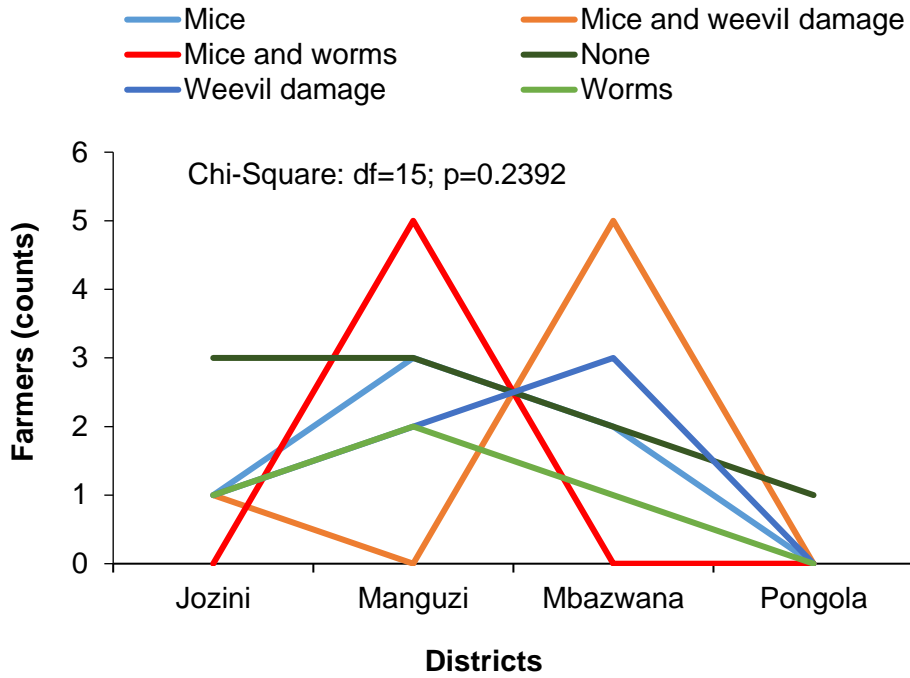
Figure 47. The relationship between problems experienced at storage and groundnut districts.
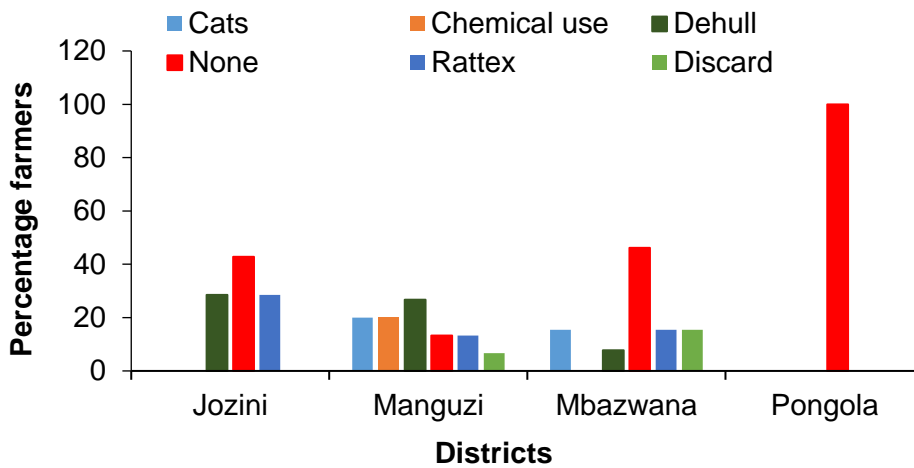
Figure 48. Control measures employed by groundnut subsistence farmers for storage-related problems in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
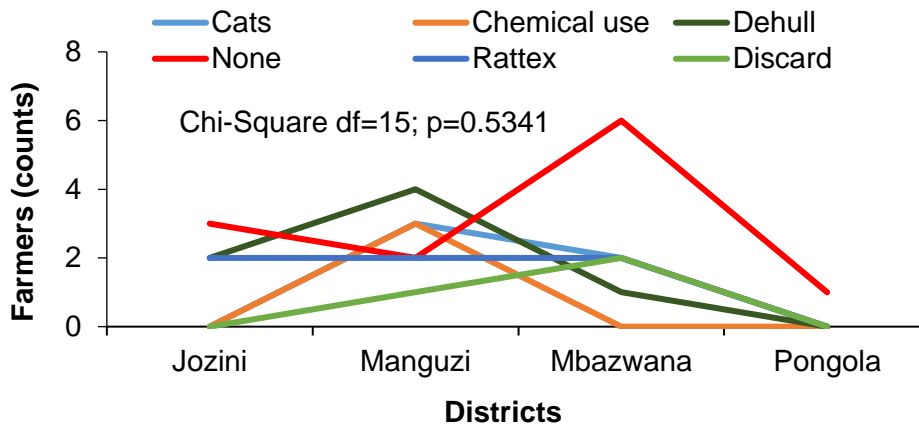
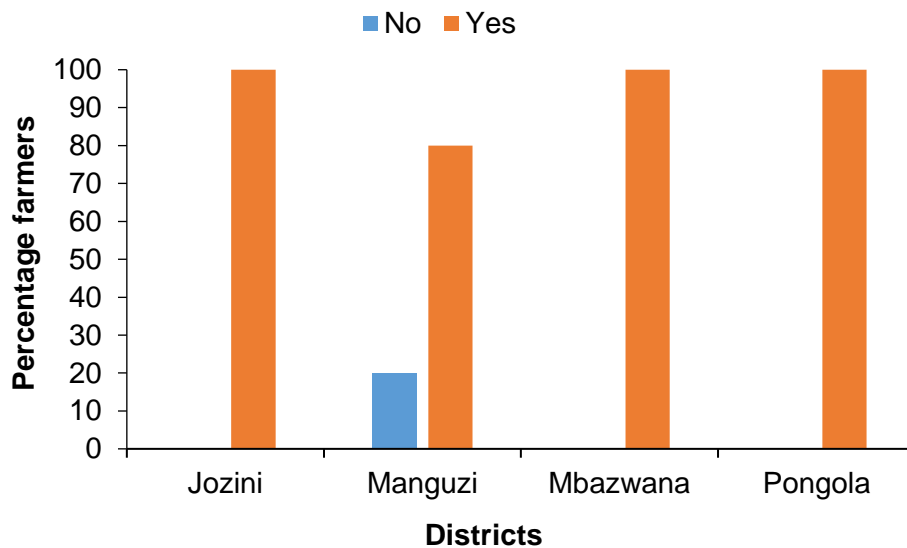**Figure 49.** The relationship between mitigating strategies and groundnut districts
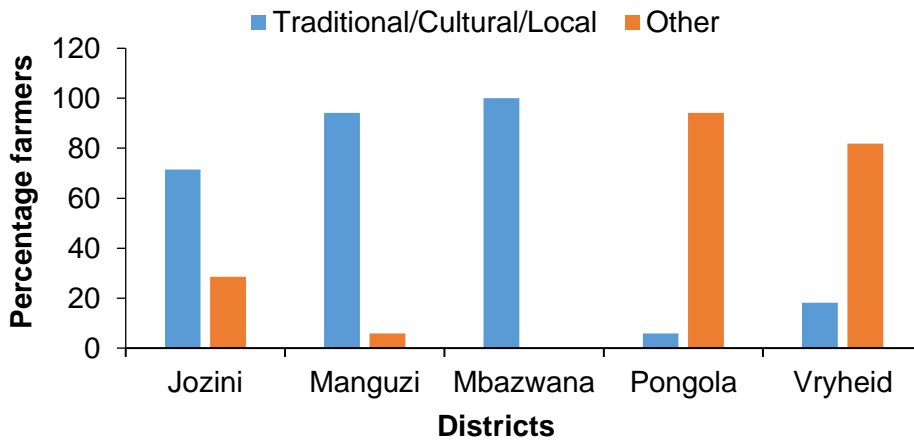
Figure 50. Sources of maize seeds used for planting by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
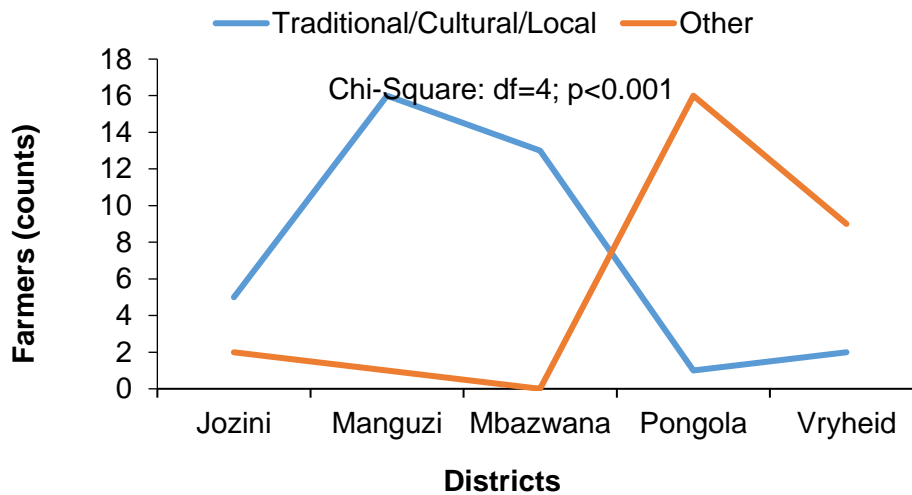
Figure 51. The relationship between sources of maize seeds and maize districts.
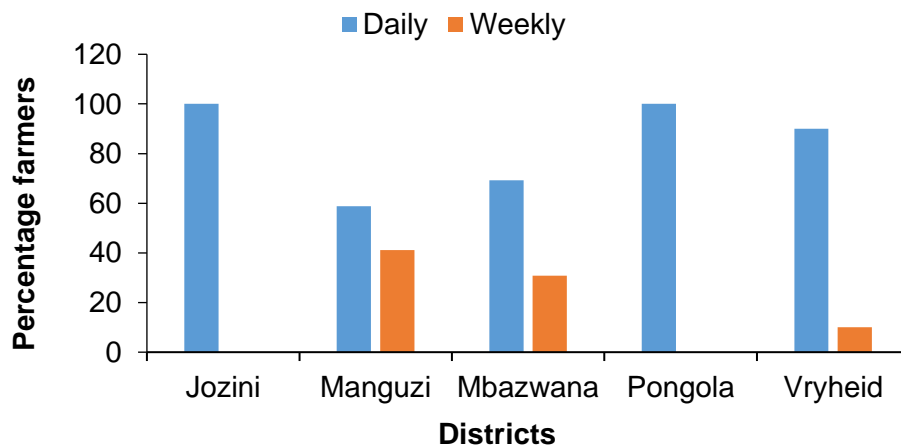
Figure 52. Period at which maize was consumed by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
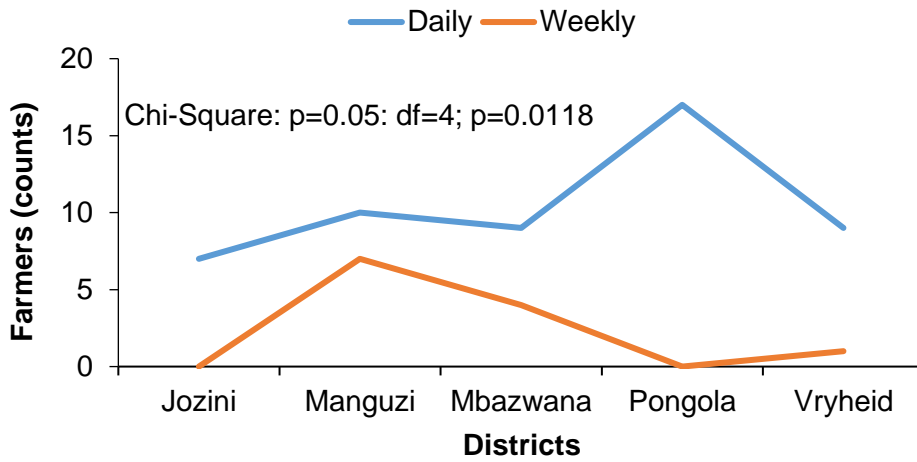
Figure 53. The relationship between maize consumption period and maize districts.
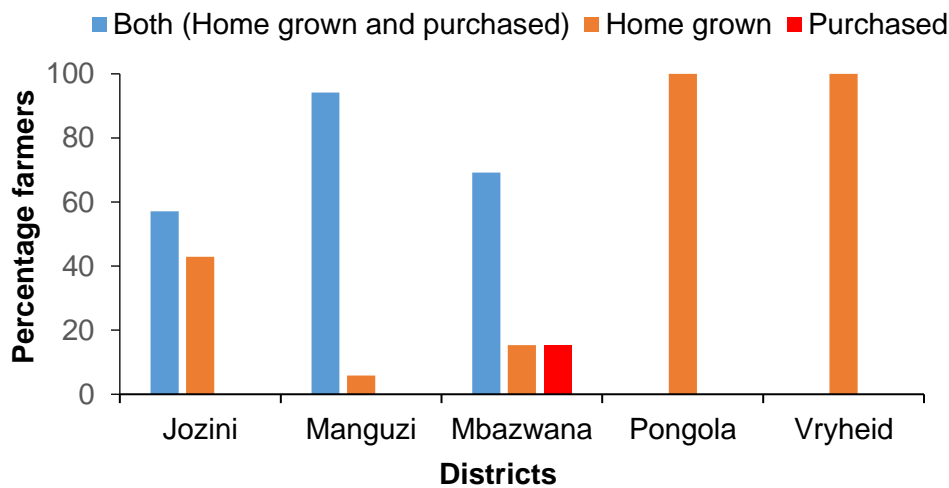
Figure 54. Home-grown and purchased maize consumed by subsistence farmers and their families in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.

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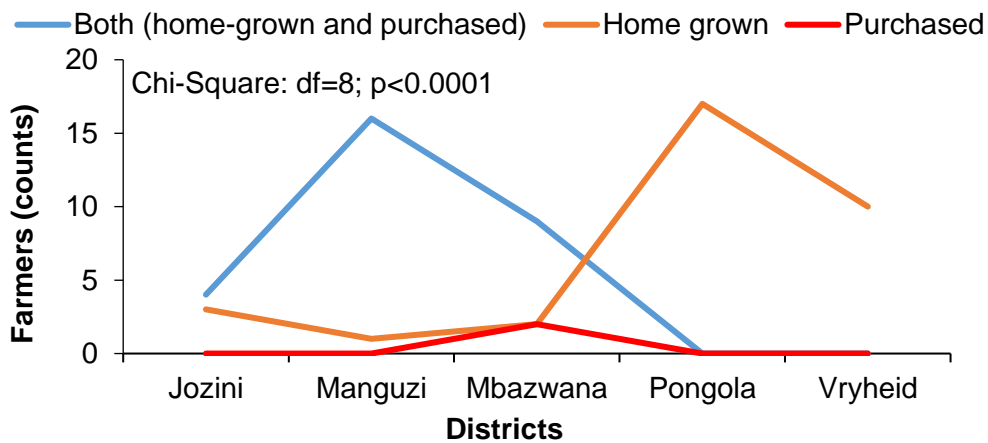
Figure 55. The relationship between maize consumption and maize districts.
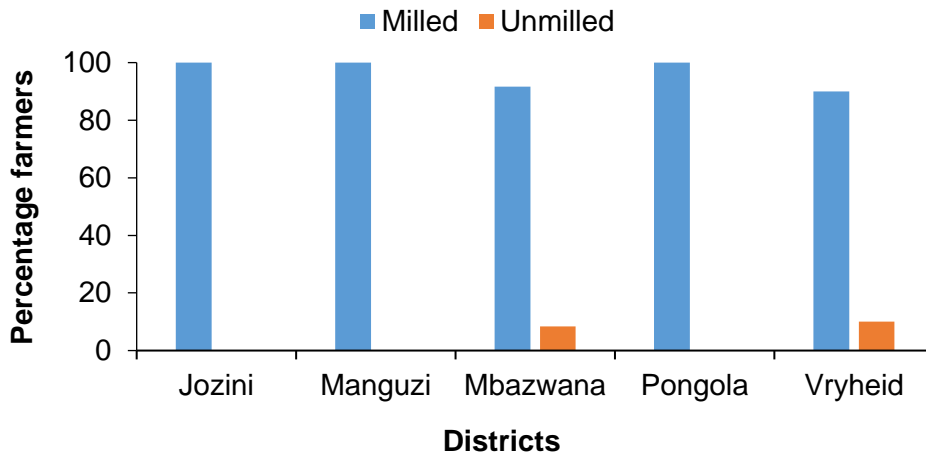
Figure 56. Differently prepared maize consumed by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
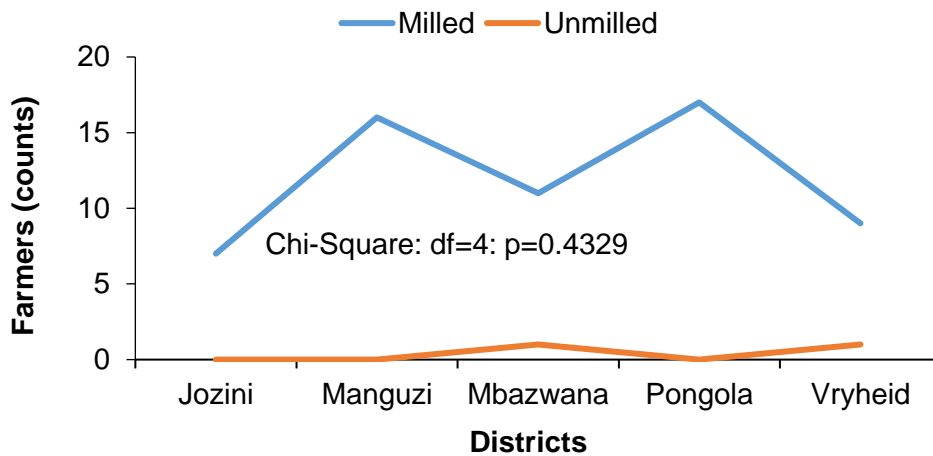
**Figure 57.** The relationship between differently prepared maize and maize districts.

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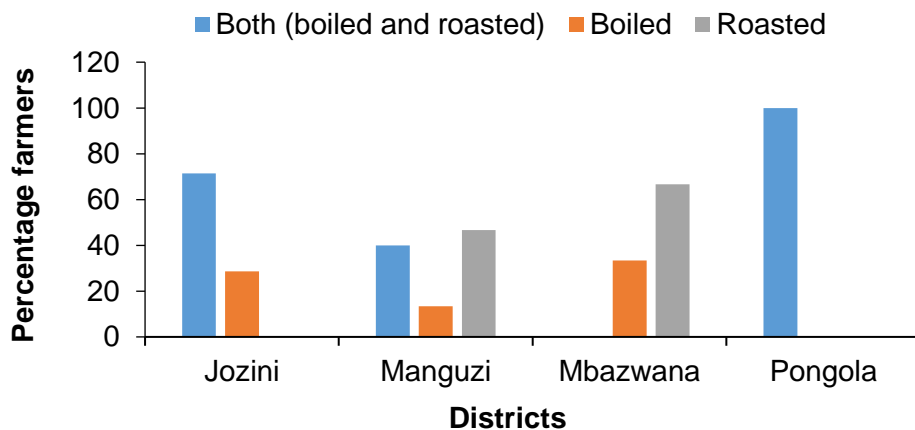
Figure 58. Preparation of groundnuts consumed by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
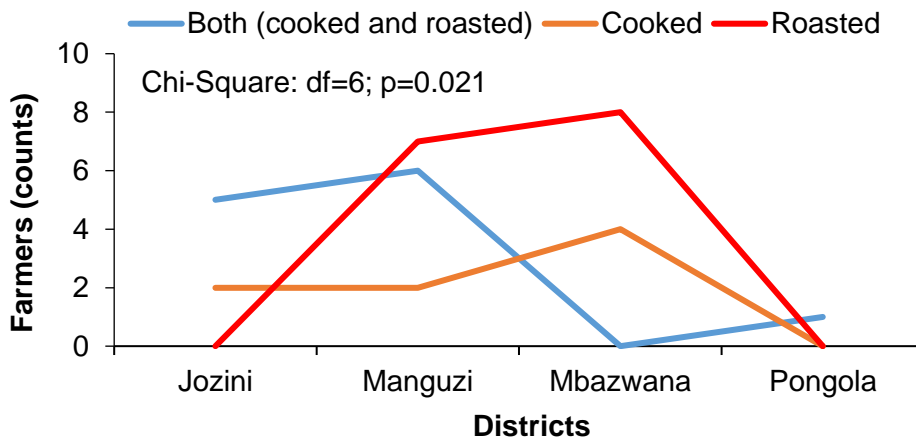
Figure 59. The relationship between differently prepared groundnuts and groundnut districts.
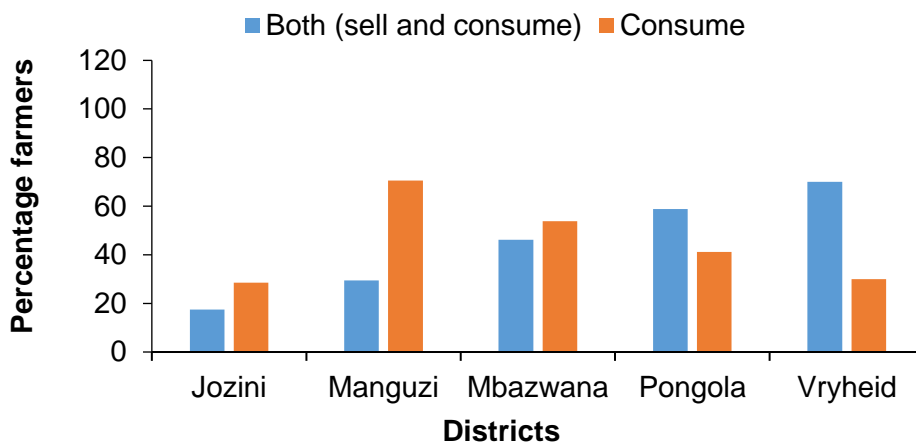
Figure 60. Consumption and trading of harvested home-grown maize by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.

