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Full Length Research Paper

Major biotic maize production stresses in Ethiopia and their management through host resistance

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Biotic stresses are recently evolving very rapidly and posing significant yield losses of maize production in Ethiopia. A number of high yielding maize hybrids, initially developed as tolerant/resistant, have been taken out of production due to their susceptibility to major maize diseases. Furthermore, recent disease and insect pest epidemics have clearly shown the importance of breeding maize for biotic stresses and study the genetics of resistance to the major maize disease pathogens, insect pests and parasitic weeds. This paper gives the general perspective of the major biotic maize production stresses in Ethiopia and the interventions made locally and globally to control these stresses using host resistance. More emphasis was given to grey leaf spot (GLS), turcicum leaf blight (TLB), common leaf rust (CLR), maize streak disease (MSD), maize lethal necrosis (MLN), maize weevil, stalk borers, fall armyworm and *Striga.* Approaches to conducting genetic analysis and achieving durable host resistance to these stresses, where applicable, are discussed. This information will be used for breeders, private and public maize seed and grain growers who are targeting to operate in Ethiopia and Eastern Africa.

Key words: Striga, biotic stress, diseases, host resistance, host - pathogen interaction, maize, pests.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important staple food crops in sub-Saharan Africa (SSA), predominantly produced and consumed directly by the smallholder farmers (Shiferaw et al., 2011). In Ethiopia, maize is one of the principal cereal crops ranking first in total production and productivity, and second to tef (*Eragrostis*

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tef) in area coverage. A total of 7.8 million tons of maize (31% of the total cereal) was produced on 2.1 million hectares (21% of the total area planted cereals) of land by nearly 11 million small households (31% of the total cereal) in 2016 (FAO, 2017). Maize is everything for the Ethiopian maize farmers. Three forth of the maize produced is consumed at the house hold level by the small-scale producers themselves (CSA, 2017). The grain is consumed in different forms of food; the stover is used as feed, fuel and construction material. Besides, it serves as a major source of income and means of employment for tens of millions of farming and business communities. Due to its widespread significance in the country, maize is one of the strategic field crops targeted to ensure food security in Ethiopia.

Despite the importance of maize as a principal food crop, its average yield in Ethiopia (3.6 tons ha^{-1}) is still lower than that of the world's average (5.6 t ha^{-1} in 2016) (FAO, 2017). A significant portion of this yield gap is attributable to biotic and abiotic stresses, slow turnover of varieties tolerant or resistant to these stresses and low level use of improved management and other inputs (Worku et al., 2012; Abate et al., 2017). Some of the main abiotic factors affecting maize production and productivity are drought, heat, soil acidity, frost and poor soil fertility mainly in N and P. Biotic stresses hindering maize production in Ethiopia are diseases (e.g., Grey Leaf Spot, TurcicumLeaf Blight, Common Leaf Rust, Maize Streak Virus, Maize Lethal Necrosis), parasitic weeds (mainly *Strigahermontica*), and insect pests (such as the maize stem borer, maize weevils and the newly emerged fall armyworm).

While more food is needed to feed the ever-increasing world population (Clover, 2003; van Ittersum et al., 2016), the effects of climate change is becoming a great challenge threatening global food security (Godfray et al., 2010). It is now well understood that agriculture faces many threats from climate change, drought, heat, irregular weather and emergence of new diseases and pests among other environmental challenges. The impact of climate change is likely to be more severe in Sub-Saharan Africa (SSA) due to the high dependence of the region on rain-fed agriculture (Cooper et al., 2008; Fisher et al., 2015). The spread of insect pests, plant diseases and invasive alien plant species to new regions, as the world's climate changes, is a threat to farmers globally, especially in Africa where climate change effects are projected to be the most severe in the world (Swaminathan and Kesavan, 2012; Biber-Freudenberger et al., 2016; Early et al., 2016). It is anticipated that the spread of diseases, insect pests and weeds will potentially cause the loss of more than 40% of the world's food supply. Of the many countries in SSA, Ethiopia is one of the climate change vulnerable countries due to its agriculture led economy (Conway and Schipper, 2011).

In this paper, the general perspective of major biotic maize production constraints in Ethiopia and the associated host resistance interventions employed locally and globally to control these constraints were reviewed. The areas of research gaps and the approaches followed to conduct genetic analysis of host-pathogen interaction were also outlined in detail. Finally, viable future research directions towards climate smart management of maize biotic stresses were suggested.

THE BIOTIC DISEASE CONSTRAINTS OF MAIZE IN ETHIOPIA

Diseases of economic significance in maize production systems of Ethiopia are TLB (caused by *Exserohilum turcicum*), GLS (caused by *Cercospora zeae-maydis*), streak disease of maize (maize streak virus), CLR (caused by *Puccinia sorghi*) (Vivek et al*.,* 2001; Mosisa et al., 2012; Tilahun et al., 2012) and the recently emerged viral disease, MLN caused by the combination of maize chlorotic mottle virus (MCMV) and sugar cane mosaic virus (SCMV)(Mahuku et al., 2015).These diseases are known to cause significant yield losses in favorable environments; especially, when the combinations of these two or more diseases affect maize.

Grey leaf spot

Grey leaf spots (GLS), caused by *C. zeae-maydis*, is an important foliar disease of maize worldwide. The disease was originally described from southern Illinois in 1925 (Tehon and Daniels, 1925; Carson and Goodman, 2006). GLS is a major economic concern in many maize growing regions of the world (Gevers et al., 1994; Nutter et al., 1999; Guo-hui, 2009). The disease was reported as a threat to maize production in the USA in the 1980s (Latterell and Rossi, 1983; Zhang et al., 2012), in South Africa in the 1990s (Latterell and Rossi, 1983; Gevers et al., 1994), and currently known to have widespread distribution in maize production areas worldwide, including South America (Pozar et al., 2009), China (Zhang et al., 2012) and Ethiopia (Wegary et al., 2001).

In Ethiopia, the disease was first reported in 1999 (Tefferi, 1999) on a few maize farms. Later, the disease was spread to all major maize producing mid altitude sub-humid agro-ecology of the country. A major epidemic occurred in early 2000s which caused considerable maize grain yield losses (Wegary et al., 2001; Tilahun et al., 2012). To date, the disease is one of the most important threats to maize production in the country, causing yield losses as high as 29.1% (Wegary et al., 2004).

No major resistance genes are known for GLS in

Table 1. Responses of parental lines of some released hybrids to turcicum leaf blight (TLB) and grey leaf spot (GLS) diseases in Ethiopia.

Inbred line	TLB (1 - 5)	Reaction type	Sources	GLS (1 - 5)	Reaction type	Sources
CML202	2.0	R	Abera et al., 2016	2.50	R	EIAR, Unpublished data
BKL004	2.0	R	Abera et al., 2016	1.8	R	EIAR, Unpublished data
144-7-b	1.9	R	Abera et al., 2016	1.2	R	Tilahun et al. 2012
BKL001	2.0	R	Abera et al., 2016	1.5	R	EIAR, Unpublished data
142-1-e	2.0	R	Abera et al., 2016	1.3	R	Tilahun et al., 2012
A7033	2.9	S	Abera et al., 2016	2.8	S	EIAR, Unpublished data
SC22	2.8	S	Abera et al., 2016	2.5	R	Tilahun et al., 2012
124-b (109)	2.9	S	Abera et al., 2016	2.7	S	Tilahun et al., 2012
CML197	3.4	S	Tilahun et al. 2012	3.2	S	Tilahun et al., 2012
CML395	2.1	R	EIAR, Unpublished data	3.0	S	EIAR, Unpublished data
CML144	1.8	R	Tilahun *et al*. 2012	2.0	R	Tilahun et al. 2012
BKL003	2.25	R	EIAR, Unpublished data	2.0	R	EIAR, Unpublished data
CML444	2.0	R	EIAR, Unpublished data	3.1	S	EIAR,Unpublished data

R, resistant; S, susceptible, GLS, grey leaf spot; TLB, turccicum leaf blight; EIAR, Ethiopian Institute of Agricultural Research. Disease scores are on a 1 to 5 scale where 1 is being highly resistant and 5 is being highly susceptible.

maize. Although there was an initial report of major genes for GLS resistance (Gevers and Lake, 1994), this was later disproved by Gordon et al*.* (2004). Resistance to GLS in maize is quantitatively inherited and is controlled primarily by additive gene action (Clements et al*.,* 2000; Menkir and Ayodele, 2005; Gordon et al., 2006; Benson et al., 2015). Many QTLs underlying GLS resistance have been identified across the 10 maize chromosomes in various mapping populations (Bubeck et al., 1993; Maroof et al., 1996; Clements et al., 2000; Lehmensiek et al., 2001; Juliatti et al., 2009; Pozar et al., 2009; Zwonitzer et al., 2010; Shi et al., 2014; Berger et al., 2014).

Some elite maize inbred lines, for example, CML444 (Okello et al., 2006; Berger et al., 2014) carry QTL conferring resistance to GLS. This inbred line is in the pedigree of high yielding and drought tolerant maize hybrid, MH140, released in many African countries including Ethiopia. However, CML444 have been found to be susceptible to GLS in Ethiopia under artificial inoculation (Table 1). This could be due to the variability in the *Cercospora species* causing the diseases, or differences in the races of the pathogen. On the other hand, there are maize inbred lines derived from ‘Ecuador573’ and CIMMYT germplasm which are resistant to the most prevalent foliar disease of maize in Ethiopia (GLS, TLB and CLR). For example, the hybrids and parents of recently released drought tolerant varieties, such as BH546, BH547 and BH661, are resistant to these three fungal diseases. These inbred lines could be used as potential sources of resistance against these major foliar diseases of maize in Ethiopia. The host-pathogen interaction, diversity and the prevalent species of *Cercospora* in Ethiopia has not been determined.

Turcicum leaf blight

Turcicum leaf blight (TLB) caused by *Exserohilum turcicum* (Pass.) is known to infect maize from the seedling stage to maturity. The initial symptoms are small elliptical spots on the leaves as grayish green with water-soaked lesions parallel to leaf margins. Later, the spots turn greenish with age and increase in size, and finally attaining a spindle shape with long elliptical grayish or tan lesions. It causes premature death of blighted leaves, if the disease starts at an early stage (Chandrashekara et al., 2014). The disease can cause loss of the nutritive value of maize as fodder (Payak and Renfro, 1968). Reductions of germination capacity, vigor, grain yield and total sugar content (Ferguson and Carson, 2004), and restriction of starch formation (Cuq et al., 1993; Henry and Kettlewell, 2012) by the pathogen have also been reported. The disease has a wide host range and a high pathogenic variability with several races already reported in different parts of the world (Pratt, 2003; Agrios, 2005). The disease has been reported throughout the world wherever maize is cultivated (Atac, 1984; Leonard et al., 1985; Adipala et al., 1993; Shiferaw et al., 2011). TLB can be severe in mid-altitude tropical regions where high humidity, low temperature and cloudy weather prevail during the maize growing season (Harlapur et al., 2000; Singh et al., 2004).

Severe grain yield losses as high as 28 to 91% due to TLB have been reported in several parts of the world

(Gowda et al., 1992; Harlapur et al*.,* 2000). The genetic nature of TLB resistance has been determined to be quantitative which can be exploited for development of resistant cultivars (Kumar et al., 2011; Abera et al., 2016; Debela et al., 2017).

TLB is one of the major maize diseases having wide distribution and high economic importance in Ethiopia. The disease is prevalent from low land humid through highland humid agro-ecologies during the wet rainy seasons. There are maize cultivars that have been made obsolete (put out of production) due to their susceptibility to TLB. For example, 'Beletech', an open pollinated variety released in 1990 for lodging resistance, and 'BH541', a high yielding hybrid variety released in 2002, were withdrawn from production due to their susceptibility to TLB (Tilahun et al., 2012). Another very recent example is 'BH543', a high yielding and N-efficient hybrid released in 2005, which is being withdrawn from most maize producing areas due to its susceptibility to this disease. There is no documented information on the existence of different races of TLB causing pathogen in Ethiopia. However, the variable reaction of maize inbred lines like '142–1–e' and '144–7–b', derived from 'Ecuador573', to the diseases when planted in different regions may imply the presence of different races of the causative pathogen within the country. These inbred lines, although known to be immune to TLB in the western maize belt of Ethiopia, are susceptible to the disease in the southern part of the country. This clearly indicates the need to study the pathogen's diversity and host–pathogen interactions under different maize growing environments, in order to develop stable maize cultivars that are more resistant to the disease than the currently available TLB tolerant/resistant hybrids (Debela et al., 2017).

Previous screening works under artificial inoculation against the disease showed that some of the commercial parental inbred lines are susceptible to TLB (Table 1). However, the national breeding program of EIAR has elite inbred lines that are resistant to the disease. Efforts have been made to introduce resistance gene(s) to a good combiner, TLB susceptible inbred line, CML197, through backcrossing with TLB resistant line, 142-1-e. The efforts were unsuccessful as the number of resistance genes carried by the donor line and their mode of inheritance was not well defined prior to the start-up of the backcrossing program. Some investigators have reported single dominant and recessive genes as well as QTLs for resistance to TLB in maize (Welz and Geiger, 2000; Poland et al*.,* 2011). When the resistance gene in the donor parent is recessive, there should be a selfing stage after backcrossing as the backcross progenies are heterozygote susceptible. This was not followed in the previous backcrossing of 142-1e and CML197. Again, when the resistance is controlled by polygenic, the backcross method may not be an efficient method. It is therefore, important to first determine the mode of inheritance of the genes involved in conferring TLB resistance to the elite inbred lines using classical Mendelian genetics. In addition, tests of allelism needs to be done to determine whether the genes carried by each of the elite inbred lines co-segregate or are independent.

Maize streak disease

Maize streak disease is caused by Maize streak virus (MSV). It is a major viral disease of maize in sub-Saharan Africa (Ininda et al., 2006; Magenya et al., 2008; Martin and Shepherd, 2009; Karavina, 2014). There are reports of serious epidemics in more than 20 African countries including Angola, Benin, Burkina Faso, Cameroon, Democratic Republic of Congo, Ghana, Kenya, Malawi, Mozambique, Nigeria Uganda, South Sudan and Zimbabwe (Wambugu and Delpuech, 2000; Lagat et al*.,* 2008; Magenya et al*.,* 2008). Similar epidemics had occurred in south western Ethiopia in 2013, which caused yield losses of 50 to 100% on farmers' fields (EIAR, unpublished data).

Eleven strains of MSV are known to exist; named from MSV-A to MSV-K (Shepherd et al., 2010; Monjane et al., 2011). Of these strains MSV-A is known to cause the most severe and economically important form of maize streak virus disease. There are also five strain variants of MSV-A; viz. MSV-A1 to MSV-A4 and MSV-A6. Among these, MSV-A1 is the most widely distributed variant (Karavina, 2014). No information is available regarding which strains and variants are prevalent in Ethiopia.

In Ethiopia, this disease was formerly known only in Gambella (the western low land sub-humid plains bordering South Sudan). But in recent years, the disease is becoming very important in the mid-altitude agro-ecology of Ethiopia and posing a significant threat to maize production in the country (personal observation). Most commercial varieties currently under production are found to be susceptible to the virus. Figure 1 depicts MSV susceptibility of BH540, the most popular hybrid that had 36% maize seed market share in 2013 (Abate et al., 2017).

A number of options have been recommended for management of maize streak disease including cultural, chemical and host plant resistance. The most effective, environmentally friendly and economically viable method of MSV management is by the use of host plant resistance (Lagat et al., 2008). To date, a gene responsible for conferring resistance to MSV is identified as *msv1* (Kyetere et al*.,* 1995; Nair et al., 2015).This gene has been fine mapped (Nair et al., 2015) to chromosome 1 and thus can be a suitable candidate locus for introgression into susceptible germplasm using

Figure 1. A popular maize hybrid, BH540, highly infected by MSV on farmers' field in Benishangul Gumuz, Western Ethiopia.

marker assisted selection (MAS) (Pratt et al., 2003; Nair et al., 2015). One of CIMMYT's elite inbred lines, CML202, is known to harbor this gene (Welz et al., 1998). This inbred line is well adapted to the Ethiopian condition; and is in the pedigrees of two high yielding drought tolerant hybrids (BH546 and BH661). CML202 can, therefore, be used as a potential source of resistance for introducing the gene into the susceptible germplasm using MAS.

There are two open pollinated maize cultivars released for MSV prone areas (Gambella Regional State); namely, 'Gambella Composite' and 'Abo–Bako'. These cultivars were developed by selecting for tolerance to the disease under natural hotspot screening. However, these cultivars were not sufficient to satisfy the recently increasing demand for hybrid maize by the emerging private sector grain and seed producers in this area. This emphasizes the need for targeted MSV tolerant/resistant hybrid maize adaptation and development endeavor in the country. Research and breeding efforts targeted to develop MSV resistant/tolerant germplasm through artificial inoculation will, however, be challenged by the requirement for well-developed insect rearing and inoculation facilities, which could be established in the long run.

Another approach is to introduce MSV resistant cultivars and breeding materials, especially from International Institute of Tropical Agriculture (IITA) where maize genotypes are specifically bred for MSV tolerance, followed by evaluation of the materials in Gambella, the area that represents MSV prone lowland sub-humid agro-ecology, for further evaluation and possible commercialization within that region. This, however, entails establishment of a quarantine site within these MSV affected areas.

Maize lethal necrosis

Maize lethal necrosis disease (MLND) is a result of a combination of two viruses, Maize Chlorotic Mottle Virus (MCMV) and any of the cereal viruses in the *Potyviridae* group, such as Sugar Cane Mosaic Virus (SCMV), Wheat Streak Mosaic Virus (WSMV) or Maize Dwarf Mosaic Virus (MDMV). The double infection of the two viruses gives rise to what is known as MLN (http://mln.cimmyt.org/mln-overview/)

In Africa, the disease was first reported in Kenya in September 2011 (Wangai et al., 2012). Since then, the disease has spread rapidly into other east African countries. To date, the presence of MLN has been confirmed in Rwanda (Adams et al., 2014), Tanzania, Uganda and South Sudan (Isabirye and Rwomushana, 2016), Democratic Republic of Congo (Lukanda et al*.,* 2016), and Ethiopia (Mahuku et al., 2015; Isabirye and Rwomushana, 2016). In Ethiopia, although its first epidemics was reported in the Central Rift Valley in 2014 main season (Mahuku et al., 2015), MLN attack has now been confirmed in all major maize growing areas of the country at varying level of severity (Demissie et al., 2016). Predictions made using genetic algorithm model estimated that Ethiopia has the potential to lose its entire maize area (Isabirye and Rwomushana, 2016) unless effective preventive measures and all available disease management options are taken. In addition to threatening food security directly, MLN has the potential to negatively impact human health and wellbeing via secondary fungal infections which lead to the production of mycotoxins.

Gowda et al.(2015) and Beyene et al. (2017) identified candidate SNPs that confer resistance to MLN. The same study identified inbred lines carrying resistance to the disease that could be used as donors for improving the susceptible germplasm by using MAS or genomic selection. Since the disease can potentially bring the maize sector in Ethiopia to a halt, there is an urgent need to search for resistant maize germplasm to use as source of resistance and/or for direct use as parents of commercial hybrids.

There is an ongoing attempt to evaluate MLN tolerant maize introduced from CIMMYT-Kenya after three years ban of any maize seed introduction from Kenya. In addition, a number of inbred lines from IITA and CIMMYT (other than Kenya) known to be tolerant to MLN are being evaluated and used in the mid-altitude maize breeding program. The recent study by CIMMYT on the inheritance of resistance to MLN in maize (Beyene et al., 2017) will enhance effective quick use of the available MLN tolerant maize germplasm in Ethiopian maize breeding program.

To determine the availability of MLN tolerant germplasm locally, the Ethiopian breeding program has sent the locally available elite maize germplasm to Kenya and screened against MLN at the Naivasha MLN screening facility in collaboration with CIMMYT. The screening included elite breeding lines, commercial OPVs, pipeline and released hybrids, and their parents. The result of screening over two years showed none of the commercial hybrids released for mid-altitude agro ecology (the high potential maize production area in the country) of Ethiopia and their parents are resistant/tolerant to MLN (unpublished data). One of the three pipeline hybrids were tolerant to the disease (average score of 4.5 across two years), however, the parental inbred lines of this hybrid were highly susceptible. While the genetics of inheritance behind such phenomenon is yet to be determined, the pipeline hybrid can hardly be recommended if the seed production is aimed in MLN prone areas.

Out of the released OPVs screened, Gibe2 (a variety tolerant to GLS and drought) and Gibe3 (a high yielding, GLS and TLB resistant variety) were found to be relatively tolerant to MLND (with an average score of 5.0 over two years) (data not shown).

Common leaf rust

Common leaf rust (CLR), caused by *Puccinia sorghi* Schwein, is another important disease of maize in Ethiopia that is widely distributed throughout the major maize growing regions of the country. However, the importance varies from place to place. It is more severe in the southern mid-altitude and the highland sub-humid maize growing agro-ecologies of the country. The first quality protein maize (QPM) hybrid variety registered in Ethiopia, 'BHQP542', was short-lived in the commercial production and seed systems due to this disease.

In maize, major race-specific resistance genes (*Rp*genes) have been used to control common rust (Pataky et al., 2001; Wisser et al., 2006). To date, eight different common rust resistance genes have been mapped (*viz. Rp1, Rp3, Rp4, Rp5, Rp6, Rp7, Rp8* and *Rp9*) (Sucher et al., 2016). However, race-specific resistance is not durable as opposed to quantitative resistance. Pyramiding these genes with the aid of molecular markers linked to the race-specific resistant loci is needed to effectively control the diseases. This approach has been effectively used in other crops to control various crop diseases (Joshi and Nayak, 2010).

MAJOR INSECT PESTS OF MAIZE IN ETHIOPIA

Insect pests are more destructive in the tropical than in the temperate environments because of the favorable climatic conditions that are more conducive for accelerated insect development with numerous overlapping generations leading to high infestation levels and losses.

Maize weevil

The maize weevils, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) are the most important postharvest pests of stored cereal grains (Abate et al., 2000; Demissie et al., 2008; Mwololo et al., 2012). The maize weevil is usually prevalent under warm and humid conditions (Barney et al., 1991); which are mostly manifested under tropical and sub-tropical climates.

Weevils attack in maize can cause both quantity and quality losses (Barney et al., 1991; Sone, 2001; Kaaya et al., 2005). Secondary grain infections by ear rot fungi that develop after weevil attack can also lead to mycotoxins accumulation (Sone, 2001).

Grain resistance against weevil infestation and damage is one of the most sustainable and cheapest methods of minimizing weevil damage in maize especially at smallholder farmer level. Weevil resistance traits have been identified and deployed in maize germplasm to enhance grain resistance against weevil damage (Tipping et al., 1989; Arnason et al., 1993; Dhliwayo and Pixley, 2003).

In Ethiopia, grain losses due to maize weevil are estimated to be 30 to 100% (Demissie et al., 2012b). Different management options including the use of chemicals, botanicals, cultural, host resistance and integrated pest management (IPM) have been recommended (Demissie et al., 2012b). Previous screening works identified a number of maize germplasm with moderate to high level of resistance to this insect pest (Demissie et al., 2012b, 2015). On the other hand, some of the popular commercial hybrid cultivars like 'BH140' and 'BH540' are susceptible to the maize weevil and farmers usually suffer from huge losses in their maize grain storage. The nature of resistance and mode of inheritance to weevil in maize remains ambiguous.

Figure 2. Picture showing typical damage by fall armyworm on maize.

Stem borers

Stem borers are major insect pest constraints to maize production in SSA causing significant yield losses and grain quality degradation. They are most damaging in the larval stages when they tunnel inside the maize stem after hatching; and therefore, very difficult to control. Successful infestation of these borers into plants, and their feeding may cause death of growing points, reduction in number of harvestable ears or may cause structural damage that increases the likelihood of lodging. These pests can also attack maize ears making the cob and the kernels vulnerable to ear rots due to fungal attacks which produce harmful mycotoxins.

There are four species of stem borers that attack the maize plant (Demissie et al., 2012a). In Africa, they are mainly the African stalk borer (*Busseola fusca* Fuller), the spotted stem borer (*Chilo partellus* Swinhoe), the pink stem borer (*Sesamia calamistis* Hampson) and the sugarcane borer (*Eldana saccharina* Walker) (Mailafiya et al., 2009). Yield losses as high as 91% due to stem borers are recorded at hot spot areas in Ethiopia (Abate, 2012). Three species of stem borers (*viz*. *B. fusca*, *C. partellus* and *S. calamistis*) are known to be distributed across maize growing agro-ecologies in Ethiopia (Abate, 2012). However, *B. fusca* and *C. partellus*are the most predominant and economically important stem borers in Ethiopia (Demissie et al*.,* 2012a).

