Effects of Compost Manure on Soil Microbial Respiration, Plant-Available-Water, Peanut (*Arachis hypogaea L.*) Yield and Pre-Harvest Aflatoxin Contamination

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ABSTRACT

Peanut production in Zambia is often characterized by low yields and high aflatoxin incidence in harvested kernels. Soil amendments such as farmyard manure have shown potential to increase yields and reduce pre-harvest aflatoxin incidence. The aim of the current study was to evaluate the effects of composted cattle manure on soil properties that relate to yield and pre-harvest aflatoxin contamination of peanut kernels. Research evaluated the effects of composted cattle manure on soil respiration, plant-available water (PAW), peanut yield and pre-harvest aflatoxin contamination in a field experiment conducted in two successive rain-fed cropping seasons starting in December, 2015 and ending in April 2017, in Chongwe District, Zambia. Six (6) levels of compost were incorporated into the top 10 cm of the soil at rates of 0, 4.5, 12.0, 19.5, 27.0, and 34.5 metric tons/ha 1 wk before planting. There was a strong positive relationship between levels of compost and soil microbial respiration (R^2=0.84) and PAW (R^2=0.86). Secondly, compost manure was associated with increases in pod (R^2=0.65) and kernel (R^2=0.61) yield. The kernel yield potential of the planted cultivar was achieved at the rate of 12 metric tons per ha. Thirdly, there was a reduction in total aflatoxin levels with increasing levels of compost (R^2=0.85). The improvement in peanut yield and the decrease in aflatoxin concentrations in kernels can be attributed to the improvement in soil moisture retention capacity and soil microbial activity arising from manure amendments. This study demonstrated the potential of compost manure to increase soil microbial activity, PAW, peanut yield and minimize aflatoxin contamination at field level.

Key Words: aflatoxin, *Aspergillus spp,* peanut, pod yield, Zambia.

Peanut (*Arachis hypogaea* L.) is an important food and cash crop of Zambia. It is grown throughout the country and is rated as the second most widely cultivated crop among small-holder farmers (Central Statistical Office, 2014-2015 Post Harvest Survey). Nevertheless, peanut cultivation is constrained by a number of factors such as low-yielding cultivars, poor quality seed, weed pressure, damage by insects, diseases, poor soil health and drought stress. (Ross and De Klerk, 2012; Mukuka and Shipekesa, 2013). In most cases, a combination of two or more of these factors often accounts for the low productivity

Some of these production constraints such as poor soil health and drought stress are strongly associated with the reportedly high levels of aflatoxin contamination in harvested kernels (Njoroge *et al*., 2017). Aflatoxins refer to toxic metabolites of toxigenic molds, predominantly *Aspergillus flavus* and *A. parasiticus* (Richard and Payne, 2003). Aflatoxin accumulation in humans has been associated with stunting in children, suppression of the immune system, liver cancer and genetic mutations (Williams *et al.,* 2004; Richard, 2008). In Zambia, stunting among children under the age of 5 yr has been associated with exposure to aflatoxin through consumption of contaminated food (Ismail *et al*., 2014).

Plant stress factors such as drought are linked to both productivity and aflatoxin contamination. Because drought stress is an important factor in the proliferation of *Aspergillus* spp. and the subsequent pre-harvest aflatoxin accumulation (Cole *et al.,* 1985; Pitt *et al.,* 2013), most pre-harvest interventions are centered on minimizing the effects of drought stress by conserving soil moisture during pod development, the most susceptible growth stage to aflatoxin formation in peanuts (Hill *et al*., 1983). Therefore, good agricultural practices such as soil water conservation during pod-development (Chalwe *et al*., 2016) should be encouraged to minimize pre-harvest aflatoxin accumulation. According to Cole *et al.*

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(1995) agronomic practices that reduce plant stress to maximize crop growth will in turn decrease pre-harvest aflatoxin accumulation.

Compost is relatively cheap and is a sustainable means of restoring soil health and promoting peanut productivity. Soil health is defined as the ability of the soil to function as a living system (Brady and Weil, 2010). Soil microbial respiration and the availability of soil water for plant use are as such important indicators of soil health. According to Waliyar *et al* (2008, 2013), farmyard manure was associated with up to 90 % reduction of total pre-harvest aflatoxin concentrations in harvested peanuts kernels. The aim of the current study was to evaluate the effects of composted cattle manure on soil factors that relate to the growth of peanuts and the activity of *Aspergillus spp*. A field experiment was conducted to evaluate the effects of compost on PAW, soil microbial respiration, selected yield components, and pre-harvest aflatoxin accumulation in kernels as key peanut crop performance indicators.