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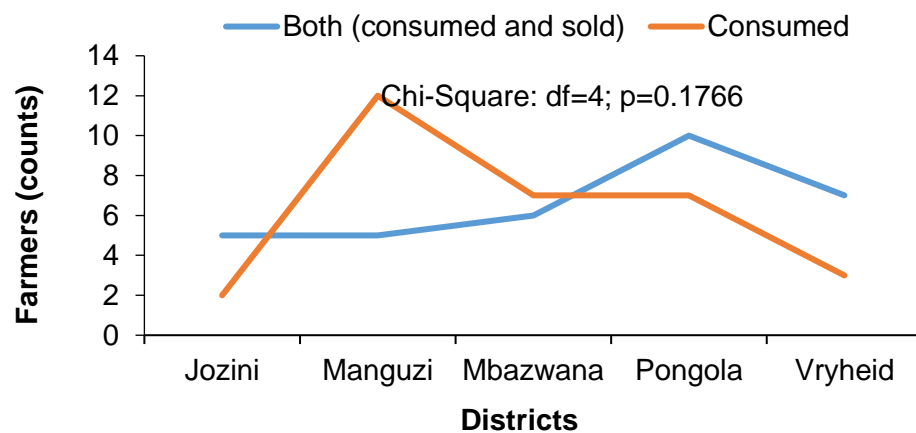
Figure 61. The relationship between consumption with trading of home-grown maize and maize districts.
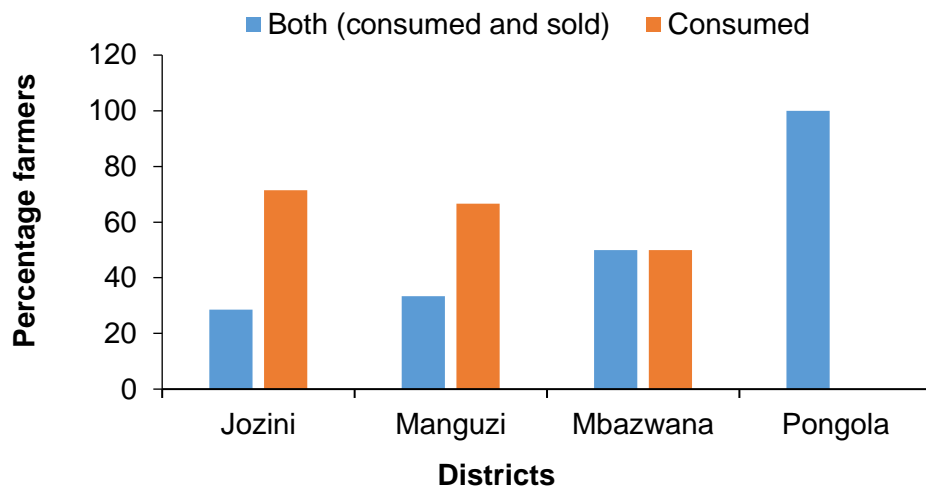
Figure 62. Consumption and trading of harvested home-grown groundnuts by subsistence farmers in four districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
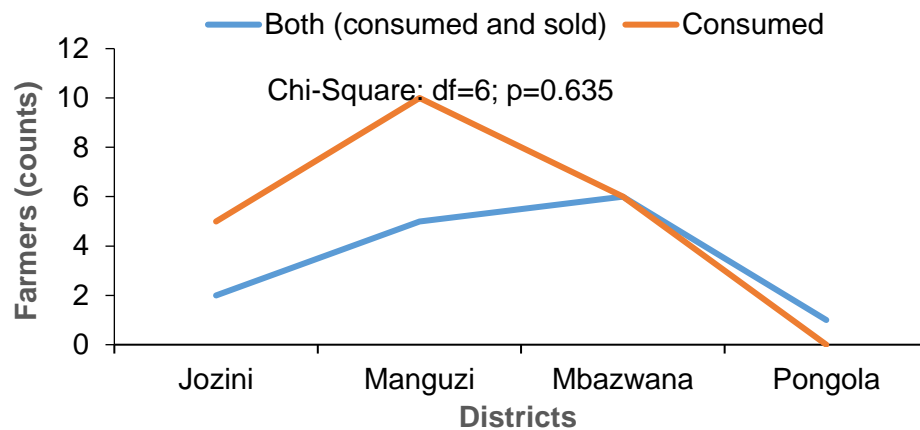
Figure 63. The relationship between consumption with trading of home-grown groundnuts and groundnut districts.
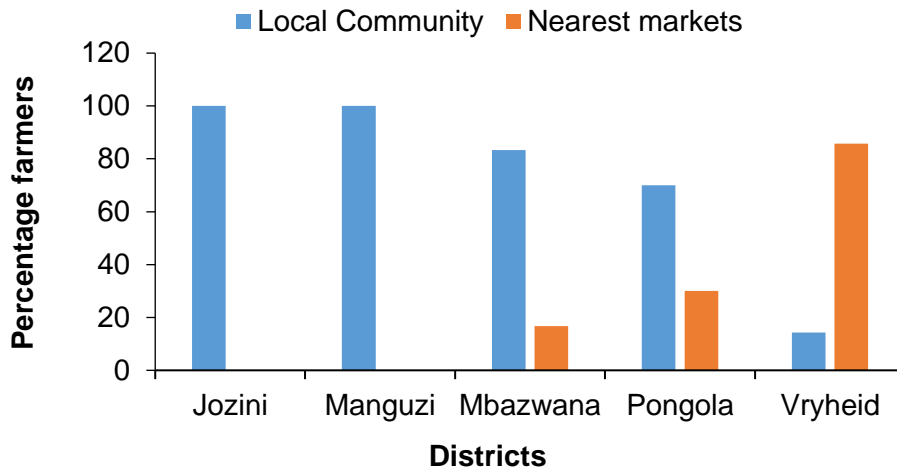
Figure 64. Areas where home-grown maize was sold (traded) by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 season.
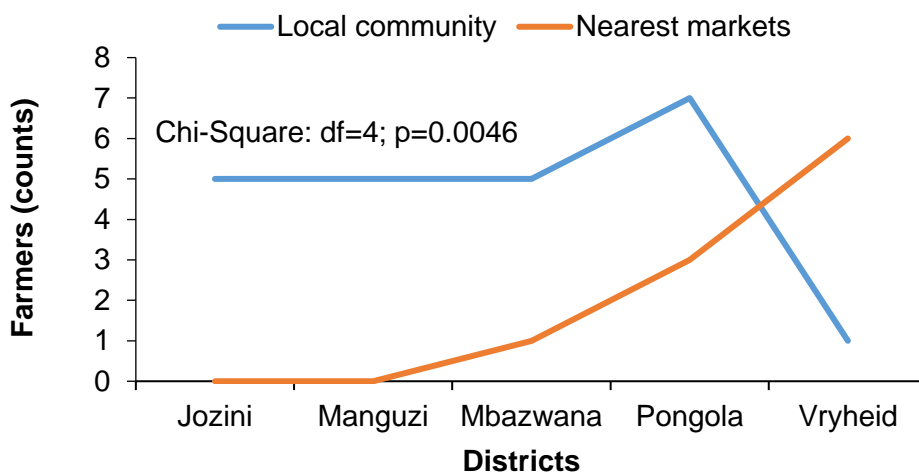
Figure 65. The relationship between trading areas and maize districts.
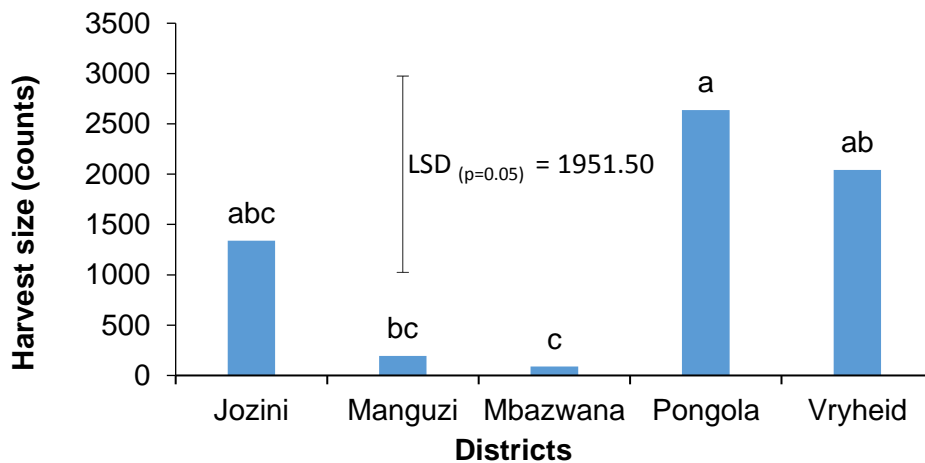
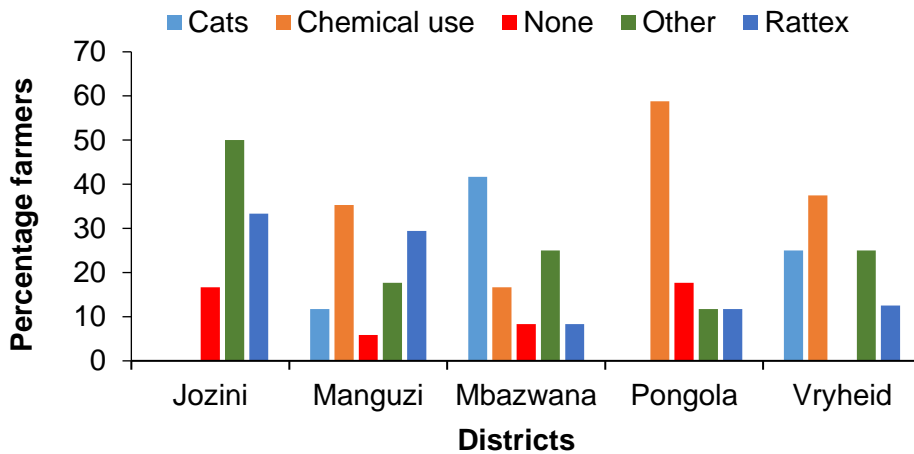**Figure 66.** The relationship between harvest size and maize districts.
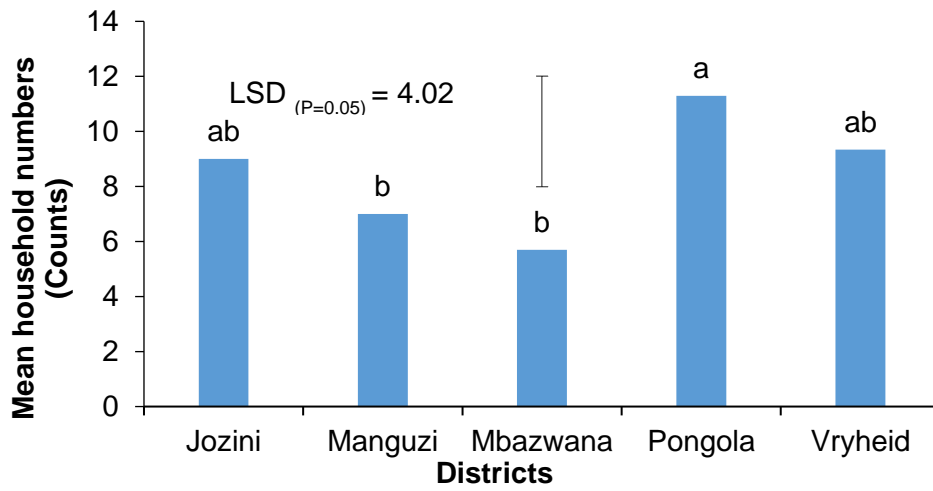
Figure 67. The relationship between mean household numbers and maize districts.
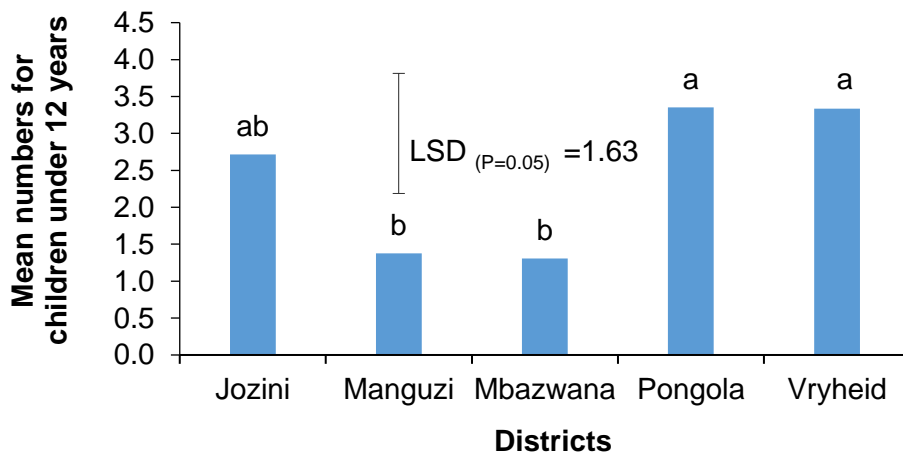
Figure 68. The relationship between mean under 12 years children and maize districts.
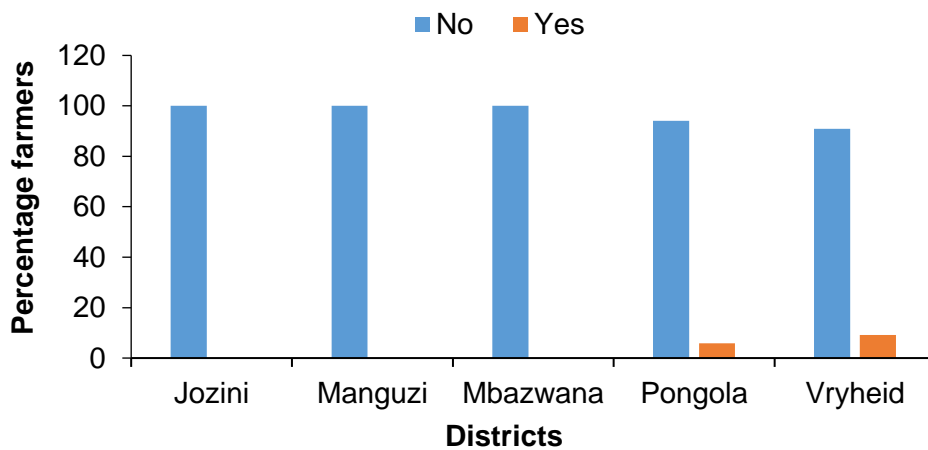
Figure 69. Mycotoxin awareness by subsistence farmers in five districts of the northern KwaZulu-Natal, surveyed during the 2012/13 seasons.
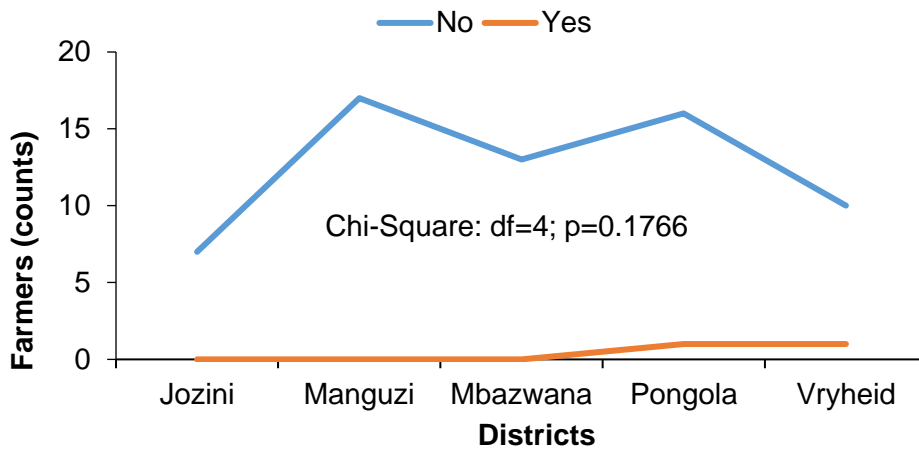
**Figure 70.** The relationship between mycotoxin awareness and maize districts.

ADDENDUM A: Maize Questionnaire

Household Details

1. Village name: ………………………. GPS location: ……………………………

2. Sample number: ……………………....

3. Contact person: ………………………………

4. How many people live in the household? ………. Children under 12 years: ………….

Storage History

1. Do you cultivate maize with other crops? YES NO

If Yes, list the crops: ……………………………………………………………………………

2. Do you store on the cob or as shelled grain? ……………………………..

3. Where and Why do you pre-store (on the
cob)?..
……
…………………………………………………..

4. When did the plant start dying out (physiological maturity)?
…………………………………………..

5. On average, how much maize do you get per harvest (size of bag or drum)?
…………………………………………

6. On average, how long does a season's harvest last? – less than a month, 3 months, 6
months, to the next harvest (1 year) ……………………………………………………….

7. Where do you store your maize (describe type of storage and take photograph):
……
…………………………………………………………………………………………………

8. In what state is the maize stored? Cob …… Loose grain ……. Milled ………. Other
……………….

9. How often do you clean the storage…………………………………………………………

10. How is the storage area cleaned? ……………………………………………………….

11. Do you sort damaged and mouldy maize before storage? YES NO

12. Do you sort damaged and mouldy maize after storage prior to use? YES NO

13. If yes, what do you do with damaged / mouldy maize?

……
……………………………………………………………………………………………….

14. Do you remove old grains from storage before putting new harvest in? YES NO

15. What other crops/items are kept in the storehouse? ……………………………….

16. What problems (if any) do you experience during storage?

………

17. When do you observe this problem?

At the beginning of storage?   After a few months?   At the end of storage

18. How do you solve the problem? …………………………………………………………

………
………….

19. What pesticides (if any) do you use? …………………………………………………..

20. Where do you source your maize seed from? …………. ….

Consumption

1. How often do you consume maize?

Daily ………… Weekly ……………… Monthly ………………..

2. How much do you consume? …………………………………………………………………..

3. Is the maize you consume home-grown or not? ………………………………………

4. How do you prepare your maize? …………………………………………………………

………

5. Do you consume all your harvested maize or do you sell some? ………………..

………
………….

6. Where do you sell your maize? …………………………………………………………….

7. Are you aware of mycotoxins?..

ADDENDUM B: Groundnut Questionnaire

Household Details

Locality:…………………………………………

Village name:………………………………….

GPS location:…………………………………………………

Sample number:……….

Contact person:…………………………………………

Storage History

When were the groundnuts planted?..

Were residues removed from the soil before planting the groundnuts? YES NO……………

Was there any crop planted on the same field before planting the groundnut (crop rotation)?

If yes, which

crop/s……………………………………………………………………………………………

Were the groundnuts planted with another crop close to each other (intercropping)? YES NO

Did you use any pesticides? YES NO...

When did the plants start drying out (month of physiological maturity)?..................................

Do you cultivate groundnuts with other crops? YES NO, if yes list the

crops……………………………………………………………………………

On average, how much groundnuts do you get per harvest? ……………………………….

Where do you store your groundnuts?..

How often do you clean the storage?..

How is the storage area

cleaned?...

Do you sort damaged and mouldy groundnuts before storage? YES NO

If yes, what do you do with damaged/mouldy groundnuts?...

Do you remove old groundnuts from storage before putting new harvest in? YES NO

What other crops/items are kept in the storehouse? …………………………………

What problems (if any) do you experience where groundnuts are stored?...........................

How do you solve the

problem?..

Do you consume all your harvested groundnut or do you sell some?...................................

In what form are the groundnuts consumed? raw/ boiled/roasted/peanut butter

Do you know anything about nematodes/fungal pathogens associated with groundnuts? YES NO

If yes, where did you get the information about these pathogens……………………………………………………..

Do you know the effects these pathogens have on animal and humans? YES NO, if yes which effects

....