Fall armyworm

Fall armyworm (FAW) (*Spodoptera frugiperda*) is another newborn challenge and pandemic to Africa's crop production (Goergen et al., 2016). The Fall Armyworm is a migratory insect pest known to cause massive destruction of maize crops under warm and humid conditions in America (RAMIREZ-CABRAL et al., 2017). The pest was first detected in Africa in 2016 in Nigeria and subsequently in southern Africa (Goergen et al., 2016). In just one year, the insect moved all the way to East Africa and reached Ethiopia in March 2017; and is now confirmed in more than 30 countries on the continent (Prasanna et al., 2018). There exists a natural variability in maize to FAW attack (Widstrom et al., 1972; Ni et al., 2011,. 2014). Owing to the fact that FAW is a new pest to Ethiopia, the responses of the commercial maize varieties widely cultivated in the country and their parental inbred lines to this pest is yet to be known. Figure 2 depicts a typical damage caused by FAW on leaves, stem and cobs of maize plant.

STRIGA, THE MAJOR PARASITC WEED OF MAIZE IN ETHIOPIA

Striga is a parasitic weed that is rapidly expanding its territory in Ethiopia. Previously, it was known to have economic importance in the Eastern (Hararghe) and Northern (Tigray and Wollo) parts of the country. However, it is now becoming a major maize production issue including the western (Benishangul Gumuz), central-western (East Wollega) and north-western (West Gojjam and Metekel) parts of the country. This parasitic weed could probably be moved to East Wollega (where it had not been known before) from the neighboring farmers in West Gojjam through informal seed exchange. Another likely introduction of *Striga* to this area is with the settler families from Hararghe and Wollo during the drought famine of 1974 and 1984 (Abate et al., 2015). It is likely that during this period the families unintentionally carried *Striga* seeds with their sorghum seed introducing the weed to the new areas. In East Wollega, the high potential maize production area with 4.5 tons ha^{-1} average productivity in 2016 (CSA, 2017), the weed has already invaded two districts and it will potentially invade the major maize belt unless immediate control measures are taken. Fasil et al. (2010) indicated the likely presence of different races of *S. hermonthica* adapted to a wide range of environments and host ranges. There have also been reports that *S. hermonthica* is going beyond its host range and affecting crops that have previously known to be unaffected (Haylom, 2014).

Host-plant resistance is the most economically feasible and environmentally friendly method of *Striga* control. Screening methodologies for the identification of sources of resistance to *Striga* in host plants has been developed (Menkir et al., 2012). And genetic resistance to *Striga* has been reported in maize (Menkir et al., 2012). IITA has been successfully developing *Striga* resistant/tolerant hybrids and open pollinated varieties and released in different western African countries (Nigeria, Benin and

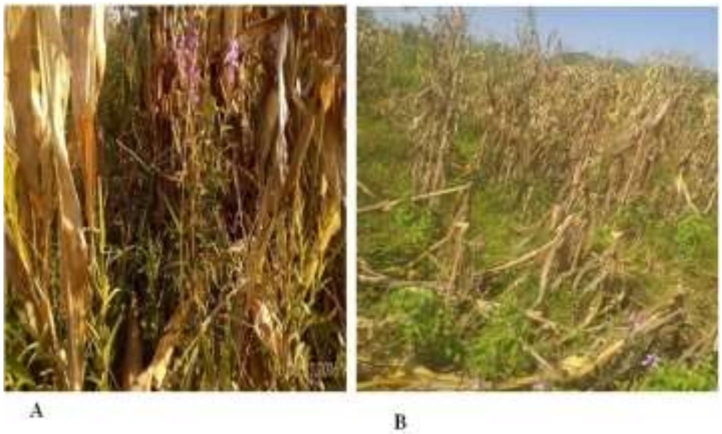

Figure 3. A, Strigainfested maze field in Pawe District (North western Ethiopia) Photo by ZigijuMesenbet; and B, Striga infested maize field in Kemashi (Western Ethiopia). Photo: By AlemuDadi.

Mali). The resistant germplasm from IITA should be exploited as sources of resistance by introgeressing into the locally adapted genotypes and/or for direct commercialization in *Striga* prone areas. Screening the currently available germplasm to this parasitic weed is also important as the reaction of the available germplasm to *Striga* has not been fully explored. In addition, incorporating herbicide resistance gene into maize varieties is another alternative to control *Striga* in maize fields (Menkir et al., 2010) (Figure 3).

CONCLUSION AND FUTURE DIRECTIONS

Maize has a versatile use in Ethiopia serving as food, feed, fuel wood and source of income, among many others. Developing biotic stress resistant maize cultivars is very important to feed the ever-increasing population in the country in the present context of climate change. Concerted research efforts have been made in developing a number of maize varieties that highly contributed to the maize productivity revolution in Ethiopia (Abate et al., 2015). These efforts should be further strengthened to develop varieties resistant to multiple biotic stresses. Stress resistant cultivars can play an important role in coping with climate variability and thus sustainably enhancing the productivity of maize in the country.

The effect of climate change in sub-Saharan Africa is becoming evident with the emergence of invasive plant diseases and insect pests. In just half a decade, Africa gave a reception to two new major pests of maize, MLN in 2011 and FAW in 2016. These pests can individually cause up to a total loss of maize yield unless appropriately managed. The pests can be controlled by application of pesticides but this will incur additional production costs to the farmers. It is also well known that application of chemical pesticides is not environmentally safe. Our understanding of the risk of pathogen and insect pest introductions is either still limited or overlooked and there is a need to improve surveillance and quarantine strategies. However, reducing the threats and impacts of such cross-boundary plant diseases and insect pests will require novel approaches to integrated research and long-term commitments from scientists and policy makers.

To effectively tackle the prevailing biotic maize production stresses and sustainably increase the rate of genetic gain, the existing breeding strategy should be transformed with the integration of modern tools and approaches. Traditional methods of crop improvement alone are not sufficient to keep abreast with the rapidly growing population and the escalating climate change threats which can potentially aggravate the biotic constraints of maize production in Ethiopia. Therefore, ways of introducing and integrating recent advances in biotechnology with the conventional maize breeding approaches should be explored and implemented. This will facilitate maize breeding for resistance to the major biotic stresses in Ethiopia through rapid and well-designed introgression of specific biotic stress tolerance genes into the already available elite maize germplasm, thus ensuring pronounced genetic gain. The conventional approach of maize germplasm screening against major biotic stresses will enable identifying sources of biotic stress tolerance genes, which can then be utilized in the breeding programs through MAS. In this paper the major biotic stresses of maize in Ethiopia and efforts in tackling this problem through genetic improvement over the years has been summarized and presented. This information will be used for breeders, private and public maize seed and grain growers who are targeting to operate in Ethiopia and Eastern Africa.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Defense response by inter-active bio-protector and chitosan to *Sclerotium rolfsii* Wilt disease on cowpea, Brazilian Oxisol

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Pathogenic microorganisms increase enzyme production and plant response, causing injuries and even plant death. Chitosan has shown potential to induce reactions in plant pathogens. An "*in vitro*" assay determines the action of chitosan and evaluates the optimal concentration to act against (*Sclerotium rolfsii*). The anti-microbial activity of chitosan and the bio-protector produced by the inter-active bacteria *Beijerinckia indica* and fungus *Caenorhabditis elegans* that contain chitosan were tested in a pot experiment against *S. rolfsii* on cowpea grown in a Brazilian Oxisol. Biofertilizer and chitosan in foliar application were used with and without *S. rolfsii*. The plants were analyzed, and the disease severity index, soluble protein and enzymatic activities were determined. The "*in vitro*" test showed chitosan effectiveness against *S. rolfsii*. The pot experiment with sterilized and non-sterilized soil confirmed the ability of the bio-protector that contains chitosan from *C. elegans* and chitosan application to increase enzymatic activities processed by *S. rolfsii*. The higher concentrations of the bio-protector and chitosan (4.0 and 6.0 mg mL^{-1}) were directly related to the catalase and peroxidase activities controlling plant resistance and the disease severity index. The bio-protector may be a viable alternative to soluble fertilizer, and recommended for organic and sustainable agriculture. These findings are important for the establishment of sustainable agriculture and to avoid the use of pesticides.

Key words: Antifungal activity, biopolymer, enzyme activities, plant resistance, postharvest analyzes, sustainable agriculture.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is a legume of socioeconomic importance in tropical countries, especially in Brazil, because it represents a popular dietary source of protein, carbohydrates, iron, potassium and phosphorus. Cowpea legumes can be used for animal feed, forage, hay, silage, flour and soil fertility as green manure; additionally, it has considerable tolerance to drought and heat stresses (Berger et al., 2016).

Sclerotium rolfsii (Sacc) is one of the most important phytopathogenic fungi in the tropical and sub-tropical regions, and it causes serious damage in more than 500 plant species, including agricultural crops; in the cowpea legume, it causes a frequent and important disease known as *Sclerotium* Wilt disease, according to Sharma et al. (2012). This pathogen affects the soil and can grow and survive for long periods in the form of chlamydospores, and the symptoms of the disease in cowpea reduce the plant growth and cause chlorosis, wilting and premature leaf fall, which almost always result in the death of infected plants (Berger et al., 2016).

Normally, the application of large amounts of chemical products (fungicides) is necessary to control plant diseases, which causes serious problems in the soil ecosystem. Natural substances studied as alternatives to control pathogenic microorganisms reduce the injuries; however, it is well-known that these products are even prejudicial to the environment and may promote food contamination (Thakkar and Sarafi, 2015).

Chitin is a natural biopolymer of N-acetyl-d-glucosamine that is present as structural elements in invertebrates, in most arthropods and in the cell walls of fungi, especially from the Mucorales Order and *Zygomycetes* classes (Bautista-Baños et al., 2016). Chitosan is a biodegradable and biocompatible polysaccharide that has antifungal activity, inhibits the growth of pathogenic fungi and induces defense mechanisms in plants. Chitosan also stimulates the accumulation of proteins and the production of reactive oxygen species, alters the metabolism of phytoalexins, induces the formation of phenolic compounds, and activates peroxidase, chitinase, β-1,3-glucanase, superoxide dismutase and catalase enzymes (Andrade et al., 2013).

The correct use of chitosan promotes a large increase in the activity of catalase and peroxidase, and these enzymes are involved in plant defense against pathogenic microorganisms, according to Dousseau et al. (2016). In recent studies, the biopolymer chitosan demonstrated antimicrobial activity against pathogenic fungi and the ability to induce mechanisms of plant defense (Romanazzi et al., 2013).

The aim of this study is to evaluate the defense response to *S. rolfsii* by the bioprotector produced from phosphate and potassic rocks mixed with organic matter inoculated with interactive diazotrophic bacteria (*Beijerinckia indica*) and *Cunninghamella elegans* (Mucorales fungi) on cowpea in a Brazilian Oxisol of the rain forest region.

MATERIALS AND METHODS

Test for chitosan: *In vitro* assay

An *in vitro* assay was conducted in Petri dishes to test the effectiveness of crustaceous chitosan applied in various concentrations against the pathogenic fungus *S. rolfsii*. The phytopatogenic fungus *S. rolfsii* used in the present study causes *Sclerotium* Wilt in cowpea, which interferes with important oxidative damage. The fungus was isolated in the laboratory from inoculated plants that presented symptoms of the disease in cowpea seedlings, transferred to Petri dishes containing potato-dextrose-agar (PDA) medium, and maintained for 15 days in a camera biochemical oxygen demand (BOD) at 28°C. The fungus pathogenicity was confirmed by characteristics of the culture when inoculated on cowpea seedlings (Berger et al., 2016).

To evaluate the mycelia growth of the *S. rolfsii* isolate, the crustaceous chitosan, purchased at a medium molecular weight from Sigma-Aldrich Chemical Company, was used. To obtain the gel chitosan, the biopolymer was mixed for 24 h in a horizontal shaker and then incorporated into potato-dextrose-agar medium, obtaining the final concentrations of 0.5, 1.0, 1.5, 2.0, 4.0 and 6.0 mg mL^{-1} according to Di Piero and Garda (2008). The absolute control contains only the PDA medium, and the relative control contains PDA and acetic acid in the same volume as the gel chitosan (Bautista-Baños et al., 2016).

The chitosan gel was sterilized for 15 min at 120°C and transferred to Petri dishes until complete growth, and then, the mycelia of *S. rolfsii* (0.5 cm) was added in the center of each Petri dish and maintained in camera BOD at 28°C. The diameter of the fungus was determined every 24 h until the control treatment reached the plaque boards. The fungal growth was observed in subsequent periods (7, 14, 21, 28 and 35 days) in the different treatments (0.5, 1.0, 1.5, 2.0, 4.0 and 6.0 mg mL^{-1}), and two control treatments were applied: (a) absolute control (PDA) and (b) relative control (PDA + Acetic acid), with six replicates.

The data were submitted to statistical calculations using the software program SAS Institute, Learning Edition 9.1, Cary, North Carolina, USA. Analyses of variance and means were compared by the Tukey test ($P \leq 0.05$), and when necessary, the regression analyses were processed to evaluate the effectiveness of the chitosan concentration on fungal growth as a function of the subsequent periods (Stamford et al., 2017).

Greenhouse experiment in soil pots

The mixed biofertilizer was produced from phosphate and potassic rock biofertilizers mixed with earthworm compost as organic matter (OM) in a PKB:OM ratio of 1:3 (v/v) following Stamford et al. (2007). The P and K analysis for rock biofertilizer showed the following results: pH = 3.8, available P = 60 g kg^{-1} and available K = 10 g kg^{-1} (Silva et al., 2009).

The earthworm compost was enriched in N by inoculation with the free living diazotrophic bacteria *B. indica* (NFB 1001) selected in the Nucleus of Nitrogen Fixation at the University Federal Rural of Pernambuco (UFRPE). The selected diazotrophic bacteria were cultured in LG liquid media (50 mL) in 125-mL Erlenmeyer flasks and shaken (180 rpm) for 96 h at ± 28°C, and 100 mL was applied per tray (6 L) according to Lima et al. (2010). Analysis of the earthworm compound showed the following: pH = 7.85; organic carbon = 120.7 g kg^{-1}; total N = 8.6 g kg^{-1}; total sulfur = 2.9 g kg^{-1}; and total P = 11.2 g kg^{-1}. The mixed organic biofertilizer analysis showed the following: pH = 6.2; organic carbon = 100 g kg^{-1}; total N

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= 20 g kg^{-1}; total sulfur = 3 g kg^{-1}; available P = 13 g kg^{-1}; and available K = 15 g kg^{-1} (Silva et al., 2009).

To produce the bioprotector, the organic biofertilizer enriched in N was inoculated with a mycelia biomass of the Mucorales fungi *C. elegans* (UCP 542) that contained chitosan and chitin in the cellular wall (Berger et al., 2016). The fungus was grown for seven days at 28°C in Petri dishes with liquid PD medium, producing a final concentration of 10^6 spores mL^{-1}, and the spore suspension was shaken at 150 rpm.

The greenhouse experiment was carried out at the University Federal Rural of Pernambuco from 03 June to 23 July 2016, using an Oxisol soil (USDA, 2014). The soil samples were collected at 0 to 20 cm deep from the rain forest region, Pernambuco, Northeast Brazil, with coordinates 7° 53′ 49″ S, 35° 10′ 48″ W. The soil was air dried sieved (5 cm), mixed and kept in pots (8 dm^3). Chemical analysis of the soil showed the following: pH (H_2O) = 5.4 and exchangeable cations (cmol$_c$ dm^{-3}): K^+ = 0.08; Ca^{2+} = 1.40; and Mg^{2+} = 0.55. Soil analyses were processed using an absorption spectrophotometer and atomic emission (Perkin Elmer 3110), and total N was determined by the Kjeldhal methodology using a N auto analyzer (Kjeltec 1030).

The experiment was realized with sterilized and non-sterilized soil to evaluate the effectiveness of the bioprotector inoculated with the Mucorales fungi *C. elegans* and the action of crustaceous chitosan (foliar application) against the pathogen *S. rolfsii* on cowpea. The soil sterilization was processed three times for 60 min at 121°C in intervals of 24 h.

The cowpea (cv. IPA 207) was surface-sterilized in 70% ethyl alcohol for 1 min, followed by immersion in $HgCl_2$ (1:500) for 0.5 min, washed six times with sterile water, sown (7 seeds pot^{-1}) at a depth of 5 mm, and after emergence, thinned to 3 seedlings per pot. Plants were inoculated (2 mL pot^{-1}) by applying a bacteria culture (10^8 UFC mL^{-1}) with *Bradyrhizobium* (BR 3267) strain.

During the experiment, the photoperiod remained close to 12 h of dark and 12 h of light, the temperature oscillated between 28 to 36°C, and the relative humidity was 60 to 80%. Seven days after germination, the crustaceous chitosan was sprayed on the first pairs of completely developed leaves (4 mL plant^{-1}). The plants were inoculated with the pathogen *S. rolfsii* by addition of a conidial suspension (10^6 mL^{-1}).

The index of disease severity was evaluated at 50 days after the planting date by a transversal cut in the stem of the plants to observe the occurrence of symptoms in the vascular system. The index of disease severity was processed as described earlier:

(1) No visual symptoms of the disease in plants and in soil;
(2) Small symptoms (necrosis in the shoot of plants) at the point of inoculation;
(3) Large symptoms (large visual lesions in the shoot of plants) and fungal growth, plants showed debilitation, and the soil has fungal growth;
(4) Plants present visual symptoms of wilt in the hypocotyls, showing fungal growth and the presence of scleroses in the plant and soil, and some plants are dead;
(5) Many plants are dead or showed large symptoms of wilt, showing fungal growth in the plant and soil.

The activities of enzymes (catalase and peroxidase) were evaluated in cowpea at 8 and 16 days after pathogen inoculation. The enzyme extract was prepared with 1.0 g of macerated leaf tissue using liquid nitrogen, 4 mL of phosphate buffer solution and 50 mg of peroxidase. This concentrate was filtered and centrifuged (10 min at 4°C, 10000 g). The supernatant was stored at -80°C and used to evaluate the activity of catalase and peroxidase via the soluble protein content according to Andrade et al. (2013). The soluble protein was determined by colorimetric analysis (Bradford, 1976).

The peroxidase activity was estimated with the evaluation of Δ absorbance provided through the oxidation of guaiacol ($C_3H_8O_2$) in the presence of hydrogen peroxide (Fatibello-Filho and Vieira, 2002). The catalase activity was analyzed according to Beers and Sizer (1952).

The pot experiment was conducted with the following treatments: (1) soluble fertilizer, without chitosan + pathogen; (2) bioprotector at a rate of 50% the recommended rate (RR) + pathogen; (3) bioprotector 100% RR + pathogen; (4) bioprotector 150% RR + pathogen; (5) earthworm compost + pathogen; (6) earthworm compost (EC) with 2 mg mL^{-1} chitosan (foliar application) + pathogen; (7) earthworm compost with 4 mg mL^{-1} chitosan (foliar application) + pathogen; (8) earthworm compost with 6 mg mL^{-1} chitosan (foliar application) + pathogen; (9) organic biofertilizer 150% RR + pathogen; (10) control 1 (soil + pathogen); and (11) control 2 (soil without pathogen).

The soluble fertilizer treatment was estimated following the recommendation for irrigated cowpea (IPA, 2008). The soluble fertilizer used N (ammonium sulfate, 20% N), P (simple super phosphate, 18% P_2O_5), and K (potassium sulfate, K_2O, 50%) corresponding to applications of 500 kg ha^{-1}, 300 kg ha^{-1} and K 80 kg ha^{-1}, respectively. The amounts of mixed biofertilizer and bioprotector were calculated based on N content (2% N), which corresponded to an application of 5000 kg ha^{-1}.

The statistical calculations were performed using the software program SAS Learning Edition 9.1. Analyses of variance and means were compared by the Scott-Knott test ($P \leq 0.05$) (SAS, 2011).

RESULTS

Mycelia growth of *S. rolfsii*: *In vitro* assay

In the mycelia growth of *S. rolfsii,* a significant difference was observed when chitosan was used at concentration of 6.0 mg mL^{-1}, and the application of chitosan showed fungicidal ability when applied in lower concentrations (2 and 4 mg mL^{-1}) with a total effect on *S. rolfsii* growth (Figure 1).

The chitosan concentration demonstrated a significant fungistatic effect on the growth of *S. rolfsii,* and an effect of the acetic acid on the fungi growth was not observed.

The control treatment affected the fungal growth after three days of incubation until the control treatment reached the plaque boards (Figure 1G). The relative control was not different from the absolute control, and the fungal growth was affected within four days of incubation (Figure 1G). The crustaceous chitosan showed fungistatic and fungicidal effects and promoted morphological changes of *S. rolfsii* during the growth period.

Bio-protector production

The strain of *B. indica* (NFB 1001 and 1003) used to produce the bioprotector was completely characterized by the Korean group Macrogen Incorporation(Geumcheon-gu

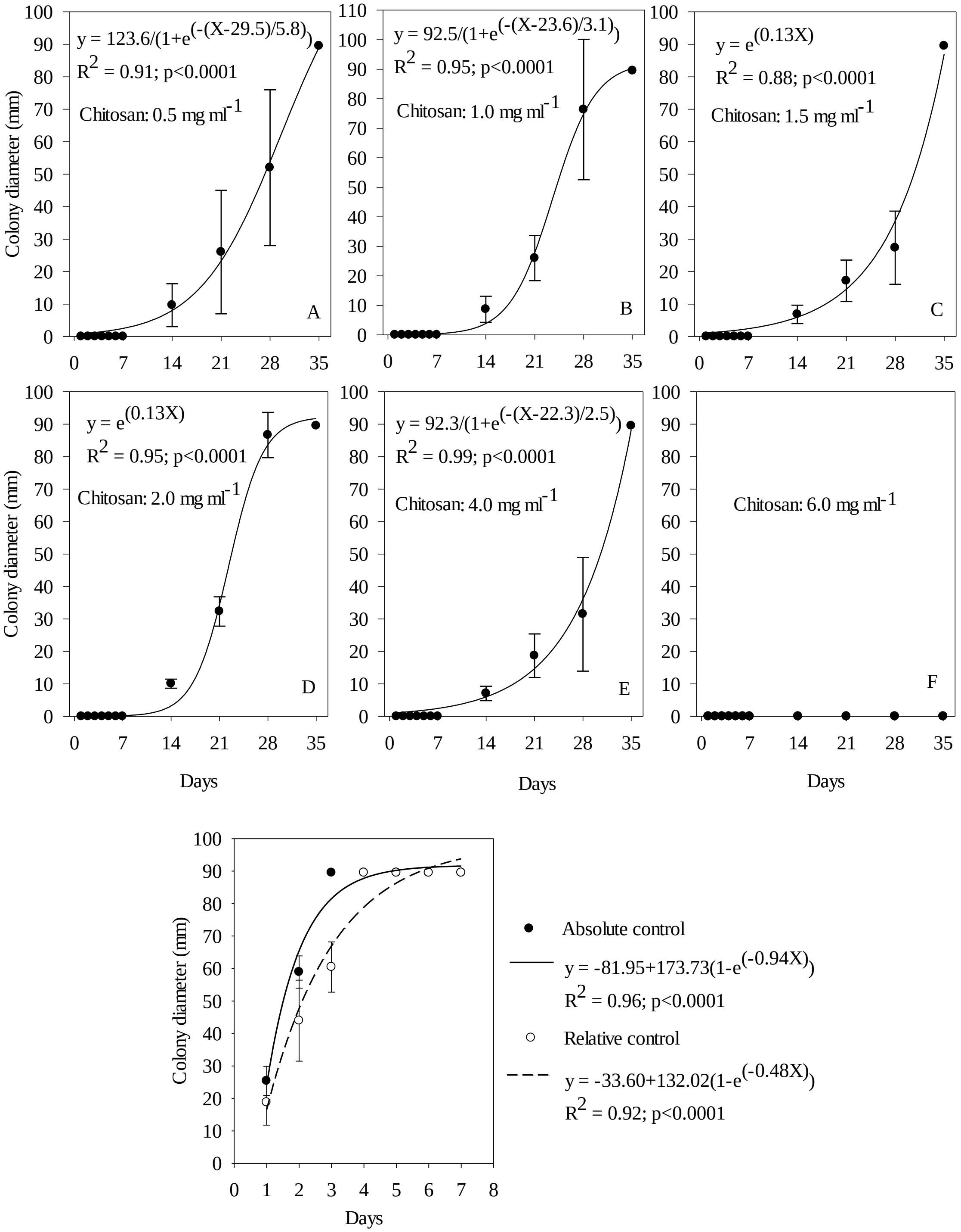

Figure 1. Mycelia growth of *Sclerotium rolfsii* with application of crustaceous chitosan at different concentrations (0.5, 1.0, 1.5, 2.0, 4.0, and 6.0 mg mL^{-1}), including absolute and relative controls at various growth times (0, 7, 14, 21, 28 and 35 days)

Seoul), and the gene sequences compared with other diazotrophic bacteria are shown in Table 1. The index of disease severity for *S. rolfsii* (%) in cowpea collected 50 days after pathogen germination is shown in Table 2. The chitosan applied in cowpea culture increased the defense against *S. rolfsii*. Plants with less damage were

Table 1. Characterization and rRNA 16S gene sequence of the diazotrophic bacteria isolated from Brazilian soils, compared with other diazotrophic bacteria deposited in GenBank (NCBI).