Materials and Methods

Study site. The field experiment was conducted under rain-fed conditions at Kasisi Agricultural Training Centre, located at 15.2498 S, 28.4836 E in Chongwe District, Zambia. The site is located in agro-ecological region IIa, which has mean annual rainfall ranging from 800 to 1000 mm (Soil Survey Branch, 2002). The cropping season is from mid-December to end of April in the following year. The average annual rainfall recorded during the two cropping seasons for this experiment was 905 mm (SASSCAL Weather data, Kenneth Kaunda International Airport). The soil at the site was classified as a chromic luvisol on the Exploratory Soil Map of Zambia (Soil Survey Branch, 1991).

Soil sampling and characterization. A composite soil sample from the study site was constituted by mixing 8 random subsamples collected from the top 20 cm soil layer using an auger. The duly constituted composite sample was air dried, passed through a 2 mm sieve and then characterized using standard laboratory soil analysis procedures. To determine soil texture, the soil was first dispersed using 5% sodium hexametaphosphate (calgon) and then determined the particle size distribution using the hydrometer method (Day, 1965). Bulk density was determined using core samples according to (Blake, 1965). Soil organic matter was determined using the wet-oxidation method (Walkley and Black, 1934). Soil reaction (pH) was determined by equilibrating soil in 0.01 M $CaCl_2$ in a 1: 2.5 soil to solution ratio and then measuring the exchangeable H^+ concentration using a pH electrode (Van Reeuwijk, 1992). The exchangeable base cations K^+, Mg^{2+} and Ca^{2+} were extracted from the soil using 1 *M* ammonium acetate at a neutral pH of 7 (Van Ranst *et al*., 1999) and then determined their concentrations using atomic absorption spectrophotometry (AAnalyst 400, PerkinElmer, USA). Available phosphorus was extracted using the Bray 1 extraction solution (Bray and Kurtz, 1945) and then measured the concentration using spectrophotometry at 882 nm. Total nitrogen was determined according to the modified Kjeldahl method (Bremner and Mulvaney, 1982).

Field Experiment

Treatments and experimental Design. Treatments for the field experiment were 6 levels of composted cattle manure (compost) applied to each experimental plot. The rates of application were 0, 4.5, 12.0, 19.5, 27.0 and 34.5 metric tons/ha. The application process involved; uniformly spreading the compost on the soil surface by hand and then mixing it with the soil in the top 10 cm using a hoe in a fine tillage operation. Compost was applied after the first continuous rains of the cropping season and was allowed to settle for one week before planting. Each rate of application was replicated 6 times resulting in 36 experimental plots. Treatments were laid out in a latin square experimental design. Each experimental plot measured 25 m^2 with a 1 m border between plots. Thus, the experiment covered an area of about 0.12 ha. The experiment was conducted for two successive cropping seasons; December 2015 to April 2016 and December 2016 to April 2017. These two growing periods are hereafter referred to as 2016 and 2017 seasons, respectively.

Preparation and characterization of compost manure. The compost for this study was prepared conventionally in compost heaps consisting of cattle manure mixed with spoiled hay arranged in windrows. These windrows were moistened when necessary and turned regularly until the materials were decomposed to stable compost. The compost was characterized for selected chemical properties using standard laboratory methods. Some of the properties of the compost included a C/N ratio of 11, pH in 0.01 M $CaCl_2$ of 7.2 and total phosphorus, potassium, calcium and magnesium levels of 1.2, 1.3, 5.4 and 1.6 mg/kg, respectively.