CHAPTER 3

Toxigenic fungi and associated mycotoxins in maize and groundnut produced by subsistence farmers in KZN

ABSTRACT

Maize (*Zea mays* L.) and groundnut (*Arachis hypogaea*) are staple foods for most subsistence farmers in northern KwaZulu-Natal (KZN), South Africa. Agricultural pests and diseases influence the growth and development of maize and groundnut crops and subsequent food production. Groundnut plants are often damaged by plant-parasitic nematode species, hence impacting adversely on yield. Furthermore, the damage caused by nematodes may promote the infection of groundnut by mycotoxigenic fungi, predominantly *Aspergillus flavus* which produces aflatoxins. Maize is also susceptible to *A. flavus* and other mycotoxigenic fungi including *Fusarium verticillioides* and *F. graminearum*. *Fusarium verticillioides* produces mycotoxins, fumonisins, while *F. graminearum* produces deoxynivalenol (DON), nivalenol (NIV) and zearelanone (ZEA). In this study, groundnut and maize grain samples were collected from Jozini, Manguzi and Mbazwana districts during the 2012/13 and 2013/14 growing seasons. Groundnut pegs, roots and surrounding soil were also sampled before harvest (in the field) during the 2013/14 season only. Additional maize grain samples were collected from Pongola and Vryheid districts in northern KZN. *Fusarium verticillioides*, *F. graminearum* and *A. flavus* were measured by quantitative PCR in grain sampled at harvest and following storage. There were no significant differences between localities within districts and the collection periods (harvest and storage) during both seasons for any of the mycotoxigenic fungi. Fumonisin contamination exceeded 2 µg/g in maize grain collected at harvest and following storage for both seasons; and in all districts except Mbazwana. Aflatoxin contamination was detected in groundnut grain both at harvest and following storage during both seasons with levels exceeding 10 µg/kg detected only in the 2013/14 season. There were no significant differences between localities within districts and collection periods (harvest and storage) during both seasons for fumonisin and aflatoxin contamination. The ZEA levels were negligible ($\leq$ 0.02 ug/g) during both seasons while DON and NIV were undetected. There were significant differences between localities within districts and collection periods (harvest and storage) during both seasons for ZEA contamination. Plant-parasitic nematodes were more often isolated in number from groundnut hulls than kernels during both seasons. *Ditylenchus africanus* was predominantly isolated from hull and kernel samples during both seasons at harvest and following storage. However, *Pratylenchus* spp. was predominant in the pegs, roots and soil. The aforementioned nematodes were more commonly found in Manguzi as

compared to other districts. During the 2012/13 season, aflatoxin levels in the groundnut kernels were undetected at harvest and insignificant following storage in all three districts. During the 2013/14 season, aflatoxin levels in groundnut grain was detected before harvest in Mbazwana and Manguzi, at harvest only in Manguzi and following storage only in Mbazwana. Linear regression demonstrated a relationship between the nematodes and aflatoxin contamination in the Manguzi, Jozini and Mbazwana districts at storage during the 2012/13 season also before and at harvest during the 2013/14 season. The high levels of fumonisins in maize grain and aflatoxins in groundnuts suggest that the subsistence farmers are at higher risk of severe health implications and also face barriers in trading their maize and groundnuts.

INTRODUCTION

South Africa produces approximately 11 million tonnes of maize (*Zea mays* L.) grain annually by means of commercial farming (www.ps-survival.com) while the average annual production of maize contributed by subsistence farming was 500 000 tons during the past 10 years (www.fao.org). Subsistence farmers in South Africa mainly produce maize for food and to earn a small income; however studies have shown a decline in subsistence agriculture (Baipheti and Jacobs, 2009). The decline is due to factors such as lack of insect-resistance maize seeds, better technologies, fertilisers and high-yielding crop varieties (Mkhabela, 2002; Mabaya *et al.*, 2009. Other factors include poor investment in irrigation and quality land (Baipheti and Jacobs, 2009). Furthermore, most subsistence farmers lack the required resources to ensure the production of quality grain (Ncube *et al.*, 2011). Crops produced subsistently in South Africa such as the groundnuts and maize are most susceptible to infection by mycotoxigenic fungi and concomitant mycotoxin contamination leading to health impacts and risks in humans and animals (Misihairabgwi *et al.*, 2017).

Mycotoxin contamination is prevalent in subsistence maize production (Ncube *et al.*, 2010, 2011; Shephard *et al.*, 2013) and is influenced by factors at harvest and at storage (Amadi and Adeniyi, 2009; Pitt *et al.*, 2013). Harvest and storage agricultural practices employed by South African subsistence farmers were found to have significant impacts in mycotoxin contamination of maize kernels (Ncube *et al.*, 2011). The mycotoxins produced by *Aspergillus* and *Fusarium* species can occur at different stages of the food chain at pre-harvest, harvest and storage (Lattanzio *et al.*, 2014; Gong *et al.*, 2015). Also, mycotoxins produced by the *Aspergillus* and *Fusarium* species were found to be carcinogenic, mutagenic and teratogenic (Niessen, 2007). Fumonisins produced by *Fusarium verticillioides* (Sacc.) Nirenberg, aflatoxins produced by *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare (Bezuidenhout *et al.*, 1988; Njapau *et al.*, 1998; Zhang *et al.*, 2011) and trichothecenes produced by *Fusarium graminearum* Schwabe amongst other mycotoxins were found to have deleterious effects on both humans and livestock (Mudili *et al.*, 2014), causing various diseases which can be acute or chronic (Coppock and Jacobsen, 2009; Nicolaisen *et al.*, 2009).

Fusarium verticillioides commonly infects maize kernels and may result in the development of Fusarium ear rot (FER) and fumonisin contamination under favourable environmental conditions (Bush *et al.*, 2004). Infection by *A. flavus* is favoured by dry conditions and elevated temperatures (Warburton *et al.*, 2011). *Fusarium graminearum* contaminates maize with mycotoxins type B trichothecenes including deoxynivalenol and nivalenol while contamination with zearalenone also occurs (Lee *et al.*, 2012).

Maximum allowable limits for major mycotoxin classes in food products have been set by the European Union (EU) established with the Commission Regulation No. 1881/2006.

Maximum levels in food set by the EU for aflatoxin B_1 and sum of aflatoxins are 2 µg/kg and 4 µg/kg, respectively (Imperato *et al*., 2011). However, the South African national regulations specified a maximum limit of 5 µg/kg for aflatoxin B_1 and 10 µg/kg for total aflatoxin in all foodstuffs. The maximum allowable limit for fumonisins in maize grain intended for human consumption has been legislated at 2 µg/g in South Africa (DOH, 2016). These maximum allowable limits are necessary to monitor and manage mycotoxin levels in foodstuffs (Imperato *et al*., 2011).

Groundnut (*Arachis Hypogaea* L.) production in South Africa varies in terms of production area and production system, from 400 kg to several tons of yield per hectare. Unlike commercial farmers, subsistence farmers mostly in the eastern and northern parts of South Africa plant groundnuts for own consumption (http://www.arc.agric.za).   Cilliers and Swanevelder, (2003) stated that groundnut production in South Africa fluctuates between 80 000 and 250 000 tons annually of which production is mainly from commercial farmers. Provinces in South Africa in which groundnuts were mainly produced during the 2010/11 season were the Northern Cape (30%), Free-State (32%) and North West (33%). However; Gauteng, Limpopo and KwaZulu-Natal were also listed as lesser groundnut production provinces during the same season (http://www.nda.agric.za).

Groundnut is an important food and oilseed crop worldwide of which its consumption has been linked to reduction of cardiovascular disease (Kamika *et al*., 2004). Also, groundnut is preferred for rotation with maize as it enriches the soil with nitrogen and its a crop of high economic value (http://www.arc.agric.za) Plant-parasitic nematodes are most important constraints to the production of groundnuts and other crops in sub-Saharan Africa (Coyne *et al*., 2009); such as the economically important peanut-pod nematode (*Ditylenchus africanus* Wendt, Swart, Vrain, and Webster) (Fourie *et al*., 2015).

Plant-parasitic nematodes such as the *Meloidogyne chitwood* Golden, O'Banon, Santo & Finley results in delayed maturity, reduced yields and quality in groundnuts (Onkedi *et al*., 2014; Fourie *et al*., 2001). The South African Plant-Parasitic Nematode Survey (SAPPNS) database records a total of 222 plant-parasitic nematode species belonging to 39 genera occurring from the KwaZulu-Natal Province (Marais and Swart, 2013) where this study was conducted. Also, the SAPPNS records *Pratylenchus* spp. and *Meloidogyne* spp., excluding *Ditylenchus africanus*, from KwaZulu-Natal.

In addition to nematode pests, mycotoxigenic fungi also pose a serious concern to the grain yield and quality of groundnut. A study by Gonçalez *et al*. (2008) reported that groundnut seeds are infected by toxigenic fungi before harvest, post-harvest curing, during drying and at storage. *Aspergillus flavus* infection occurring in the field mostly leads to the infection of groundnut kernels at storage (Vijayasamundeeswari *et al*., 2010). The drawback is that *A. flavus* infection on the groundnut crop cannot be visually determined as the fungus

has no pathological effects on the groundnut plant (Pitt and Hocking, 2006). Of more immediate concern is the production of mycotoxins, aflatoxins, which are carcinogenic, hepatotoxic and mutagenic (Khayoon *et al*., 2012) by *Aspergillus* species. Amongst the aflatoxins produced by *A. flavus*, aflatoxin B_1 is the most carcinogenic and was classified as a group one human carcinogen (Mupunga *et al*., 2014). Groundnuts from two districts in the northern KwaZulu-Natal province of South Africa were found to be highly contaminated with aflatoxins (Ncube *et al*., 2010). This was reported to be above the maximum limit of 10 µg/kg as set by the department of health in South Africa. However, there was no positive correlation between *A. flavus* contamination and aflatoxin production as reported from the study by Ncube *et al*. (2010).

The use of atoxigenic *A. flavus* strains to control the toxigenic fungi at timely harvest has been suggested as a biological control method (Hell *et al*., 2010). Competitive atoxigenic *A. flavus* strains were found to be very effective in reducing *A. flavus* infection in the field (Pitt and Hocking, 2006). This intervention has been successfully used in Africa on maize and groundnuts with 77 to 98% reduction in aflatoxin contamination being recorded (Horn and Donner, 2009). After the development of the groundnut canopy, a grain substrate with fungal conidia is applied directly to the soil surface remaining in close contact with the groundnut pods. The toxigenic strains are competitively eliminated by the atoxigenic fungal strains (Horn and Donner, 2009).

In terms of soil-borne microorganisms, reported to be antagonistic to economically important nematode pests of groundnut, fungi are particularly known to occur in disease complexes with root-knot and cyst nematodes (Akhtar and Malik, 2000). However, interactions between plant-parasitic nematodes and *A. flavus* on groundnut have not been recorded to date under South African environmental conditions. The objectives of this study were to evaluate maize produced by subsistence farmers in northern KZN for multi-mycotoxin contamination as well as quantify target DNA mycotoxin-producing fungi at harvest and at storage over the 2012//13 and 2013/14 seasons. Moreover, groundnut samples were evaluated for plant-parasitic nematodes of groundnuts in hulls and kernels. Aflatoxin contamination of groundnut kernels and atoxigenic fungi were quantified in groundnut kernels over 2012/13 and 2013/14 seasons.

The objective of this study relating to maize was to quantify the most common mycotoxigenic fungi and determine multi-mycotoxin contamination levels in maize grain, collected at harvest and following storage, during the 2012/13 and 2013/14 seasons from subsistence farmers in northern KwaZulu-Natal. The objectives relating to groundnuts were to i) determine plant-parasitic nematodes of groundnuts collected at harvest and storage during the 2012/13 and 2013/14 seasons and before harvest during the 2013/14 season; ii) determine parasitic nematodes in the pegs, roots and surrounding soil before harvest during the 2013/14

season and iii) quantify aflatoxin levels in groundnut kernels collected at harvest and following storage during the 2012/13 and 2013/14 seasons. Lastly, this study sought to determine the relationship between plant-parasitic nematodes and aflatoxin contamination of groundnut kernels during the 2012/13 and 2013/14 seasons.

MATERIALS AND METHODS

Sampling of maize and groundnuts (including pegs, roots and rhizosphere soil)

Maize samples were collected in Vryheid, Pongola, Jozini, Manguzi and Mbazwana districts of northern KZN from subsistence farmers during the 2012/13 and 2013/14 seasons. Maize and groundnut collection was carried out at the same time with the survey of agricultural practices. Grain was collected at harvest and after three months in storage. Maize cobs were randomly picked both at harvest and following storage with some cobs clearly showing fungal growth (Fig. 1). Groundnut samples were also randomly collected in Jozini, Manguzi and Mbazwana during both seasons at harvest and after three months in storage. Most of the groundnut samples collected were apparently healthy (Fig. 2). Maize samples were collected as either cobs or loose grain and groundnut samples were collected as groundnut pods (kernels with intact hulls). None of the farmers in Vryheid planted groundnuts while only one interviewed farmer in Pongola planted groundnuts and was excluded from all groundnut analyses to reduce any bias. Maize and groundnut samples were collected at harvest during both seasons from the same farmers, however, stored grain samples were not collected from all farmers as the produce was either consumed or sold.

Bulk groundnut and maize samples were collected, respectively, per field. A total of 52 and 45 maize samples were collected at harvest and after three months at storage, respectively, during the 2012/13 season. At Vryheid and Mbazwana, equal number of maize samples (10) were collected for both districts at harvest and following storage, also in Pongola equal number of maize samples (17) were collected at harvest and following storage during the 2012/13 season. However, in Jozini and Manguzi some maize samples were used up following storage, in Jozini (6 and 5) and in Manguzi (9 and 3) maize samples were collected at harvest and following storage, respectively during the 2012/13 season. During the 2013/14 season a total of 38 and 37 maize samples were collected at harvest and after three months at storage, respectively. In Vryheid (8), Pongola (17), Jozini (3) and Manguzi (8) districts equal number of maize samples were collected both at harvest and following storage during the 2013/14 season. In Mbazwana, 2 and 1 maize samples were collected at harvest and following storage during the 2013/14 season. During the 2012/13 season, 30 and 29 groundnut samples were collected at harvest and after three months of storage, respectively. Equal number of groundnut samples were collected in Pongola (1), Jozini (2) and Manguzi (16) districts at harvest and following storage during the 2012/13 season. In Mbazwana 11 and 10 groundnut

samples were collected at harvest and following storage and no groundnut samples were collected in Vryheid both at harvest and following storage during the 2012/13 season. A total of 18, 17 and 11 groundnut samples were collected during the 2013/14 season before harvest, at harvest and following storage, respectively in the three districts. Before harvest, groundnut samples were collected in Jozini (5), Manguzi (9) and Mbazwana (4) during the 2013/14 season. At harvest and following storage, groundnut samples were collected in Jozini (1 and 1), Manguzi (13 and 7) and Mbazwana (3 and 3), respectively during the 2013/14 season. The groundnut pegs, roots and rhizosphere soil were collected only before harvest during the 2013/14 season. GPS co-ordinates were used to identify the localities within the districts were both maize and groundnuts were collected. During collection, the samples were labelled clearly with specific sample number, name and surname of the farmer, locality, district, collection period and season. Subsequently, rainfall and maximum daily temperatures were obtained for locations within the different districts.

Maize grain processing

After collection, the moisture content of the loose maize samples was determined before analysis. Moisture content was determined using the Twist Grain Moisture Meter (Draminski, Olsztyn, Poland). Maize cobs were shelled if needed and a 250 g grain sample was weighed for milling. The samples were milled using the Cyclotec 1093 sample mill (Foss Tecator, Hoganas, Sweden) and the resultant maize flour was stored at -20°C for further analyses. The remaining kernels were stored in the cold-room at -4°C.

Plant (maize) and fungal DNA extraction

Deoxyribonucleic acid (DNA) was extracted from 0.5 g milled maize flour using the DNeasy® Plant Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. *Fusarium verticillioides* MRC 826 (Medical Research Council, Tygerberg, South Africa), *F. graminearum* MRC 1125 (Medical Research Council, Tygerberg, South Africa) and *A. flavus* MRC 3954 (Agricultural Research Council, Potchefstroom, South Africa) were grown on potato dextrose agar (PDA) for 7 days in the dark at 25°C. The resulting mycelia was transferred to 2 mL Eppendorf tubes and frozen at -20°C overnight until DNA isolation was performed.

Fusarium graminearum, F. verticillioides and *A. flavus* DNA were extracted using a DNA isolation method employing cetyl trimethylammonium bromide (CTAB) according to Guertler *et al*. (2013). Before extraction, the CTAB (5 M NaCl (pH 8.0), 0.5 M EDTA (pH 8.0), 1 M Tris-Cl, Polyvinylpyrrolidone (PVP) (MW 40 kDa), β-Mercaptoethanol and H_2O) buffer was warmed at 95°C in the water bath. The frozen mycelia were ground using a warm glass rod. A volume of 900 μL CTAB buffer was added to each sample and vortexed. The samples were

then frozen in liquid nitrogen for 30 seconds where after they were placed in a 95°C water bath for 5 min and then cooled on ice. Two µL of RNase A (Life Technologies, Fairland, South Africa) was added to each sample and the samples were vortexed. Samples were placed in a 37°C water bath for 30 min. A volume of 800 µL of phenol: chloroform:isoamylalcohol (25:24:1) (Sigma-Aldrich, Aston Manor, South Africa) was added to the samples, followed by centrifugation at 12 000 revolutions per minute (rpm) for 10 min at 4°C. The resulting supernatant from each sample was transferred into 2 ml Eppendorf tube without disturbing the bottom layer. A volume of 500 µL of another phenol: chloroform:isoamylalcohol was added to the supernatant followed by centrifugation at 12 000 rpm for 10 min at 4°C. Ice-cold isopropanol (added amount calculated 150 µL less than the resulting supernatant) was added to each sample. The samples were incubated for 30 min at -20°C. After addition of the isopropanol, the samples were centrifuged at 12 000 rpm for 10 min. The supernatants were discarded and the tubes were blotted on paper towel to remove the remaining ethanol. A volume of 500 µl of 70% ethanol was added to the pellet followed by centrifugation at 12 000 rpm for 5 minutes at 4°C. The supernatant was discarded and the tubes were again blotted on paper towel. The tubes containing the pellets were opened and air dried for 30 minutes at room temperature. The dry pellets were re-suspended in filtered TE buffer to desired concentration.

Determination of maize DNA concentration and dilutions

The concentration of the DNA was determined using the NanoDrop® ND-1000 machine (NanoDrop Technologies, Wilmington, DE). Autoclaved DNase/RNase/Protease-free water was used as a blank solution. A volume of 2 µL was loaded for the blank solution and also for the measured sample DNA. The average of three readings was taken as the final DNA concentration. For qPCR, only a DNA concentration of 10 ng/µL was used. The purity of the DNA was evaluated using the A260/280 and A260/230 ratios. Ten-fold serial dilutions from the initial DNA were prepared to produce standard curves for the qPCR.

Real-time PCR (quantitative PCR) of maize

Quantitative PCR was performed on a MicroAmp Optical 96-well reaction plates (Applied Biosystems) sealed with optical adhesive covers. The reactions were carried out in triplicates, similar to the study done by Tellenbach *et al*. (2010). The standard curve was used to calculate the amount of fungal target DNA from the cycle threshold (Ct) according to Picot *et al*. (2012). The slope of the linear regression ranged between -3.1 and -3.6, corresponding to a PCR efficiency of 80-100 %, and R^2 values of ≥0.98 according to Scauflaire *et al*. (2012). A positive control used was the 18.2MΩ PCR Grade Bioline Water (Celtic Molecular Diagnostics, Wynberg, Cape Town).

Quantification of F. verticillioides *target DNA*: For the amplification of *F. verticillioides*, a forward primer (Taqman-2F) with nucleotide sequence: 5'-ATGCAAGAGGCGAGGCAA-3' and a reverse primer (VPgen-3R) with nucleotide sequence: 5'GGCTCTCRGAGCTTGGCAT-3' were used together with a FUM-probe 1 with nucleotide sequence: 5'-/56-FAM/CAATGCCATCTTCTTG/36-TAMSp/-3' (Waalwijk *et al.*, 2008). The Taqman targets a conserved gene, the polyketide synthase gene fum1, responsible for the production of fumonisins (Waalwijk *et al.*, 2008).

The master mix reaction was prepared to a volume of 25 µL per sample containing: 83 nM FUM-probe1 (Whitehead Scientific, Fairland, South Africa), 333 nM of each primer (Whitehead Scientific, Fairland, South Africa), 1x Quantace SensiMix II Probe (Celtic Molecular Diagnostics, Wynberg, Cape Town), 18.2MΩ PCR Grade Bioline Water (Celtic Molecular Diagnostics, Wynberg, Cape Town) and 10 ng/µL of *F. verticillioides* DNA. The following cycling conditions were used: pre-incubation step of 10 min at 95°C, followed by 40 cycles at 95°C for 15 seconds (s) of primer annealing, then enzymatic chain extension at 60°C for 15 s and last extension step at 72°C for 15 s.

Quantification of F. graminearum *target DNA*: The quantification of *F. graminearum* in maize grain was performed according to Boutigny et al. (2012). The primers consisted of FgramB379 (Whitehead Scientific, Fairland, South Africa) with nucleotide sequence: 5'-CCATTCCCTGGGCGT-3' and FgramB411 (Whitehead Scientific, Fairland, South Africa) with nucleotide sequence: 5'-CCTATTGACAGGTGGTTAGTGACTGG-3' (Nicolaisen et al., 2009). The master mix reaction was prepared up to a volume of 25 µl per sample containing: 1x iTaq™ Universal SYBR® Green Supermix, 200 nM of each primer, 18.2MΩ PCR Grade Bioline Water and 10 ng/ µL *F. graminearum* DNA. The following cycling conditions were used: 10 min at 95°C, followed by 40 cycles at 95°C for 15 s, then 60°C for 15 s and lastly 72°C for 15s.