Strain	Description	Similar sequence	Coverture (%)	Identity (%)	E-value	Score
NFB 1003	CP 011534.1	*Bacillus subtilis strain UD1022, complete genome*	55	99	0.0	27042
NFB 4	AJ 295079.1	*Mesorhizobium plurifarium 16S rRNA gene, strain ORS1096 (LMG 15298) complete genome*	92	99	0.0	2553
NFB 6	NR 044403.1	*Paenibacillus castaneae strain Ch-32 16S ribosomal RNA gene, complete sequence*	96	96	0.0	2468
NFB 1001	CP 001016.1	*Beijerinckia indica subsp. indica ATCC 9039, complete genome*	73	99	0.0	7714

exclusively those that received crustaceous chitosan.

The results for the index of disease severity were higher in sterilized soil than in non-sterilized soil. The treatments with crustaceous chitosan at 6.0 and 4.0 mg mL^{-1} in sterilized and non-sterilized soil, respectively, resulted in a lower index of disease severity in cowpea plants. In non-sterilized soil, crustaceous chitosan at 4.0 mg mL^{-1} showed a lower index of disease severity that was significantly different in the cowpea plants. A low index of disease severity was obtained when the bioprotector with an increased fertilizer concentration was applied, especially at the highest rate (150% RR), which presented an index of disease severity of 1.5% in sterilized soil and 1.8% in non-sterilized soil.

The bioprotector applied at the different concentrations produced higher soluble protein content compared with the treatments of earthworm compost without chitosan, earthworm compost with chitosan (rates 2.0 and 6.0 mg mL^{-1}) and mixed biofertilizer with chitosan (Table 3).

An interaction effect was observed when the mixed biofertilizer was applied at higher rates and increased the protein content. However, the treatments using mixed biofertilizer with pathogen and the control treatment presented the higher protein content in sterilized soil.

In the second period, an effect of the control treatment with lower protein content ($P<0.05\%$) was observed compared with the other treatments (Table 3). In non-sterilized soil, the mixed biofertilizer showed a higher soluble protein content, and the bioprotector, independent of the concentration, showed a lower protein content ($P<0.05$). In the sterilized soil, the treatment with earthworm compost and chitosan (4.0 and 6.0 mg mL^{-1}) with pathogen inoculation produced higher protein content.

Peroxidase activity

Peroxidase activity in cowpea leaves collected eight days after inoculation with *S. rolfsii* using sterilized and non-sterilized soil is shown in Table 4. The peroxidase activity was activated in the leaves of cowpea plants when crustaceous chitosan was applied at higher levels (bioprotector + chitosan at 6 mg mL^{-1}). Consequently, the plants that received this treatment showed less damage than the control treatment.

In non-sterilized soil, the plants inoculated with *S. rolfsii* that received treatments with bioprotector + chitosan (4 mg mL^{-1}) and bioprotector + chitosan (6 mg mL^{-1}) showed a higher peroxidase activity compared with the plants in the absence of this pathogen. The application of these treatments also resulted in the lowest index of disease severity of *S. rolfsii* wilt in the cowpea plants. Consequently, the relationship between increases in peroxidase activity and increases in plant protection in the presence of a biotic stress is confirmed.

The results of peroxidase activity in the cowpea leaves collected 14 days after inoculation with *S. rolfsii* are shown in Table 4. The enzyme peroxidase showed low activity in the plants with the bioprotector + chitosan (6.0 mg mL^{-1}) and bioprotector + chitosan (4.0 mg mL^{-1}) treatments with sterilized and non-sterilized soil, respectively. In sterilized soil, it was observed that the treatments induced higher peroxidase activity, especially when compared with the control treatment that used soil without pathogen addition.

Catalase activity

In the first period, independent of the sterilized and non-sterilized soil, the treatment of earthworm compost with the treatment with the bioprotector in a higher rate (150% RR) presented higher catalase activity.

The catalase activity in cowpea leaves collected eight days after inoculation with *S. rolfsii* is presented in received the bioprotector + chitosan (6.0 mg mL^{-1}) showed higher catalase activity.

Table 2. Index of disease severity for Sclerotium rolfsii on cowpea in Oxisol soil in the Brazilian rain forest region, evaluated at 50 days after seed germination in sterilized and non-sterilized soil submitted to different fertilization treatments.

Fertilization treatment	Sterilized soil	Non-sterilized soil
	Index of disease severity (%)	
Soluble fertilizer + Pathogen	4.3±0.6^a	4.0±0.1^a
Bioprotector 50% + Pathogen	2.3±0.6^b	2.3±0.6^b
Bioprotector 100% + Pathogen	1.6±0.6^b	2.3±0.6^b
Bioprotector 150% + Pathogen	1.5±0.6^b	1.8±0.6^b
Earthworm Compost (EC)+ Pathogen	4.0±1.7^a	3.3±1.2^a
EC + Chitosan 2 mg mL^{-1}+ Pathogen	2.9±0.6^b	2.3±0.6^b
EC + Chitosan 4 mg mL^{-1}+ Pathogen	2.3±0.6^b	2.0±0.3^b
EC + Chitosan 6 mg mL^{-1}+ Pathogen	1.6±0.6^b	1.6±0.6^b
Biofertilizer + Pathogen	3.6±0.6^a	3.6±0.6^a
Control- Soi1 + Pathogen	1.0±0.1^b	1.0±0.1^b
Control- Soil without Pathogen	5.0±0.2^a	4.6±0.6^a
CV- Coefficient of variation (%)	21.32	20.41

Means followed by the same letter are not different in treatments according to the Scott-Knott test (P≤0.05).

Table 3. Soluble protein in leaves collected at 14 and 28 days after pathogen inoculation (DAPI) on cowpea in an Oxisol of the Brazilian rain forest region in sterilized and non-sterilized soil submitted to different fertilization treatments.

Treatment	Sterilized soil		Non-sterilized soil	
	14 DAPI	28 DAPI	14 DAPI	28 DAPI
	Soluble protein (mg g^{-1})			
Soluble fertilizer + Pathogen	8.9±0.2^a	11.6±0.2^b	8.3±0.4^b	11.8±0.2^a
Bioprotector 50% + Pathogen	6.8±0.3^c	11.1±0.1^c	7.5±0.1^b	10.6±0.4^b
Bioprotector 100% + Pathogen	7.3±0.3^c	11.2±0.1^c	8.3±0.4^b	10.8±0.1^b
Bioprotector 150% + Pathogen	8.2±0.1^b	11.6±0.4^b	8.4±0.1^b	11.9±0.6^a
Earthworm Compost (EC) + Pathogen	8.6±0.3^a	11.0±0.2^c	9.0±1.2^b	10.3±0.3^c
EC + Chitosan 2 mg mL^{-1} + Pathogen	8.7±0.2^a	11.6±0.3^b	8.0±0.3^b	10.5±0.2^b
EC + Chitosan 4 mg mL^{-1} + Pathogen	8.2±0.1^b	11.4±0.3^b	8.7±0.7^b	11.4±0.1^a
EC + Chitosan 6 mg mL^{-1} + Pathogen	8.0±0.1^b	11.5±0.2^b	6.4±0.1^c	11.8±0.7^a
Biofertilizer + Pathogen	8.9±0.5^a	12.1±0.1^a	9.8±0.1^a	11.8±0.2^a
Control - Soil + Pathogen	7.1±0.3^c	10.5±0.2^d	9.8±0.2^a	10.9±0.7^b
Control - Soi1 + (without Pathogen)	8.6±0.3^a	10.4±0.3^d	6.7±1.1^c	10.8±0.4^b
CV – Coefficient of variation (%)	3.22	2.06	6.99	3.84

Means followed by the same letter are not different in treatments according to the Scott-Knott test (P≤0.05).

This result probably contributed to the lowest index of disease severity for *S. rolfsii* wilt in the cowpea plants. The same relationship between the increase in enzyme activity and the reduction in the disease severity index was already discussed with respect to the greater peroxidase activity of the bioprotector with chitosan (4.0 and 6.0 mg mL^{-1}) treatments observed in the non-sterilized soil.

In the non-sterilized soil, the low index of disease severity in the plants inoculated with *S. rolfsii* treated with chitosan at higher concentration showed a reduction in the catalase activity after the pathogen application. The sterilized soil show a significant decrease in the catalase activity when compared with the results in leaves collected eight days after inoculation with the pathogen.

The reduction in catalase activity after the pathogen inoculation (Table 5) compare the results in Table 4 may occur due to the reduction in H_2O_2 in the plants, and the activation of the antioxidant enzyme may be unnecessary. The low activity of catalase is possible due

Table 4. Peroxidase activity in leaves of cowpea collected at 14 and 28 days after pathogen inoculation (DAPI) in an Oxisol of the Brazilian rain forest region, using sterilized and non-sterilized soil submitted to different fertilization treatments.

Treatment	Sterilized soil		Non-sterilized soil	
	14 DAPI	28 DAPI	14 DAPI	28 DAPI
	Peroxidase activity (mmol AsA g^{-1} Protein min^{-1})			
Soluble fertilizer+ Pathogen	2.58±0.2^{e}	8.66±0.3^{c}	0.90±0.1^{d}	3.22±2.6^{d}
Bioprotector 50%+ Pathogen	2.61±0.3^{e}	12.21±1.7^{a}	1.83±0.1^{c}	6.93±0.4^{c}
Bioprotector 100%+ Pathogen	2.36±0.1^{e}	8.24±0.6^{c}	5.48±0.2^{a}	10.50±1.1^{b}
Bioprotector 150%+ Pathogen	2.79±0.1^{e}	8.89±0.1^{c}	1.28±0.4^{c}	12.26±0.9^{a}
Earthworm Compost (EC)+ Pathogen	5.61±0.1^{b}	5.73±0.2^{d}	5.79±0.4^{a}	9.22±0.4^{c}
EC + Chitosan 2 mg mL^{-1}+ Pathogen	1.68±0.1^{f}	5.16±0.2^{d}	0.91±0.3^{e}	7.17±0.7^{c}
EC + Chitosan 4 mg mL^{-1}+ Pathogen	2.24±0.1^{e}	6.24±0.3^{d}	5.64±0.6^{a}	9.45±0.6^{b}
EC + Chitosan 6 mg mL^{-1}+ Pathogen	7.43±1.0^{a}	6.41±0.5^{d}	3.77±1.1^{b}	9.44±0.8^{b}
Biofertilizer+ Pathogen	4.65±0.2^{c}	5.66±0.9^{d}	2.43±0.5^{c}	8.54±0.9^{c}
Control- Soi1 + Pathogen	0.91±0.1^{g}	2.73±0.1^{e}	0.93±0.1^{d}	1.48±0.1^{d}
Control- Soil without Pathogen	3.41±0.2^{d}	11.01±0.5^{b}	1.73±0.2^{c}	12.66±3.6^{a}
CV- Coefficient of variation (%)	10.47	9.35	15.77	21.90

Means followed by the same letter are not different in treatments according to the Scott-Knott test ($P \leq 0.05$)

Table 5. Catalase activity in leaves of cowpea collected at 14 and 28 days after pathogen inoculation (DAPI) in an Oxisol of the Brazilian rain forest region using sterilized and non-sterilized soil submitted to different fertilization treatments.

Treatment	Sterilized soil		Non-sterilized soil	
	14 DAPI	28 DAPI	14 DAPI	28 DAPI
	Catalase activity (µmol AsA g^{-1} Protein min^{-1})			
Soluble fertilizer+ Pathogen	141±7.6^{b}	229±75.0^{a}	86±5.2^{d}	150±28.7^{b}
Bioprotector 50% + Pathogen	111±3.2^{c}	227±19.3^{a}	137±5.4^{c}	173±70.9^{b}
Bioprotector 100% + Pathogen	141±4.2^{b}	241±13.6^{a}	170±9.5^{b}	176±13.7^{b}
Bioprotector 150% + Pathogen	108±5.4^{c}	278±22.0^{a}	187±15.6^{b}	208±19.9^{a}
Earthworm Compost (EC)+ Pathogen	139±7.6^{b}	137±18.2^{b}	126±7.8^{c}	174±40.0^{b}
EC + Chitosan 2 mg mL^{-1}+ Pathogen	159±9.1^{a}	171±13.4^{b}	221±46.0^{a}	146±14.9^{b}
EC + Chitosan 4 mg mL^{-1}+ Pathogen	145±9.6^{b}	172±15.5^{b}	155±12.2^{c}	98±2.1^{c}
EC + Chitosan 6 mg mL^{-1}+ Pathogen	104±3.5^{c}	154±22.0^{b}	172±26.8^{b}	96±9.3^{c}
Biofertilizer + Pathogen	120±6.9^{c}	139±17.7^{b}	140±20.7^{c}	87±5.0^{c}
Control - Soi1 + Pathogen	92±7.5^{c}	123±21.0^{b}	64±14.4^{d}	84±3.8^{c}
Control - Soil without Pathogen	160±8.6^{a}	162±15.6^{b}	245±56.4^{a}	217± 7.3^{a}
CV – Coefficient of variation (%)	8.53	15.91	16.55	18.97

Means followed by the same letter are not different in treatments according to the Scott-Knott test ($P \leq 0.05$).

to the low amount of ROS in the sites where the enzymes act, which are sites of the peroxidase action.

DISCUSSION

Effectiveness of chitosan *in vitro*

The effectiveness of chitosan on *Botrytis cinerea* was reported by Camili et al. (2007), who described the *in vitro* effects of chitosan concentrations equivalent to 0.5, 1.0, 1.5 and 2.0% over a period of five days at 22°C. Similar results were reported by Berger et al. (2016) with crustaceous chitosan, and the authors observed a fungistatic effect in the control of *Fusarium oxysporum* f.sp. *tracheiphilum*.

The dependence of the effect of chitosan on the concentration was also reported by Freddo et al. (2014)

in a study with *Rhizoctonia solani* to evaluate the fungistatic effect of chitosan applied in various concentrations (0.0, 0.25, 0.5, 1.0 and 2.0%). Liu et al. (2006) observed a fungicidal effect of chitosan inhibiting the mycelia growth and spore germination of *Botrytis cinerea* and *Penicillium expansum* fungi.

Many authors suggested that the antimicrobial activity of chitosan is due to the amino group in a polycationic form in the presence of low pH, as generally occurs in chitosan solutions. In these conditions, the cationic structure may interact with the negative charges of the anionic groups of the cellular membrane of the microorganisms and change the permeability, promoting the decrease in intracellular components (Di Piero and Garda, 2008). Prapagdee et al. (2007) agree that the effect of chitosan is a fungistatic effect, because this biopolymer is the principal active compound that can promote structural and morphological modifications and can disorganize the molecules of the fungi.

Gene sequence of the diazotrophic bacteria

Most of the genes involved in the nitrogen fixation process by the diazotrophic bacteria (strain NFB 1001) are distributed into two genomes (10 kb and 51 kb) regarding the gene *nif* that responds to the codification of cysteine desulfurase (Tamas et al., 2010). The strain NFB1001 showed 99% similarity and covertures of 73% with the *B. indica* species. It is found to have lower similarity with the others diazotrophic bacteria deposited in “GenBank” compared with the diazotrophic isolates used in this study. The sequence of the gene *nif* may involve the best information about the diazotrophic bacteria because this gene sequence can confirm 100% of the diazotrophic isolates. However, the gene *nif* has several limitations to its use in phylogenetic analyses and depends on the microbial group (Gaby and Buckley, 2012).

Effectiveness of the bio-protector and chitosan in foliar application

Several studies in greenhouse conditions have shown that chitosan is a polymer that could potentially be used to control plant disease (El Hadrami et al., 2010; Soleimani and Kirk, 2012). When chitosan is applied as a root or seed dressing and in foliar spray, such as in this experiment, the biopolymer can hinder pathogen growth in the plant host tissue. Lowe et al. (2012) observed that chitosan, applied as a foliar spray, reduced the disease symptom severity in strawberry plants infected by *Bacillus subtilis.* The biopolymer induces a number of defense reactions, including structural barriers and different biochemical activities during the plant-pathogen interaction. In response to chitosan application, the host plants showed cellular lignifications and fungal toxin accumulation at sites of attempted pathogen penetration. The chitosan created a barrier that impeded the flux of nutrients between the host and the pathogen, and these results are in accordance with Bautista-Banõs et al. (2006).

In a study to evaluate the effectiveness of crustaceous chitosan, Berger *et al.* (2016) reported no effect of the biopolymer on the growth of *F. oxysporum* f.sp. *tracheiphilum* when used at low concentrations, and a great effect in high concentration (4 and 6 mg mL^{-1}).

Most likely, the chitosan contained in the bioprotector inoculated with *C. elegans* that is present in fungi as chitosan in the cellular walls may act against pathogens. In accordance with Stamford et al. (2017), this biopolymer induces resistance against pathogens. Di Piero and Garda (2008) demonstrated the effectiveness of crustaceous chitosan in the control of *Colletotrichum lindemuthianum* on *Phaseolus vulgaris*, with an increase in the activity of the enzyme β-1,3-glucanase that directly affects the glucose present in the cellular walls, inhibiting the fungal growth. According to Thakkar and Sarafi (2015), chitosan affects the biological control of pathogens because the biopolymer increases the plant resistance.

It is very important to observe that when plants grow in sterilized soil show a low response to the chitosan application against phytopathogenic microorganisms and a non-significant change compared with the control treatment. This behavior may occur in relation to the alterations verified in the soil biota, probably due to the soil autoclaving process. Soil exposed to high temperatures during the autoclave process has reduced native microorganisms, and the competition against *S. rolfsii* increases the pathogen growth and directly contributes to an increase in the index of disease severity in accord with (Stamford et al., 2017).

Enzymatic activity

The results of enzymatic activity suggested that the increase in peroxidase activity provides protection against *S. rolfsii.* On the other hand, chitosan can induce peroxidase activity in plants in the absence of a pathogen. The same treatment with bioprotector + chitosan (6 mg mL^{-1}) also resulted in greater peroxidase activity in cowpea plants in the absence of a pathogen. Falcón-Rodríguez et al. (2009) demonstrated the increase in peroxidase activity in leaves and roots of tobacco plants treated with chitosan in the absence of pathogens. These authors suggested that chitosan induces defensive enzyme activity and reported that the plants accumulate secondary metabolites and form barriers to enhance plant resistance against pathogens.

The peroxidase activity may directly influence the control of pathogens because it liberates nonspecific compounds that increase plant defenses (Van Loon et al., 2006). In the same way, chitosan has been characterized as a signal in the plant response and may minimize the plant response against pathogens (Mejía-Teniente et al., 2013). In addition, the performance of peroxidase activity in plant resistance against pathogen attacks is based on hydrolytic activities in the cellular walls, where direct or indirect effects may increase antifungal activities (Stangarlin et al., 2011).

No significant difference was observed in the peroxidase activity in plants inoculated with the pathogen. However, the plants that received the bioprotector + chitosan (2 mg mL^{-1}) shows a decrease in the peroxidase activity, and it may be conclude that the crustaceous chitosan at this concentration (2 mg mL^{-1}) does not induces peroxidase activity in cowpea plants.

The chitosan can induce the antioxidant system and increase the peroxidase activity and increases the plant protection against some stressors, as described by Ortega-Ortiz et al. (2007). These authors suggested that the effects of chitosan on the disease control and quality maintenance in peach fruit may be associated with their antioxidant properties and the elicitation of defense responses.

Mazaro et al. (2012) reported that chitosan treatment reduced *Mycosphaerella dendropoma* in strawberry plants and activated peroxidase activity. The authors suggested that the alteration in peroxidase activity may be reflected by a metabolic response of the plant that leads to lignin formation.

The antioxidant enzyme peroxidase is also responsible for the control of pathogenic microorganisms because it increases the production of ROS and is a signal of the plants to biotic stress promoted by a pathogen (Sharma et al., 2012). Furthermore, avoiding cellular damage is important to control the equilibrium between ROS formation and the detoxification of the antioxidant enzymes such as peroxidase and catalase.

In accordance with Dousseau et al. (2016), peroxidase and catalase enzymes can increase the oxidation of phenol groups as a precursor of lignin synthesis and increase the resistance of the cellular wall for pathogenic entrance in infected plants. These authors reported the effect of different chitosan concentrations (2.5, 5.0 and 10.0 g L^{-1}) on the catalase activity in jaborandi (*Piper mollicomum*), and the best results were obtained using the concentration of 5 g L^{-1}.

The results of catalase activity suggest that the chitosan concentration reflects an increase in the enzyme activity and that the higher enzyme activity in cowpea directly influences the plant age. Ortega-Ortiz et al. (2007) also find the same relationship in the peroxidase and catalase in tomato fruit during different growth states of the fruit. The authors agree that the resistance inductor chitosan has different effects according to the stage of development of the fruit.

Conclusions

The *in vitro* test demonstrates that the crustaceous chitosan promotes fungicide and fungistatic effects on the mycelia growth of S. *rolfsii*. The experiment in soil pots showed that the bio-protector and crustaceous chitosan applied in different concentrations reduces the index of disease severity of *S. rolfsii* wilt on cowpea and induces mechanisms of defense in cowpea, increasing soluble protein, peroxidase, and catalase activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Use of biochar for increased crop yields and reduced climate change impacts from agricultural ecosystems: "Chinese farmers' perception and adoption strategy"

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Biochar is a carbon-rich product from pyrolysis of biomass at relatively low temperatures in a closed system with limited oxygen. The product has been shown to have economic and environmental benefits, ranging from improved soil moisture retention for carbon sequestration, reduced pollution and dependence on inorganic fertilizers. Additionally, biochar can be produced on-farm by small scale farmers using locally made stoves as well as on industrial scale in bioenergy plants. Chinese government is fast tracking commercial production of biochar based fertilizers from pyrolysis of crop straw. However, most of the work on biochar is confined to universities and other research institutions mainly through field trials. Yet, without understanding of farmers' roles as the main stakeholders in generation and use of this innovation, use of biochar is unlikely to be effective. Using survey data collected in the Henan region, Central China where major biochar industries are located, this study assesses farmers' perspectives and adoption decisions on the use of biochar in agricultural production. A binary logit model is used to analyze the factors influencing biochar adoption. Higher probabilities of adopting biochar are observed among farmers with more contact with extension officers and other sources of information, higher levels of education, credit access and those belonging to farmers' groups. Furthermore, the perceived positive aspects of biochar increased the probability of biochar adoption. These results strongly suggest that, government interventions in these areas are needed to realize the full potential of biochar production and use by farmers'.

Key words: Biochar adoption, logit model, smallholder farmers', agriculture, China.

INTRODUCTION

As China strives to ensure sustainable food production for the increasing population, it is faced with the challenge of reducing land degradation and greenhouse gas emissions of which agriculture is a major contributor (Chen and Zhang, 2010). Chinese farming is characterized by higher yielding, greater external-inputs and agricultural technologies which have played a considerable food production (Kibue et al., 2014; Tong et al., 2003). This result in increased greenhouse gas emissions, pesticide residues, environmental degradation,

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salinity, and reduced biodiversity (Gomiero et al., 2011; Lichtfouse et al., 2009). Additionally, more than 600 million tons of crop straw generated annually (Liu et al., 2008), presents a huge challenge of bio-wastes management. Farmers are skeptical of returning the straws to the soil due to concerns of pest control and labor input (Li et al., 2013; Kibue et al., 2014). Therefore, most farmers are compelled to burn the crop straws in the fields (Kibue et al., 2014). These rampant burning activities aggravate the environmental situation as it results in release of pollutants such as carbon dioxide (CO_2), carbon monoxide (CO), nitrogen oxides (NO_x), and trace amounts of sulphur dioxide (SO_2) (Gadde et al., 2009). These pollutants in turn accelerate global warming and cause health problems (Mishra and Shibata, 2012). Moreover, the burning process reduces soil nutrient availability and nutrient turnover (Wang et al., 2010) and consequently reducing crop yield. These consequences have propelled both farmers and the government to start looking at strategies of managing the bio-wastes. One option that is being supported nationally is conversion of agricultural bio-wastes to value-added products such as biochar and/or renewable energy (MF-SBEMOA, 2012).