Field preparation, management, harvesting and drying of pods. Soil tillage was done by hand using a hoe. Thereafter, the ploughed area was levelled into a fine seedbed. A red-colored, virginia, bunch type peanut cultivar known as MGV 4, was then planted in rows at the recommended planting spacing of 75 cm x 10 cm, inter-row by intra-row spacing, respectively. Each plot had a total of 6 plant rows giving a net plot of 4 rows after subtracting the 2 border rows. Crop management practices were done manually and included regular weeding whenever weeds appeared and ridging at the on-set of the pegging stage. Major weeding was done four times per growing season. The crop was harvested by digging out plants with a hoe at physiological maturity, 130 d after planting. The pods were stripped from the plant by hand, packaged in polythene bags and then dried in an electric vacuum oven (D-6450 Hanau, Heraeus Instruments, Germany) set at 45 C to a gravimetric moisture content of about 10%, which took about 72 hr of continuous drying.

Determination of pod yield and preparation of laboratory samples. At harvest, the number and weight of mature grain-filled fresh pods per individual plant were determined from 6 representative plants randomly selected from the middle rows of each plot. After weighing, the pods were mixed with the other pods from plants in the middle rows for drying as described above and then shelling upon drying to approximately 10% w/w moisture content. Dry pods were shelled by hand and then temporarily stored in air-tight plastic jars at room temperature. Shelled kernels were sub divided into four equal subsample lots of about 500 g. Laboratory samples were collected by scooping 50 g samples per scoop for 4 times (200 g per 500 g subsample) from each quarter of the lots. A 120 mL plastic cup (1/2 standard cup) was used as a scooper. The samples were shaken before each scoop of sub-sample was taken. The scooped samples were added together and homogenized by shaking. The mixed sample constituted the laboratory sample, which was then ground into fine flour using an ordinary kitchen grinder (LM2211BM, Moulinex, China).

Determination of total aflatoxins in dry kernels. The total concentration of aflatoxins in dried kernels was determined using Neogen Afla Reveal® Q+ aflatoxin kit (Neogen Corporation, USA) within 1 wk after shelling. Ground samples were homogenized by thorough shaking. For each treatment 18 samples (6 replicates by 3 subsamples) each weighing 10 g were assayed for total aflatoxin using 30 ml of 65% ethanol (diluted from 95% ethanol, UN1170, Xilong Scientific Co., Shantou City, China) by shaking on a rotary shaker (ISO-9001-2000, Navyug, India) at a speed of 120 rpm for 3 min. The extract was then filtered through Whitman 42 filter paper. Five hundred uL of the diluent buffer solution was pipetted into a sample dilution cup using a standard 500 μL pipette and then thoroughly mixed with 100 µl of each extract using a clean sterile micro pipette. One hundred μL of the mixture was then transferred into a measuring cup into which one Afla Reveal® Q+ test strip per sample was placed and allowed to develop for 6 min. After 6 min, the aflatoxin content was read by placing the strip into the strip holder of a computer tablet (K011, ASUS Corporation, USA) installed with a mycotoxin reader application.

Determination of soil respiration and PAW. Soil microbial respiration and PAW were determined at 90 d (during pod-development stage) after planting as once-off soil health indicators in each of the two seasons. To determine soil respiration, composite soil samples each weighing 2 kg per plot were collected from 3 to 4 random sampling points in the top 10 cm of soil using a bucket soil auger. The samples were transported in air-tight plastic jars stored in a cooler box filled with ice blocks between jars containing soil samples. To determine carbon evolution due to microbial respiration, the evolved carbon dioxide was trapped in 1 M KOH (Landa and Fang, 1978) and quantified by titrating samples with 1 M HCl.

To determine plant-available-water, 3 undisturbed soil samples per plot were collected from the top 10 cm of soil using standard core rings. The samples were then placed in the pressure plate apparatus to determine water content at field capacity (FC) and permanent wilting point (PWP). The samples were subjected to -10 kPa and -1500 kPa pressure for FC and PWP, respectively.

Data management and statistical analysis. All the data collected in the experiment were managed in Microsoft Excel and SPSS version 20 statistical program. The effects of compost on PAW, soil microbial respiration, peanut pod and kernel yield and aflatoxin content in harvested kernels were determined year by year for the two cropping seasons. Each data set was checked for extreme outliers defined according to SPSS as data points with a magnitude of 3 times the inter-quartile range by first plotting box plots and removing flagged data points. There were no outliers in all the data sets. Scatter plots were used to establish whether or not there were linear relationships between the independent and the outcome variables. The central limit theorem was applied to assume normal distribution since all data sets had more than 30 observations. Simple linear regression analysis was then performed to estimate the response to each level of treatment.