Quantification of A. flavus *target DNA*: Amplification of *A. flavus* target DNA also involved the use of a probe, known as the Norprobe 1 with nucleotide sequence: 5'-/6-FAM/TGTCTTGATCGGCGCCCG/36-TAMRA/-3'. Primer sequences which were used was Nortaq 1 and Nortaq 2 were 5'-GTCCAAGCAACAGGCCAAGT-3' and 5'-TCGTGCATGTTGGTGATGGT-3', respectively (Mayer *et al.*, 2003). The reaction mixture for *A. flavus* was made to a final volume of 25 µl per sample containing: 1x Quantace SensiMix II Probe (Celtic Molecular Diagnostics), 0.5 nM Norprobe 1 (Whitehead Scientific, Fairland, South Africa), 25 µM of each primer (Whitehead Scientific, Fairland, South Africa), 18.2MΩ PCR Grade Bioline Water and 10 ng/ µL of *A. flavus* DNA. The thermal cycling conditions for

A. flavus included 95ºC for 4 min followed by 40 cycles at 95ºC for 30 s, 53ºC for 30 s and 72ºC for 20 s.

Multi-mycotoxin analyses from maize

Multi-mycotoxins were extracted from maize (5 g) grain using a 70% methanol (Microsep, Sandton, South Africa) and 30% water (HPLC grade) solution. The extraction buffer of 20 ml was added to each sample at ratio 4:1. Following the addition of the extraction solution, the samples were put in a shaker at 25ºC for 30 min at a speed of 200 rpm. After shaking, the samples were centrifuged at 4ºC for 10 min at a speed of 500 relative centrifugal force (rcf) An extract of 2 mL was withdrawn from the tubes. A 0.25 µm filter was used to filter the extract into a 2 mL Eppendorf tube. The filtered samples were placed in a refrigerator overnight and were then sent to mass spectrometry unit at the central analytical facility at Stellenbosch University for analyses using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Nematode extractions from groundnut

Groundnut samples collected from the same field were classified as one batch and from each batch of samples, 20 apparently healthy and 20 apparently diseased groundnut kernels were selected for nematode extractions. Groundnuts collected during the 2013/14 season were treated the same as groundnuts collected during the 2012/13 season. The groundnut pods were shelled into hulls and kernels and both hulls and kernels were cut into smaller parts of 1 cm size. Five grams of hulls and kernels, respectively, were soaked separately in 120 ml tap water to allow plant-parasitic nematodes to exit hull and kernel tissue. Immersion in tap water took place for 24 hours at room temperature to allow extraction of nematodes. The water suspension, containing the nematodes was washed through a 53 µm-aperture sieve nested on top of a 25 µm-aperture sieve. The supernatant containing the nematodes was subsequently collected from the 25-µm-aperture sieve by washing it into a 50-ml beaker. These extraction methods were described by Bolton et al. (1990), with some modifications being made during this study as compared to the study by Bolton et al. (1990) whereby the suspension of nematodes was washed through a 750 µm-aperture sieve nested on top of a 45 µm-aperture sieve.

Rhizosphere soil samples surrounding sampled groundnut crops were also taken during the 2013/14 season before harvest in the three groundnut districts, marked and put in plastic bags. The soil samples taken from one household were first mixed thoroughly before nematode extraction. Nematodes were extracted from 100 g of each soil sample by first using the decanting and sieving methods followed by use of sugar-flotation method according to Christie and Perry (1951). Nematodes were also extracted from root and peg samples using the sugar centrifugal-flotation and sieve methods (Coolen and D'Herde, 1972). Extraction of

nematodes from soil samples requires a different technique from the extraction of plant material samples, excluding the Baermann technique but which is ineffective when used to retrieve large nematodes (Hooper *et al.*, 2005). The extracted nematodes were fixed in a 4% formaldehyde solution of 90°C (Bridge and Starr, 2007) and stored until counting commenced. Prior to nematode counting and identification, the formaldehyde solutions containing the nematodes were decanted on a 25 µm-aperture sieve and rinsed with tap water. The tap water solutions containing the nematodes were washed from the sieve into a 50-ml sample bottle and then decanted into a De Grisse (De Grisse, 1963) counting dish for nematode counting and identification using a stereo microscope (100x magnification). Morphological and morphometrical characters were used to identify the nematodes (Mirghasemi *et al.*, 2014).

Preparation of nematode specimens for species identification

Fixation: A formaldehyde-propionic-acid-water (FPG) mixture was prepared by adding 100 ml of a 40% formalin solution, 10 ml propionic acid, 890 ml distilled water and a pinch of picric acid to produce a citrus yellowish fixative solution. Nematodes were isolated from the De Grisse counting dishes using a dissecting needle and suspended in Syracuse dishes containing tap water. Following this, the tap water was removed using a glass pipette and approximately 1 ml of the FPG fixative solution was placed in a test tube and heated to 65°C. The hot FPG fixative was poured onto the nematodes in the Syracuse dish, which were placed in a closed petri dish inside a desiccator saturated with FPG (50 ml). The desiccator with the petri dish containing the nematodes was incubated for 3 days at 38°C.

Hydration: An initial hydration solution (1) was prepared by adding 200 ml of a 95% alcohol (ethanol) solution and 10 ml of glycerin to 790 ml distilled water. The FPG fixative was drawn off using a glass pipette after removing the Syracuse dish. One ml of hydration solution 1 was added to the nematodes and the Syracuse dish was placed in an open Petri dish in a desiccator, saturated with 95% alcohol to allow slow evaporation to occur. The desiccator was once more incubated for 12 hours at 38°C.

Hydration solution 2 was prepared by adding 950 ml of a 95% alcohol solution and 50 ml of glycerin. Half of hydration solution 1 was drawn off carefully from the glass dish and replaced with hydration solution 2 of 1000 ml. The Syracuse dish was placed in an open Petri dish, incubated at 38°C for approximately three days to allow the alcohol solution to completely evaporated. Thereafter, the Syracuse dish was then removed and glycerin added to cover the nematodes. The dish was transferred to a desiccator again for 24 hours at 38°C after which mounting of nematodes took place.

Nematodes suspended in the small drop of anhydrous glycerine were isolated and mounted onto glass slides with wax rings as described by Ryss (2003). Five nematodes were

mounted per slide and viewed using a stereo microscope (60x magnification). Slides were sent to nematode taxonomists at the ARC-Plant Protection Research Institute for morphological identification to species level.

Aflatoxin extractions from groundnut kernels

Aflatoxins were extracted from one gram of the groundnut kernel sample using 4 ml of Methanol (Microsep (Pty) Ltd (Sandton, SA): water (70:30, v/v %). The extract was shaken at 25°C for 30 minutes and centrifuged at 3 000 rpm for 10 minutes in sterile 50 ml falcon tubes. Regenerated Cellulose (RC) filters (Microsep (Pty) Ltd (Sandton, SA) of 0.2 µM were used to filter the extract into 2 ml Eppendorf tubes. The samples were submitted to the Central Analytical Facility, Stellenbosch University for analysis using LC-MS/MS. Certified standards of aflatoxins B_1, B_2, G_1 and G2 and aflatoxin columns (Sigma-Aldrich, Missouri, USA) were used for sample preparation prior to analyses.

Data analyses

Fungal and mycotoxin levels were subjected to the Least Significant Difference (LSD) test at a significance level at $P = 0.05$, if $P > 0.05$ the results are non-significantly different and if $P \leq 0.05$ then results are significantly different. For aflatoxin analyses, the Fisher's exact test was used due to the small total sample size. The test was used to test for independence whereby the tested null hypothesis (H_o) was that the one factor (aflatoxin production) is independent on the different districts and the alternative hypothesis (H_a) was that the one factor is dependent on the different districts. When the null hypothesis is accepted then $P > 0.05$ and when the null hypothesis is rejected then $P < 0.05$.

Plant-parasitic nematode calculations

Prominence values (PV) for each nematode genera and/or species identified were calculated as follows (Bolton & DeWaele, 1989; De Waele & Mc Donald, 2000; Fourie *et al.*, 2001; Ntidi *et al.*, 2012):

(i) Population density at each locality =

$$\frac{\text{Total number of nematodes present per field (genera/species/family)}}{\text{number of localities at which the nematode genera/species/family occurred}}$$

(ii) Frequency of occurrence (FO) =

$$\frac{\text{Number of localities on which the nematode species/genus/family occurred} \times 100}{\text{total number of localities sampled}}$$

(iii) Prominence value (PV) = population density x $\sqrt{\text{frequency of occurrence}/10}$

Simple linear regression using the coefficient of determination (r^2) was used to determine the relationship between the number of plant-parasitic nematodes and aflatoxin production. The closer the r^2 is to one, the closer the relationship between number of plant-parasitic nematodes and aflatoxin production.

RESULTS

Mycotoxigenic fungal contamination of maize

Fusarium verticillioides *target DNA:* During the 2012/13 and 2013/14 seasons, *F. verticillioides* target DNA was detected in all five districts at harvest and following storage (Fig. 4). During the 2012/13 season, the highest levels of *F. verticillioides* target DNA were observed at harvest as compared to storage in Jozini, Manguzi and Vryheid, levels increased in these districts following storage as compared to harvest during the 2013/14 season (Fig. 4). Maize grain collected from Jozini district was the most infected with *F. verticillioides* and grain from the Mbazwana district was the least infected (Figs. 4 and 5). There were no significant differences between the districts and the grain collection periods. In all five districts collectively, the highest levels of *F. verticillioides* target DNA were observed during the 2013/14 season and specifically at harvest (Fig. 6). However, a significant decrease in *F. verticillioides* target DNA levels at storage was observed during the 2013/14 season (Fig. 6). The quantity of *F. verticillioides* target DNA measured did not differ significantly between localities and grain collection period during both seasons with the exception of significantly higher levels in grain collected from Mngamanzi (Pongola district) following storage (11800.59 pg/uL) when compared to all other localities (Table 1). Grain sampled from Ndlandla (Vryheid district) following storage contained the least *F. verticillioides* target DNA (9.29 pg/uL) (Table 1).

Fusarium graminearum *target DNA*: Target DNA levels of *F. graminearum* exceeded 1 000 pg/µL in all five districts both at harvest and storage during the 2012/13 and 2013/14 seasons (Fig. 7). *Fusarium graminearum* target DNA levels decreased significantly at storage as compared to harvest in Jozini and Mbazwana districts. In Pongola and Vryheid districts, *F. graminearum* target DNA levels increased significantly at storage (Fig. 7). In Manguzi district, the levels of *F. graminearum* did not differ significantly between grain collected at harvest and following storage (Fig 7). There were no significant differences between the districts and the collection periods during both seasons (Fig. 7). *Fusarium graminearum* target DNA levels were the highest during the 2012/13 season as compared to the 2013/14 season, with levels highest

in grain following storage (Fig. 8). All the localities within the five districts had *F. graminearum* target DNA levels detected during both seasons at harvest and at storage. There were significant differences in *F. graminearum* target DNA levels between localities and taking the grain collection periods, over the two seasons into account (Table 2). Significantly higher levels were found in grain collected from Ezidulini (Vryheid district) (6895.61 pg/uL), Zwaailagte (Vryheid district) following storage (7432.22 pg/uL) and Mngamanzi (Pongola district) following storage (6455.34 pg/uL) when compared to all other localities (Table 1). The quantity of *F. graminearum* target DNA measured in grain collected from Impala (Jozini district) following storage (111.03 pg/uL) was the lowest measured but did not differ significantly from a number of localities whose concentrations ranged from KwaZondo (Pongola district) at harvest (383.32 pg/uL), Msuzwaneni (Pongola district) following storage (615.05 pg/uL), Belgrade (Pongola district) at harvest (1779.37 pg/uL) to Manzabomvu (Pongola district) at harvest (2053.92 pg/uL) (Table 2).

Aspergillus flavus *target DNA*: *Aspergillus flavus* target DNA levels were the lowest as compared to *F. verticillioides* and *F. graminearum* target DNA levels, collectively in all districts during the 2012/13 and 2013/14 seasons (Fig. 9). Maximum levels of 200 pg/μl were detected in all districts during both seasons with Manguzi, Mbazwana and Pongola districts having detected levels of below 50 pg/L both at harvest and following storage during both seasons (Fig. 9). There were significant differences between districts and collection periods (Fig. 9). *Aspergillus flavus* target DNA levels were the highest during the 2013/14 season as compared to the 2012/13 season; and levels during the 2013/14 increased significantly at storage (Fig. 10). During the 2012/13 season, the quantity of *A. flavus* target DNA measured differed significantly between localities and grain collection period with the highest levels found in grain collected from Manyandeni (Pongola district) at harvest (445.78 pg/uL) and Impala (Jozini district) at harvest (388.30 pg/uL) which differed significantly from each other as well. Grain collected at harvest from Mngamanzi at harvest had the lowest (1.35 pg/uL) *A. flavus* contamination and did not differ significantly from Othungwini (Mbazwana district) (stored; 3.59 pg/uL) and Thelezini (Vryheid district) (harvest; 3.92 pg/uL) (Table 3). During the 2013/14 season significantly higher levels of *A. flavus* were measured in grain collected from Impala (Jozini district) following storage (877.44 pg/uL) when compared to all other localities evaluated. Grain collected from Mdonini (Pongola district) at harvest (0.68 pg/uL) contained the least *A. flavus* DNA but did not differ significantly from several localities evaluated including Thelezini (Vryheid district) (storage; 4.84 pg/uL) (Table 3).

Multi-mycotoxin analyses

Fumonisin contamination: During the 2012/13 season maize grain collected at both harvest and following storage contained fumonisin levels of above 2 µg/g in Jozini, Manguzi, Pongola and Vryheid districts and above this limit only in the Jozini district during the 2013/14 season (Fig. 11). Fumonisin levels in Jozini during the 2013/14 season were the highest (above 9 µg/g), collectively at both harvest and storage (Fig. 11). During both the 2012/13 and 2013/14 seasons, fumonisins levels were still the highest in the Jozini district moreover at harvest with levels above 11 µg/g (Fig. 12). Mbazwana was least contaminated with the fumonisins as compared to other districts both at harvest and storage during both seasons (Fig. 12). There were no significant differences between the districts and collection periods (Fig. 12).Significantly higher fumonisin levels in grain collected from Mngamanzi (Pongola district) during the 2012/13 season (43.27 µg/g) and Myeni (Jozini district) during the 2012/13 season (39.64 µg/g) was measured when compared to all other localities (Table 4). Several localities contained no or very little fumonisins with only Thelezini (Vryheid district) (2012/13; 11.45 ug/g) and Lundini (Jozini district) (2013/14; 13.11 µg/g) containing fumonisin levels that differed significantly to such localities (Table 4).

Aflatoxin contamination: Aflatoxin contamination levels in maize samples collected in all five districts during the 2012/13 season did not reach the maximum specified limit (10 µg/kg) both at harvest and storage; with no aflatoxins detected in Manguzi and Mbazwana (Fig. 13). However, during the 2013/14 season aflatoxin contamination levels were above the maximum quantification limit in all five districts both at harvest and storage (Fig. 13). The highest aflatoxin contamination levels were detected in Jozini above 800 µg/kg and the lowest aflatoxin contamination levels were detected in Mbazwana at 13 µg/kg both at harvest and storage (Fig. 13). There were no significant differences between the districts and the seasons (Fig. 13).

Focusing separately on harvest and storage during both seasons, the Jozini district had the highest aflatoxin contamination levels at harvest and at storage as compared to the other four districts (Fig. 14) Aflatoxin levels in Jozini were higher at storage as compared to harvest (Fig. 14). Aflatoxin contamination in Mbazwana was negligible, interestingly, aflatoxin contamination levels in Pongola were detected at harvest (17 µg/kg) but not detected at storage (Fig. 14). There were no significant differences between the districts and the collection periods during both seasons (Fig. 14). The aflatoxin levels differed significantly between localities and grain collection season with significantly higher levels in grain collected from Lundini (Jozini district) (2000 ug/kg), Impala (Jozini district) (1000 ug/kg), Myeni (Jozini district) (1000 ug/kg) and Ndumu (Jozini district) (1000 ug/kg) all collected during the 2013/14 season and all above the limit of quantification which is 500 µg/kg (Table 5). Grain sampled from Mngamanzi (Pongola district) during the 2012/13 season (0.01 µg/kg) and from Intuthuko

(Pongola district) during the 2013/14 season (1.83 µg/kg) contained the least aflatoxins as compared to other localities (Table 5).

Zearalenone contamination: During the 2012/13 season, zearalenone contamination levels in maize were detected only in the Pongola district and during the 2013/14 season in the Pongola and Jozini districts (Fig. 15). Detected zearalenone contamination levels in different districts during both seasons were below 0.02 µg/g (Fig. 15). There were significant differences between the districts and the seasons (Fig. 15). In both the Jozini and Pongola districts, zearalenone contamination levels were detected only at harvest during both seasons (Fig. 16). There were significant differences between the districts and the collection periods (Fig. 16). Within the Pongola district, 0.02 µg/g and 0.01 µg/g zearalenone contamination levels were detected in the Dlomololo (Pongola district) and Mngamanzi (Pongola district) localities, respectively. In Jozini, 0.18 µg/g zearalenone levels were detected only in the Myeni locality during the 2013/14 season. There were significant differences between the localities and the seasons and there were also significant differences between the localities and the collection periods during both seasons (data not shown).

Deoxynivalenol and nivalenol contamination: During both the 2012/13 and 2013/14 seasons, deoxynivalenol and nivalenol were undetected in maize samples collected in the five districts.

Nematode identification in groundnut hulls and kernels at harvest and storage during the 2012/13 season

Four plant-parasitic nematodes, namely *D. africanus* (the peanut-pod nematode), *Pratylenchus* spp*.* (lesion nematodes), *Meloidogyne* spp*.* (root-knot nematodes) and *Helicotylenchus* spp*.* (spiral nematodes), were identified from groundnut samples obtained during the 2012/13 season at harvest (Table 6). The head and tail of *D. africanus* (Fig. 17) and *Pratylenchus* spp*.* (Fig. 18) was identified by microscopy. Except for *Meloidogyne* spp., individuals from all other nematode species were identified from stored groundnut grain samples during 2012/13. Nematodes were more frequently isolated from groundnut hulls than from kernels irrespective of the grain collection time (Table 6). *Ditylenchus africanus* was predominant in Manguzi, with the highest prominence values (PV) of 427 and 31 and highest mean population densities of 565 and 44 in grain sampled at harvest. Following storage, *D. africanus* was also predominant in Manguzi with the highest prominence values (PV) of 2106 and 125 and highest mean population densities of 4711 and 198 in the sampled grain (Table 6). The frequency with which *D. africanus* was isolated in hulls (57) was lower when compared to *Pratylenchus* spp*.* (79) in Manguzi, at harvest. Conversely, *Pratylenchus* spp*.* (7) was less frequently isolated in kernels when compared to *D. africanus* (50) in grain samples following

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storage. Nematodes of the *Pratylenchus* spp*.* occurred most frequently (FO) in all three districts in grain sampled at harvest. Jozini had the least *D. africanus* PV, FO and mean population density levels both in the hulls (0.5, 25, 1) and kernels (0, 0, 0) at harvest when compared to the other districts evaluated (Tables 6). Moreover, no other nematodes were present in kernels from Jozini at harvest but *Pratylenchus* spp. were also obtained from the hulls (Table 6). In contrast, *D. africanus* and *Pratylenchus* spp. were not present in hulls from Jozini following storage and only *D. africanus* was identified from stored kernels (Table 6). *Meloidogyne* spp. occurred more frequently in the hulls and kernels in Manguzi as compared to Mbazwana at harvest; and did not occur following storage in kernels or hulls from both these districts. *Helicotylenchus* spp*.* were only found in the Manguzi district following storage with high mean population density in the hulls (837) than in kernels (7) although PV (3) and FO (20) in both hulls and kernels were the same.

Nematode identification in groundnut pegs, roots and soil before harvest during the 2013/14 season

Pratylenchus spp. were recorded from Jozini and Manguzi with Manguzi having the highest PV (55, 61 and 23), FO (100, 56 and 44) and mean population density (55, 82 and 35) in pegs, roots and the soil, respectively as compared to Jozini (Table 7). Also, *Pratylenchus* spp. were recorded in pegs (PV: 64, FO: 50, mean population density: 9) and roots (PV: 41, FO: 75, mean population density: 47) from Mbazwana but with no PV and mean population density in the soil, the nematode occurred least frequently (25) in the soil (Table 7). *Ditylenchus africanus* were only recorded from Manguzi, in the pegs (PV: 12, FO: 11, mean population density: 35) and *Helicotylenchus* spp. were only recorded from Jozini, in the soil (PV: 11, FO: 40, mean population density: 18) (Table 7). *Tylenchus* spp. were recorded from Manguzi and Mbazwana with PV (2 and 4), FO (11 and 50) and mean population density (6 and 6) in association with the pegs only, respectively (Table 7).

Nematode identification in groundnut hulls and kernels before harvest during the 2013/14 season

Fungivorous nematode genus *Tylenchus* were identified from groundnuts collected before harvest at Manguzi and Mbazwana districts (Table 8). In Manguzi, this genus occurred only in the kernels with PV, FO and mean population density of 1, 6.25 and 4, respectively (Table 8). *Tylenchus* spp. occurred equally frequent (9) and also with equal mean population densities (4) and PVs (1) in both the kernels and hulls of Mbazwana. The *Helicotylenchus* spp*.* only occurred in Manguzi, in the hulls with PV, FO and mean population density of 205, 6 and 837, respectively (Table 8). *Pratylenchus* spp. dominated in the hulls than in the kernels in all three

districts. Mean population density (402 and 76) and PV (298 and 56) of *Pratylenchus* spp., respectively were the highest in Mbazwana in both hulls and kernels although the FO (76) was the highest in Manguzi in the hulls (Table 8). *Ditylenchus africanus* levels also dominated in the hulls than in the kernels in Manguzi and Mbazwana, this species did not occur in Jozini groundnut hulls but occurred in the kernels with PV, FO and mean population density of 8, 20 and 17, respectively. Contrary to *Pratylenchus* spp., the mean population density (1486 and 207), PV (963 and 137) and FO (42 and 44) of *D. africanus* were the highest in Manguzi in both hulls and kernels, respectively (Table 8).

Nematode identification in groundnut hulls and kernels at harvest and storage during the 2013/14 season

In Manguzi, *D. africanus'* frequency of occurrence, PV and mean population density were higher in the hulls than in kernels both at harvest and storage. *Ditylenchus africanus* was recorded only in the hulls at harvest and only in the kernels following storage in Mbazwana, with PV, FO and mean population density of 8, 33 and 14, respectively at harvest and 5, 14 and 12, respectively following storage (Table 9). Interestingly, groundnuts from the Jozini district were free of plant-parasitic nematodes at harvest but low levels of *D. africanus* infestations were recorded following storage with PV (8.3 and 4), FO (100 and 100) and mean population density (8.3 and 4) in the hulls and kernels, respectively (Table 9). Similar to the 2012/13 season, *Helicotylenchus* spp*.* were only found in the Manguzi district following storage but only in the hulls with PV, FO and mean population density of 7, 75 and 8, respectively (Table 9). In Mbazwana, *Pratylenchus* spp. were identified only in the hulls both at harvest and storage with PV (105 and 6), FO (100 and 50) and mean population density higher (105 and 9) at harvest than at storage, respectively. Also in Manguzi, *Pratylenchus* spp. were identified only in the hulls at storage and at harvest, levels were identified in both hulls and kernels. Similar to *D. africanus*, the *Pratylenchus* spp. frequency of occurrence (69 and 15), PV (143 and 7) and mean population density (172 and 18) were higher in the hulls than in kernels at harvest, respectively in Manguzi (Table 9).