Biochar is a carbon-rich product from pyrolysis of biomass at relatively low temperatures in a closed system with limited oxygen (Lehmann and Joseph, 2009). It differs from common charcoal in that it is primarily produced for use as soil amendment aimed at improving soil productivity and enhancing soil carbon storage (Lehmann and Joseph, 2009). Biochar has a number of economic and environmental benefits, ranging from increased yields, reduced pollution and dependence on fertilizers, improved soil moisture retention to carbon sequestration. Beneficial effects of biochar have been reported through intensive research in the laboratory and field experiments in China (Liu et al., 2010; Zhang et al., 2010, 2012; Lashari et al., 2013) and elsewhere (Whitman and Lehmann, 2009; Mulcahy et al., 2013; Joseph et al., 2013).

Biochar can be produced on-farm by small scale farmers using locally made stoves (Whitman and Lehmann, 2009) as well as on large scale in bioenergy plants (Wang et al., 2013). Furthermore, the Chinese government is fast tracking commercial production of biochar from pyrolysis of crop straw under the national project of green energy with agricultural bio-wastes (MF-SBEMOA, 2012). In addition, biochar based fertilizers from pyrolysis of crop straw are being developed nation-wide (Pan et al., 2011). However, the role of farmers, who are the main stakeholders in generation and use of this innovation, has not been adequately addressed. Besides, for adoption of biochar to be effective, policy makers require an understanding of elements that motivate farmers to use biochar innovation. Farmers adoption of biochar will not only solve the problem of waste management but will also confer environmental benefits. The aim of this study is therefore to assess the factors that may influence biochar adoption.

Adoption of innovations

The uptake of new technologies or agricultural practices has attracted considerable interest over the years. Hence, there is a vast literature on the adoption and diffusion of technologies in agriculture (Feder, 1985). The uptake of biochar innovation follows adoption and diffusion process similar to other agricultural technologies. In order to explain what influences the adoption of new technologies, several researchers have examined the influence of various determinants on adoption decisions. These studies show that technology adoption or disadoption is largely dependent on a technology's capacity to meet social expectations (Frey et al., 2012; Reimer et al., 2012), and adopters' perceptions of all the technical, cultural and economic costs, and benefits of the innovation (German et al., 2006; Kiptot et al., 2007; Rogers, 2003; Khan et al., 2008; Frey et al., 2012). Farmers' perceptions of a technology may be influenced by farm size, age and gender (Marenya and Barrett, 2007; Mwirigi et al., 2009; Gachango et al., 2015). Several adoption studies yield evidence on the importance of information access and particularly the role of informal information sources and social networks (German et al., 2006; Frey et al., 2012; Kibue et al., 2016). Besides, farmers' educational background is critical for adoption. For instance education and training greatly increased the likelihood of initial uptake of polythene mulch, contour cultivation, sweet chestnut, and intercropping in china (Subedi et al., 2009). The role of extension in bridging the gap between new innovations and actual uptake by the farmers is underscored. Rahman (2003) and Mariano et al. (2012) have shown that adoption is higher for farmers with contacts with extension agencies working on technologies. Other factors may impede adoption of a technology, for example: loss of social status for being associated with negative outcomes of a technology and lack of access to credit facilities (He et al., 2007; Mariano et al., 2012) and lack of economic incentives (Shi et al., 2008).

In the current study, it is expected that farmer-specific characteristics have a positive relationship with the adoption of biochar innovation. More educated farmers are in a better position to evaluate the relevance of new technologies. It is expected that farmers with off farm income are more financially capable to invest in technologies than poor farmers. Farmers who own bigger farm sizes are more likely to adopt biochar technologies than farmers owning small sizes of land because they can afford to devote sections of their lands to experiment the innovation. Since smallholder farmers often have insufficient capital to invest in a new innovation, availability of credit will encourage technology adoption.

Farmers receiving extension services, those attending on-farm demonstrations of new technologies, seminars and workshops, and farmers having positive attitudes towards gathering information and with high frequency of contact with information sources are also expected to adopt biochar. Lastly, if farmers consider the innovation beneficial and they are perceptive about climate change, they are likely to adopt it.

MATERIALS AND METHODS

Study area

The study area is located in Xieji Township (34°31’11”N115°28’33”E), Liangyuan district, Shangqiu Municipality, Henan province, China. The area lies on the North China Plain, easternmost region of Henan province. It is mostly flat, with elevations ranging from 30 to 70 m (98 to 230 ft), and has a monsoon-influenced humid subtropical climate with four distinct seasons. The annual average temperature is 14.1°C (57.4°F). Precipitation mainly occurs from June to September. The region is characterized by highly fertile soil and convenient irrigation facilities that greatly help the production of crops and other plants. The most important agricultural products are wheat, maize, cotton, sesame, vegetables, fruit, tobacco, and livestock. The region hosts major biochar trial fields managed by Nanjing Agriculture University and Sanli New Energy Company, a key biochar producer in China. Most of the biochar feedstock is provided by the farmers’, who give their crop straw for free to Sanli company, while others exchange it for biochar. Field experiment trials have been undertaken on salt affected land in Xieji village (Lashari et al., 2013) and more trials are ongoing.

Sampling questionnaire and field survey

The study is based on a regional survey of factors influencing biochar adoption by farmers in Xieji municipality, which was conducted between April and May, 2013. The sampling frame constituted households in three villages where biochar companies are located since significant numbers of farmers knowledgeable and/or using biochar, necessary for an empirical analysis, can be found in this area. A total of 287 households were randomly sampled (Marshall, 1996) for interview. A standard questionnaire was formulated in English and translated into Chinese by a native bilingual English speaker who also back-translated it to ensure accuracy. The Chinese version of the survey tool was then pre-tested in a different site and necessary adjustments made before the actual survey. The dataset contained information on: (1) Farmers socioeconomic characteristics (farm size, age and level of education, (2) Contact with sources of biochar information and perceived benefits of biochar innovation, and (3) Access to credit facilities. Before the commencement of interviews, respondents were thoroughly briefed about the purpose of the study and asked if they were willing to participate. After giving consent, all interviews and discussions were recorded (Bordens and Abbott, 2008). The questionnaire was administered face to face by 4 enumerators each spending approximately 30 min to interview each farmer. The dependent variables are shown in Table 1.

Binary choice model

Most of the technology choices that farmers consider in their decision making are of a ‘use or not use’ nature. Similarly, respondents-farmers were asked whether they used biochar or not. The aim was to investigate which factors influence the decision process and by how much each factor affects the farmers’ choice. As in most empirical studies, the observed yes/no decision to use biochar technology is viewed as the outcome of a binary choice model. Two models, logit and probit are widely used. They differ from each other only in the assumption about the functional form of F. This happens when sample sizes were large and certain extreme patterns are observed in the data (Chambers and Cox, 1967). The models assume that a variable *Y* has only two possible outcomes, in this case, adopt and not adopt. They also assume a discrete vector of regressors *X*, which are assumed to influence the outcome *Y*, in this case factors that influence farmers’ decision to adopt biochar (Table 1). The observations (adopt or not adopt) are the outcome of the binary choice model, meaning each farmers choice to adopt is a dummy variable defined as:

$$y_i = \begin{cases} 1 & \text{if thefarmeradoptsbiochar} \\ 0 & \text{if thefarmerdoesnotadoptbiochar} \end{cases}$$

This study adopts a logit model. This model has been used in similar studies (Burton et al., 1999; Bryan et al., 2009; Läpple and Rensburg, 2011; Mariano et al., 2012). All the equations used in this work have been derived and modified from Greene (1997). The parameters of farmers’ decision to adopt or not adopt biochar are defined by latent variable (U_i^*), that is, related to factors that influence the farmers’ decision (X_i):

$$\bigcup_i = X_i\beta + \ell_i \quad \mathrm{i} = 1,2,3,\ldots\ldots \mathrm{N}$$

where β is a vector of adoption parameters and ℓ_i is a random error term. The observed pattern of adoption is then represented by the dummy variable (y_i) where these observable values of (X) are related to y^*:

$y_i = 1$ if $U_A^*(\pi) > U_N^*(\pi)$, otherwise $y_i = 0$

The probability that a farmer adopts biochar is:

$$P[y_i = 1] = P(\ell_i > -X_i\beta) = 1 - F(-X_i\beta) = F(X_i\beta)$$

where *F* is the cumulative distribution function (CDF). The β parameter can be estimated using maximum likelihood ratio. Using the logit model, the farmers’ probability of adopting biochar can be predicted as:

$$P_i = P[y_i = 1] = \ell^{x_i\beta} / 1 - \ell^{x_i\beta}$$

The maximum likelihood estimates of the model can be used to predict the probability that a farmer adopts biochar is based on his/her characteristics, estimate change in probability for change in farmers’ characteristics X_i. For instance if the farmer changes the frequency of obtaining advisory information from $X_a = 1$ to $X_2 = 2$, then, the change in probability of adapting changes can be computed as:

$$P = F(\beta_0 + \beta_1 X_b + \beta_2 X_2 + \ldots\ldots\ldots + \beta_k X_k) - F(\beta_0 + \beta_1 X_a + \beta_2 X_2 + \ldots\ldots + \beta_k X_k)$$

Table 1. Definition of variables and their measurement.

Variable	Definition and measurement
Age	Age of the farmer. Measured in years
Gender	Sex of the respondent. Measured in percentage and described as 1 if male, and 0 otherwise
Education	Highest level of schooling attained by the farmer. 1= none, 2= primary, 3= Secondary, 4= College. 1 if educated, and 0 otherwise
Farm size	Total agricultural area of the farm measured in Chinese Mu
Off farm income	Proportion of farmers earning income from other sources. If the farm household has an off-farm income=1, =0 otherwise,
Info frequency	Mean number of times of consultation with source of information on biochar
Contact with extension services	Proportion of farmers in contact with extension worker. 1 if the farmer access to advice from extension workers; 0 otherwise
Belonging to association	Proportion of farmers belonging to farmers associations. 1 if the farmer belongs to a farmers association; 0 otherwise
Info seeking attitudes	Attitudes towards seeking information. Rated on a scale of 1-5. Higher value = higher interest in information gathering
Access to credit facilities	Proportion of farmers obtaining credit. 1 if the farmer has access to credit; 0 otherwise
Perceived benefits of biochar use	Proportion of farmers viewing biochar innovation as beneficial. 1 if the farmer views biochar use as beneficial; 0 otherwise

The estimates of marginal effects, that is, the effect of a unit change in X_i if all other factors are held constant, on the farmer's probability to adapt can be shown as:

$$\Delta P_i / \Delta X_i = \partial P_i / \partial X_i$$

Data analysis

Two hundred and eighty responses were used for analysis since 7 respondents declined to be interviewed. Analysis was done using Statistical Package for the Social Science (SPSS) version 16.0. Descriptive statistics were used to summarize the data. The data were tested for their reliability through scale creation process (using a 'reliability analysis'; Alpha = 0.77). The value of computed Cronbach alpha was 0.421 (unweighted variables) and 0.306 (weighted variables). Analysis of factors that influence farmer's adoption of biochar was done using the logit model. In order to derive the magnitude of the impact of the independent variables on the probability of adoption, marginal effects at the mean for continuous variables and for a change from zero to one for dummy variables were estimated (Greene, 1997).

RESULTS

Descriptive statistics

Table 2 shows the descriptive statistics of the variables for adopters and non-adopters of biochar technology and the total sample. The results show that most farmers had attained secondary school education. Most farmers had mean age of farmers to be 49 years, owned 11 Chinese Mu of land, relied purely on agriculture as the only sources of income and perceived biochar as beneficial. The table further reveals that compared to non-adopting farmers, adopting farmers had more contact with extension workers, sought information more frequently, had other sources of income and have higher level of education among others.

Besides the conventional sources of information (print and electronic media), most farmers (46%) sought information from fellow farmers, 22% did not seek information anywhere, 13% from friends and family members, 12% from extension officers, and 7% from nearby field demonstration stations.

Results of logit model

The estimated coefficients of the parameters and the marginal effects in the binary logit model are shown in Table 3. The chi-squared test statistic is significant at the 5% level. The power of prediction of the estimated model is 0.702. The estimated marginal effects indicate that for every unit increase in the variables, the probability of biochar adoption increases by: farm size (8%), frequency of gathering information (6%), credit access, information gathering attitudes and contact with extension officer (5%) each, farmers education and belonging to farmers

Table 2. Descriptive statistics for variables; adopters (N=84), non adopters (N=196) and total farmers (N=280).

Variable	Biochar adoptors Means (St. dev)	Biochar non-adoptors Means (St. dev)	Total sample Means (St. dev)
Age of household head	49.48 (16.02)	48.78 (13.02)	48.93 (12.51)
Gender (% males)	68 (0.55)	63 (0.66)	65 (0.45)
Education of HH head (level)	3.47 (0.99)	2.82 (0.97)	3.17 (1.09)
Farm sizes (Chinese Mu)	13 (0.77)	11 (0.91)	11.83 (0.87)
Off farm income (%)	39 (0.48)	19 (0.87)	23.80 (1.00)
Frequency of obtaining information (count)	3.80 (0.86)	1.99 (1.57)	2.74 (1.72)
Contact with extension services (count)	2.80 (1.71)	1.64 (1.89)	2.17 (2.69)
Information seeking attitudes (level)	3.3 (1.62)	2.70 (1.79)	3.07 (1.97)
Access to credit facilities (%)	37 (0.08)	18 (2.23)	27 (1.01)
Belonging to farmers associations (%)	43 (0.81)	27 (1.91)	35.6 (1.27)
Perceived benefits of biochar (%)	65 (0.42)	38 (0.81)	57.2 (0.70)

Table 3. Results of logit model (N=280)

Variables	Coefficient	Marginal effects
Age of household head	0.200	0.860
Education of household head	0.001**	0.040
Gender	0.607	0.503
Farm sizes	2.36**	0.082
Off farm income	0.741	0.161
Information frequency	0.997**	0.061
Contact with extension services	0.864**	0.049
Belonging to association	0.329**	0.040
Information seeking attitudes	0.216**	0.045
Access to credit facilities	0.735**	0.054
Perceived benefits	1.630**	0.0271
Likelihood ratio	0.70228	
Pseudo R- square	.4180	

** Test statistics is significant at the 0.05 level.

association (4%)-each and perceived benefits (3%). As expected biochar adoption is influenced by farmer's formal education, farm size, contact with extension services, frequency of gathering information, access to credit, farmers associations, perceptions that biochar is beneficial, and information seeking attitudes. However, off farm income had no influence on biochar adoption.

With regard to information availability, the results in Table 3 show that belonging to a group, association with access and diffusion of information increase the likelihood of biochar adoption. Farmers who are in more contact with extension services are also more likely to adopt biochar innovation. In line with this, better attitudes towards seeking information and high frequency of obtaining information more also plays an important role for the adoption decision. The findings are in line with results of previous studies on technology adoption that identify informal information exchange between peers to be an important determinant of technology diffusion (Montalvo, 2003; German et al., 2006; Frey et al., 2012) and that contact with information, farmers' associations as well as farmers' attitudes toward actively seeking information influence adoption decisions (He et al., 2007; Mariano et al., 2012).

With respect to socioeconomic characteristics, Table 3 also shows that household head's education levels, farm sizes and perceived benefits of biochar have a likelihood to influence biochar adoption. In line with He et al. (2007), Marenya and Barrett (2007), and Mwirigi et al. (2009), education has positive influence on adoption. Farmers who have higher levels of education are more able to embrace an innovation that can enhance their livelihoods by easing farm management cost. Similarly, farmers with bigger farm sizes are likely to adopt biochar because they can devote some section of their farms for trials. On the contrary, due to their vulnerability in an event of crop failure or any other eventuality, the farmers with small farm sizes are likely to watch and learn from other farmers about the outcome of the innovation rather than take the risk of taking up the innovation. This observation is corroborated by other studies on adoption (Marenya and Barrett, 2007; Mwirigi et al., 2009).

Farmers' perception of biochar innovation as beneficial influences its adoption. This is for the reason that the adoption results in social or economic gains. This is in agreement with other adoption studies (German et al., 2006; Kiptot et al., 2007; Khan et al., 2008; Frey et al., 2012; Rogers, 2003) that perceived benefits of an innovation influences its adoption. Finally, as expected, access to credit facilities influences farmers' likelihood of biochar adoption. This is because the farmers are able to meet costs of adoption. This observation is in line with Shi et al. (2008), that lack of economic incentives and lack of credit facilities (He et al., 2007; Liu and Huang, 2013) are barriers of adoption.

DISCUSSION

Biochar innovation is relatively new and the focus of the research by universities has been on field trials with little extension from agricultural departments. Subsequently, few agricultural extension experts have received detailed training or written information about the method of production, application and potential benefits. First and foremost, this presents a gap in information diffusion which, in other similar studies, has been blamed for limiting the widespread use of information (Hansen et al., 2011). Additionally, lack of knowledge among extension officers has been blamed for poor adoption of organic agriculture and communication failure (Wheeler, 2008).

Social ties among the farmers belonging to groups and the perception that biochar innovation can offer them a solution to manage their crop residues by converting them to a value-added product with other benefits is indicative of adoption. Besides, it is established, in the same region by Li et al. (2013) that farmers find crop residues as a big challenge because of increased labor and chemical inputs to kill insects and pests attracted by the residues. This is well corroborated by similar studies that show that adoption is dependent on farmers recognition that an innovation is beneficial and can meet social expectations (Frey et al., 2012; Reimer et al., 2012; D'Antoni et al., 2012) belonging to social groups facilitates spread of information and social learning (Rahman, 2003; Mariano, 2012).

Having confirmed the factors that influence biochar adoption and the vast literature on beneficial use of biochar and its large scale production in China, this study seeks to discuss some policy perspectives based on the findings.

Information/Awareness

As revealed by the survey data, information is key to adoption of biochar. As such, the government should ensure that farmers are in constant contact with each other. These extension officers should be equipped with both technical and application aspects of biochar. Successful agricultural extension can help to overcome the gap between newly invented technologies and changes in the farmer's field. That is, extension specialists supply farmers with the required knowledge, thus assisting in a shift to more efficient production techniques and thereby enhancing the diffusion process of technologies (Birkhaeuser, 1991). Hence, the farmers will have the advantage of not only using biochar but also establishing onfarm biochar production. Biochar can be produced on-farm by small scale farmers using locally made stoves (Whitman and Lehmann, 2009).

Incentives for farmers to give some land for demonstrations and trials

The government should encourage more farmers in the region to devote some sections of their land for on-farm trials and demonstrations. Few farmers in this region have devoted some portions of their land for biochar field trials that are managed by Nanjing Agriculture University and Sanli New Energy Company, a key biochar producer in China. This acts as a capacity building initiative by bringing research and innovations to the farmers and empowering them to learn practically and acquire technical skills. Studies associate farmers with contacts with extension agencies working on technologies and for those belonging to farmers' groups to higher adoptions (Rahman, 2003; Mariano et al., 2012).

Education

Government should liaise with higher institutions of learning to develop short courses to meet farmers' needs and also to keep the extension officers updated with the latest developments in their fields. In addition to short courses, research institutions should be facilitated to conduct more workshops for farmers. Of great importance also, the government should take advantage of existing social groups and networks and non-governmental organizations to educate and train farmers on new and best practices in agriculture.

Credit facilities and incentives

Lack of affordable credit facilities and lack of incentives remain a huge barrier to adoption of innovations. This is because given the inherent risks in agricultural projects, the lending bodies (micro finances, saving cooperatives, banks, etc). A study of farmers access to credit (Corpuz, 2008) finds that lending processed are complicated by farmers lack of collaterals, high transaction cost and interest rates among others. To attempt to solve this problem, the government should create a fund specifically for smallholder farmers to make them access their agricultural needs. The government could also negotiate lending rates with the lenders to make it affordable for the farmers and in return waive some taxes for the specific lenders. Finally, the government should give incentives and relevant technical support to the big companies that manufacture biochar.

Conclusion

Biochar innovation is a relatively new innovation whose beneficial impacts in agriculture are known to few farmers. This study shows that biochar adoption is influence by individual farmer's characteristics and institutional factors, a fact consistent with adoption literature.

Based on the estimated marginal effects, farm sizes and information-related variables have the biggest impact on technology adoption. This makes it imperative to involve farmers as major stakeholders in trials and other developments in agricultural matters. In addition, the government should invest in efficient ways of communicating with farmers besides exploiting the social groups. For instance, through training more extension workers and intensifying media campaigns to educate the farmers on new ideas. This is of paramount importance because precise and timely information will help farmers make important decisions to adopt innovations. In regard to farm sizes, government should work closely with researcher to develop high yielding varieties and high value crops that can give satisfactory returns under the farm sizes. A follow-up survey is important to provide a more comprehensive analysis of farmers' behavior in adopting new technology over a longer period of time.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Full Length Research Paper

Determinants of mobile phones usage in sweet potato vine business in Gulu district northern Uganda

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Seed systems that provide farmers with planting materials may be divided into formal and informal systems. For the sweet potato sector, the informal seed system is the main provider of planting materials to small and medium sized farmers in developing countries. This informal system provides planting materials to smallholder farmers at the right time, place and quantity. However, for it to be effective there must be an appropriate means of communication between multipliers and buyers of sweet potato vines. This may be achieved with the use of mobile phones. Mobile phones are said to have great impact in improving trade through provision of accurate and timely information directly to farmers. However mobile phones usage in agriculture is still low. Many farmers still rely on the traditional way of accessing information which seems inadequate. This study characterized users and non-users of mobile phones and assessed factors that influenced mobile phones usage in sweet potato vines trade. A cross sectional causal comparative survey research design was used. The study obtained primary data from 140 randomly selected respondents in three purposively selected project sub-counties in Gulu district. Descriptive statistics and probit regression were used to analyse the data. Our results indicated that users and non-users of mobile phones in sweet potato vines marketing differed in a number of socio-economic characteristics. Users of mobile phone were on average younger, better educated and had bigger sweet potato fields. The Probit regression results revealed that the farmers' age negatively influenced the decision to use mobile phones in sweet potato vines trade. On the other hand, education and household income positively influenced the decision to use mobile phones in sweet potato vine trade. Mobile phones therefore have potential for improving the livelihoods of smallholder farmers and should be specifically targeted to lure the youths into agriculture.

Key words: Mobile phones, sweet potato vines, seed system, marketing, Gulu district.

INTRODUCTION

Sweet potato is vegetatively propagated using cuttings preferably from the tip of the growing plant (Wilson, 1988). Long dry seasons destroy the planting material and require an effective seed system to facilitate early planting and to assure farmers of a good crop (Gibson et al., 2009). A seed system refers to an organised, formal or informal mechanism through which farmers get planting materials (Louwaars et al., 2010). There are two major seed systems. The formal seed system which involves production of seeds by institutions following specific rules and regulations, maintaining distinctiveness, uniformity of seeds and following an organized channel of distribution (FAO, 2004). While the informal seed system sometimes referred to as the traditional or farmer seed system is where farmers select, produce and manage their own seeds usually local varieties and the distribution is through farmer to farmer networks (Wekundah, 2012). The informal seed system accounts for over 80% of the total area planted under subsistence crops (FAO, 2004). In Uganda, sweet potato planting materials spread through the informal seed system which is always affected by dry seasons. However, closer to the equator at 1˚ a sweet potato crop planted in the previous rainy season can survive the short dry season, allowing farmers to obtain planting materials from their own surviving crops or from their neighbours', but in Gulu just at 2˚ north of the equator, farmers need to conserve sweet potato planting materials in wetlands or shade during the long dry season in northern Uganda (Gibson et al., 2009). The trend in the agricultural seasons in northern Uganda therefore creates opportunities for those near wetlands, to conserve and multiply sweet potato vines in dry seasons and sell at the onset of the rainy seasons. However, this can only benefit farmers far from wetlands when mobile phones are put into good use to communicate information on the availability of sweet potato vines and make business transactions.

Mobile phones are known to speed up the pace at which farmers access and use different agricultural information (Nyamba and Mlozi, 2012). However, farmers always seek information from one another on: Where to buy agricultural inputs and who pays the highest output price in the market, but rarely find answers to these questions even if related ones arise yearly (World Bank, 2011). This has been linked to poor access to production and market information especially in rural areas where farmers rely on neighbours and friends to access market information. These traditional methods of accessing and disseminating information seem inadequate and do not provide timely information and hence affect crucial decisions including planting time of sweet potatoes. It is therefore important to find and use alternative ways to access production and market information and mobile phones could be the answer.