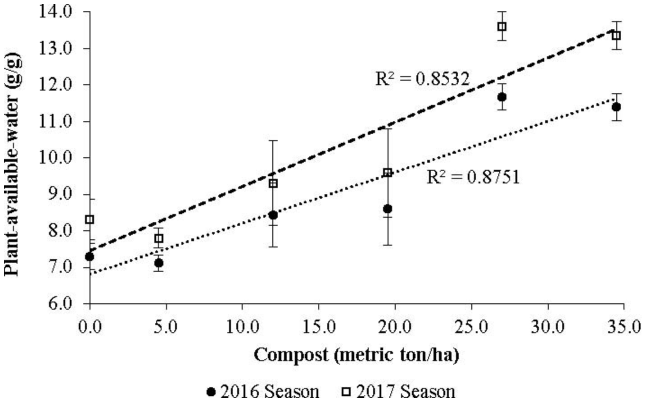

Fig. 1. Effect of compost on plant-available-water. Error bars represent standard error of the mean. Plotted values represent means of 6 replicates. The R-square values for the fitted regression lines are as indicated on each line.

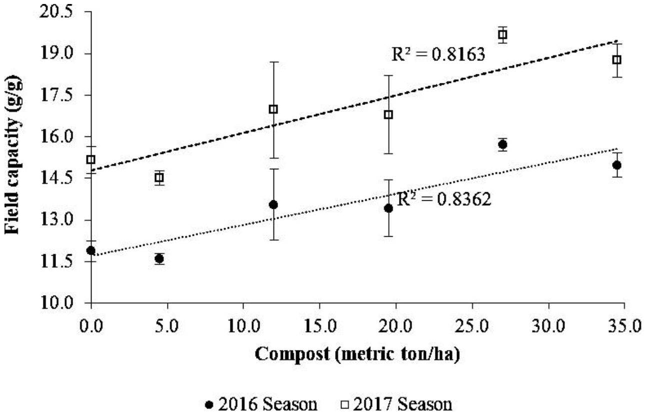

Fig. 2. Effect of compost on field capacity. Error bars represent standard error of the mean. Plotted values represent means of 6 replicates. The R-square values for the fitted regression lines are as indicated on each line.

Results and Discussion

Soil characteristics. The soil at the research site was a strongly acidic (pH = 4.22) sandy loam (19 % clay, 11.4 % silt and 69.6 % sand), with very low available phosphorus (0.56 mg/kg), low soil organic matter content (0.7 %) and low exchangeable calcium (0.06 cmol/kg). No measures were taken to correct neither the acidity nor nutrient deficiencies for the sole purpose of evaluating groundnut performance on marginal soils (control) common to local farmers.

Effects of compost manure on plant-available-water. The plant-available-water (PAW) in soil treated with compost manure increased with increasing levels of compost manure applied (R^2=0.86) (Fig. 1). Plant-available-water is the fraction of soil water between field capacity (FC) and the permanent wilting point (PWP). This is the water that is available for plant uptake after natural drainage (Brady and Weil, 2010). The increase in PAW can be attributed to the increase in soil moisture content at FC (-10 kPa suction pressure) at each level of compost applied to the soil (Fig. 2), while the moisture content at PWP (-15 000 kPa) did not vary with the level of compost manure ($P > 0.05$).

The role of organic matter on soil moisture retention capacity relates to its role on aggregate stability and soil structure. Aggregate stability relates to the capacity of the soil aggregate to maintain its physical structure/shape when subjected to a given pressure while soil structure relates to the distribution of the solid phase and the pore space (liquid and gaseous phases) in a given mass of soil. Stable aggregates tend to have more pore space and are able to hold more water than weak aggregates. According to Yazdanpanah *et al.* (2015) organic amendments were associated with higher aggregate stability and soil microbial respiration. In a related study, Ramos (2017) reported an improvement in structure and moisture holding capacity at different suction pressures with the addition of composted cattle manure at a rate of 40 Mg per ha. It is worth noting that the capacity of organic amendments to increase the soil's moisture holding capacity partly depends on the type of organic matter. Organic matter with high quantities of hydrophobic components such as humic substances tends to promote aggregate stability and hence good soil structure (Piccolo and Mbangwu, 1999). In this context, a more humified organic material such as compost would be more appropriate for moisture retention purposes than fresh organic wastes such as green manures and raw animal manure.