Aflatoxin contamination in groundnut kernels

During the 2012/13 season, none of the groundnut kernels from all three districts were contaminated with aflatoxins at harvest. However, relatively low levels of aflatoxins ranging from 0.00114 to 0.84758 ug/kg were detected after 3 months of storage with the highest level of 0.84758 ug/kg contamination observed at Manguzi (Fig. 19). Aflatoxin contamination levels both at harvest and storage for the 2012/13 were detected at very low levels of below 1.0 ug/kg. Aflatoxin contamination was independent of the different districts surveyed (Table 10).

During the 2013/14 season, groundnuts from Jozini collected before harvest, at harvest and following storage were free of aflatoxins. At Mbazwana, aflatoxin contamination was detected before harvest; no aflatoxins were detected at harvest while aflatoxin levels increased following storage. This result could be due to uneven sampling at harvest. The aflatoxin contamination before harvest, at harvest and following storage at Mbazwana was 24.12, 0 and 41.08 ug/kg, respectively (Fig. 20). Aflatoxin contamination before harvest at Manguzi was the highest during the 2013/14 season with samples measuring in excess of 500 ug/kg (limit of quantification). The aflatoxins levels decreased at harvest and following storage with zero contamination observed (Fig. 20). There were significant differences between aflatoxin production before harvest, at harvest and following storage in Jozini and Manguzi during the 2013/14 season (Table 11). There were no significant differences between aflatoxin contamination before harvest and Mbazwana district, however, significant differences were observed at harvest and at storage during the 2013/14 season (Table 11).

Relationship between plant-parasitic nematodes and aflatoxin production in groundnut kernels

Ninety-nine percent of the variance (R^2 = 0.9936) in aflatoxin contamination following storage could be explained by the changes in the number of plant-parasitic nematodes following storage in Manguzi, Mbazwana and Jozini districts during the 2012/13 season (Fig. 21). During the 2013/14 season, before harvest, 73% of the variance (R^2 = 0.7299) in aflatoxin contamination could be explained by the changes in the number of plant-parasitic nematodes in Manguzi, Mbazwana and Jozini districts (Fig. 22). During the 2013/14 season at harvest, the total variation (R^2 =1.000) in aflatoxin contamination could be explained by the changes in the number of plant-parasitic nematodes in the Manguzi, Mbazwana and Jozini districts (Fig. 23). Seventy-five percent of the variation in aflatoxin levels, measured in grain following storage, could be ascribed to changes in the number of plant-parasitic nematodes in the Manguzi, Mbazwana and Jozini districts (Fig. 24).

Climate data

Rainfall and maximum daily temperatures were obtained for the districts surveyed in this study (Table 12 and 13). Rainfall and temperature levels from other weather stations varied substantially from weather station 30982 (Tables 12). No rainfall or temperature data was recorded for 2012 with levels recorded only in November and December 2013. Rainfall and maximum day temperature increased in during 2014 (Tables 12 and 13). As expected, rainfall was the highest between December and January, as the area is a summer-rainfall area with increasingly less rainfall being measured toward April (Table 12).

DISCUSSION

This study highlighted significant differences in subsistence-produced maize and groundnut mycotoxin contamination between localities representing various districts of northern KZN. Moreover, the study provided valuable data on mycotoxin levels in these grains before harvest (groundnuts only), at harvest and following storage indicating the great risk for mycotoxin exposure in the immediate and surrounding communities. The South African government implemented new regulations since 2016 for deoxynivalenol and fumonisins B_1 and B_2 limits in maize. Maximum levels of 2 000 ug/kg for deoxynivalenol and 4 000 ug/kg for fumonisins B_1 and B_2 were set (Government Gazette, 2016). South African commercially produced maize is tested at grain silos, however there is a lack of such facilities available to subsistence farmers (www.sagl.co.za).

Contamination of maize grain with *Fusarium graminearum*, at harvest and following storage during both seasons, was the highest as compared to fungal levels of *F. verticillioides* and *A. flavus*. This study demonstrates for the first time under South African conditions, higher natural infection levels of *F. graminearum* at harvest and following storage than other commonly associated fungi. Interestingly, *F. verticillioides* and *A. flavus* levels were higher in grain collected at harvest than stored grain during 2012/13. However, higher fungal levels of *F. verticillioides* and *A. flavus* were measured in stored grain during the 2013/14 season. Therefore, seasonal effects, which can be caused by variation in temperature and rainfall, as well as storage conditions, play a major role in fungal infection of maize (Marin *et al.*, 2012). Higher rainfall levels observed in April 2012/13 as compared to 2013/14 could have contributed to the increased *F. verticillioides* and *A. flavus* levels observed in grain at harvest. However, increased *F. verticillioides* and *A. flavus* following storage suggests that other factors such as the extra grain moisture content and storage facilities may have contributed to the increased fungal contamination of grain.

In this study, maize grain sampled from Jozini district contained the highest *F. verticillioides*, *F. graminearum* and *A. flavus* levels. Moreover, maize sampled in this district had the highest fumonisin and aflatoxin contamination in maize grain and may represent a particular hotspot within northern KZN. This may be attributed to the fact that Jozini had the lowest percentage of farmer participation in this study, as determined in Chapter 2. Nonetheless, the majority of the farmers surveyed in Jozini practiced crop rotation but more than half the farmers did not remove plant residues before planting the next season's crop (Chapter 2). Although 100% of farmers sorted their maize grain, the fungal and mycotoxin contamination was still significant. The poor to moderate correlation between visual disease symptoms caused by *F. verticillioides* and *A. flavus* and mycotoxin contamination of maize grain is well established (Afolabi *et al.*, 2007; Small *et al.*, 2012; Rose *et al.*, 2017). The significantly higher fungal and mycotoxin levels indicates that communities in the Jozini district

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is at a higher risk for mycotoxin exposure as farmers surveyed indicated they consume their grain or sell it locally (Chapter 2).

Maize grain obtained from the Mbazwana and Manguzi districts were the least contaminated with mycotoxigenic fungi and their associated mycotoxins. The highest percentage of farmer participation was recorded in these districts with most farmers intercropping with groundnuts. In Manguzi, more than half the farmers surveyed removed plant residues from the field prior to planting while no residues were removed in Mbazwana (Chapter 2). All farmers sorted their maize grain with mouldy/diseased kernels generally being used as feed for chickens. Interestingly, Mbazwana, Manguzi and Jozini farmers used traditional, cultural or local maize seeds for planting (Chapter 2). The maize varieties may differ significantly between districts and their genetic background (resistance) as well as adaptation to the particular district may all contribute significantly to the differences in mycotoxin contamination between Jozini, Mbazwana and Manguzi. Unfortunately, no information on the maize varieties planted by farmers was obtained in this study.

The inability to detect deoxynivalenol (DON) and nivalenol (NIV) was contrary to the expectations associated with the high *F. graminearum* levels observed, considering the fungus can produce both these mycotoxins. Similarly, Abia *et al.* (2013) reported only fumonisins, aflatoxins, zearalenone and ochratoxin analysed by LC-MS/MS when assessing the occurrence of multi-mycotoxins in maize under natural infection. Fumonisin and aflatoxin contamination were the highest both at harvest and following storage during the 2012/13 and 2013/14 seasons, respectively. The natural co-occurrence of multi-mycotoxins in maize has been previously reported (Chilaka *et al.*, 2012; Pleadin *et al.*, 2012). Furthermore, the fungal quantification and multi-mycotoxin analyses showed that the presence of fungal target DNA doesn't necessarily correlate to mycotoxin production, under natural infection. However, further research is needed to validate this statement. There are many factors that could influence mycotoxin production by fungi including the prevailing environmental conditions (Lazzaro *et al.* 2012).

In this study, *D. africanus* was the most commonly isolated nematode species associated with groundnut hulls and kernels. This result is supported by earlier reports that documented *D. africanus* as the most common plant-parasitic nematode associated with groundnut hulls and kernels. (Venter *et al.*, 1992; Venter *et al.*, 1995; Mc Donald *et al.,* 2005; Steenkamp *et al.*, 2010). This may be due to the presence of *D. africanus* in all groundnut-producing areas in South Africa (McDonald *et al.*, 2005; Steenkamp *et al.*, 2010). The population density of *Ditylenchus africanus* obtained from groundnut hulls was higher than any other plant-parasitic nematode obtained from hull or kernel samples. The findings of this study also correlates with previous studies which reported low *D. africanus* population levels in soil as compared to high population levels in hull and kernel samples (Venter *et al.*, 1992; Venter

et al., 1995; McDonald *et al.,* 2005; Steenkamp *et al.*, 2010). Occurrence of *D. africanus* in groundnuts was restricted to South Africa and is a major problem as it downgrades the quality of groundnut kernels by 32-64% (McDonald *et al.*, 2005). However, another report by Steenkamp *et al.* (2010) stated that *D. africanus* may also occur in other southern African countries.

Pratylenchus spp. (root lesion nematode) was recently listed as the third economically most important plant-parasitic nematode genus worldwide (Jones *et al.*, 2013), although associated with groundnut during this study the species also has a wide host range (Singh *et al.*, 2013). *Pratylenchus brachyurus* Filipjev & Schuurmans Stekhoven (Castillo and Vovlas, 2007) is known to be a major pest of groundnuts worldwide (Singh *et al.*, 2013), including in South Africa (Van den Berg, 1971; Kleynhans *et al.*, 1996). This nematode pest invades, creates a path and travels through the cortex of the crop roots (Back *et al.*, 2002), and in groundnut hulls it causes lesions that adversely affect the development of these structures (Dickson and De Waele, 2005). Furthermore, low rainfall was mentioned as a factor leading to high economic damage by *Pratylenchus* spp. (Jones and Fosu-Nyarko, 2014) and could hence explain the high *Pratylenchus* spp. numbers in the Manguzi district that had very low rainfall levels when compared to other districts during both seasons. *Pratylenchus* spp. is also commonly associated with groundnut as it occurred in both seasons and all three districts. This nematode was found to occur more frequently and in high numbers as compared to *Meloidogyne* spp., *Helicotylenchus* spp. and *Tylenchus* spp.

The presence of relatively low population levels of *Meloidogyne*, ranked as the economically most important nematode pest worldwide (Jones *et al.*, 2013), from groundnut hull and kernel samples demonstrate their pest status towards groundnuts. This genus has been documented by Kleynhans *et al.* (1996) and Fourie *et al.* (2001) as a plant-parasitic nematode that infects the groundnut crop. Furthermore, although *Meloidogyne incognita* is not listed as one of the three major root-knot nematode species being associated with groundnut (Dickson & De Waele, 2005), this species was recorded to parasitise groundnut in South Africa (Kleynhans *et al.*, 1996). Also, *Meloidogyne Chitwood*, *Meloidogyne fallax* Karssen (1996) and *Meloidogyne hapla* Chitwood (1949) (Fourie *et al.*, 2001) have been recorded in association with groundnut under local environmental conditions. Jones *et al.* (2013) mentioned that suitable temperature and moisture is needed for the reproduction of *Meloidogyne* spp. and that, in certain cases root execudates and generation number in one season can influence the response of second-stage juveniles hatching. These factors could have impacted on the limited occurrence of these nematode pests at localities sampled during this study.

Helicotylenchus spp, also identified in this study, commonly occur in local soils where a range of agricultural crops are planted, including groundnut (Kleynhans *et al.*, 1996). Although their pathogenicity to such crops have not been investigated, this genus is not

suggested to be of concern to local farmers (Personal communication, Prof D. Fourie, Nematologist, North-West University) However, the species *Helicotylenchus dihystera* Cobb (1893) were found to occur in groundnut amongst other crops as a pathogen with a broad host range (Singh *et al.*, 2013). This study reports for the first time on the isolation of the fungivore genus *Tylenchus* associated with groundnut in South Africa. This was confirmed by using the South African Plant-Parasitic Nematode Survey (SAPPNS) database (Personal communication, Dr M. Marais, Nematologist, ARC-Plant Protection Research). Interestingly, *Tylenchus* spp. was only present in groundnut sampled before harvest during the 2013/14 seasons and its association with *A. flavus* and aflatoxin contamination requires further elucidation.

Groundnuts collected from the Manguzi district had more aflatoxin contamination as compared to other districts. Factors such as high soil temperatures and drought stress could have contributed to this result (Timper *et al.*, 2004). Poor to no rainfall was recorded at Tembe (30982) weather station which includes the Manguzi district suggesting drought conditions during the evaluation period. A positive correlation was found between number of plant-parasitic nematodes and aflatoxin contamination in Jozini, Manguzi and Mbazwana districts. This relationship was observed at harvest and following storage during the 2012/13 and 2013/14 seasons and also before harvest during the 2013/14 season. In terms of nematode-pathogen disease complexes, Abdel-Momen and Starr (1998) observed that *M. incognita* and *Rhizoctonia solani* relationship occurs in groundnut. Also, Timper *et al.* (2013) recently observed a nematode-pathogen disease complex between *Meloidogyne arenaria* Neal (1889) and *A. flavus*. However, these nematode-pathogen disease complexes were observed under controlled environments. Relationships between plant-parasitic nematodes and aflatoxin production found during this study, is supported by Timper *et al.* (2004) whereby a similar relationship in which aflatoxin production was influenced by nematode infection was observed. The study by Timper *et al.* (2004), however, established this relationship under drought stress conditions while the current study was conducted under natural conditions.

A correlation between a plant-parasitic nematode and fungal pathogen can be found in an environment where several biotic and abiotic factors are present (Akhtar and Malik, 2000). However, in terms of correlations between the fungivorous nematode genus *Tylenchus* and fungi (e.g. *Aspergillus flavus* and *Aspergillus parasiticus*), no literature is available (Back *et al.*, 2002). However interrelationships were found to exist between other plant-parasitic nematodes and fungi (Powell, 1971). Hence a future prospect would be to conduct glasshouse trials of traditional/local groundnut varieties from the three districts of the northern KZN and inoculate the seedlings with varying inoculum levels of the different nematode pests which were identified during this study. Different stress patterns could also be applied to the growing groundnuts, such as drought stress and high temperature which are known to favor aflatoxin

contamination of groundnuts (Timper *et al.*, 2013). For this reason, the breeding of drought-tolerant groundnut cultivars could be of great benefit. Korayem and Bondok (2013) stated that the groundnut cultivar type, environmental conditions and nematode population densities influences the nematodes severity to cause yield loss in groundnuts. Back *et al.*, (2002) also mentioned that plant cultivars and lines may be an important factor to consider during nematodes and fungal interactions.

Due to the extent of variation between nematodes at harvest and following storage at Jozini, Manguzi and Mbazwana districts, it would be of interest to further investigate these differences. Differences in soil types, concomitant environmental conditions and their effect on nematode population density may contribute to an increased understanding of nematode-disease/mycotoxin associations. The identification of some species in grain following storage but the lack of these at harvest within the same season could be attributed to nematode egg masses at harvest that may have hatched during storage.

The conduction of this survey on maize and groundnut grain quality, produced by subsistence farmers, yielded numerous experimental challenges that influenced statistical analyses and subsequent interpretations. Follow-up studies should attempt to obtain an equal number of samples from all districts so that the results may be more representative of the target population. Increasing the sample size or number of samples will reduce sampling error, providing better data on which to draw conclusions. Additionally, other statistical approaches may provide further insights into relationships between environments, pests and disease complexes.

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Table 1. *Fusarium verticillioides* target DNA levels in maize from different localities at harvest and storage during the 2012/13 and 2013/14 seasons.

District	Locality x Collection period	*F. verticillioides* target DNA	District	Locality x Collection period	*F. verticillioides* target DNA
	Stayland x Harvest	22.47 e		Belgrade x Harvest	735.76 de
	Stayland x Storage	32.72 e		Belgrade x Storage	61.96 e
	Maqweshe x Harvest	71.77 e		Intuthuko x Harvest	10.75 e
	Maqweshe x Storage	19.98 e		Intuthuko x Storage	35.57 e
	Bhobozana x Harvest	984.80 de		Ncotshane x Harvest	360.71 de
Vryheid	Bhobozana x Storage	909.34 de		Ncotshane x Storage	297.32 de
	Zwailaagte x Harvest	202.98 de		Madibheni x Harvest	386.09 de
	Thelezini x Harvest	304.64 de		Madibheni x Storage	512.61 de
	Thelezini x Storage	28.77 e		Manzabomvu x Harvest	143.04 e
	Ezidulini x Harvest	53.34 e		Manzabomvu x Storage	78.49 e
	Ezidulini x Storage	18.73 e		Mkhwakhweni x Harvest	106.21 e
	Hlahlindlela x Harvest	1692.81 c-e		Mkhwakhweni x Storage	89.56 e
	Hlahlindlela x Storage	68.45 e		Mdonini x Harvest	1839.02 b-e
	Ndlandla x Storage	9.29 e		Mdonini x Storage	1343.25 c-e
	Mangumeni x Harvest	406.72 de	Pongola	New stand x Storage	175.612 e
Mbazwana	Mbhulu x Harvest	1502.74 c-e		Msuzwaneni x Harvest	1279.00 c-e
	Othungwini x Harvest	46.25 e		Msuzwaneni x Storage	924.03 de
	Thusazana x Harvest	113.01 e		Khiphunyawo x Harvest	902.76 de

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	Othungwini x Storage	114.30 e		Khiphunyawo x Storage	19.35 e
Jozini	Impala x Harvest	2291.14 b-e		Kortnek x Harvest	1145.97 de
	Impala x Storage	4046.08 e		Kortnek x Storage	543.41 de
	Lundini x Harvest	34.47 e		Ngwabi x Harvest	525.91 de
	Lundini x Storage	33.54 e		Ngwabi x Storage	293.83 de
	Manyiseni x Harvest	1386.48 c-e		Dlomololo x Harvest	39.59 e
	Manyiseni x Storage	75.45 e		Kwa-Zondo x Harvest	31.03 e
	Lundini x Harvest	1762.64 b-e		Manyandeni x Harvest	860.34 de
Manguzi	Engozini x Harvest	308.72 de		Manyandeni x Storage	244.54 de
	Engozini x Storage	397.72 de		Mbomoba x Harvest	502.14 de
	Manguzi x Harvest	886.74 de		Mbomoba x Storage	80.08 e
	Thengani x Harvest	204.63 de		Mngamanzi x Harvest	67.17 e
	Makhanya x Harvest	2528.65 b-d		Mngamanzi x Storage	11800.59 a
	Thengani B x Harvest	89.64 e			
	Thengani x Storage	304.32 de		**LSD $_{(p= 0.05)}$ = 2352.4**	

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Table 2. *Fusarium graminearum* target DNA levels in maize from different localities at harvest and storage during the 2012/13 and 2013/14 seasons.

District	Locality x Collection period	*F. graminearum* target DNA	District	Locality x Collection period	*F. graminearum* target DNA
Vryheid	Bhobozana x Harvest	669.572 i-n	Pongola	Belgrade x Harvest	1779.37 c-n
	Bhobozana x Storage	1093.56 f-n		Belgrade x Storage	2702.79 b-h
	Ezidulini x Harvest	2910.56 b-f		Dlomololo x Harvest	3419.11 b-d
	Ezidulini x Storage	6895.61 a		Khiphunyawo x Harvest	2670.48 b-h
	Hlahlindlela x Harvest	2362.86 b-j		Khiphunyawo x Storage	2592.73 b-i
	Hlahlindlela x Storage	2445.46 b-j		Kortnek x Harvest	1540.19 d-n
	Stayland x Harvest	2122.02 b-m		Kortnek x Storage	2428.54 b-j
	Stayland x Storage	1484.13 d-n		Intuthuko x Harvest	1637.78 d-n
	Zwailaagte x Harvest	2454.03 b-j		Intuthuko x Storage	2166.98 b-m
	Zwailaagte x Storage	7432.22 a		Msuzwaneni x Harvest	2917.70 b-g
	Thelezini x Harvest	1428.64 e-n		Msuzwaneni x Storage	615.05 i-n
	Thelezini x Storage	2705.91 b-h		Ncotshane x Harvest	1202.96 e-n
	Maqweshe x Harvest	1014.94 g-n		Ncotshane x Storage	3656.49 bc
	Maqweshe x Storage	3865.32 b		Madibheni x Harvest	798.75 h-n
	Ndlandla x Storage	2001.93 b-n		Madibheni x Storage	2331.88 b-k
Mbazwana	Othungwini x Harvest	1538.06 d-n		Mbomoba x Harvest	502.14 de
	Othungwini x Storage	1514.06 d-n		Manzabomvu x Harvest	2053.92 b-n

Jozini	Impala x Harvest	1005.95 g-n	Manzabomvu x Storage	1436.12 e-n
	Impala x Storage	111.03 n	New stand x Harvest	3040.34 b-f
	Lundini x Harvest	1271.23 e-n	New stand x Storage	3773.13 b
	Lundini x Storage	2736.78 b-h	Mdonini x Harvest	2553.62 b-i
	Manyiseni x Harvest	1581.13 e-n	Mdonini x Storage	2658.00 b-h
	Manyiseni x Storage	1474.61 d-n	Mkhwakhweni x Harvest	1960.90 b-n
	Ndumu x Harvest	2246.75 b-k	Mkhwakhweni x Storage	2086.56 b-m
	Lundini x Harvest	961.82 g-n	Mngamanzi x Harvest	529.73 j-n
Manguzi	Engozini x Harvest	2136.71 b-m	Mngamanzi x Storage	6455.34 a
	Engozini x Storage	1661.05 d-n	Ngwabi x Harvest	1121.55 f-n
	Makhanya x Harvest	926.72 h-n	Ngwabi x Storage	2135.71 b-m
	Makhanya x Storage	3057.28 b-f	Mbhulu x harvest	1502.74 c e
	Thengani x Harvest	838.49 h-n	Mangumeni x Harvest	222.40 l n
	Thengani x Storage	949.39 g-n	Manyandeni x Harvest	1616.76 d n
	Thengani B x Harvest	1553.37 d-n	Manyandeni x Storage	2357.13 b k
	Manguzi x Harvest	1415.27 e-n	**LSD** $_{(p=0.05)}$ **= 1978.70**	

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Table 3. *Aspergillus flavus* target DNA levels in maize from different localities at harvest and storage during the 2012/13 and 2013/14 seasons, respectively.