Many development partners have been trying to introduce the use of mobile phones in the agriculture sector to improve access to production and market information (Akiiki, 2006), but their efforts end with the projects due to poor targeting as characteristics of people likely to adopt and use the intervention are not clear. The factors that influence use of phones in agriculture seems not clear and identifying these factors will enable stakeholders devise possible strategies to address the issues limiting mobile phone usage in agriculture. This study characterized users and non-users of mobile phones for information access and investigated factors influencing smallholder farmers' decision to use mobile phones' in sweet potato vine marketing business in Gulu district of northern Uganda.

HISTORY OF TELEPHONE/MOBILE PHONE IN UGANDA

South-eastern African region collectively provided the telephone services to their members by the government of Uganda, Kenya and Tanzania before the year 1977 (Econ One Research, 2002). However in 1977 Uganda left the regional shared services model by establishing the Uganda posts and telecommunication corporation (UPTC) as a state owned monopoly provider of telecommunication services. The services provided by UPTC by then were poor with limited innovation (Econ One Research, 2002). To address the above gap, transformation of the industry was done through privatization and liberalization. In 1993 Celtel, was issued a license to provide telecommunication services other than UPTC in Uganda. However, the license was for cellular not fixed-line services. Celtel Uganda started providing services in 1995. In 1998, Uganda telecommunication limited (UTL) was established as state owed company but was privatized in 2000 and started operating in 2001. In the same year (1998), mobile telephone network (MTN) and started operating in October the same year. These were then followed by other companies (Warid, Orange, Africel) among others.

The introduction and establishment of the many telephone companies in Uganda increased competition and rapid development of mobile phone use inn Uganda from 1% in 2000 to 48% in 2012 (World Bank 2, 2013). This also came with a lot of initiative such as mobile money services which enables one to save, send, money to another, pay bills through ones' subscriber identification module- card (SIM-Card) (Aker and Mbiti, 2010) and mTrac that was initiated by ministry of health together with United Nations International Children Efficiency Fund (UNICEF) (Maree et al., 2013), alongside voice and SMS application.

Mobile phone in agriculture

Mobile phones serve a number of purposes. Phones have shifted from being just voice device to a multimedia

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Table 1. Variables used in the study.

Variable	Description
Dependent variable	
Decision to use a mobile phone for vine marketing	1if used mobile phones for sweet potato vine business, 0 otherwise
Independent variables	
Age	Age of the farmer in years
Gender	1if Male 0 otherwise
Marital Status	1 if married, 0 otherwise
Education level	Number of years spent at school
Occupation	1if farming, 0 otherwise
Group affiliation	1 if belong to a group, 0 otherwise
Income level	Total income earned in a year in Uganda shillings
Household size	Number of household members
Field size	Total acreage under sweet potato for vines
Distance to market	Distance from home to Market in Kilometres
Vine marketing experience	Number of years spent in sweet potato vine marketing
Sweet potato production experience	Number of years spent in sweet potato production
Ownership of mobile phone	1if own a phone, 0 otherwise
Volume of sweet potato Vine sold	Total of sweet potato vine small bundles sold in one year
Source of sweet potato vines sold	1 if grow, 0 otherwise
Frequency of sweet potato vine sales	1 if once a week, 0 otherwise
Ease of getting buyers	1 if easily get, 0 otherwise
Quantity of vines lost	Total number of sweet potato vines lost in a year in small bundles
Price	Unit price for each small bundle of sweet potato vines

communication tool capable of downloading, uploading text, can be used as a wallet, calculator, television, alarm clock, camera and many more (World Bank, 2011). Farmers use mobile phones for obtaining agricultural related information (Muto and Megumi, 2009; Mwakaje, 2010; Lashgarara et al., 2011). Mobile phones usage facilitates transactions and provides producers access to relevant and timely information, allowing them to make wise decision (Dhaliwal and Joshi, 2012). Mobile phones can be used by farmers in coordinating access to agricultural inputs; including agricultural training, seeds, livestock and pesticides from local dealers, governmental, non-governmental organizations, agriculture extension agents and community members without any physical contact and access to the market (Martin and Abbott, 2011). In the past individuals would travel to seed dealers only to find all seeds had been sold but today, individuals call or send SMS and make appointments before travelling and payments can be made through mobile money services provided by telephone companies (M-pesa, Airtel money, MTN mobile money) (Olwande et al., 2013). Information according to Lashgarara et al. (2011) is needed by smallholders to enable them make an informed decision at each stage of the agricultural production cycle.

Limited information constrains farmers to know about prices in only few nearby markets within a village or town since they cannot move to more than one market a day due to high transport costs (Jensen, 2007). Improved information access through mobile phones may help to improve poorly performing markets through reduction of price disparity within markets (Jensen, 2007; Abraham, 2007) and reduction of transport cost and other transaction costs.

MATERIALS AND METHODS

This study was carried out in three sub-counties in Gulu district. Bungatira, Unyama and Koro. These sub-counties are characterized by presence of wetlands and year round flowing streams that can be used to support dry season sweet potato growing and reliance on sweet potato to earn a living.

A sampling frame was drawn up and respondents were randomly selected using random numbers table in proportion to the number of sweet potato vine multipliers in each sub-county. All the town sweet potato vine sellers were surveyed because they were only 9. The procedure resulted into 140 respondents with; 131 multipliers and 9 town sellers.

The study employed a cross sectional causal comparative research design. Data were collected in 2015 using personally administered pre-tested questionnaires. Data were entered in SPSS version 20 and analysed using STATA package version 13. Variables used in the study are presented in Table 1.

Conceptual framework

The need to improve smallholder farmers' access to agricultural and

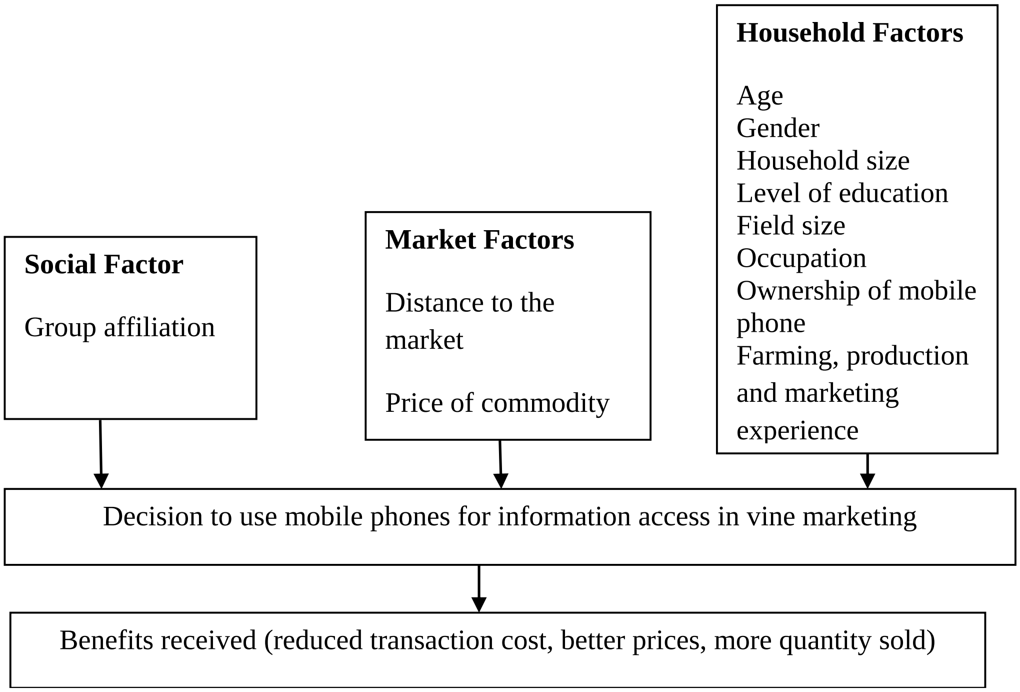

Figure 1. Factors influencing mobile phone usage in sweet potato vines marketing.

market information which in the past relied on extension agent and yielded limited impact has led to development of new models. The new models of information dissemination include the use of radio, television, newspapers, computers, and mobile phones (Aker, 2011). Of the above, mobile phones stand out as a cheap, timely and direct means of informing farmers (Dhaliwal and Joshi, 2012). This study uses random utility theory, to analyse adoption of innovations under uncertainty (Pannell, 2003). The concept of utility was first put forward by Bentham in 1748 (Read, 2007). Following Bentham's idea a farmer will only use a mobile phone in sweet potato vine marketing transactions if he/she expects to benefit from it.

The decision to use a mobile phone is likely to depend on a number of factors such as market, household and social factors (Figure 1). Household factors such as age and primary occupation positively influence farmers' decision to use ICTs including mobile phones (Okello et al., 2012), gender has both negative and positive influences (Mwakaje, 2010). Males and high income earners are more likely to use mobile phones for agricultural purposes than their counterparts; phone ownership also increased its use (Nyamba and Mlozi, 2012). Group members are also more likely to use ICT/mobile phones to access agricultural information than non-group members (Okello and Mensah, 2011).

Furthermore, market factors for example expected price and distance to the market positively influenced mobile phone use in agriculture. People far away from the market use more of mobile phones to access market information than their counter parts closer to the markets. They can also sell more and receive better prices through better connections to markets (Mwakaje, 2010). Mobile phones can also benefit farmers by reducing transaction time, reducing market and inputs search cost (Mittal and Mehar, 2012).

Model specification

Usage of mobile phone is associated with costs and benefits. Costs may arise from purchase of airtime (credit/data), charging of phone batteries and maintenance. Benefits on the other hand may arise from better access to information, reduced transaction costs, higher sales volume, minimal losses, and better prices. The decision to use a mobile phone in agriculture by a farmer can therefore be modelled building on random utility theory model framework mainly used to analyse adoption of innovations under uncertainty (Pannell, 2003). A farmer uses a mobile phone if the expected benefit out weights the cost of using a mobile phone. The decision to use a mobile phone is therefore a binary choice variable assuming utility maximization subject to household resource constraint(s), social factors and market factors. The actual utility level of each individual farmer $\mathbf{U}_i$ is unobserved. The part of the utility function that is observed can be expressed as a function of the vector of exogenous variables X_i and a vector of parameters β to be estimated. $V_i^{(\beta X_i)}$, where:

$$\mathbf{U}_i = V_i^{(\beta X_i)} + u_i \qquad (1)$$

The vector X_i includes farm and household characteristics like income, age, group affiliation, etc., part of the farmer's utility which is unobserved is represented by an error term u_i. The farmer chooses to use a mobile phone if the utility U_i^m derived from mobile phone usage is greater than the utility U_i^n derived from non-usage. The probability of a farmer using a mobile phone is given by Pr(u_i < βX_i). This can be estimated using probit or logit models. The Probit model was selected because the error term assumes a normal distribution (Dow and Endersby 2004). The probit model was estimated as follows:

Table 2. Differences in characteristics of users and non-user of mobile phones in sweet potato vine marketing.

Variable	Users (n=87)	Non-users (n=53)	Mean difference (non-user-users)
	Mean (SD)	Mean (SD)	
Age	35.474 (12.468)	41.396 (15.105)	5.922**
Education	6.038 (3.694)	3.962 (3.311)	-2.076***
Household size	7 (2.692)	8 (3.911)	1.000*
Farming experience	15.256 (9.955)	20.491 (14.888)	5.234**
Sweet potato production experience	13.795 (9.898)	19.774 (14.820)	5.979***
Vine marketing. experience	7.051 (6.418)	6.094 (6.792)	-0.957
Income level	2610995 (3330786)	1699057 (977511)	-911938*
Field size	0.679 (0.659)	0.418 (0.382)	-0.261**
Quantity sold in year	3393 (1051)	1149 (135)	-245.000
Price per small bundle	367 (122)	379 (140)	13.000
Quantity lost in year	49 (75.296)	31 (57.540)	-18.000
Distance	4.281 (1.882)	3.796 (1.953)	-0.485
Categorical variable			**Chi-square**
Gender			0.0027
Marital status			10.7127
Primary occupation			0.0768
Group affiliation			1.4758
Freq. of sales			25.8520***
Labour hire			7.8229**

Significant level: *10%, **5%, ***1% level.

$$\Pr(M_i = 1) = \Pr(u_i < \beta X_i) = \beta X_i + u_i \qquad (2)$$

Where $M_i = 1$ if $U_i^m > U_i^n$ and $M_i = 0$ if $U_i^m < U_i^n$

RESULTS AND DISCUSSION

Differences in characteristics of users and non-user of mobile phones in sweet potato vine marketing

Users and non-users of mobile phones in sweet potato vine marketing were compared; t-tests and chi-square test were used for continuous and categorical variables respectively. Significant differences were observed between users and non-users of mobile phones in sweet potato vine marketing (Table 2). Results further indicate that there were significant differences in the mean age, mean number of years spent in school, farming and sweet potato production experience, annual income, household size and sweet potato field size between users and non-users of mobile phones. Mobile phone users were on average younger, spent more years at school, had less farming and sweet potato production experience, and had higher annual incomes and bigger sweet potato fields but with smaller household sizes. There was no significant difference in the mean quantity of sweet potato vines sold in a year and mean quantity lost in a year between users and non-users of mobile phones. There were also no significant differences between users and non-users of mobile phones in-terms of, vine marketing experience, prices received and distance from home to the markets. For categorical variables, gender, marital status, primary occupation and group affiliation were not significantly different between users and non-users of mobile phones in sweet potato vine marketing business. Frequencies of sweet potato vine sales and labour hire were significantly different between users and non-users (X^2=25.8520, *P*= 0.000 and X^2=7.8229, *P*=0.020, respectively).

Demographic characteristics of respondents

Of the 131 multipliers surveyed, 106 (80.9%) were female while 25 (19.1%) were male. On the other hand all the 9 town sweet potato vine sellers surveyed were women. Majority of those involved in sweet potato vine marketing business are females. Most multipliers surveyed were young (youth) aged < 36 years 68 (51.9%) and middle aged from 36-55 years 47 (35.9%) categories. The other category considered old was aged > 55 years 16 (12.2%). On the other hand, of the 9 town sellers surveyed 3 (33.3%) were young aged < 36 years, 5 (55.6%) were middle aged (36-55 years) and 1 (11.1%) was old aged > 55 years. The minimum age of those surveyed was 18 years and maximum was 78 year.

Production and sale of sweet potato vines are mostly done by youth and middle aged people, probably because dry season growing and marketing of sweet potato vines are so demanding in terms of time and labour and need energetic and patient people. Of the 131 multipliers surveyed, 64 (48.9%) had spent 0-5 years in school, 56 (42.8%) had spent from 6-10 years in school and 11(8.4%) had spent from 11-15 years in school. On the other side, of the 9 town sellers, 3 (33.3%) had spent 0-5 years in schools and 6 (66.7%) had spent from 6-10 years in school. Majority of sweet potato vine multipliers and town sellers could have stopped in primary education. In case of marital status, 114 (87%) of multipliers surveyed were married, 2 (1.5%) were single, 6 (4.6%) were divorced and 9 (6.9%) were widowed. However, for the 9 town sellers, 5 (55.6%) were married, 2 (22.2%) were divorced and 2 (22.2%) were widows. On household size, of the 131 multipliers surveyed, 31 (23.7%) had < 5 people, 88 (67.2%) had household size of 5-10 people, 10 (7.6%) had size of 11-16 people and 2 (1.5%) had household size of > 16 people. On the other hand, of the 9 town sellers surveyed 2 (22.2%) had household size of < 5 people and 7 (77.8%) had household size of 5-10 people. Majority of respondents had household size of more than 5 people, probably because most of the people in the study areas marry when they are young and stay as extended families.

As expected, nearly all respondents 129 (98.5%) and 5 (55.6%) for multipliers and town sellers respectively reported that they rely on farming as their primary occupation and 2 (1.5%) multipliers and 4 (44.4%) town sellers said they rely on small businesses. This is likely because most of them did not attained high level of education and most likely they cannot be hired to do formal jobs and probably have limited capital to venture into medium and large size businesses.

Mobile phone ownership in the study area

Slightly less than half 62 (47.3%) of the 131 multipliers owned mobile phones. However, all the town sellers surveyed owned mobile phones, probably because they were given mobile phones by Commercialization of sweet potato Planting Materials in Northern Uganda –project. Of the 62 multipliers who owned phones, 47 (75.8%) were females and 15 (24.2%) were male. This is probably because sweet potato vine marketing business is done mostly by women. Of the 47 females that owned mobile phones, 43 (91.5%) used it in sweet potato vine marketing business. On the other hand, of the 15 males that owned mobile phones, 13 (86.7%) used them in sweet potato vine marketing business.

Use of mobile phones

All the respondents had used mobile phones in the last one year mainly for social communication (calling loved ones). However, in the study, 53 (40.5%) of the multipliers used mobile phones exclusively for social communication while the rest 78 (59.5%) used mobile phones for both social communication and sweet potato vine marketing purposes. On the other hand, all the 9 town sellers used mobile phones for both social communication and sweet potato vine marketing purposes. This shows that social communication still takes the larger portion of activities for which mobile phones are used. This finding coincides with other findings for instance that of (Mittal and Mehar, 2012). Of the multipliers that used mobile phones for conducting sweet potato vine marketing business, 65 (83.3%) were females and 13 (16.7%) were males. This could be because more females than males are involved in sweet potato vine marketing business.

Other mobile phone services and applications used other than voice calls

Of the 78 multipliers who used mobile phones, 16 (20.5%) of them used other services and applications. Of the 16 multiplies, 10 used mobile money services only, 7 used both mobile money services and SMS, 4 used SMS only, none used both mobile money services and mobile phones as calculator, 2 used mobile phones as calculator only. The total number of users of other mobile phones services and application exceeds 16 because of multiple uses (one person using more than one services and applications). On the other hand, of the 9 town sellers surveyed only 4 used other mobile phone services and applications. Of the 4, 3 had used mobile money services only and 1 used both mobile money service and SMS. The low number could be due to lack of knowledge and confidence to operate mobile phones.

Volume of calls and districts where calls were made from

The average numbers of calls received and made by multipliers were approximately 2 in a week in each case. On the other end, town sellers received on an average 22 calls and made 28 calls in a week. This could be liked to limited income among multipliers of sweet potato vines. The town sellers received and made more calls probably because they are connected to many customers, sell more and get better income. Of the multipliers who used mobile phones in sweet potato vine marketing 24 (30.8%) received calls from other districts as orders for sweet potato vines. In the case of town sellers 8 (88.9%) received calls from other districts as orders for sweet potato vines. The districts where calls were made include: Apac, Kitgum, Nwoya, Amuru, and Pader among others. However, most calls were made from Kitgum and Pader districts. This could probably be because of high sweet potato vines demand in the two districts.

Table 3. Probit estimates of the factors that influence the decision to use mobile phones in sweet potato vine marketing.

Variable	**Probit regression**	**Marginal effect**
	Coefficient	**Coefficient**
Distance to market (Km)	0.127 (0.077)	0.048 (0.029)
Household income (Ugx)	0.493*** (0.143)	0.185*** (0.055)
Age in years	-0.025** (0.011)	-0.009** (0.004)
Marital Status (1 if Married 1, 0 Otherwise)	0.245 (0.157)	0.092 (0.059)
Vine marketing experience in years	0.038 (0.024)	0.014 (0.009)
Group Affiliation (1 if group member, 0 Otherwise)	0.187 (0.353)	0.071 (0.137)
Primary Occupation (Farming = 1, 0 Otherwise)	-1.074 (1.703)	-0.403 (0.640)
Education (No. of years spent in school)	0.089** (0.041)	0.033** (0.015)
Gender (Male = 1, 0 Otherwise)	-0.389 (0.371)	-0.053 (0.142)
Quantity of vines lost in a year (in small bundle)	0.002 (0.002)	0.001 (0.001)
Price (Ugx)	0.001 (0.001)	0.001 (0.0004)
Frequency of Vine sale (Once a week = 1, 0 Otherwise)	0.226** (0.167)	0.085** (0.186)
Source of vine sold (1 if grow, 0 Otherwise)	0.223 (0.167)	0.083 (0 .063)
Model characteristics		
No. of observation	131	
Pseudo R^2	0.258	
Log likelihood	-65.56	
p- Value	0.0000	

Payments for sweet potato vines ordered through mobile phone calls were received by multipliers in the following ways: Through bodaboda riders (people who use motorcycles to carry passengers), 72 (92.3%) of multipliers, 4 (5.1%) went and picked their money and 2 (2.6%) received payment through mobile money services. Surprisingly, no town market sellers received payment through mobile money services but physically from transporters. The mode of payment made through transporters is risky because they are likely not to deliver the money to the intended owners.

Benefits of using mobile phones in sweet potato vine marketing

Use of mobile phones in sweet potato vine marketing has been of benefit. This is in line with Mittal & Tripathi, (2009). There were increased incomes through increased sales, enabling multipliers and sellers of sweet potato vines to meet their basic needs. Mobile phones made connection easer, improved the relationship between the trading parties by reducing conflicts, reduced losses and transaction costs and improved the quality of sweet potato vines sold to customers as they were delivered fresh on call.

Challenges of using mobile phones in sweet potato vine marketing

A number of challenges limit the use of mobile phones in sweet potato vine trade; inability to operate mobile phones, poor telephony network in the areas, mobile phone number of the other party being off/unavailable on the network and charging difficulties due to lack of power are the major challenges of using mobile phones in the business. Charging difficulties was also reported by (Syiem and Raj, 2015) among the tribal farmers in Meghalaya State in North-East India.

Factors influencing smallholder farmers' decision to use mobile phones in sweet potato vine marketing

In Table 3, a number of factors were revealed to influence smallholder farmers' decision to use mobile phones in sweet potato vine marketing. Farming experience and sweet potato production experience were highly correlated with age of respondents and therefore were excluded from the model. Similarly, quantity of sweet potato vines sold in a year and field size were also excluded because of their high correlation with income. Town market sellers were also excluded here because they were given mobile phones by the project. The probit model fit the data with 63.8% ($p < 0.0000$) correct prediction of users and non-users of mobile phones in sweet potato vine marketing. The likelihood and pseudoR^2 of the probit model are also presented. Income significantly influences farmers' decision to use mobile phones in sweet potato vine marketing ($P < 0.001$). Farmers with higher incomes are more likely to use mobile phones than their counterparts. A unit increase in income increases the likelihood of using mobile phones by a marginal effect of 0.185, probably because with high income a farmer can afford to buy a phone, buy airtime vouchers, re-charge battery and pay phone maintenance cost. This finding agrees with that of Nyamba and Mlozi (2012). Age has significant but negative influence ($P < 0.023$) on the decision to use mobile phones in sweet potato vine marketing, this was anticipated. An increase in age by one year decreases the probability of using mobile phones in sweet potato vine marketing business by a marginal effect of 0.009. This finding indicates that the use of mobile phones is grater among younger sweet potato vine sellers and multipliers than their older counterparts, probably because they are better educated. Also as one grows older, one may start experiencing visual problem making it difficult to operate mobile phones. Other scholars found that age negatively influences adoption and use of new technologies (mobile phones). This was found in Ali and Erenstein (2013). Frequency of sweet potato vine sale positively influences ($P < 0.015$) one's decision to use a mobile phone in sweet potato vine marketing. Sweet potato vine multipliers who sell once a week are more likely to use mobile phones by a marginal effect of 0.085 than their counterparts who sell more than once a week. Probably because they need to look for market and get more customers to be able to sell more frequently. Furthermore, as was expected education has significant and positive influence ($P < 0.029$) on the use of mobile phones in sweet potato vine marketing. Educated people are more likely to use mobile phones in sweet potato vine marketing by a marginal effect of 0.033 compared to their other uneducated counterparts. This is likely because educated people may find it easier to operate mobile phones than uneducated. This finding is in disagreement with that of Nyamba and Mlozi (2012) who found that education has no significant influence on mobile phone use in communicating agricultural information.

Quantity of sweet potato vines lost, primary occupation, group affiliation (group membership), sweet potato vine marketing experience, marital status, gender, source of sweet potato vine sold, distance to the market and price of sweet potato vines have no significant influence on the decision to use mobile phones in sweet potato vine marketing. The findings probably mean both users and non-users of mobile phones fairly have the same sweet potato vine marketing experience and receive the same price for each small sweet potato vines bundle.