Effects of compost manure on soil microbial respiration. There was an increase in microbial respiration (R^2=0.84) with increasing levels of compost (Fig. 3). The increase can be attributed to the addition of soil microorganisms contained in the manure and the activation of native soil microorganisms in the soil through the supply of nutrients. Soil microbial respiration is an important indicator of soil microbial activity, which also relates to the decomposition of soil organic matter.

Results similar to our study have been reported by several authors. For instance, Chaoui *et al.* (2002) evaluated the effects of earthworm casts and compost manure on nitrogen mineralization rates, soil microbial biomass and microbial respiration revealed that compost manure was superior in all the three aspects of the study. In a recent study, Yazdanpanah *et al.* (2015), observed higher mineralization rates and soil microbial respiration with the addition of organic matter. The same study also reported increased soil aggregate stability, which

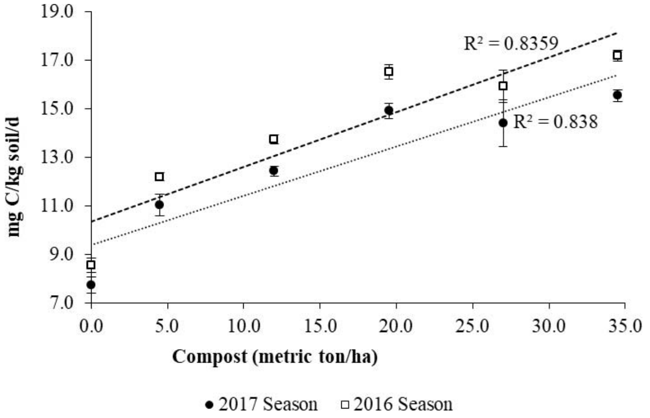

Fig. 3. Effect of compost on soil microbial respiration. Error bars represent standard error of the mean. Plotted values represent means of 6 replicates. The R-square values for the fitted regression lines are as indicated on each line.

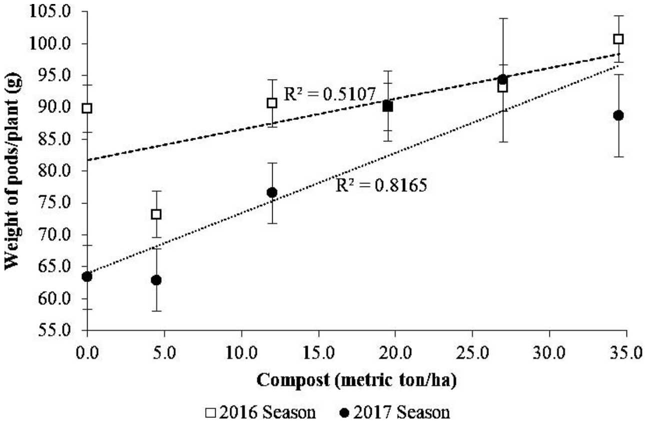

Fig. 5. Effect of compost on weight of pods per plant. Error bars represent standard error of the mean. Plotted values are means of 6 representative plants per replicate. The R-square values for the fitted regression lines are as indicated on each line.

contribute to improved hydraulic conductivity and soil aeration. An active soil biota requires a good supply of nutrients and oxygen. A study on a black soil of Northeast China revealed that compost significantly contributed to the buildup of soil organic matter, increased electrical conductivity and the availability of major plant nutrients nitrogen, potassium, phosphorus and calcium (Yang *et al.*, 2017). A 4-yr application of cattle manure in a commercial vineyard in Italy was associated with higher soil organic matter content and microbial biomass (Gaiottia *et al.*, 2017).

Effects of compost manure on pod and kernel yield. There were significant increases in pod (R^2=0.65) and kernel yield (R^2=0.61) with an increase in compost in each cropping season (Fig. 4, 5 and 6). However, a comparison of yield data between the two seasons showed that both pod and kernel yields in 2016 tended to be lower than in the 2017 season especially at lower levels of compost. As shown in Fig. 6, kernel yield was lower than the potential yield of 1.5 ton/ha for the MGV 4 cultivar across all levels of compost in 2016 while this was achieved in the second season at the rate 12 metric ton/ha and higher. The lower yield in the 2016 season could be attributed to poor rainfall distribution of 1 rainfall event per 2.5 days and lower total rainfall of 592.2 mm during the growing period compared with better rainfall distribution of 1 rainfall event per 1.6 days and more total rainfall of 916 mm received during the growing period in the 2017 season (SASSCAL weather data, Kenneth Kaunda International Airport).