District	Locality x Collection period for the 2012/13 season	*A. Flavus* target DNA		District	Locality x Collection period for the 2013/14 season	*A. Flavus* target DNA	
Vryheid	Thelezini x Harvest	3.92	d	**Vryheid**	Thelezini x Storage	4.84	e
	Impala x Harvest	388.30	b				
	Ndumu x Storage	22.86	c		Impala x Storage	877.44	a
Jozini				**Jozini**	Ndumu x Storage	498.63	b
					Manyiseni x Harvest	4.15	e
	Manyandeni x Harvest	445.78	a		Msuzwaneni x Harvest	0.87	e
Pongola	Mngamanzi x Harvest	1.35	d	**Pongola**	Msuzwaneni x Storage	124.89	c
					Mangumeni x Harvest	1.48	e
					Mdonini x Harvest	0.68	e
Mbazwana	Othungwini x Storage	3.59	d	**Mbazwana**			
Manguzi				**Manguzi**	Engozini x Harvest	2.34	e
					Thengani x Storage	2.04	e
LSD$_{(p = 0.05)}$ = 18.7324 (2012/13 season)				**LSD$_{(p = 0.05)}$ = 4.9215 (2013/14 season)**			

All other localities had a 0.0 d mean *A. flavus* target DNA value for the 2012/13 season and 0.0 e mean *A. flavus* target DNA value for the 2013/14 season (data not shown).

Table 4. Fumonisin levels in maize from different localities during the 2012/13 and 2013/14 seasons, respectively.

District	Locality x Season	Fumonisins	District	Locality x Season	Fumonisins
	Bhobozana x 2012/13	9.62 c-e		Belgrade x 2012/13	1.12 e
	Bhobozana x 2013/14	0.74 e		Belgrade x 2013/14	0.00 e
	Hlahlindlela x 2012/13	7.10 c-e		Intuthuko x 2012/13	0.11 e
	Hlahlindlela x 2013/14	0.00 e		Intuthuko x 2013/14	0.00 e
	Maqweshe x 2012/13	0.08 e		Khiphunyawo x 2012/13	1.08 e
	Maqweshe x 2013/14	0.00 e		Khiphunyawo x 2013/14	10.16 c-e
Vryheid	Ndlandla x 2012/13	0.10 e		Kortnek x 2012/13	1.51 de
	Ndlandla x 2013/14	0.00 e		Kortnek x 2013/14	6.89 c-e
	Stayland x 2012/13	0.15 e		Manyandeni x 2012/13	2.90 c-e
	Thelezini x 2012/13	11.45 cd		Manyandeni x 2013/14	0.95 e
	Thelezini x 2013/14	0.00 e		Manzabomvu x 2013/14	0.00 e
	Ezidulini x 2012/13	8.53 c-e		Msuzwaneni x 2012/13	0.85 e
	Othungwini x 2012/13	0.15 e		Msuzwaneni x 2013/14	3.00 c-e
Mbazwana	Othungwini x 2013/14	0.54 e	**Pongola**	Kwa-Zondo x 2013/14	0.20 e
	Impala x 2012/13	8.32 c-e		New stand x 2012/13	3.85 c-e
	Impala x 2013/14	10.10 c-e		New stand x 2013/14	0.02 e
Jozini	Lundini x 2012/13	0.13 e		Madibheni x 2012/13	2.56 de
	Lundini x 2013/14	13.11 c		Madibheni x 2013/14	0.08 e
	Myeni x 2012/13	39.64 a		Mkhwakhweni x 2013/14	0.38 e

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	Myeni x 2013/14	24.67 b		Manzabomvu x 2012/13	1.44 de
	Manyiseni x 2012/13	0.56 e		Ngwabi x 2012/13	0.23 e
	Manyiseni x 2013/14	6.36 c-e		Ngwabi x 2013/14	0.30 e
	Ndumu x 2012/13	2.00 de		Mbhulu x 2013/14	0.00 e
	Ndumu x 2013/14	7.22 c-e		Ncotshane x 2012/13	7.59 c-e
	Engozini x 2012/13	0.15 e		Mngamanzi x 2012/13	43.27 a
	Engozini x 2013/14	1.19 de		Mngamanzi x 2013/14	1.45 de
	Makhanya x 2012/13	8.35 c-e		Mangumeni x 2013/14	0.36 e
	Thengani x 2012/13	2.10 de		Thusazana x 2012/13	2.14 de
Manguzi	Thengani x 2013/14	0.20 e		Thusazana x 2013/14	0.00 e
	Manguzi x 2012/13	9.09 c-e		Dlomololo x 2013/14	0.33 e
	ThenganiB x 2012/13	0.03 e		Mdonini x 2012/13	0.40 e
	Thengani B x 2013/14	0.00 e		Mdonini x 2013/14	1.23 de
	LSD $_{(p = 0.05)}$ = 10.329			Mbomoba x 2013/14	1.63 de

Table 5. Aflatoxin levels in maize from different localities during the 2012/13 and 2013/14 seasons, respectively.

District	Locality x Season	Aflatoxins	District	Locality x Season	Aflatoxins
Vryheid	Bhobozana x 2013/14	14.90 fg		Belgrade x 2012/13	0.05 g
	Ndlandla x 2013/14	13.91 fg		Belgrade x 2013/14	250.00 d
	Thelezini x 2013/14	203.02 de		Mngamanzi x 2012/13	0.01 g
	Thengani x 2013/14	97.92 e-g		Mngamanzi x 2013/14	3.60 g
	Manqweshe x 2013/14	22.51 fg		New stand x 2012/13	0.03 g
	Ezidulini x 2012/13	0.02 g		Mdonini x 2013/14	44.70 fg
Mbazwana	Othungwini x 2013/14	13.03 fg		Mkhwakhweni x 2013/14	39.12 f
Jozini	Impala x 2012/13	0.09 g		Manyandeni x 2013/14	141.21 d-f
	Impala x 2013/14	1000.00 b		Madibheni x 2013/14	5.50 g
	Lundini x 2013/14	2000.00 a	Pongola	Intuthuko x 2013/14	1.83 g
	Myeni x 2013/14	1000.00 b		Kortnek x 2013/14	500.00 c
	Ndumu x 2013/14	1000.00 b		New stand x 2013/14	28.60 fg
	Manyiseni x 2013/14	100.60 e-g		Msuzwaneni x 2013/14	138.02 d-f
Manguzi	Engozini x 2013/14	80.70 e-g		Ngwabi x 2013/14	2.35 g
	ThenganiB x 2013/14	39.84 fg		LSD $_{(p=0.05)}$ = 132.39	

All other localities during the 2012/13 and 2013/14 seasons had 0.0g mean aflatoxin values.

Table 6. Prominence value (PV), frequency of occurrence (FO) and mean population density of *Ditylenchus africanus, Pratylenchus* spp., *Meloidogyne* spp. and *Helicotylenchus* spp. from groundnut hulls and kernels at three districts in northern KwaZulu-Natal during the 2012/13 season at harvest and at storage**.** Values based on 5.0 g of hulls and kernels, respectively.

Districts	Plant parasitic nematodes	At harvest						At storage					
		Prominence value (PV)		Frequency of occurrence		Mean population		Prominence value (PV)		Frequency of occurrence		Mean population	
		Hulls	*Kernels*	*Hulls*	*Kernels*	*Hulls*	*Kernels*	*Hulls*	*Kernels*	*Hulls*	*Kernels*	*Hulls*	*Kernels*
Jozini	*Ditylenchus*	0.5	0	25	0	1	0	0	3	0	20	0	7
	Pratylenchus spp.	13	0	50	0	19	0	-	-	-	-	-	-
Manguzi	*Ditylenchus*	427	31	57	50	565	44	2106	125	20	40	4711	198
	Pratylenchus spp.	60	0.3	79	7	67	1	32	16	60	20	41	35
	Meloidogyne spp.	11	5	43	14	17	13	-	-	-	-	-	-
	Helicotylenchus	-	-	-	-	-	-	3	3	20	20	837	7
Mbazwana	*Ditylenchus*	96	12	27	18	183	28	-	-	-	-	-	-
	Pratylenchus spp.	17	0.9	100	18	17	2	364	0	67	0	445	0
	Meloidogyne spp.	21	35	9	9	69	117	-	-	-	-	-	-

"-" indicates no nematodes were identified

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Table 7. Prominence value (PV), frequency of occurrence (FO) and mean population density (per 5g of hulls and kernels, respectively) of nematode species isolated from groundnut pegs, roots and rhizosphere soil at three districts in northern KwaZulu-Natal during the 2013/14 season before harvest.

Districts	Plant parasitic nematodes	Prominence value (PV)			Frequency of occurrence (FO)			Mean population density		
BEFORE HARVEST		*Pegs*	*Roots*	*Soil*	*Pegs*	*Roots*	*Soil*	*Pegs*	*Roots*	*Soil*
Jozini	*Pratylenchus* spp.	0.4	8.0	3.0	20.0	40.0	20.0	1.0	12.0	6.0
	Helicotylenchus spp.	0.0	0.0	11.0	0.0	0.0	40.0	0.0	0.0	18.0
Manguzi	*Ditylenchus africanus*	12.0	0.0	0.0	11.0	0.0	0.0	35.0	0.0	0.0
	Pratylenchus spp.	55.0	61.0	23.0	100.0	56.0	44.0	55.0	82.0	35.0
	Tylenchus spp.	2.0	0.0	0.0	11.0	0.0	0.0	6.0	0.0	0.0
Mbazwana	*Pratylenchus* spp.	64.0	41.0	0.0	50.0	75.0	25.0	9.0	47.0	0.0
	Tylenchus spp.	4.0	0.0	0.0	50.0	0.0	0.0	6.0	0.0	0.0

Table 8. Prominence value (PV), frequency of occurrence (FO) and mean population density (per 5g of hulls and kernels, respectively) of nematode species isolated from groundnut hulls and kernels at three districts in northern KwaZulu-Natal during the 2013/14 season before harvest.

Before harvest

Districts	Plant parasitic nematodes	Prominence value (PV)		Frequency of occurrence (FO)		Mean population density	
		Hulls	*Kernels*	*Hulls*	*Kernels*	*Hulls*	*Kernels*
Jozini	*Ditylenchus africanus*	0.0	8.0	0.0	20.0	0.0	17.0
	Pratylenchus spp.	9.0	0.0	20.0	0.0	21.0	0.0
	Ditylenchus africanus	963.0	137.0	42.0	44.0	1486.0	207.0
Manguzi	*Pratylenchus* spp.	199.0	7.0	76.0	19.0	228.0	15.0
	Helicotylenchus spp.	205.0	0.0	6.0	0.0	837.0	0.0
	Tylenchus spp.	0.0	1.0	0.0	6.3	0.0	4.0
	Ditylenchus africanus	375.0	14.0	27.0	9.0	722.0	46.0
Mbazwana	*Pratylenchus* spp.	298.0	56.0	55.0	55.0	402.0	76.0
	Tylenchus spp.	1.0	1.0	9.0	9.0	4.0	4.0

Table 9. Prominence value (PV), frequency of occurrence (FO) and mean population density (per 5g of hulls and kernels, respectively) of *Ditylenchus africanus, Pratylenchus* spp., *Meloidogyne* spp. and *Helicotylenchus* spp. from groundnut hulls and kernels at three districts in northern KwaZulu-Natal during the 2013/14 season at harvest and following storage.

Districts	Plant parasitic nematodes	At harvest						At storage					
		Prominence value (PV)		Frequency of occurrence (FO)		Mean population density		Prominence value (PV)		Frequency of occurrence (FO)		Mean Population density	
		Hulls	*Kernels*	*Hulls*	*Kernels*	*Hulls*	*Kernels*	*Hulls*	*Kernels*	*Hulls*	*Kernels*	*Hulls*	*Kernels*
Jozini	*Ditylenchus africanus*	-	-	-	-	-	-	8.3	4	100	100	8.3	4
Manguzi	*Ditylenchus africanus*	143	7	69	15	172	18	105	5	50	33	149	8
	Pratylenchus spp.	40	1	100	8	40	4	98	0	75	0	113	0
	Helicotylenchus spp.	-	-	-	-	-	-	7	0	75	0	8	0
Mbazwana	*Ditylenchus africanus*	8	0	33	0	14	0	0	5	0	14	0	12
	Pratylenchus spp.	105	0	100	0	105	0	6	0	50	0	9	0

"-" indicates no nematodes were identified

Table 10. Fisher's exact test results for mean aflatoxin levels in groundnut kernels from Jozini, Manguzi and Mbazwana districts at storage during the 2012/13 season.

P-value (Two-tailed)	1.000
alpha	0.05

As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.

Table 11. Fisher's exact test (significance by cell) results for mean aflatoxin levels in groundnut kernels from Jozini, Manguzi and Mbazwana districts before harvest, at harvest and at storage during the 2013/14 season.

	Aflatoxins (ug/kg^{-1}) before harvest	Aflatoxins (ug/kg^{-1}) at harvest	Aflatoxins (ug/kg^{-1}) at storage
Jozini	<	<	<
Manguzi	>	>	<
Mbazwana	<	<	>

Arrows displayed in red are significant at the level alpha=0.05

Table 12. Daily average and monthly total rainfall for seasons 2012/13 and 2013/14 including the months during which the farmers started planting (November -December) until physiological maturity of grain (April).

Station code	Year	2012 season						2013 season						2014 season					
	Rain	*Jan*	*Feb*	*Mar*	*Apr*	*Nov*	*Dec*	*Jan*	*Feb*	*Mar*	*Apr*	*Nov*	*Dec*	*Jan*	*Feb*	*Mar*	*Apr*	*Nov*	*Dec*
30109	Av	3.6	4.4	1.9	0.5	4.6	11.3	4.3	4.7	2	2.9	3.5	7.2	1.4	3.4	4	0.1	0.1	3.8
	Total	111.	126.	59.4	13.5	139.	350	134.	132.	61.5	86.6	104.	223.	42.4	95.8	123.	3.3	3.6	117.9
30535	Av	2.5	3	2.3	0	2.5	5.1	8.4	2.4	4.1	2.7	3.4	5.7	0.6	1.7	8	0.6	2.6	4.2
	Total	76.5	88.1	72.6	0.1	75.4	157.	126.	68.1	125.	80.2	100.	175.	18.9	46.3	247.	18.5	77.4	124.6
30621	Av	1.8	5.4	1.4	0.6	2.6	4.4	5	3.6	1.4	2.8	3.5	4	1.4	1.3	3.8	0.4	3.2	3.2
	Total	56.9	155.	42.2	17.5	77	135.	156	99.8	41.9	82.8	105.	123.	44.7	36.1	118.	11.2	94.8	98.3
30681	Av	2.1	3.4	4.7	0.5	1.6	2.1	5.1	0.3	0.9	0.7	2.5	5.2	0.9	2.7	8.4	1.2	2.3	0.7
	Total	64.8	97.8	147.	15.2	48.5	65.5	156.	8.1	29.2	19.6	75.7	160.	28.2	74.2	260.	35.6	69.9	21.3
30729	Av	0.5	2.2	3.7	0.3	2.1	3	5.5	0.3	0.9	0.9	2.9	4.5	1.5	2.4	9.1	0.6	2.2	0.9
	Total	15.1	65	113.	7.4	63.1	91.9	170.	8.1	26.2	26.1	87.6	139.	46	67.6	282.	18.4	65.8	27.8
30836	Av	1.7	3	2.2	0.6	4.1	4	4.2	4.6	2.3	2.6	4.2	4.8	3	1.9	3.6	0.2	3.8	4.0
	Total	52.8	86.4	69.3	17.5	121.	122.	129	130.	70.4	78.7	124.	147.	92.5	52.8	110.	6.6	114.	123.4
30982	Av	-	-	-	-	-	-	-	-	-	-	2.6	8.1	2.1	2.3	11	1.2	1.8	3.6
	Total	-	-	-	-	-	-	-	-	-	-	77.2	249.	65.5	63.5	339.	35.8	55.1	110.0

Districts were covered within different stations represented by a specific code e.g. 30109: Dundee Res Station (Vryheid); 30535: Pongola; SASRI Experimental Farm (Pongola); 30621: Piet Retief; Sulphur Springs (Pongola); 30681: Mkuzi (Mbazwana); 30729: Makatini (Jozini); 30836: Bloodriver (Vryheid); 30982: Tembe (Manguzi).

Table 13. Daily average and monthly maximum temperatures for seasons 2012/13 and 2013/14 including the months during which the farmers started planting (November -December) until physiological maturity of grain (April).

Station code	Year / Maximum temperature (ºC)	2012 season						2013 season						2014 season					
		Jan	Feb	Mar	Apr	Nov	Dec	Jan	Feb	Mar	Apr	Nov	Dec	Jan	Feb	Mar	Apr	Nov	Dec
30109	Av	27.9	29.8	27.3	23.9	26.1	26.7	26.6	28.5	25.7	23.6	27.3	24.8	28.1	27.7	25.8	23.9	25.1	26.8
	Total	862.5	924.6	846.3	716.4	783.6	827.9	825.7	797.6	797.2	871.7	819.9	767.8	870.4	776.3	799.3	716.8	752.5	831.0
30535	Av	31.3	31.6	29.8	28.3	27.5	30.2	30.7	30.9	29.5	27.4	29.5	27.3	31.9	32.7	29.7	28.4	27.3	29.5
	Total	969.7	978.7	924.9	651.3	826.1	936.9	460.2	865.3	914.9	1,012.5	884.6	844.7	988.7	916.5	919.8	852.5	819.1	884.6
30621	Av	26.9	28.1	26.7	24.7	24.5	26.6	26.4	26.9	26.0	24.9	26.3	24.9	27.9	29.1	27.1	25.4	24.1	26.6
	Total	860.3	871.0	828.0	739.7	735.1	825.4	817.5	644.7	805.4	922.0	789.3	770.3	1,005.8	959.0	974.8	888.2	722.1	824.2
30681	Av	32.8	32.8	30.9	28.3	28.3	31.4	31.4	32.3	31.0	29.3	29.6	28.3	32.2	32.9	30.6	28.7	28.1	31.2
	Total	1,016.9	1,016.1	957.8	847.5	848.5	972.2	971.8	903.4	959.9	1,084.7	888.4	878.1	997.9	921.6	949.3	861.9	842.1	965.9
30729	Av	33.4	33.7	31.8	29.5	29.3	32.0	32.2	33.4	32.3	29.8	30.5	29.3	33.4	34.4	31.2	29.3	28.7	32.0
	Total	1,034.6	976.7	986.8	883.6	880.2	991.2	998.6	934.4	1,000.7	1,104.2	913.7	908.9	1,034.1	963.0	965.7	880.0	860.0	992.6
30836	Av	31.2	32.5	30.1	26.5	28.2	29.4	29.3	31.5	28.2	25.4	29.2	26.9	31.0	30.7	27.9	26.6	26.5	29.0
	Total	966.9	1,006.5	933.3	794.7	845.9	910.2	908.4	880.8	874.7	941.0	875.1	833.1	960.1	858.2	865.8	780.0	795.7	897.6
30982	Av	-	-	-	-	-	-	-	-	-	-	31.7	30.1	33.7	34.4	32.7	31.0	29.8	32.1
	Total	-	-	-	-	-	-	-	-	-	-	950.2	933.2	1,045.5	964.1	1,012.3	928.9	893.5	995.9

Districts were covered within different stations represented by a specific code e.g. 30109: Dundee Res Station (Vryheid); 30535: Pongola; SASRI Experimental Farm (Pongola); 30621: Piet Retief; Sulphur Springs (Pongola); 30681: Mkuzi (Mbazwana); 30729: Makatini (Jozini); 30836: Bloodriver (Vryheid); 30982: Tembe (Manguzi).

Figure 1. Maize cobs selected from samples collected from subsistence farmers in northern KwaZulu-Natal. Mould growth suspected to be due to *F. verticillioides* and *A. flavus* is apparent on the maize cobs (Photos by S. Phokane).