Conclusions

This study characterised users and non-users of mobile phones in vine marketing and assessed factors that influence the decision for one to use phones in sweet potato vine marketing business. Results indicated that users were younger, more educated had more income, had less farming experience, sweet potato production experience and slightly more experienced in vine marketing. Social communication is still the main reason for buying mobile phones. Reasons for not using mobile phones in sweet potato vines business were; non-ownership of mobile phones, nearness to markets, expenses associated with using mobile phones, inability to operate mobile phones, no phone contact of buyers and others sold through a focal person who liked them to buyers. Our results further revealed that 30.8% of the multipliers and 88.9% of the town market sweet potato vine sellers who used mobile phones in sweet potato vine business received calls from others districts as orders for sweet potato vines.

Mobile phones use in sweet potato vine trade was limited by: Battery charging difficulties due to lack of electricity, inability to operate mobile phones, poor telephony network and choice of channels to pass information to farmers. Despite the challenges, using mobile phones also had benefits. There was reduced conflict between the trading parties, reduced losses, reduced transport costs and improved connectedness and networking. Sweet potato vine multipliers called markets before cutting vines and got to know market situations prior to sending sweet potato vines to the market. Factors that significantly influenced the decision to use mobile phones in sweet potato vine business include; age, income, education and frequency of sweet potato vine sales.

This study therefore contends that use of mobile phones in agriculture has a great role in rural areas. Farmers received timely information by contacting the market without physically traveling to the market; they can receive payment for their products sent to the market while home through mobile money services and can find markets for their products elsewhere without leaving home as long as they have contacts of people in those places. The freshness of the product is not compromised since sweet potato vines are cut on request. Conflict between the trading parties and losses are reduced when full use of mobile phones is put to practice.

Poor access to information limits the economic potential of farmers as market participants and value chain actors. Use of mobile phones seemed to make information available to sweet potato vine sellers and improved their livelihood and position in the business, by improving their skills and knowledge. As such, mobile phones were said to have a great contribution in vine business. Multipliers/sellers were able to communicate and received payments for their products directly in their mobile phones without any geographical hindrance. The social aspect of getting people change to mobile money services is important; one can accumulate large sums of money by saving in his/her mobile money account, which can then be used to pay large bills. Saving in mobile money account also reduces unnecessary spending which is the case with physical cash. Women whose husbands are problematic are saved from their husbands taking money forcefully from them. Use of mobile phones in agriculture can help solve some of the market failures that smallholder farmers face as a result of lack of access to market information especially in rural areas. Our findings allude to the fact that investment should be made in programs that make it easy for smallholder farmers to acquire and use mobile phones. Basic education/training is much needed to boost up smallholder farmers' confidence to operate mobile phones. Also to encourage use of mobile phones in promoting agriculture, government should introduce and promote the teaching of ICT skills including mobile phone used in business in secondary schools and vocational institutes. Specific interventions targeting use of phones in agriculture should focus on luring the youth into agriculture and hence contribution to the reduction of unemployment.

Our results also show that lack of electricity in rural areas also limits use of mobile phones by smallholder farmers. Therefore interventions like rural electrification and solar phone charging stations are match needed so as to unlock the full potential of mobile phones.

Using mobile phones reduces transaction costs of conducting business. However, it is still not clear to what extent use of mobile phones in agriculture reduces transaction cost of conducting business. Further research needs to be done to investigate the extent to which mobile phones use in agriculture reduces costs of conducting business.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of biochar and gypsum soil amendments on groundnut (*Arachis hypogaea* L.) dry matter yield and selected soil properties under water stress

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The effects of amending soil with gypsum and biochar on groundnut chlorophyll concentration, water use efficiency (WUE), biomass yield and selected soil properties were investigated under water stress. Gypsum ($CaSO_4.2H_2O$) was applied at 0 and 200 kg/ha, groundnut shell biochar at 1, 2 and 4% *w/w* of soil, and water at 100, 70 and 40% of daily plant water requirement (PWR) as main, sub and sub-sub plots, respectively, in a split-split-plot design. Biochar neutralized the acid soil, significantly raising soil pH from 5 to 7.15 and increasing cation exchange capacity by 75%. Biochar amended at 1 and 2%, increased groundnut dry matter yield by 28%. The optimum biochar application rate for dry matter yield was 1.4% w/w. Biochar application at 4% and irrigation at 40% of PWR reduced the WUE by 45 and 50%, respectively. Chlorophyll concentration index was highest at 40% of PWR. The results suggest that biochar has potential to raise soil pH, increase moisture retention and improve crop performance. Applying water at 100% PWR can increase groundnut dry matter yields, while higher gypsum application rates may be required to affect crop performance.

Key words: Biochar, dry matter, crop evapotranspiration, groundnut gypsum, water use efficiency.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is nutritious, rich in protein, carbohydrate, fibre, unsaturated fats, and minerals such as phosphorus (P), calcium (Ca), magnesium (Mg), vitamins E, B complex and vitamin K (Settaluri et al., 2012). It is the second most cultivated crop after maize in Zambia, consumed as a major source of protein, used as animal feed, and an important fertiliser crop because it fixes nitrogen, making it a very lucrative cash crop (Mukuka and Shipekesa, 2013). However, production and yields are low (690 kg/ha) and approximately only 50 to 70% of the potential yield under rainfed conditions (FAOSTAT, 2016). This is mainly due to climatic and soil constrains such as; poor rainfall distribution, soil acidity, low soil Ca and low soil moisture

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retention. In addition, pests, disease, poor agronomic practices such as late planting, weeding, and planting uncertified poor quality seeds also reduce crop yields (Chabala et al., 2014: Tunwari et al., 2018). Traditionally, it is a rain-fed crop, with most groundnut farmers being small-holder; they have limited resources to and seldom use inputs (fertilisers, pesticides, lime, and supplemental irrigation systems) to address these production constraints (Mukuka and Shipekesa, 2013).

Groundnut is sensitive to drought stress at flowering and pegging which reduces fruit set. The availability of Ca in the soil for plant uptake is largely dependent on the soil moisture levels in the geocarposphere; so during periods of drought, groundnuts show deficiencies of Ca and cracks in pod shells tend to develop resulting in reduced phytoalexin production, increased number of pops, cracked pods, and increased susceptibility of kernels to aflatoxin contamination by *Aspergillus* spp. infection (Njoroge et al., 2013). Ca can be supplied in several forms, for example, as a sole lime application which has been shown to reduce aflatoxin contamination by 72% (Waliyar et al., 2013). Gypsum application rates for groundnuts range from 200 to 1000 kg/ha when less than 0.25 cmol/kg Ca is present in the soil (Nyambok, 2011).

Biochar is the product after any organic material is charred in the presence of limited O_2, by a process called pyrolysis (Abel et al., 2013), and can be used as a soil amendment. Studies have shown that biochar as a soil amendment has unique properties which allow it to offset some climatic and soil constraints brought about by changing climate. When amended to soil, biochar can improve the fertility of soil by buffering against temperature fluctuations, neutralizing acidity, increasing cation exchange capacity (CEC), increasing base saturation, increasing organic matter content, sequestering carbon, improving nutrient retention and increasing moisture retention (Cornelissen et al., 2013). Martinsen et al. (2014) and Xu et al. (2015) found that incorporation of biochar to soils planted with maize and groundnuts significantly increased yields.

Constraints affecting groundnut yield such as low soil Ca, soil acidity and exposure to prolonged dry spells have generally been investigated independently. However, these soil constraints rarely occur independently. This paper reports the sole and combined effects of gypsum and biochar on leaf chlorophyll concentration index (CCI), biomass dry matter (DM), water use efficiency (WUE), crop evapotranspiration (ETc) of the groundnut crop under water stress, and on the effects of biochar on soil pH and CEC.

MATERIALS AND METHODS

Site description and soil sampling

Soil was collected from the experimental site at the Agricultural Technology Development Centre (ATDC) of the University of Zambia Agricultural Demonstration Centre, located in Chongwe, Zambia (latitude 15° 21’ 25” South and longitude 28° 27’ 25” East, 1,260 m above sea level). The field was used to grow maize in the previous cropping season (2015/2016). This site falls in Agro-ecological region IIa of Zambia (receives an average rainfall of 800 to 1,000 mm/y during the cropping season that runs from November to March). The soil is sandy loam, belongs to the Chromic Luvisol taxonomy based on the World Reference Base (WRB) Classification System (IUSS Working Group WRB, 2015). For the greenhouse pot experiment and soil characterization, subsamples were collected randomly across the field at a depth of 0 to 20 cm to form a composite soil sample.

Soil characterization

The fine earth fraction of the composite soil sample was analysed for selected chemical and physical properties. The soil reaction (pH) was measured in 0.01 M $CaCl_2$ with a 1:2.5 soil: solution ratio (Van Reeuwijk, 1992) read on a pH meter (Hanna, HI2210-01 Benchtop pH/mV Meter). The EC was measured in a 1:5 soil: solution ratio (Richards, 1954) using a conductivity meter (Hanna, HI98312 DiST® 6 EC/TDS/temperature Tester). Available P was determined by the Bray 1 Method (Bray and Kurtz, 1945) read on a spectrophotometer (UV/Visible, Jenway 6305). The total N and organic matter were determined by Kjeldahl method (Bremner and Mulvaney, 1982), and Walkley and Black (1934) chromate reduction method, respectively. Exchangeable acidity (Al^{+3} and H^+) was determined by the titration method (McLean 1965), while exchangeable bases (K^+, Mg^{+2} and Ca^{+2}) and CEC were determined by the leaching method (Rowell, 1994). The bases were read on a Flame Atomic Absorption Spectrophotometer (AAS Perkin Elmer Analyst 400). The hydrometer method (Day, 1965) was used to determine the soil texture, and bulk density was determined according to the Blake (1965) Core Ring Method.

Biochar production, characterization and gypsum characterization

Groundnut shells underwent pyrolysis in a homemade kiln to produce biochar. The biochar was pounded with a mortar and pestle and passed through a 1 mm sieve for characterization. The CEC was determined by the leaching method (Rowell, 1994), total C (Walkley and Black, 1934), total N was determined using the Kjeldahl method (Bremner and Mulvaney, 1982) and neutralizing value (NV) using the titrimetric method (Faithfull, 2002). Dry ashing (Campbell and Plank, 1998) and 30 ml of 1 M HNO_3 was used for the extraction of total P and total bases Ca, Mg, K, and Na. The P was read on a Jenway 6305 UV/Visible spectrophotometer and the bases were read on a Perkin Elmer Analyst 400 flame AAS. The ash content was determined according to the American Society for Testing and Materials (ASTM) D1752-84 (2007) at 750°C. Gypsum was characterized for Ca% and S% according to the American Society for Testing and Materials (ASTM) C 471M – 01(2002), where Ca% was read on the AAS and S% was determined by weight using barium chloride.

Greenhouse experiment

At the University of Zambia (UNZA) in the school of Agricultural Sciences (at 15° 23’ 24” S and 28° 19’48” E, at an altitude of 1260 m) a greenhouse pot experiment was set up in a spilt-split plot experimental design. The treatments comprised a combination of three factors; gypsum ($CaSO_4.2H_2O$ which contained 28% Ca and 11% S) at 0 and 200 kg/ha, groundnut shell biochar at 0, 1, 2 and

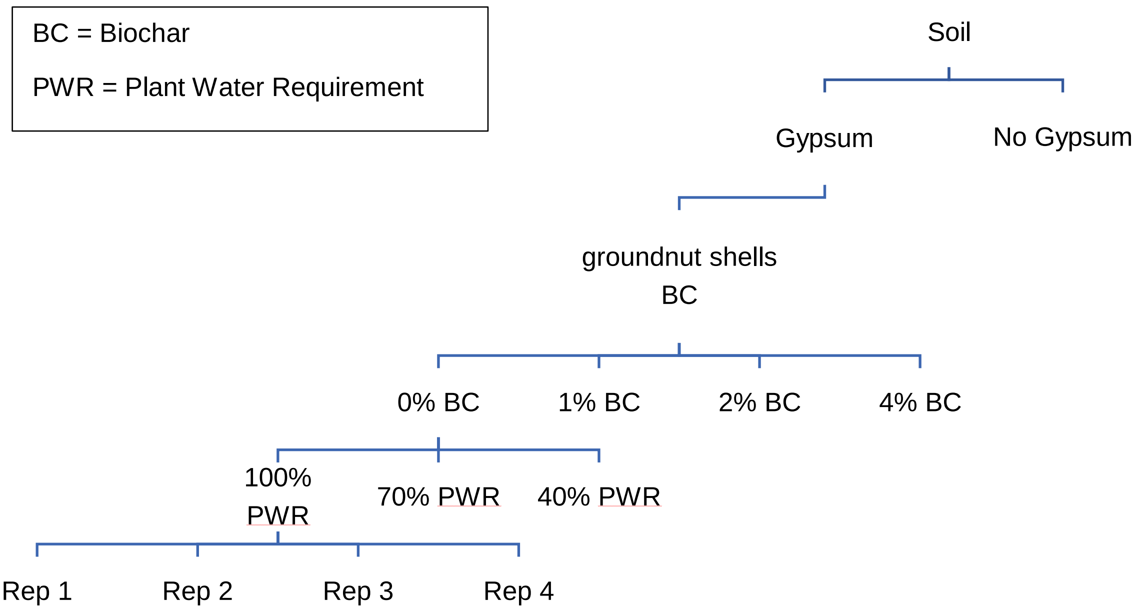

Figure 1. Treatment combinations in greenhouse pot experiment.

Table 1. Selected soil characteristics.

USDA textural class	Soil reaction (pH) in 0.01 $CaCl_2$	Exchangeable acidity (cmol/kg)	EC (mS/cm)	Total N (%)	Available N (mg/kg)		Plant available P	Organic matter	C: N ratio
					NH_4^+	NO_3^-	(mg/kg)	(%)	
Sandy loam	4.02	0.26	0.13	0.05	13.58	17.66	12.26	0.98	10:01
Critical levels	6.5		≤ 3.2	0.2	-	-	10.0	2.0	20:1

4% (*w/w* in 5 kg soil), and daily plant water requirement (PWR) at 100, 70 and 40%, giving a total of 24 treatments (Figure 1). Each treatment combination had 4 replications giving a total sample size of 96 plants. Biochar was homogeneously mixed into the soil at planting and all treatments received the optimum PWR (100%) calculated using the Food and Agriculture Organization (FAO) irrigation scheduling program CROPWAT version 8.0. The MGV 5 groundnut variety was pre-germinated by incubating the seed at 25°C for 7 days on moist Petri dishes. Four germinated seeds were planted per pot, and then thinned to 1 plant per pot after 10 days. Gypsum was applied when the first flowers appeared (40 days after planting (DAP), while plant water stress treatments were introduced at full bloom (59 DAP) until maturity.

The drainage, change in water storage (by weight), evaporation (evaporation-pan), maximum and minimum daily ambient temperatures (maximum-minimum thermometer) were measured throughout the growing season. The soil pH readings were taken at 35 DAP, by inserting a direct pH meter electrode (SCT-pH-PEN-5, Boston, USA) into the soil. The chlorophyll meter readings (SCMR) were taken at the vegetative stage (V3), first reproductive stage (R1) and third reproductive stages (R3) which were at 41, 54, 99 DAP, respectively; each recorded between 10 and 11 AM using a portable SPAD Chlorophyll Meter (CCM-2000 plus). Fungal diseases and insect pests were controlled by periodical spraying of insecticides and fungicides. At maturity (182 DAP), the biomass and pods were harvested. For each plant, the roots, shoots and pods were separated, and sun dried for 4 days and then weighed.

Statistical analysis

Analysis of variance (ANOVA) was used to compare the effects of biochar on the soil pH and CEC. A 3-way ANOVA was used to determine the effects of biochar, gypsum and water on the CCI, DM, water balance components and WUE of the crop under water stress. Fisher's least significant difference (LSD) was used to separate the treatment means. Simple correlation was also done where the ANOVA showed significant differences. The data was analysed using R Statistical Package (Version 3.3.2) as a split-split plot design.

RESULTS

The soil used in the study was a sandy loam with a low soil pH of 4.02. Some selected soil properties are presented in Table 1. The biochar had a high pH of 10.34 and high ash content (24.48%). The total N and organic carbon content were 1.24 and 18.7%, respectively, giving a C:N ratio of 15:1. Important biochar properties are

Table 2. Selected biochar characteristics.

Total N (%)	Total P (mg/kg)	Total cations (g/kg)				CEC (cmol(+)/kg)	Organic carbon (%)	C: N ratio	Ash (%)
		Na^+	K^+	Ca^{2+}	Mg^{2+}				
1.24	5.32	6.30	10.99	11.92	10.00	11.25	18.7	15 :1	24.48

Table 3. Effect of biochar on soil bulk density, exchangeable bases, cation exchange capacity (CEC) and soil reaction (pH).

Treatment	Bulk density (g/cm^3)	Exchangeable bases (cmol(+)/kg)				CEC (cmol(+)/kg)	Soil reaction (pH) in water
		Na^+	K^+	Ca^{2+}	Mg^{2+}		
Soil	1.31	0.22	0.04	0.51	0.24	2.00^c	5.00^d
Soil + 1 % Biochar	1.40	0.24	0.08	0.45	0.26	3.20^{bc}	6.03^c
Soil + 2 % Biochar	1.36	0.27	0.10	0.48	0.30	3.25^{ab}	6.38^b
Soil + 4 % Biochar	1.35	0.28	0.16	0.48	0.32	3.50^a	7.15^a
CV (%)	2.26	2.16	2.16	2.16	2.16	2.18	1.99
P- value	0.84	0.43	<0.001***	0.71	<0.001***	0.04*	<0.001***

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 Means followed by the same letter in a column are not significantly different at α=0.05.

presented in Table 2.

Incorporation of biochar into the soil at 1, 2 and 4% biochar, had very little effect on the bulk density, Na^+ and Ca^{+2} but significantly raised the pH (P <0.001), K^+(P <0.001), Mg^{+2}(P <0.001) and CEC (P=0.04) (Table 3). The exchangeable bases in the biochar were found to be at 0.69, 5.89, 0.5, 0.38 cmol (+)/kg for Na^+, K^+, Ca^{+2} and Mg^{+2}, respectively. The biochar contained a higher level of K^+ as compared to Na^+, Ca^{+2} and Mg^{+2}, which raised the soil K^+ from 0.04 cmol (+)/kg to 0.16 cmol (+)/kg at 4% biochar. The soil CEC was also raised from 2 to 3.5 cmol (+)/kg at 4% biochar.

The results show that applying water at 100, 70 and 40% PWR only had a significant effect on the chlorophyll concentration at the third reproductive stage (R3) (99 DAP)(Figure 2). Water applied at 40% PWR increased CCI by 22% at R3 as compared to water applied at 100% PWR.

Biochar application had a significant effect on the chlorophyll concentration at all three stages of crop growth. The chlorophyll concentration readings ranged from 23.3 to 32.99, 22.9 to 33.47 and 20.4 to 33.7 CCI at V3, R1 and R3, respectively with CCI differences of 42, 46 and 40% at V3, R1 and R3, respectively among biochar treatments. Gypsum had no significant effect on the CCI at all stages of growth.

The groundnut dry biomass yield increased with the addition of biochar and was highest at 1% (97.25 kg/ha) and lowest at 4% (43.58 kg/ha) (Figure 3). Applying biochar at 1% increased DM by 28%, while 4% reduced the DM by 43% and the optimum application rate of biochar was at 1.42% w/w. Applying 100% PWR gave DM of 2 and 3-fold greater than at 70 and 40% PWR, while gypsum had no significant effect. The pooled effect of biochar, gypsum and water on dry matter yield (DM) of the groundnuts at maturity (182 days) is presented in Figure 4.

Gypsum application did not directly or indirectly affect the change in soil water storage (dS), drainage (D), crop evapotranspiration (ETc) as displayed in Figure 5. Application of biochar also had no significant effect on dS and ETc (P = 0.896 and 0.563, respectively) but significantly affected the D (P = 0.0076) (Figure 6). The water application rates had no significant effect on dS (P = 0.394) but significantly affected the D and ETc components (P < 0.001 and P < 0.001, respectively (Figure 7). The D and ETc components were highest at 100% PWR while at 40% PWR resulted in a 35% decrease in ETc.

The effect of biochar, gypsum and water on water use efficiency of root (WUE_R), shoot (WUE_S) and total (WUE_T) biomass is displayed in Figure 9. Biochar at 1 and 2% had no effect on WUE_T, while 4% biochar significantly reduced the WUE_T by 45%. Water applied at 40, 70 and 100% had a significant effect (P<0.001) on the WUE as it ranged from 0.018 to 0.036 g/mm, representing a 50% reduction in WUE at 40% PWR.

DISCUSSION

The soil used in this study belongs to the Chromic Luvisol taxonomy (IUSS Working Group WRB, 2015). The pH of the soil was 4.02 which is extremely acidic (Hazelton and Murphy, 2007) and below the optimal pH range of pH 5.5 (slightly acidic) to 7.0 (neutral) suitable for growing groundnuts (Nyambok, 2011). This might have resulted in deficiencies in exchangeable bases (K, Ca, and Mg), P and molybdenum. Even at this low pH, P (12.3 mg/kg) was adequate for cop production. This could be attributed

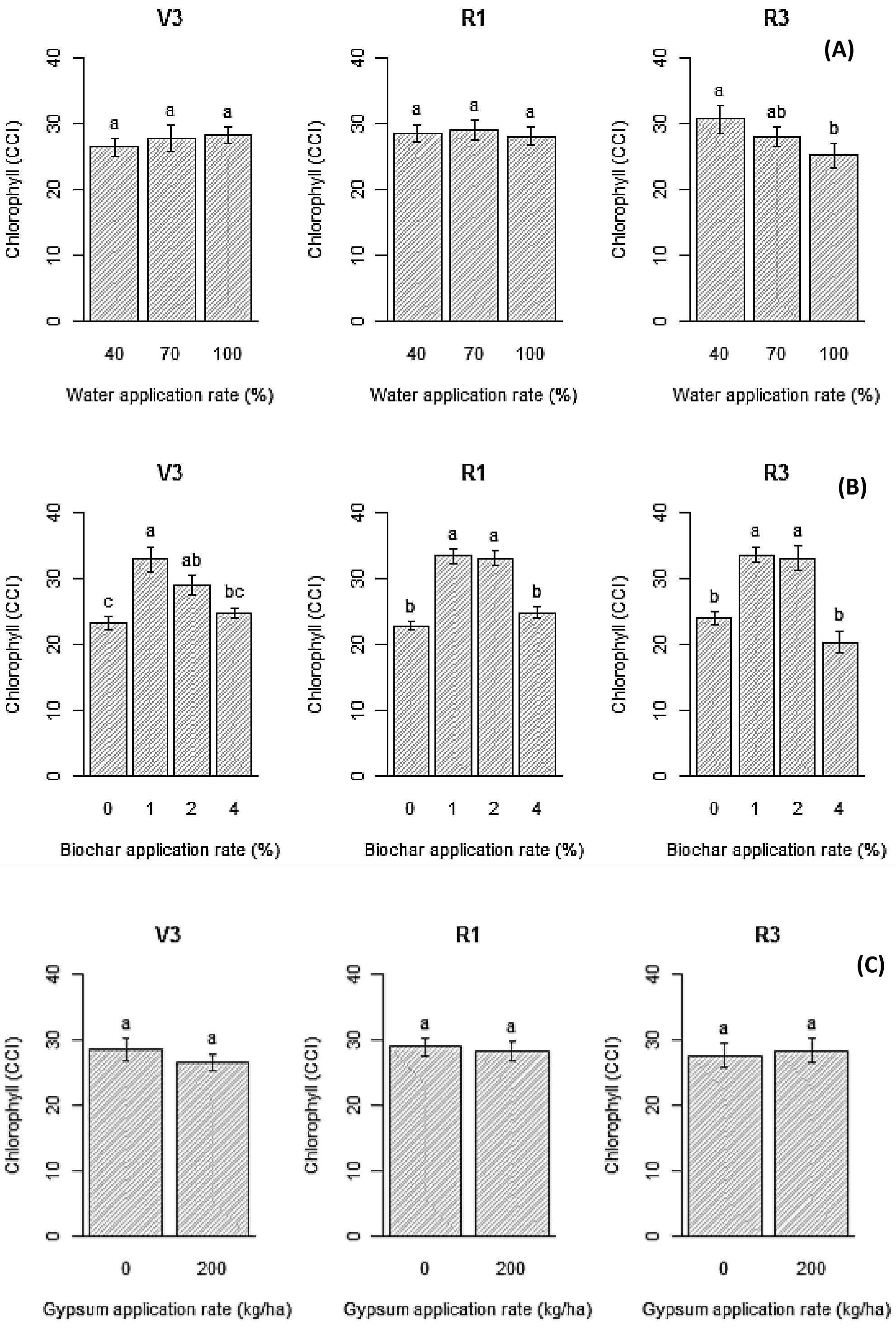

Figure 2. (A) Effect of water application rates on chlorophyll concentration at V3, R1 and R3. (B) Effect of biochar application rates on chlorophyll concentration at V3, R1 and R3. (C) Effect of gypsum on chlorophyll concentration at V3, R1 and R3.

to residual P from fertilizer application in the previous growing season. Sub-optimal levels of P and molybdenum inhibit early root development in legumes (Muhati et al., 2011).