Amending soils low in organic matter with compost is one of the sustainable means of improving soil fertility and crop productivity. Compost manure can enhance the growth of crops either by supplying plant nutrients or enhancing the supply and recycling of plant nutrients (Brady and Weil, 2010). The compost used in this study contained significant quantities of macro-nutrients in plant-available form and may have contributed to the improved yield. In a study in Senegal, the use

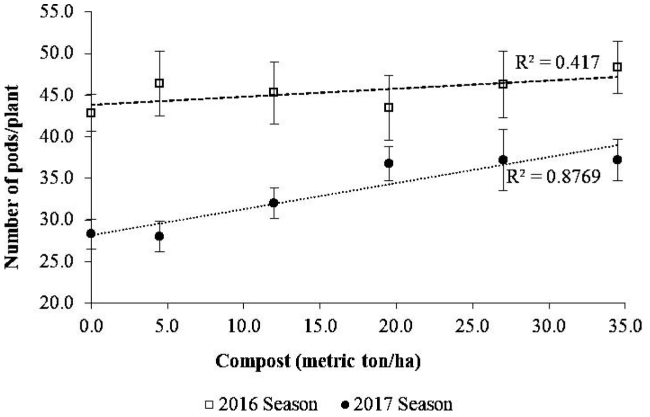

Fig. 4. Effect of compost on number of pods per plant. Error bars represent standard error of the mean. Plotted values are means of 6 representative plants per replicate. The R-square values for the fitted regression lines are as indicated on each line.

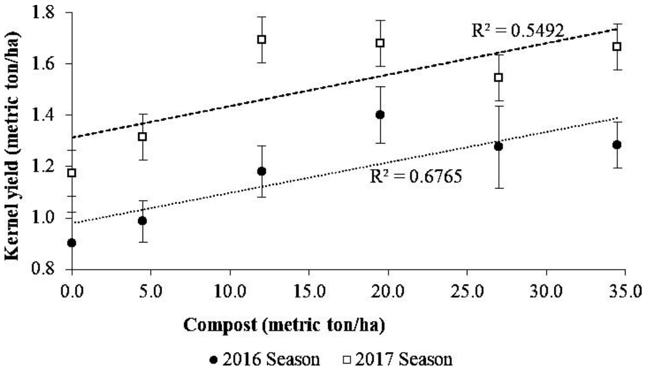

Fig. 6. Effect of compost on kernel yield. Error bars represent standard error of the mean. Plotted values are means of 6 replicates. The R-square values for the fitted regression lines are as indicated on each line.

of compost manure was associated with an increase in the effective cation exchange capacity and plant nutrients K and Mg and higher pod yield of groundnuts in amended soils (McClinton and Diop, 2005). A higher cation exchange capacity in a soil increases its nutrient retention capacity. At the same time, the increased charge enhances soil water retention, which contributes to nutrient availability by bringing dissolved elements into solution (Brady and Weil, 2010).

Effects of compost manure on pre-harvest aflatoxin contamination. Total aflatoxin concentrations in kernels decreased with increasing levels of compost (Fig. 7). This result can be attributed to the observed increments in PAW (Fig 1) and soil microbial respiration (Fig. 3). According the R-square values of the fitted regression lines, the reduction in mean total aflatoxin concentrations was higher in the second cropping season ($R^2 = 0.89$), than in the first one ($R^2 = 0.81$).

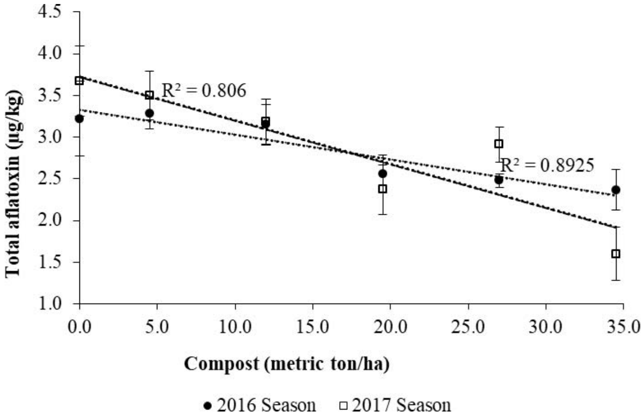

Fig. 7. Effect of compost on total aflatoxin contamination in kernels. Error bars represent standard error of the mean. Plotted values are means of 6 replicates. The R-square values for the fitted regression lines are as indicated on each line.