Figure 2. Groundnut kernels with intact hulls (a) and groundnut kernels with hulls removed (b). These groundnut samples were collected from subsistence farmers in three districts of the northern KwaZulu-Natal (Photo by S. Phokane).
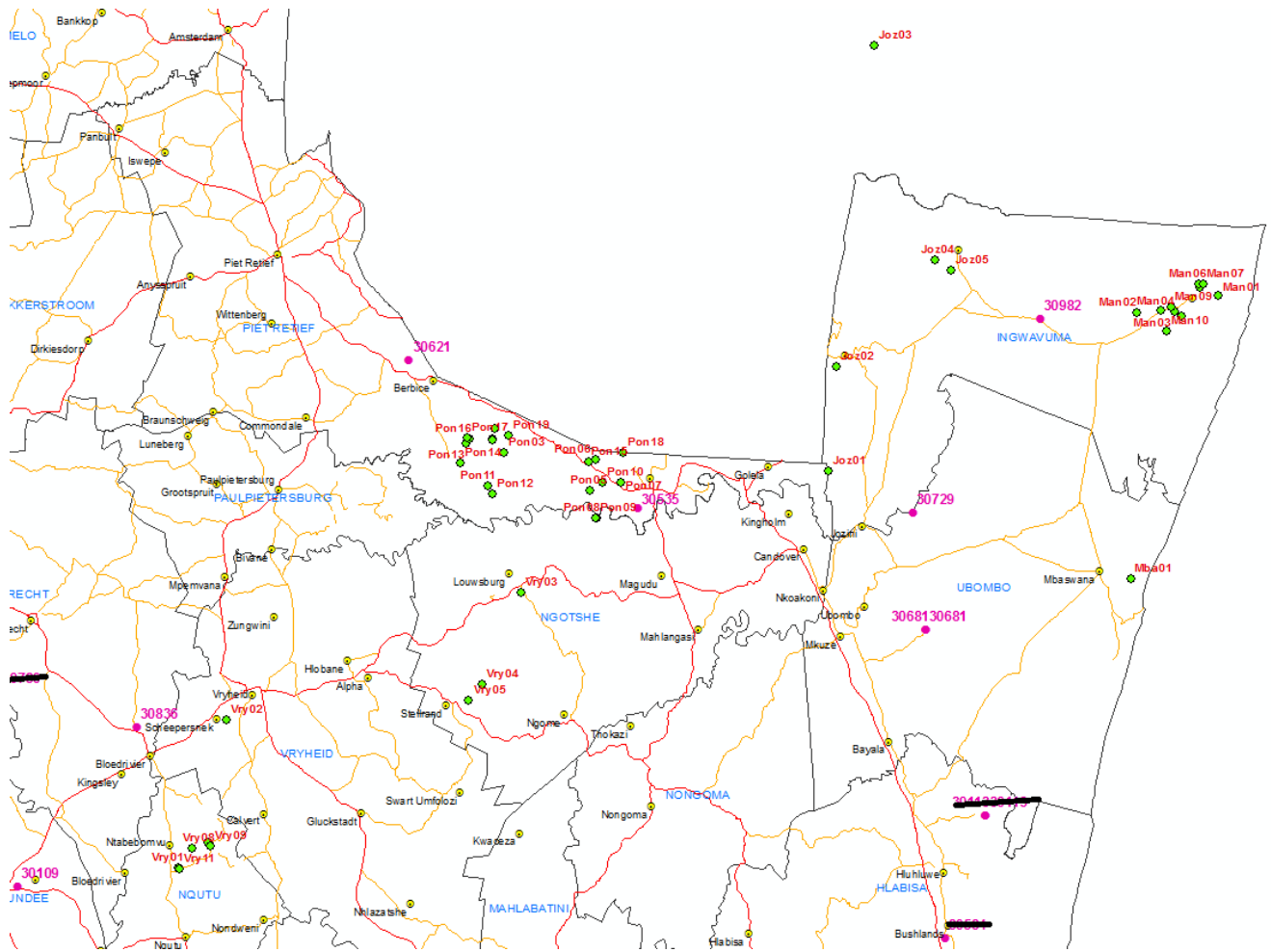
Figure 3. Northern KwaZulu-Natal map showing different districts, GPS locations (e.g. Pon 12) and codes (e.g. 30621). The codes were used to distinguish between different weather stations with each code containing information about rainfall and maximum daily temperature.
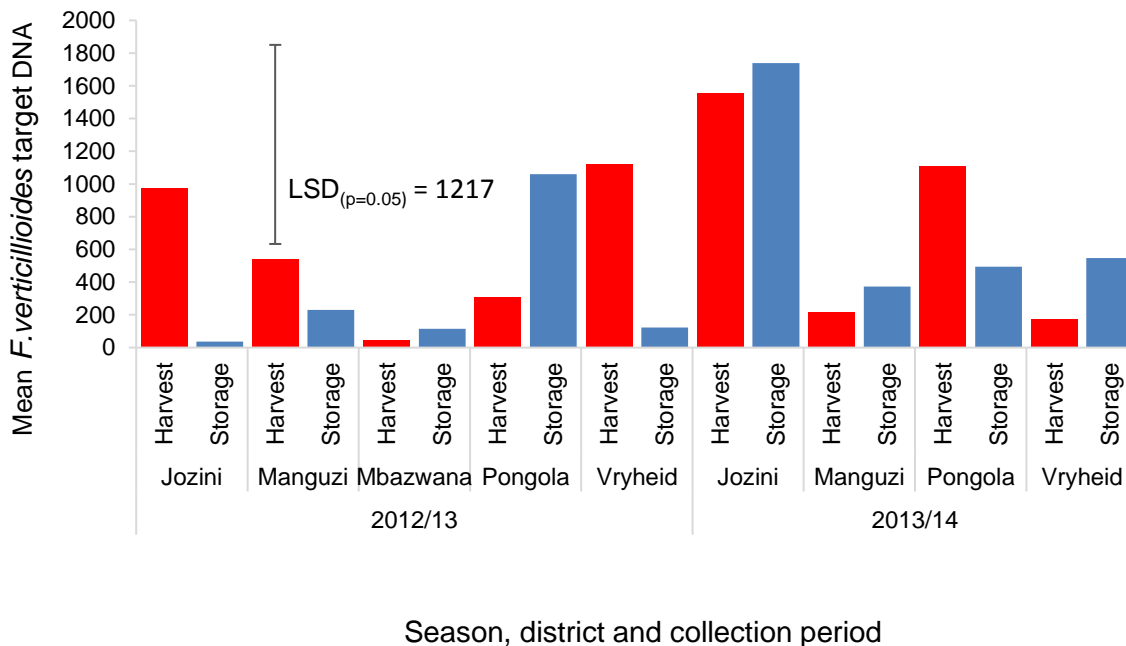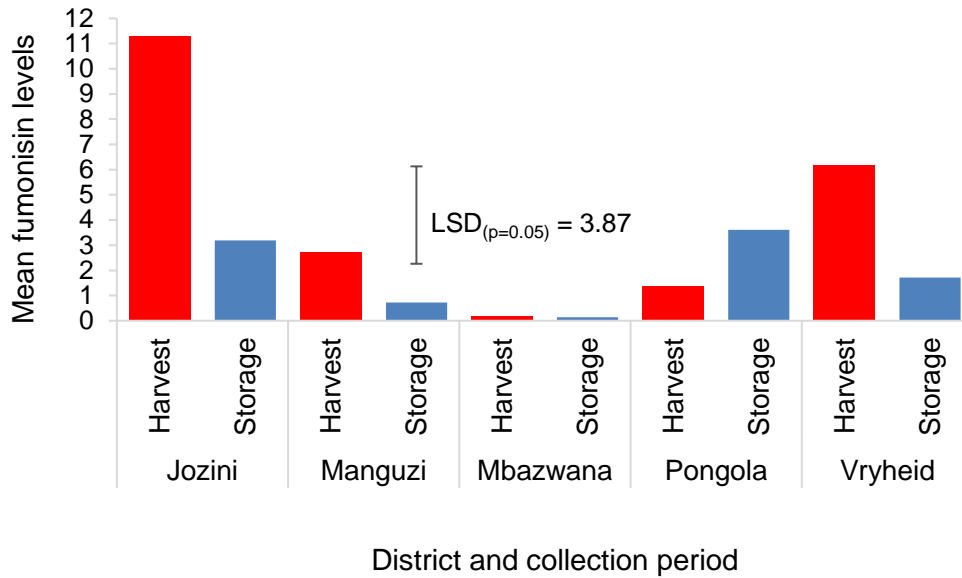
Figure 4. Mean *Fusarium verticillioides* target DNA levels in maize grain in five districts at two collection periods (harvest and storage) during the 2012/13 and 2013/14 seasons.
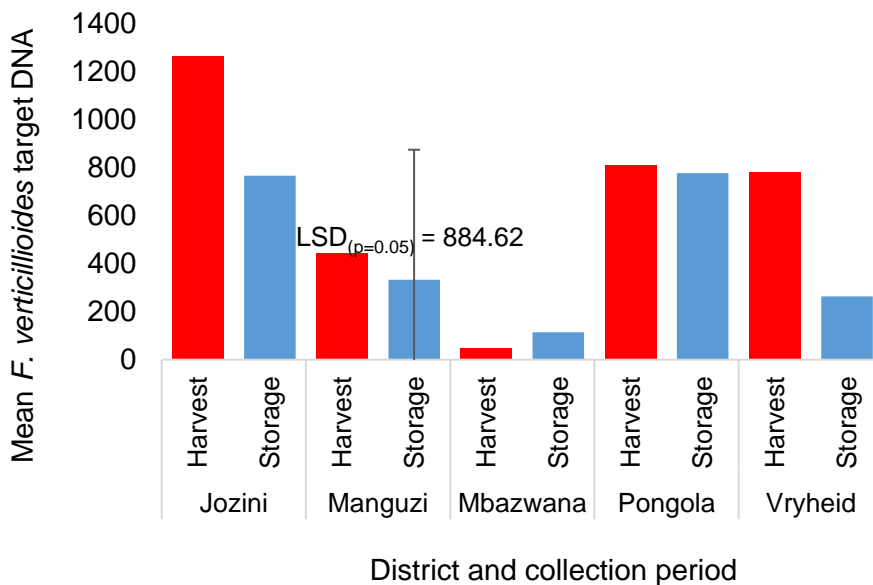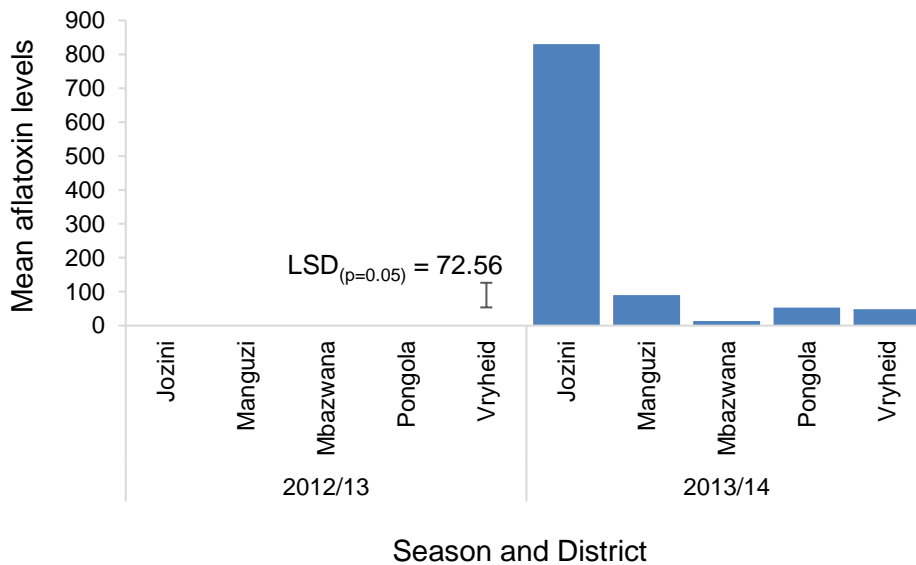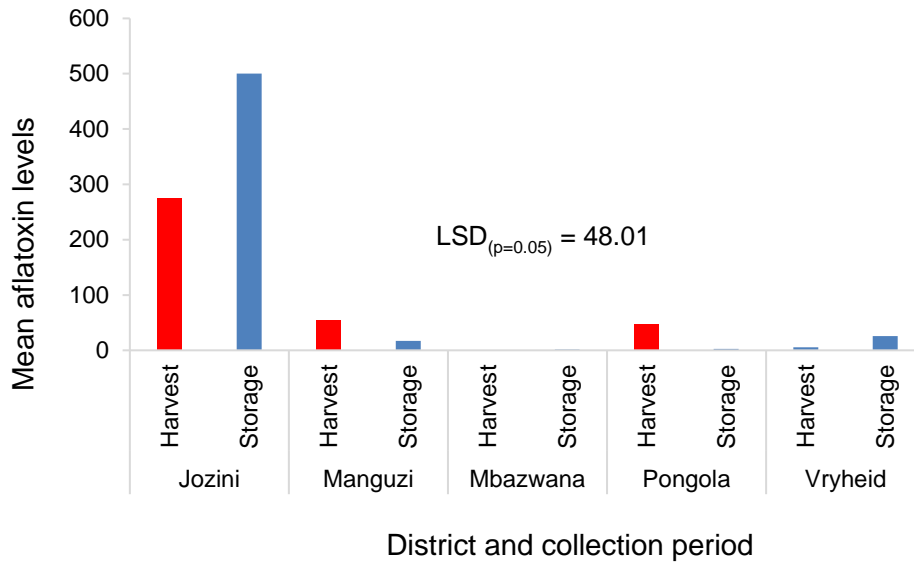

Figure 5. Mean *Fusarium verticillioides* target DNA levels in maize grain in five districts at two collection periods (harvest and storage) across the 2012/13 and 2013/14 seasons.

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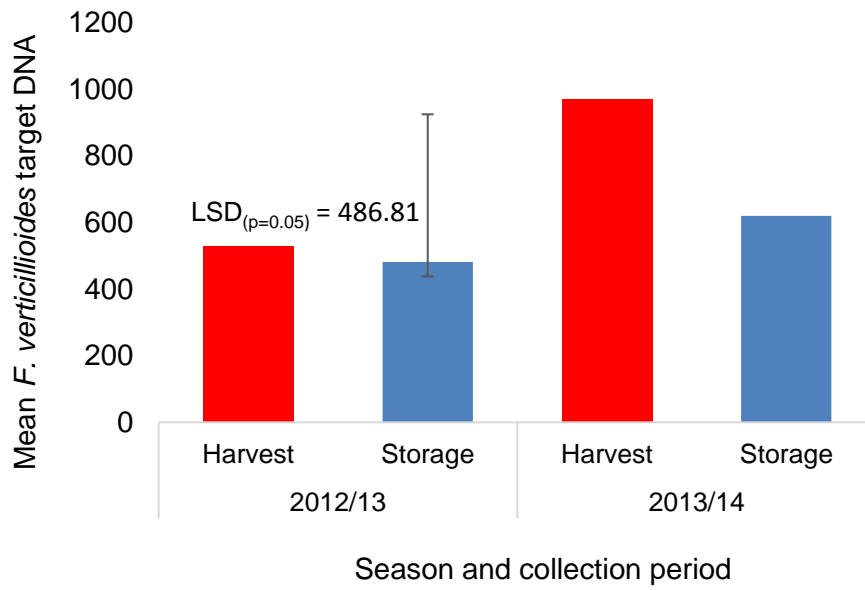
Figure 6. Mean *Fusarium verticillioides* target DNA levels in maize grain in five districts at two collection periods (harvest and storage) during the 2012/13 and 2013/14 seasons.
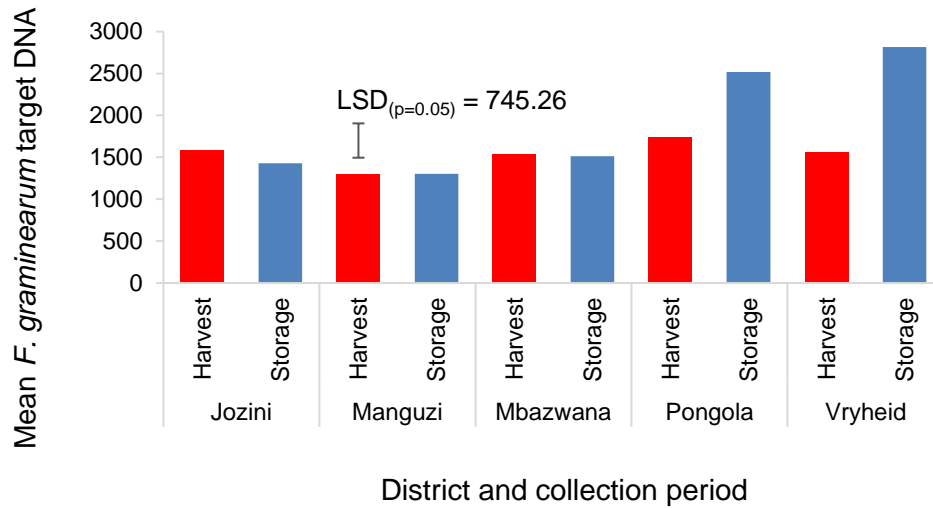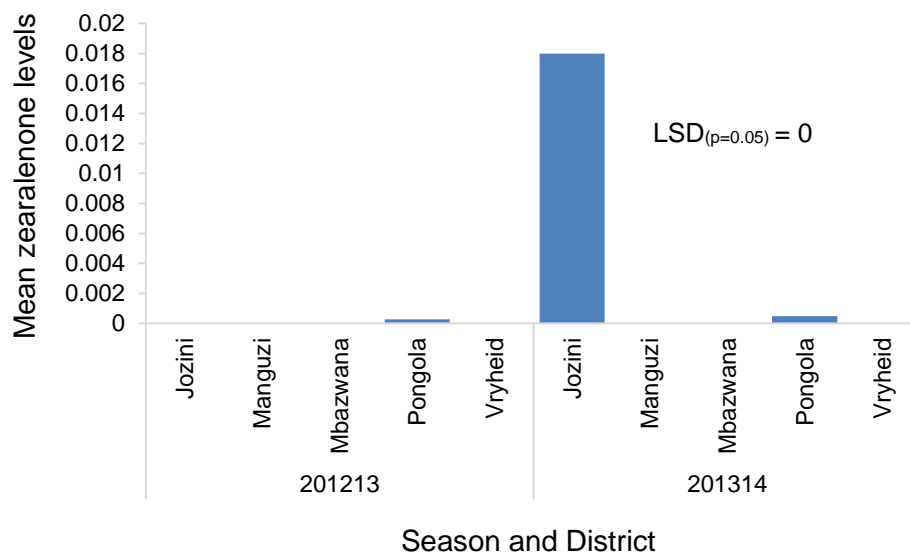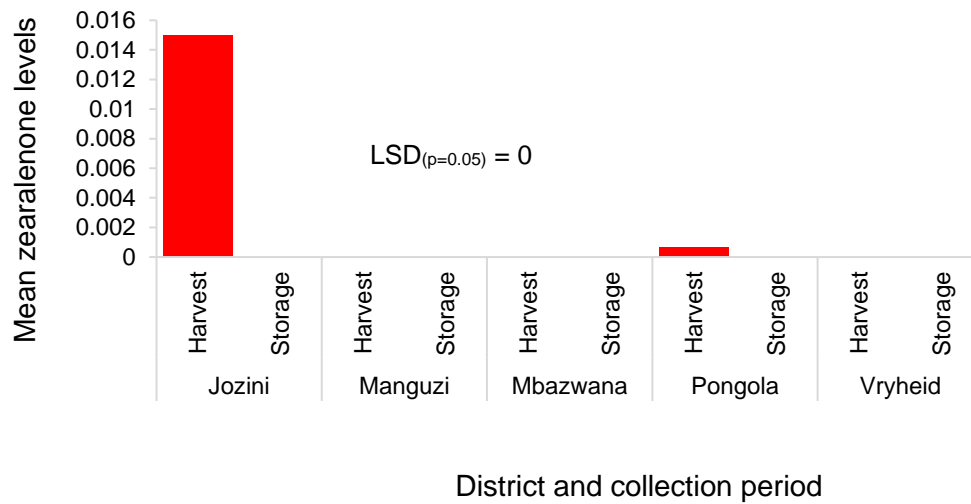

Figure 7. Mean *Fusarium graminearum* target DNA levels in maize grain in five districts at two collection periods (harvest and storage) during the 2012/13 and 2013/14 seasons.
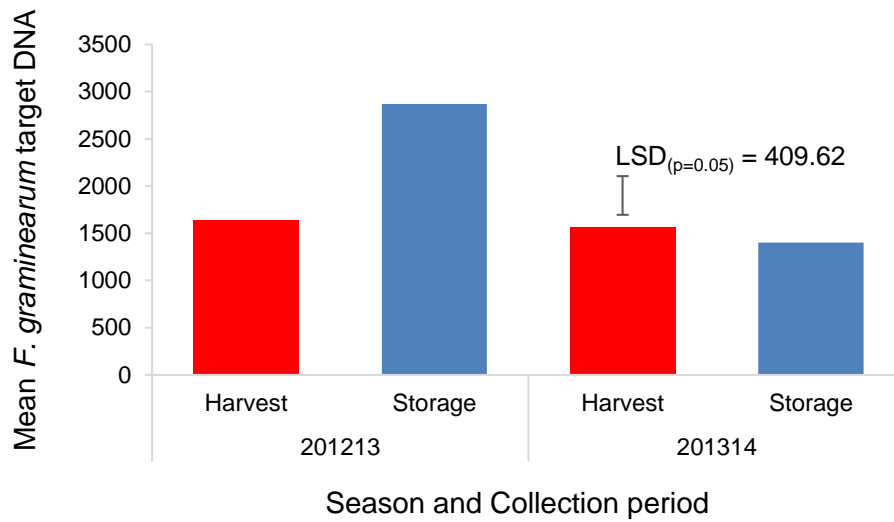
Figure 8. Mean *Fusarium graminearum* target DNA levels in maize grain in five districts at two collection periods (harvest and storage) during the 2012/13 and 2013/14 seasons.
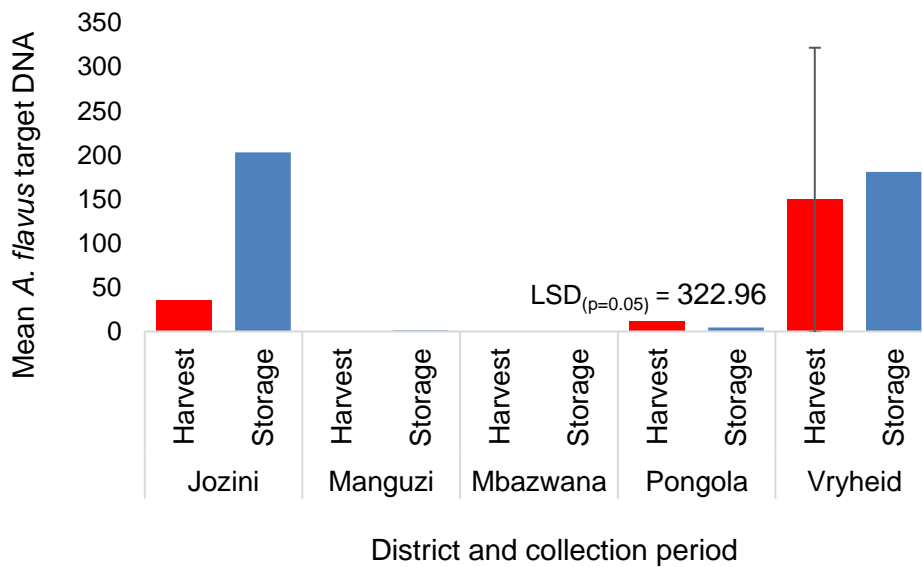
**Figure 9.** Mean *Aspergillus flavus* target DNA levels in maize grain in five districts at two collection periods (harvest and storage) during the 2012/13 and 2013/14 seasons.