The biochar was notably higher in K, Ca and Mg as compared to Na. High levels of Na are not desirable as this leads to the destruction of soil structure and additionally, this is not a plant nutrient. The ash content

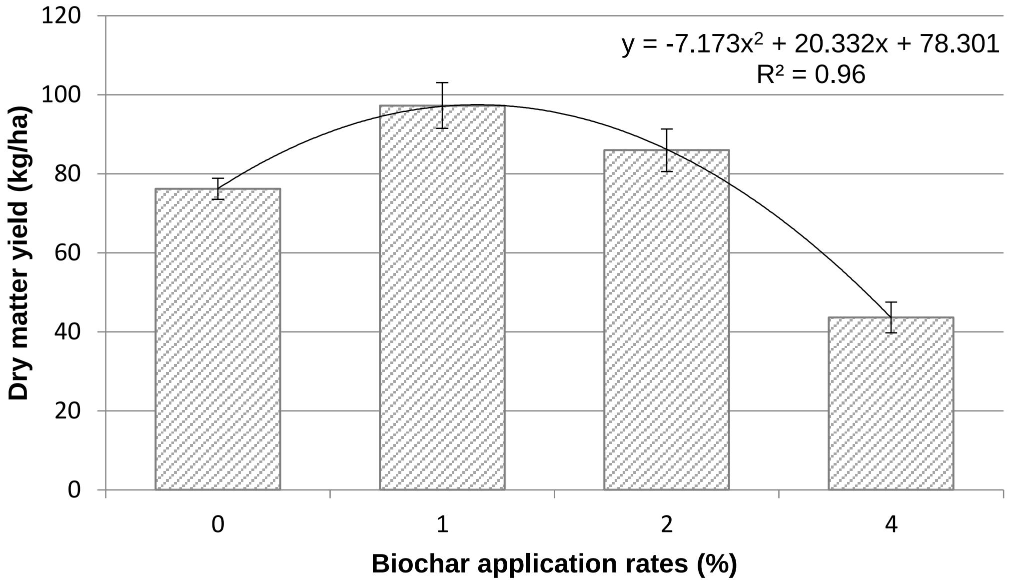

Figure 3. Effect of biochar application rate on the groundnut dry matter yield at maturity (182 days).

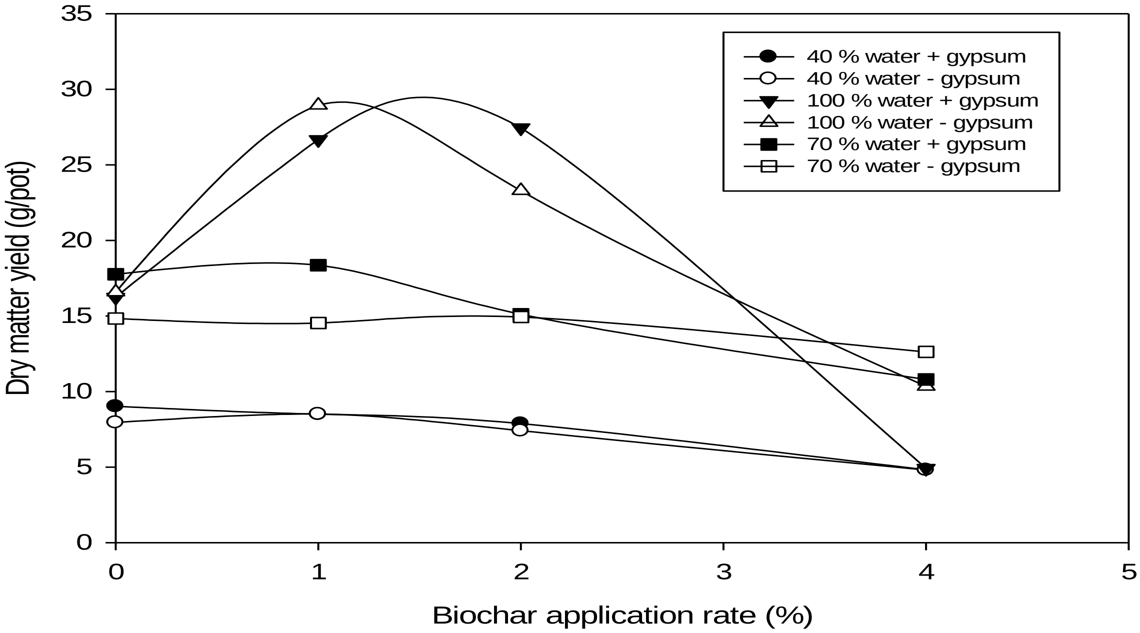

Figure 4. Effect of biochar, gypsum and water application rates on groundnut dry matter yield at maturity (182 days).

was rather high at 24.5%, indicating a high mineral content. The C/N ratio was 15 which is within the general range of 7 and 500 for biochar C/N ratios (Herbert et al., 2012). This narrow C/N ratio indicates a potential for this biochar to supply N to the groundnut crop (Singer and Munns, 1987). The neutralizing value was too low to be measured. The biochar neutralized the acidic soil by raising the soil pH from 5 to 7.15 at 4% biochar. The characteristic large specific surface area of the biochar (Lehmann et al., 2011) and high concentration of total bases (Table 2) readily displace the H^+ ions on the soil colloids by adsorption, thereby raising the low pH of the soil. There was a positive linear relationship that gave R^2 = 0.8698 between CEC and soil pH at different rates of biochar applied (Martinsen et al., 2014).

Water and gypsum had no effect on the chlorophyll

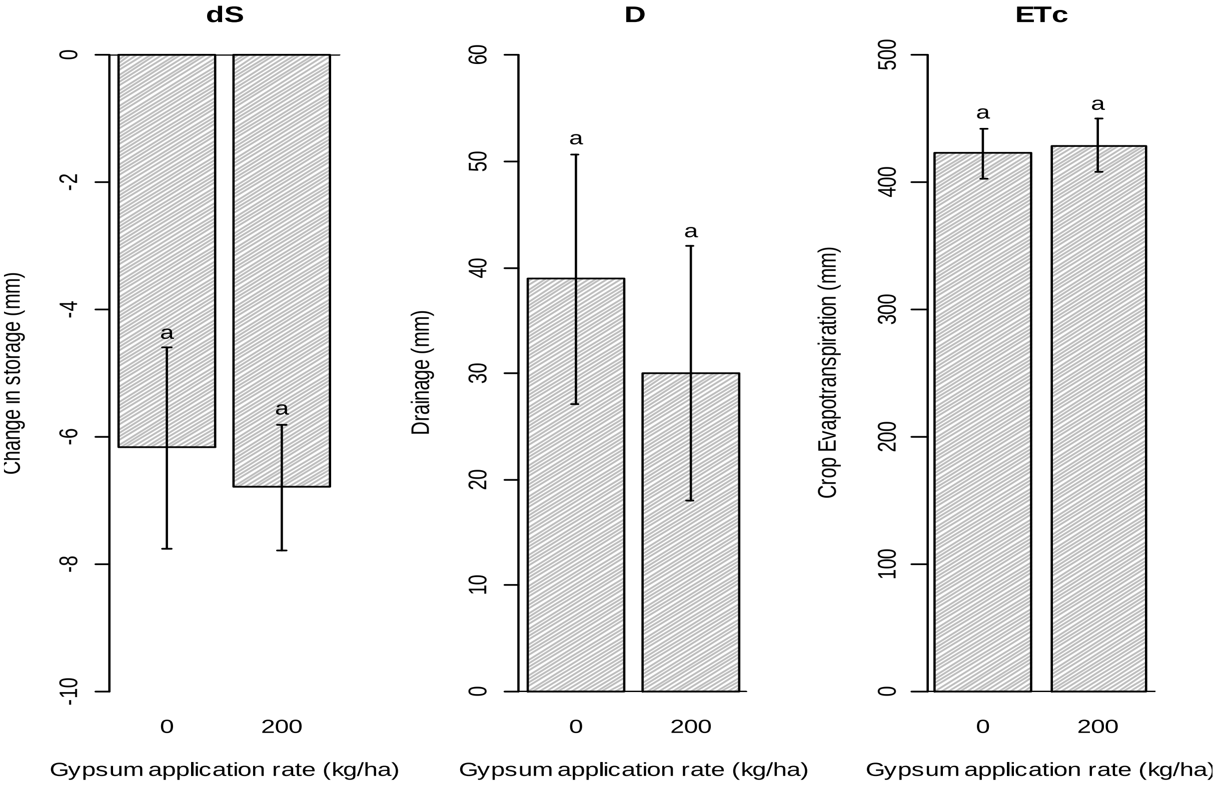

Figure 5. Effect of applying gypsum on change in storage (dS), drainage and crop evapotranspiration (ETc).

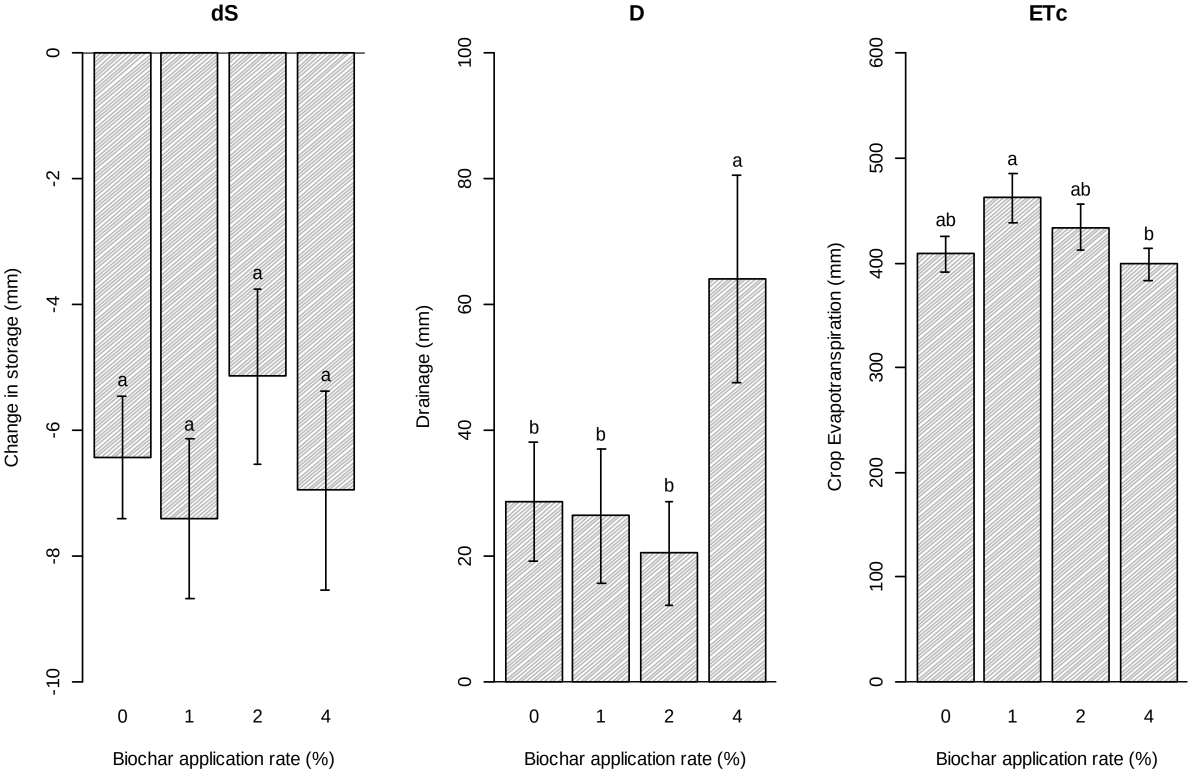

Figure 6. Effect of biochar application on soil water balance components; change in storage (dS), drainage (D) and crop evapotranspiration (ETc).

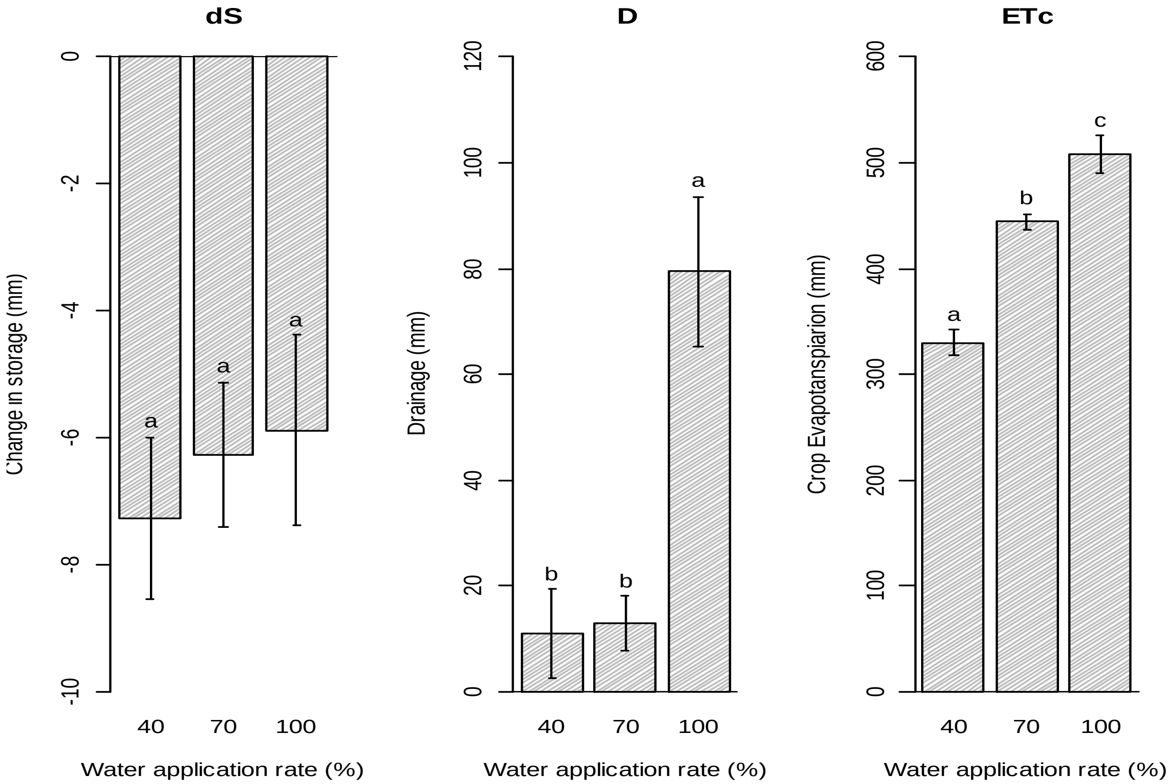

Figure 7. Effect of water application on soil water balance components; change in storage (dS), drainage (D) and crop evapotranspiration (ETc).

concentration at V3 and R1 because the treatments had not yet been initiated at these two stages. At R3, increase in water application rate resulted in a reduction (P=0.02) in chlorophyll concentration, where the chlorophyll readings were 30.71, 28.10 and 25.22 CCI for 40, 70 and 100% PWR, respectively. The highest leaf chlorophyll reading was in the 40% PWR treatments. This observation contradicts research findings by Akhkha et al. (2011) in wheat under deficit water conditions, where the chlorophyll concentration reduced with an increase in water stress. The drop-in chlorophyll production as the water application rate increased may have been due to leaching of ions essential for chlorophyll formation such as N, Mg and S (Marschner, 2012; Mathowa et al., 2012) associated with high drainage rate. Gypsum had no effect on the CCI at R3 possibly due to the low rate of application (200 kg/ha). Some studies have shown that trial application of gypsum at 50 mg/kg results in the maximal chlorophyll content in lettuce (Prasit et al., 2009)

Application of biochar had significant effect on the chlorophyll concentration at all three stages of crop growth (V3, R1 and R3). The CCI readings were lowest at 0 and 4% biochar and highest at 1 and 2%. The low CCI at 0% biochar was because of the low soil pH which affected nutrient availability, thus inhibiting plant growth and chlorophyll production. Nutrients such as N, P and Mg are directly involved in chlorophyll production, these nutrients tends to be low in acidic soil. At 4% biochar, the low CCI may have been because of 4% biochar having had the highest drainage across the biochar treatments; therefore, nutrient leaching was also highly likely. This may have resulted in nutrient deficiency which retarded crop growth and reduced chlorophyll production (Mathowa et al., 2012).

At 40% PWR, the application of gypsum to the soil had no effect on the DM regardless of the level of biochar incorporated in the soil (Figure 4). Moisture is a key factor for crop development and also affects availability of nutrients in the soil solution such as Ca (Gascho and Davis, 1994). The lower CCI observed at 4% biochar and 100% PWR could either be attributed to high moisture levels in the root environment leading to anaerobic conditions or to leaching of nutrients through drainage. The excess water resulted in retardation of crop development and growth at 4% biochar.

Application of biochar at 1 and 2 % was beneficial to the plant as it increased the dry matter yield. At 100% PWR and biochar application rate of 1.42% w/w the highest DM yield for groundnuts was achieved which agrees with a study by Xu et al. (2015), where groundnut biomass and pod yields increased by 2- and 3- times on the red ferrosol and redoxi-hydrosol when biochar was

Figure 8. The effect of biochar application at 0, 1, 2 and 4% (left to right) on groundnut plant growth at 100 PWR.

incorporated at 0.375 to 6.00% w/w, respectively. Martinsen et al. (2014) also detected a similar effect with addition of biochar resulting in an increase in maize yield though not of groundnuts because the presence of biochar increased plant available water (PAW), CEC, available K^+, pH, in the acid tropical soils. Overall only biochar and water influenced the crop development.

Application of biochar had no significant effect on dS, ETc and D. Although gypsum supplies the soil with Ca and P, its application did not enhance crop growth or affect the dS, D and ETc components. Typically Ca and P are known to be involved in plant cell elongation and protein synthesis (Jain et al., 2011; Kumar and Sharma, 2013), but gypsum did not enhance crop growth, therefore had no effect on the dS, D or ETc. The higher the biochar application rate, the higher the soil moisture retention between irrigation intervals was observed, leaving less space in the soil pores to hold more water at each irrigation; allowing more drainage at higher biochar application rates (4%). At the highest biochar application rate, smaller groundnut plants were observed. This diminished stature resulted in reduced water uptake and subsequent larger volumes of excess water loss as drainage. On the other hand, 0, 1 and 2% biochar drained the least amount of water as the soil was not saturated and plants were larger which took up more water (Figure 8).

The drainage was highest where 100% PWR was applied because more water was applied to the soil each day. The ETc was highest where 100% PWR was applied, followed by 70%. The trend of decrease in ETc with a decrease in water applied to the soil was expected. As aC3 crop species, water stress leads to an increase in photorespiration; in order to prevent more water loss through transpiration stomata begin to close (Akhkha et al., 2011). Application of gypsum did not affect the WUE_R, WUE_S and WUE_T. This could be because the gypsum applied to the low Ca soil was too low to enhance crop growth as earlier alluded to concerning the DM. On the other hand, application of biochar had a substantial effect on the WUE_R, WUE_S and WUE_T. Biochar application at 0, 1 and 2% resulted in higher WUE, while application at 4% had a negative effect. Excess water at 4% could have inhibited plant growth due to nutrient leaching and anaerobic conditions, as previously mentioned. Water, like biochar had a notable effect on the WUE_R, WUE_S and WUE_T. The general trend was that the less water applied, the lower the WUE_R, WUE_S and WUE_T, because the more moisture stress the crop experienced, the less it was able to use water efficiently (Songsri et al., 2013).

Conclusion

Incorporation of biochar in the soil significantly increased the pH of the strongly acidic soil to neutral and also significantly increased the CEC of the soil. Applying biochar to the soil at rates of 1 and 2% w/w biochar significantly increased leaf CCI (at V3, R1 and R3 growth stages) and DM yield. The results suggest that biochar application at 1.42% could give the best response for groundnut production. Applying biochar to the soil had no significant effect on the ETc. Gypsum applied at 200 kg/ha did not affect the leaf chlorophyll concentration,

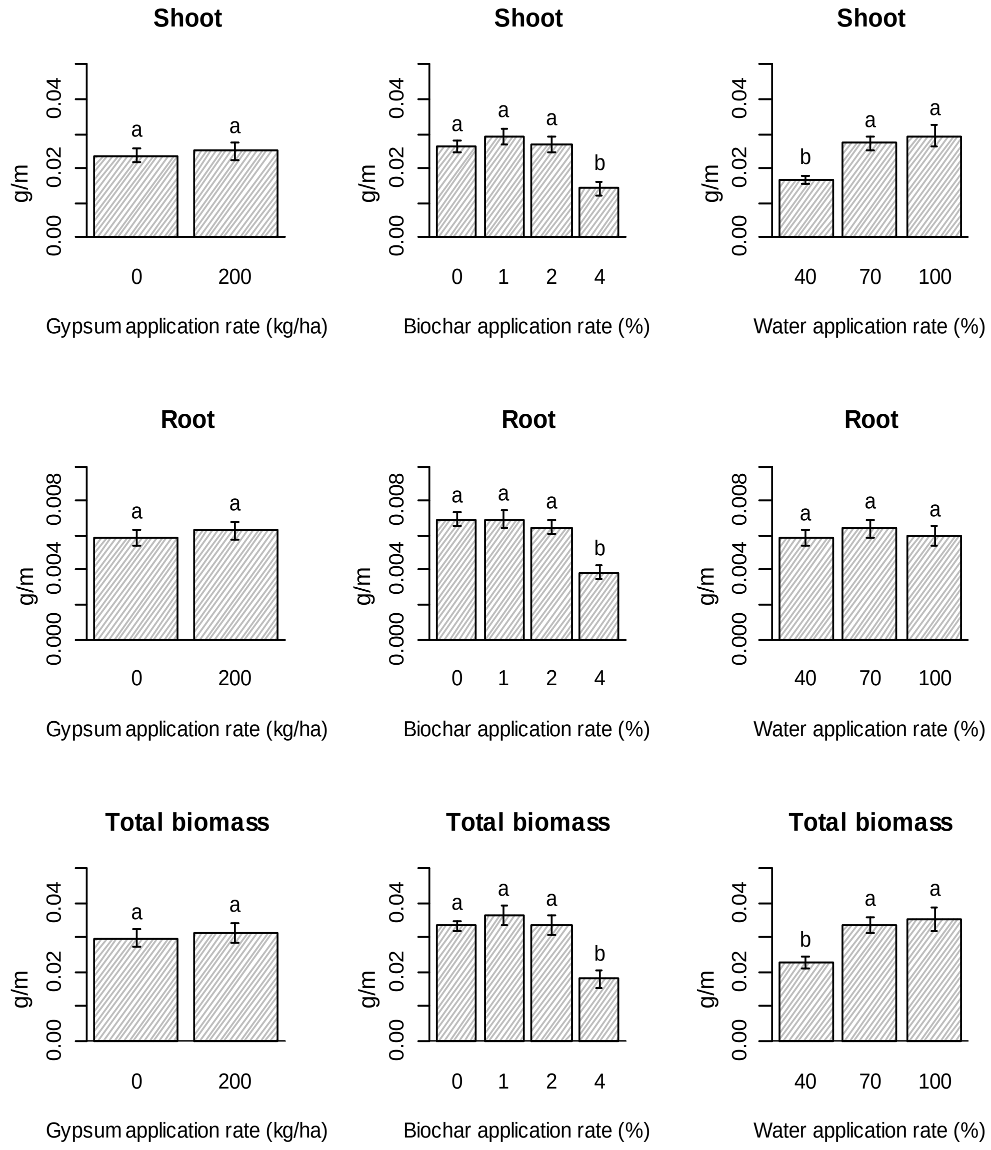

Figure 9. Effect of biochar, gypsum and water on water use efficiency (WUE) of shoot, root and total biomass.

DM, ETc and WUE as it was not sufficient to notably contribute to the crop growth in this low calcium soil. There was no significant interactive effect of applying gypsum and biochar on the CCI, DM, ETc and crop WUE under water stress conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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African Journal of Agricultural Research

Full Length Research Paper

Use of organic alternatives in the production system of habanero pepper (*Capsicum chinense* Jacq.) under greenhouse conditions

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Cultivation of pepper has become the fastest growing practice in recent years, and specifically, habanero pepper which has surpassed its traditional area of cultivation, and has expanded to other areas, conquering the markets of the rest of the country and the world. In the present study, 3 doses of solarized manure (40, 60 and 80 t ha^{-1}) and an absolute control were evaluated for the production of habanero pepper (*Capsicum chinense* Jacq). The results indicated a significant difference between treatments for the plant height at 30 and 90 days after transplantation. However, for the variables: fruit number and fruit weight (kg per experimental unit, kg m^{-2} and kg per plant), the results indicated that there was no significant difference among the treatments evaluated. For the variables: polar diameter of the fruit, a significant difference was observed. However, the treatments did not show a significant effect on the equatorial diameter of the fruit.