The soil moisture content of the soil during pod-development in peanuts is not only crucial for minimizing pod colonization by *Aspergillus flavus*, but also promotes the formation of sound kernels. On the contrary, soil moisture deficit during pod development is strongly associated with higher levels of aflatoxin contamination in groundnut kernels (Hill *et al.*, 1983; Cole *et al.*, 1985; Pitt *et al.*, 2014; Sibakwe *et al.*, 2017). Although *A. flavus* and *A. parasiticus*, the two major toxigenic fungi are soil-borne and saprophytic in nature (Richard and Payne, 2003), their capacity to produce aflatoxins is influenced by the soil moisture status and temperature. Being xerophitic in nature, the *Aspergilli* species become active and produce aflatoxins under severe moisture deficits often associated with elevated soil temperature (Bowen and Hagan, 2015).

Adequate soil moisture is important to minimise soil temperature, an equally important factor influencing pre-harvest aflatoxin contamination in groundnuts (Hill *et al.*, 1983; Dorner *et al.*, 1989; Bowen and Hagan, 2015). According to Hill *et al.* (1983) soil temperatures lower than 25 C in the geocarposphere did not encourage aflatoxin contamination even under low soil moisture conditions. As such, enhancing soil moisture retention capacity using organic amendments, which often keeps the soil temperatures low, seems to override the effect of the high presence of *Aspergillus flavus* on aflatoxin production in soils that are rich in organic matter (Zablotowicz *et al.*, 2007).

An increase in soil microbial respiration is indicative of improved microbial activity in the soil. Compost manure inoculates the soil with microorganisms and adds nutrients to the soil (Gaiottia *et al.*, 2017). Adequate moisture and nutrients are essential for microbial activity. As reported by Cole *et al.,* (1985) high microbial activity minimized pod colonization by the *Aspergillus flavus,* the causal agent for aflatoxin contamination in kernels. These results are consisted with studies by Waliyar *et al.* (2013) who reported 42% reduction in total aflatoxin levels following an application of farmyard manure at the rate of 2.5 metric ton/ha.

It is noteworthy that although there were significant differences in aflatoxin levels, the observed concentrations were markedly low for the warm climatic region in which the experiment was conducted. Soil temperature data from a weather station situated within 8 km southeast of the study site indicated average soil temperatures of 23.2 C in the last 6 wk to harvesting of the peanuts coupled with a fairly distributed average annual rainfall of 905 mm during the two growing seasons (SASSCAL Weather data, Kenneth Kaunda International Airport). The weather conditions were more favourable for plant growth and development than for pre-harvest aflatoxin development and hence the low levels of aflatoxin observed across treatments. According to Cole *et al.* (1995) agronomic practices that reduce plant stress and meant to maximise crop growth would in turn decrease mycotoxin occurrence. In both planting seasons, the field was free of weeds and diseases throughout the growing period and there were no physical signs of water stress such as wilting during the pod development stage of the crop.

Conclusions

Results from this study demonstrate the potential of compost manure to increase soil respiration, PAW, pod and kernel yield, and minimize aflatoxin development in peanut kernels at field level. There were significant increases in soil microbial respiration ($R^2 = 0.84$) and PAW ($R^2 = 0.86$) with increasing levels of compost. An improvement in soil microbial respiration is an important indicator for improved soil health. In terms of crop performance, compost had a strong positive correlation with kernel yield ($R^2 = 0.61$) and a strong negative correlation with aflatoxin content in kernels ($R^2 = 0.85$). With the potential yield achieved at the rate of 12 metric ton/ha, the study showed that compost can be used by local farmers for better crop performance. Additionally, the decline in aflatoxin levels with increasing levels of compost is an improvement in the quality of kernels. We therefore recommend the use of compost for better yield and lower pre-harvest aflatoxin content.

Acknowledgements

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