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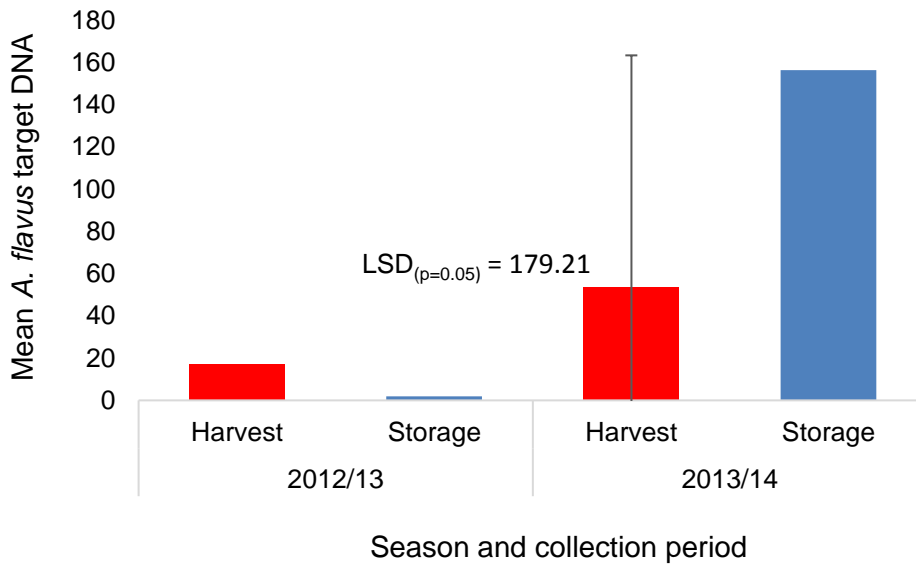
Figure 10. Mean *Aspergillus flavus* target DNA levels in maize grain in five districts at two collection periods (harvest and storage) during the 2012/13 and 2013/14 seasons.
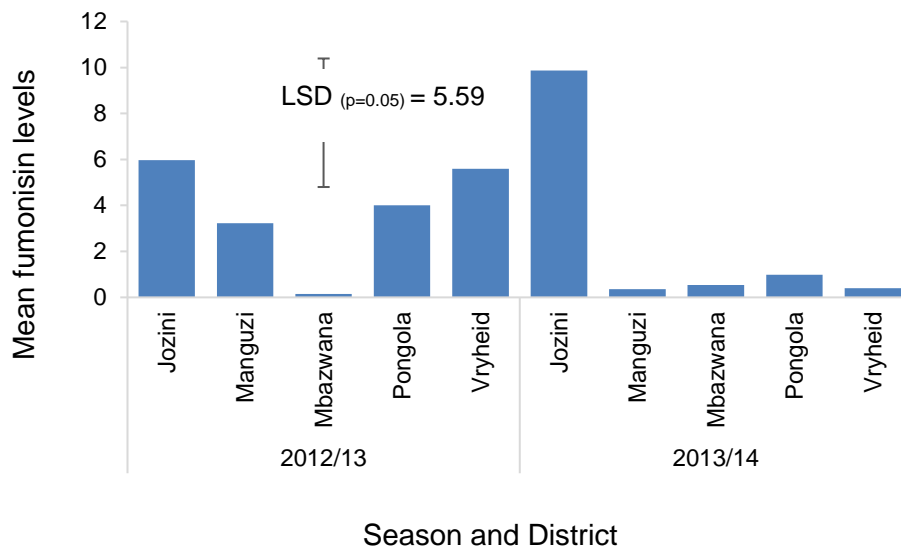
Figure 11. Mean fumonisin levels in maize grain in five districts during the 2012/13 and 2013/14 seasons.

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Figure 12. Mean fumonisin levels in maize grain in five districts at two collection periods (harvest and storage) during the 2012/13 and 2013/14 seasons.

Figure 13. Mean aflatoxin levels in maize grain in five districts during the 2012/13 and 2013/14 seasons.

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Figure 14. Mean aflatoxin levels in maize grain in five districts at two collection periods (harvest and storage) during the 2012/13 and 2013/14 seasons.

Figure 15. Mean zearalenone levels in maize grain in five districts during the 2012/13 and 2013/14 seasons.

Figure 16. Mean zearalenone levels in maize grain in five districts at two collection periods (harvest and storage) during the 2012/13 and 2013/14 seasons.
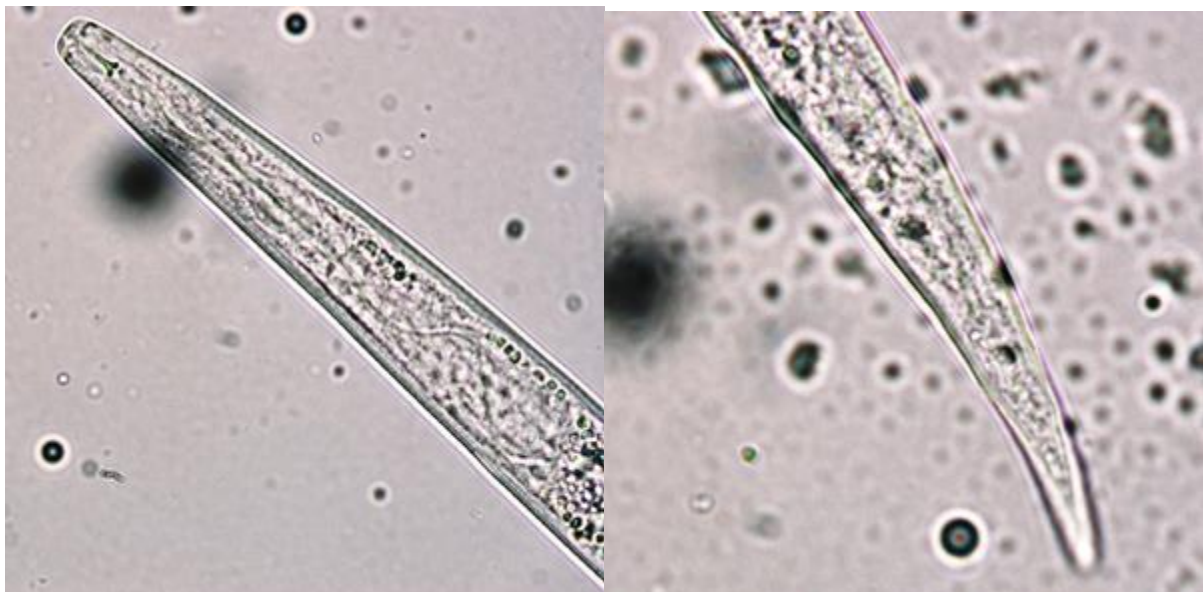
Figure 17. The head and tail of the most predominant nematode species (*D. africanus*) identified on groundnuts (Photos by S. Phokane).

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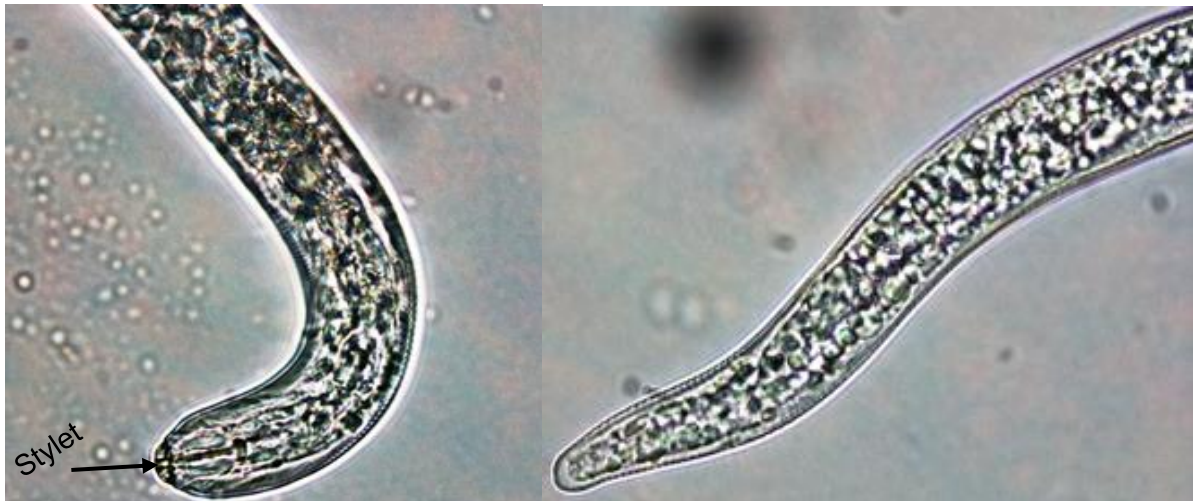
Figure 18. The view of *Pratylenchus spp.* clearly showing the stylet with the tail shown on the right-hand side (Photos by S. Phokane).
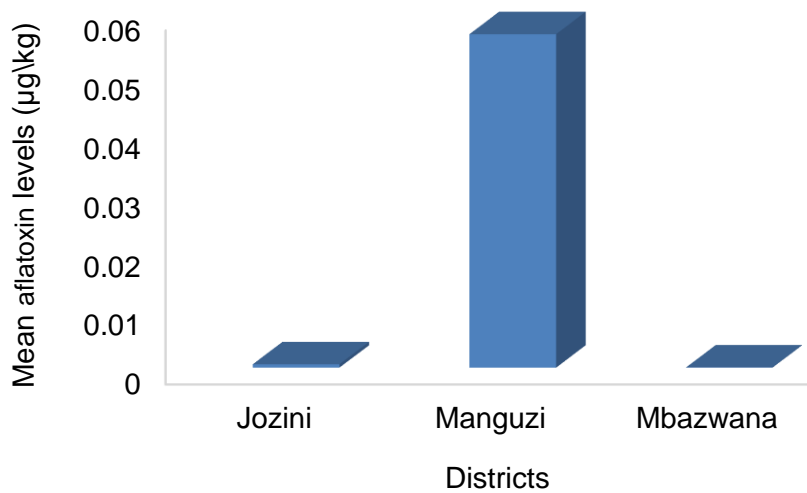
Figure 19. Mean aflatoxin levels in groundnut kernels from Jozini, Manguzi and Mbazwana districts following storage during the 2012/13 season.
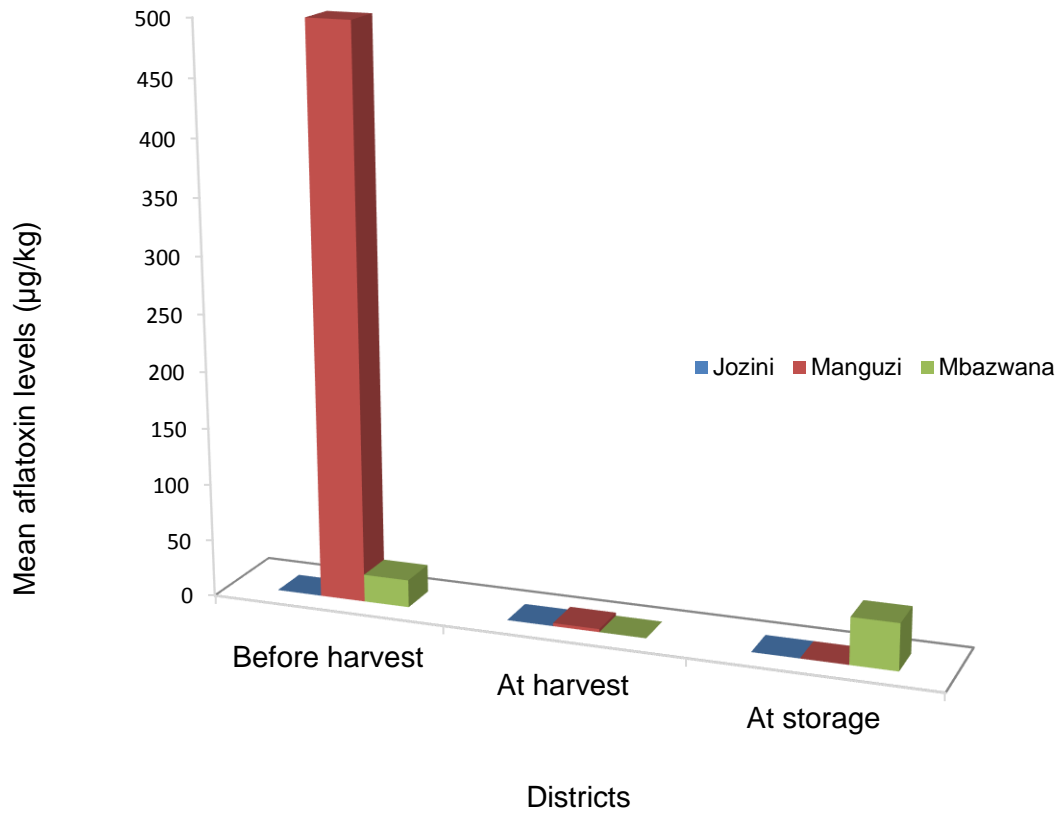
Figure 20. Mean aflatoxin levels in groundnut kernels from Jozini, Manguzi and Mbazwana districts before harvest, at harvest and following storage during the 2013/14 season.
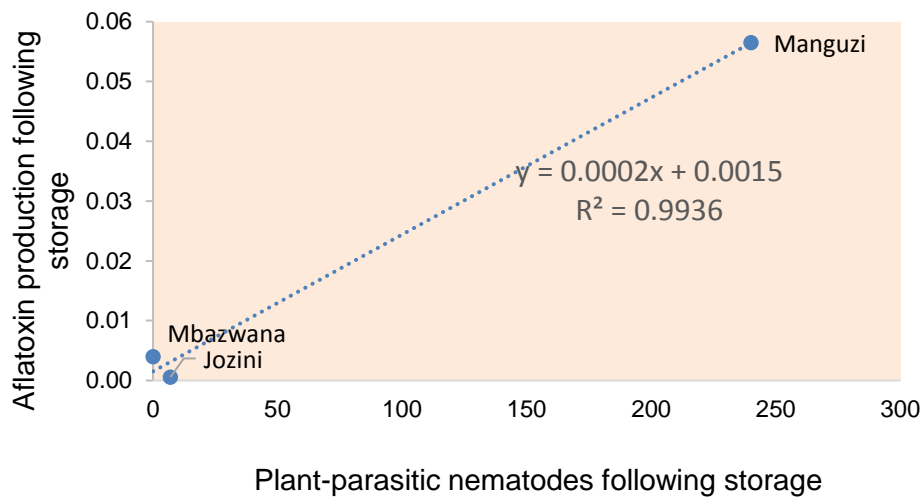
Figure 21. The relationship between plant-parasitic nematodes and aflatoxin contamination in groundnut kernels, following storage, from Jozini, Manguzi and Mbazwana districts during the 2012/13 season.
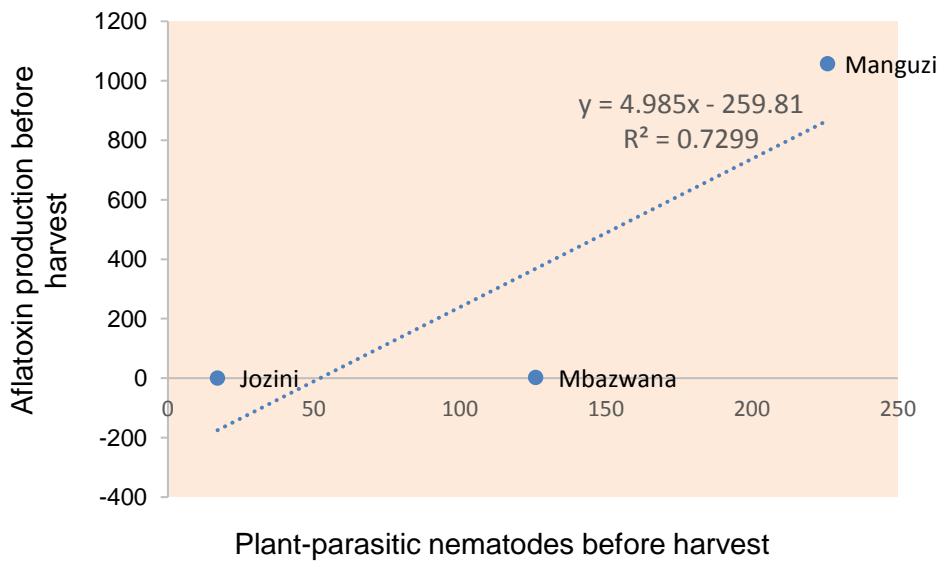

Figure 22. The relationship between plant-parasitic nematodes and aflatoxin contamination in groundnut kernels, collected before harvest, from Jozini, Manguzi and Mbazwana districts during the 2013/14 season.
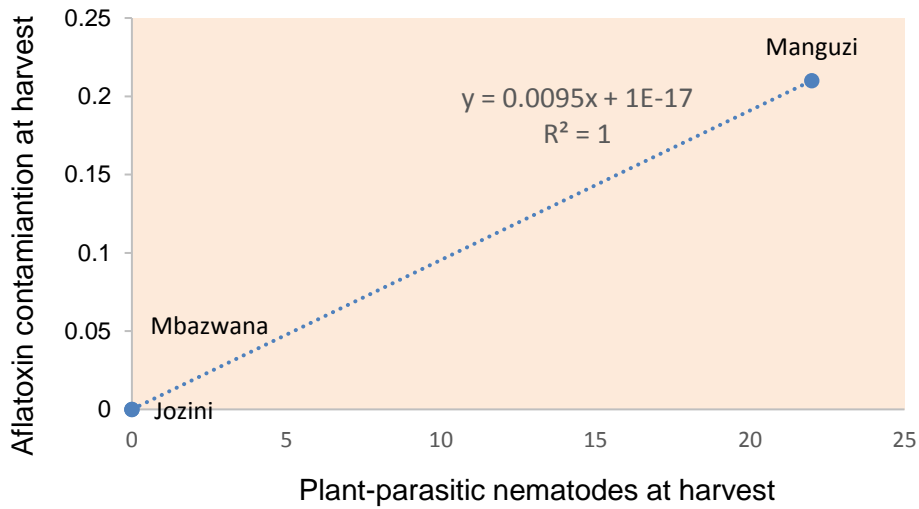
Figure 23. The relationship between plant-parasitic nematodes and aflatoxin contamination in groundnut kernels, collected at harvest, from Jozini, Manguzi and Mbazwana districts during the 2013/14 season.
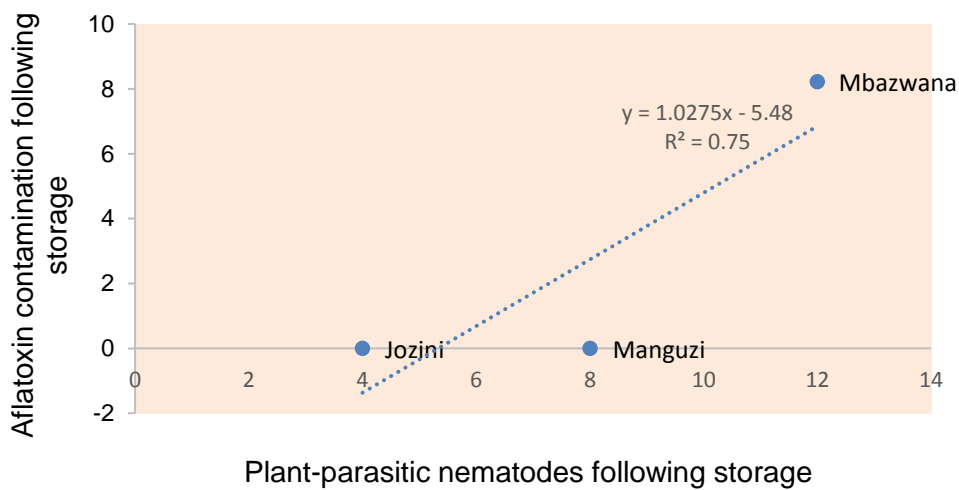
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Figure 24. The relationship between plant-parasitic nematodes and aflatoxin contamination in groundnut kernels, following storage, from Jozini, Manguzi and Mbazwana districts during the 2013/14 season.

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