Key words: Solarized manure, habanero pepper, organic fertilizers.

INTRODUCTION

Cover cropping in Mexico has been booming in recent days, and by 2010, 12,000 hectares of land for horticultural crops, such as tomato, cucumber, peppers and habanero pepper were quantified, with habanero pepper having greater profitability in the domestic market and in the export market (Macías et al., 2013).

According to Ramírez and Vázquez (2007), peppers have become the fastest growing vegetable in recent years, and the habanero pepper has surpassed its traditional area of cultivation, conquering the markets of the rest of the country and the world, achieving an average yield of 14 t ha^{-1}. Habanero pepper originates from the Amazon basin, but dispersed to Peru, Colombia, Venezuela, Guyana, Suriname, French Guiana and the Caribbean Antilles during the pre-Hispanic period (Salaya, 2010).

In 2004, the production of habanero pepper in the state of Veracruz, as observed by Ruiz (2009) was not greater than 7 t ha^{-1}. Borges et al. (2010), pointed out that for the same year, yields of habanero pepper in the states of

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Table 1. Solarized manure (SM) effect on the mean values of plant height at 30, 60 and 90 DAT (days after transplanting).

Treatments (t SM ha)	Plant height (cm)		
	30 DAT	60 DAT	90 DAT
Absolute control	12.7^{b}	33.1^{a}	59.9^{b}
40	16.3^{ab}	46.5^{a}	86.2^{ab}
60	20.8^{a}	57.9^{a}	84.7^{ab}
80	19.4^{ab}	58.6^{a}	96.3^{a}

Means followed by the same letter in the column do not differ significantly by the Tukey test ($p < 0.05$).

Yucatan, Tabasco, Campeche, Quintana Roo, Veracruz and Chiapas were 5.0, 7.7, 7.1, 10.9, 6.3, 15 and 5.9 t ha^{-1}, respectively, and Macías et al. (2013) reported that in states of Yucatan, Campeche and Quintana Roo, yields were 10 to 40 t ha^{-1} in the open field and 7 to 12 kg m^{-2} under protected agriculture conditions.

Habanero pepper is the main commercial species exploited in the Yucatan Peninsula. This species is of great commercial interest, as a result of the high content of capsaicinoids in the fruit, which can be influenced by the conditions of water stress or nutritional management of the culture (Borges et al., 2010). However, as Sánchez et al. (2010) pointed out, these capsaicinoids are alkaloids that have great importance in human, alimentary and pharmaceutical health, and they are only produced by plants of the genus Capsicum, synthesized in the cells of the surface of the placenta of the mature fruits, and are later deposited in the seeds and walls of the endocarp, as pointed out by González et al. (2006). Pacheco (2005) mentioned that it is used in some drugs for combating gastrointestinal atony and some cases of diarrhea, and this species is used to make capsules that improve blood circulation.

According to Herencia et al. (2011), organic nutrition can improve with practices such as rotating crops with legumes. However, according to Castro et al. (2009), organic fertilizers have high concentrations of macronutrients, and this concentration may vary due to moisture content, so, the analysis of total contents may be a reference for the richness of organic fertilizers. According to Durán and Henríquez (2007), Potassium (K) is an element that is characterized by its low affinity to organic fertilizers; nevertheless, some organic fertilizers contain appreciable amounts of this element.

MATERIALS AND METHODS

The present study was carried out under greenhouse conditions, at the Universidad Tecnológica de la Costa, in the municipality of Santiago Ixcuintla, Nayarit, Mexico. Three doses of solarized manure (40, 60 and 80 t ha^{-1}) and an absolute control were evaluated on the production of habanero pepper (*Capsicum chinense* Jacq). One week prior to transplantation, bovine manure with a moisture content of 56% was incorporated in each of the experimental units (one row of 6 m length or 6 sq m), according to the dose established for the different treatments. It should be noted that the solarized manure was covered with a transparent plastic and kept in a place where it was exposed to the sun most of the day for a period of 6 weeks (May 21, 2014 to July 1, 2014). The transplantation was performed on July 7, 2014, in furrows of 6 m long, 1 m between furrows, and 0.5 m between plants. A randomized block experimental design with three replicates per treatment was used.

The variables that were measured were: plant height (cm) at 30, 60 and 90 days after transplantation, number of fruits and fruit weight (kg) per experimental unit, fruit weight per m², fruit weight per plant, polar and equatorial diameter of the fruit. The analysis of variance and the Tukey comparison of means of treatments of the measured variables were performed with the statistical package SAS 9.1.

For nutritional management, foliar sprays of worm humus leachate were weekly applied at a rate of 10 L ha^{-1} from 15 days after transplanting. Also, weekly applications of compound tea mixed with water, at the rate of 1: 1 v/v, directed to the base of the plant in drench. Wormhole leachate solution was prepared by mixing vermicompost and water (1:3 v/v) in a cement stack with a slope of 5%, with an exposure time of 4 weeks; after the time the leachate was ready to be applied.

Compost tea was prepared as follows: a sack filled with 20 kg of compost was placed in a 100-L plastic drum, water was added to make up the total volume of the drum and left to rest for 3 days. Irrigation was done by dripping, using irrigation ribbons with a distance between drippers of 0.15 m. For weed control and moisture conservation, seed beds were quilted with reused newspaper. Pest management was carried out with weekly applications of the biological insecticide made of *Bacillus thuringiensis*, and botanical extracts from garlic, onion and soap.

RESULTS AND DISCUSSION

In Table 1, it is observed that at 30 days after transplantation, there was a significant difference among the evaluated treatments on plant height. The application of 60 t of solarized manure per hectare (60 t SM ha^{-1}) had the highest plant height of 20.8 cm, as compared to the treatments of 80 and 40 t SMha^{-1} and the absolute control, which obtained 19.4, 16.3 and 12.7 cm of plant height, respectively. However, at 60 days after transplantation, the results indicated that for this same variable, there was no significant difference between treatments, but at 90 days, a significant difference was observed between treatments. The treatment of 80 t SM ha^{-1} reached the highest plant height with 96.3 cm, followed by the application of 40 and 60 t SMha^{-1} with 86.2 and 84.7, respectively. The absolute control had the lowest (59.9 cm). The results were higher than those obtained by López et al. (2012), when evaluating the effect of manure, compost, bocashi, vermicompost tea on habanero pepper growth and yield of fresh fruit. In fact, their results indicated that the infusion of manure significantly increased those variables than other evaluated treatments, especially for plant height with 52 cm after 90 days of transplantation, while the lowest height was obtained by the control with a value of 17 cm.

In relation to the variables, fruits numbers and fruit

Table 2. Effect of solarized manure on habanero pepper yield variables, fruit number and weight.

Treatments (t SM ha^{-1})	Fruits numbers	Fruit weight (kg/m^2)	Fruit weight (kg m^{-2})	Fruit weight (kg $plant^{-1}$)
Absolute control	998.7^a	12.3^a	2.05^a	1.03^a
40	771.7^a	9.1^a	1.52^a	0.76^a
60	888.8^a	12.8^a	2.13^a	1.07^a
80	1183.5^a	15.1^a	2.52^a	1.26^a

SM = Solarized manure; means followed by the same letter in the column do not differ significantly by the Tukey test ($p < 0.05$).

Table 3. Solarized manure (SM) effects on polar and equatorial diameter of habanero pepper fruit.

Treatments (t SM ha^{-1})	Polar diameter (cm)	Equatorial diameter (cm)
Absolute control	4.70^{ab}	3.17^a
40	4.37^b	2.90^a
60	4.47^b	3.10^a
80	5.07^a	3.10^a

Means followed by the same letter in the column do not differ significantly by the Tukey test ($p < 0.05$).

weight (kg per experimental unit, kg per square meter and kg per plant) (Table 2) showed that there was no significant difference between the evaluated treatments. These observations may be due to the fact that the availability of nutrients from organic fertilizers is usually low and inconstant as compared to inorganic or mineral fertilizers, since organic requires previous mineralization that can last from weeks to several months, according to Soto (2003). Evanylo et al. (2008), also pointed out that organic fertilizers are considered as soil improvers and that their properties depend on the degree of mineralization of the material used.

However, the results of fruit weight per plant, found in some treatments of the present experiment, such as the applications of 80 and 60 t SM ha^{-1}, which obtained 1.26 and 1.07 kg per plant were higher than those found by López et al. (2012) when evaluating different organic fertilizers on the yield of habanero pepper; the used manure infusion treatment, obtained 949 g per plant. In contrary, the results of the present experiment were lower than those reported by Macías et al. (2013) from a greenhouse study in the states of Yucatan, Campeche and Quintana Roo, where they obtained 7 to 12 kg m^{-2} of fruit**.**

The treatments evaluated had a significant effect on polar diameter of the fruit (Table 3). The applications of 80 t SM ha^{-1} showed the highest diameter of 5.07 cm which was followed by absolute control treatment with a diameter of 4.70 cm, and the applications of 60 and 40 t SM ha^{-1}, with 4.47 and 4.37 cm in diameter, respectively. For the equatorial diameter of the fruit, the results showed that there was no significant difference between the evaluated treatments.

Conclusions

The results of the present study indicate that there was a significant difference between the evaluated treatments, absolute control, 40, 60 and 80 t SM ha^{-1} for plant height at 90 days after transplant, and fruit size (polar diameter). However, there was no significant difference for the yield components: Number and weight of the fruit. This suggests some other evaluations to observe the effect of the solarized manure on later cycles derived from the additive effect in the soil.

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Full Length Research Paper

Comparative microscopic observations of arbuscular mycorrhizal fungi after colonization of five Tunisian olive cultivars

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Arbuscular Mycorrhizal Fungi (AMFs) are known as a symbiotic microorganism which ameliorates growth and tolerance against stressful conditions of their host plants. It is characterized by morphological, physiological and biochemical events that are controlled by the interaction of both plants and fungus. The aim of this research was to evaluate the intensity (M%) and the Arbuscules abundance (A%) of mycorrhizal infection with *Glomus intraradices* in olive (*Olea europaea* L.) tree roots of five Tunisian local cultivars (cvs *Oueslati*, *Meski*, *Jarboui*, *Chemlali* and *Chetoui*). After 2 weeks of acclimatization in a greenhouse, the olive plantlets were inoculated with "*G. intraradices*". The estimate of percentage of root colonization was calculated using an adequate method. This method consists of putting fragments of colored roots from each cultivar and observing them in a light microscope. These fragments were noted in a grid based on a scale class. Differences obtained in values were detected in all cultivars. This paper suggests the presence of cultivar-dependent differences in both the M% and A% that can be modified by AM fungi as well as by its interaction with olive roots. Results confirm also that *oueslati* cv has the best compatibility with *G. intraradices*. These findings open up many opportunities for mycorrhiza inoculation in the oleaster plants production in Tunisia.

Key words: *Olea europaea* L., *Glomus intraradices,* arbuscules, *Oueslati* cv.

INTRODUCTION

AMF is a beneficial form of symbiosis microorganism for majority of plants because of their positive effect on plant growth, rhizosphere volume (Al-Karaki, 2000) and a sustainable symbiosis with which to improve drought tolerance in horticultural crops, including fruit trees, vegetables and flowers (Jayne and Quigley, 2014; Baum et al., 2015). Bonfante and Genre (2015) described the 'plantish' or 'fungish' signals process as a hot topic in the field of plant–microbe interactions. However, plants and microbes exchange signals control each other's development and metabolism and ultimately alter their interactions.

In general, lack of nutrients limits plant growth. Therefore, plants usually interact with microorganisms provided by their environment (Raaijmakers et al., 2009; López-Ráez et al., 2011). By this, they are able to establish symbiosis with some of microorganisms present in their rhizosphere. One of the most well studied beneficial plant-microorganism interactions is that established with Arbuscular Mycorrhizal Fungi (AMF). Smith and Read (2008) reported that this symbiotic association affects 95% of the plants. A good thing is that, AMF are well known in agriculture because they increase root surface area for water and nutrients absorption (Ortas and Ustuner, 2014). Infact, they enhance significantly, plant growth and tolerance, against biotic and abiotic stresses (Pozo and Azcón-Aguilar, 2007) and also soil texture (Smith, 1997). The mycorrhizal hyphae grow from roots to soil, producing roots which come in contact with additional area of soil thus increasing water absorption and nutrients uptake (Soriano-martı, 2009).

The olive (*Olea europaea* L.) is one of the oldest cultivated fruit trees in Tunisia, where it is grown traditionally in rain-fed conditions (Mechri et al., 2014). In order to stop the use of chemical fertilizers, there has been an increasing interest in the use of mycorrhiza in agricultural applications. Indeed, AMF interferes well with olive tree (Soriano-martı 2009), especially, Tunisian olive cultivars which showed the best compatibility with of *Glomus* species in enhancing production and growth (Khabou et al., 2014) and antioxidant activities by the accumulation of flavonoids and phenolic compounds (Mechri et al., 2015).

Soriano-martı (2009) and Calvente et al. (2004) agreed that AM fungi induced significant increases in the growth, water relations, nutrient uptake, photosynthesis and tolerance to salinity in olive trees. Combination of AM and the *Arbequina* cv enhanced plant development and production while with *Mission* cv it increased root and leaf phenolic contents (Ganz et al., 2002). On the other hand, the inoculation of *Glomus mosseae* in *Morailo* and *Frantoio* cultivars increased plant growth, but did not show any significant difference for *Leccino* cv. When inoculated with *G. intraradices*, *Arbequina* cv showed higher development in terms of growth as compared to *G. mosseae* (Estaun et al., 2003). Study provided by Estaun et al. (2003) demonstrated that different olive cultivars have different responses to the same fungal species. Similarly, the same cultivar can respond differently to different fungal species.

In the present study, the main purpose was to obtain a general idea about AMF symbiosis established with olive plantlets of Tunisian cultivars. It aimed to compare microscopic observations of *G. intraradices* colonized olive roots of each local cultivar (cvs *Oueslati*, *Meski*, *Jarboui*, *Chemlali* and *Chetoui*) and to quantify the variation of mycorrhizal colonization. For this reason, focus was on the degree of M% and A% colonizations, of which no previous study has been carried out in Tunisia to examine the myccorhizal status for this case.

MATERIALS AND METHODS

Experimental design

The experiment was carried out at the Olive institute of Sousse in Tunisia (35°50'N; 10°37'E). Olive (*O. europaea* L.) plantlets (15 cm long and three pairs of leaves) from cvs *Oueslati*, *Meski*, *Jarboui*, *Chemlali* and *Chetoui* were well watered and maintained in greenhouse. The air temperature of the greenhouse and relative humidity were about 25°C and 54%, respectively. After 2 weeks of acclimatization in a greenhouse, the olive plantlets were inoculated with "*G. intraradices*". 1000 spores of *G. intraradices* were deposited directly below the roots of the olive plantlets (Mechri et al., 2014). Spores of "*G. intraradices*" used in this experiment were purchased from Agronutrition (France). Six months after planting, the plants were harvested and only roots of each cultivar were prepared for microscopic observations. Results were compared with uninoculated control plants for each cultivar.

Microscopic observations

The presence of an AMF infection was determined visually by clearing washed roots in 10% KOH and staining the preparation with 0.05% (vol/vol/vol) trypan blue in lactoglycerol and distilled water. According to the method of Trouvelot et al. (1986), the estimate of percentage of root colonization was calculated. The method involved putting five fragments of colored roots between strips in glycerol and then observing them in a light microscope. For each cultivar, six observations were prepared. Therefore, at least 30 replicates were used. Then, the observed fragments were noted in a grid based on a scale class. Calculation of the Intensity of mycorrhizal colonization in the root system (M%) and the Abundance of arbuscular in the root system (A%) were processed with the "Mycocalc" online program available freely from: http://www2.dijon.inra.fr/mychintec/. Photos of arbuscules and vesicles were taken with a digital camera coupled to the inverted microscope using "image J software".

Statistical analysis

Results were statistically analyzed using the SPSS statistical software version 18.0. The significance of differences between mean values was determined by one way analysis of variance.

Duncan's test was used to compare the means. Differences

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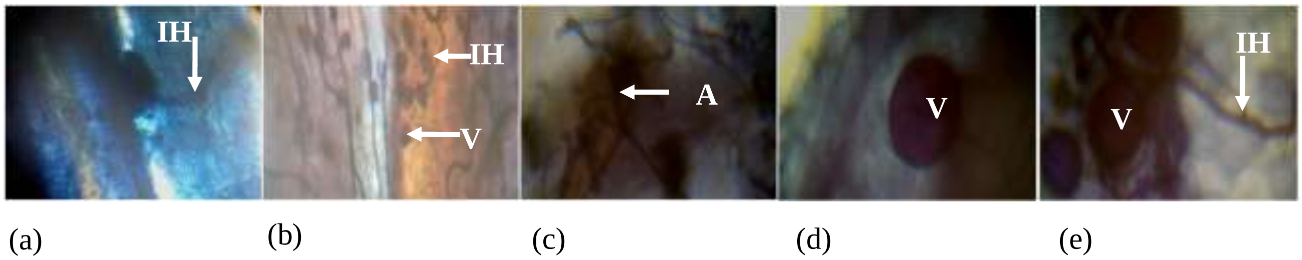

Figure 1. Structure of *Glomus intraradices* colonizing *Oueslati* (a), *Meski* (b), *Jarbouí* (c), *Chemlali* (d) and *Chetoui* (e) cultivar roots. Fungal storage organs: Arbuscules (A), Intraradical hyphae (IH) and vesicles (V) are indicated in photos.

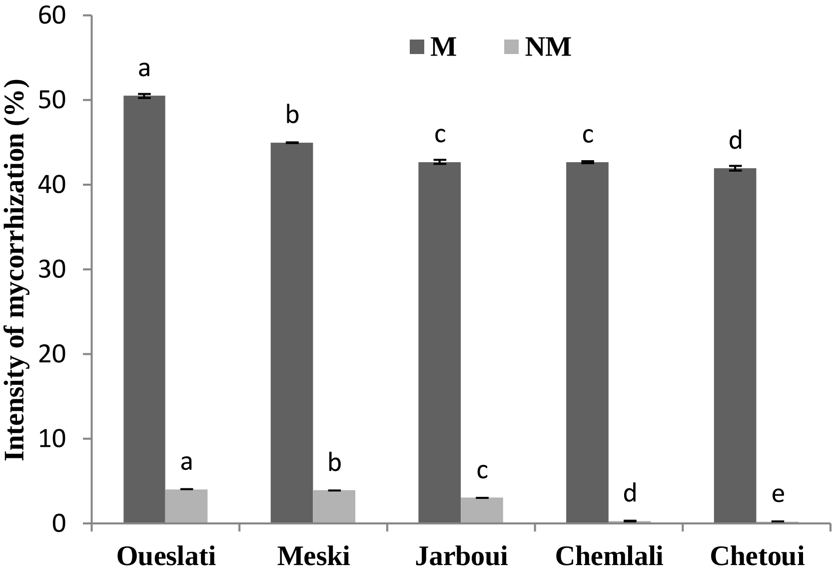

Figure 2. The intensity of mycorrhizal infection (M%) in olive (*O. europaea* L.) tree roots of five Tunisian local cultivars (cvs *Oueslati, Meski, Jarboui, Chemlali and Chetoui*) (M) compared with non mycorrhizal plants (NM). Each value is the mean of three replicates ± S.E. Means followed by the same letter are not significantly different at p<0.05, as determined by Duncan's test.

between parameter means were considered significant when the *P* values of the ANOVA Duncan test were less than or equal to 0.05.

RESULTS AND DISCUSSION

This study highlighted olive root colonization by arbuscular fungi "*G. intraradices*" in Tunisia. Microscopic observations shown (Figure 1) revealed the presence of mycelium on one hand, and arbuscules (A) and vesicles (V), on the other. The "mycocalc" program was used to evaluate mycorrhizal colonization. Indeed, it is apparent that olive roots of *Oueslati* cv colonized by "*G. intraradices*" maintain higher colonization levels compared to the other four cultivars. Results presented in Figures 2 and 3 showed a significant difference in the percentage of root colonization (M%) and the arbuscules abundance (A%) between the five cultivars. *Oueslati* cv (50.47%) (Figure 2) has more colonization in terms of M%, compared to *chetoui* cv which has the lowest (41.90%). Similarly, the results showed higher percentage of Arbuscules abundance (A%) (Figure 3) in *Oueslati* cv (43.44%) compared to *Meski* (41.46%), *Jarboui* (41.40%) *Chemlali* (34.17%) and *Chetoui* (35.02%) cultivars and non-inoculated control plants.

Wu and Zou (2010) reported that the AMF symbiosis established induces modifications in the root architecture of plants, in particular root length, diameter, density and the number of secondary roots. The present results demonstrated the dependence of the olive tree to arbuscular mycorrhizal fungi. Root colonization induced significant changes in the root structures of olive tree for *Oueslati*, *Meski*, *Jarboui*, *Chemlali* and *Chetoui* cultivars,

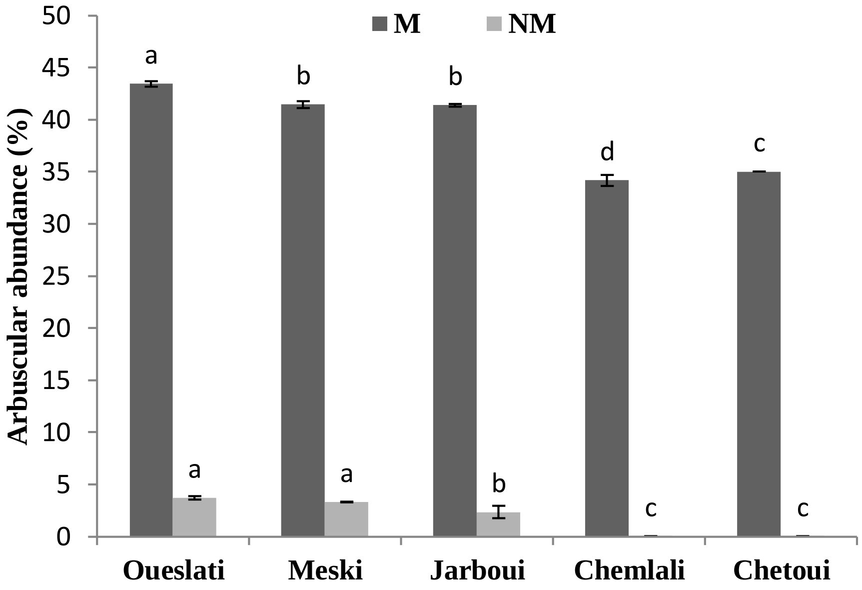

Figure 3. The Arbuscules abundance (A%) in olive (*Olea europaea* L.) tree roots of five Tunisian local cultivars (cvs Oueslati, Meski, Jarboui, Chemlali and Chetoui) (M) compared with non mycorrhizal plants (NM). Each value is the mean of three replicates ± S.E. Means followed by the same letter are not significantly different at p<0.05, as determined by Duncan's test

compared to uninoculated plants (Figure 1). In this case, study reported by Estaun et al. (2003), proved that different cultivars show different responses to the same fungus. Eftekhari et al. (2012) also found differences in the percentage of root colonization among four grape cultivars. Similarly, data recorded in this study reported a cultivar dependent impact of *G. intraradices* on olive cultivars that induced changes in the roots.

In other cases, Augé (2001) and Staddon et al. (2003) reported that symbiotic efficiency between the two partners is indirectly influenced by environmental factors at the community, organism and cellular level. In addition, Smith et al. (2004) claimed that the nutrient uptake and transport capacity of the symbionts can influence the symbiotic efficiency. The present study evaluated microscopic observations but not physiological and agronomic parameters, and this may be explained by the differences detected in both M% and A% between the five cultivars.

Differences in values of M% and A% were detected in the five Tunisian olive cultivars and to our knowledge the increase in each of the two parameters is of special interest and may provide important information. The present research proved also that the local cultivars of olive (*O. europaea* L.), *Oueslati,* is the most compatible cultivar with "*G. intraradices*" AM fungi compared to the other cultivars. This finding may be related to the genotypes of these cultivars and/or other factors when symbiosis is established. As usual, when discussing functional diversity, the gene expression is often touched. The recognition and the initiation of the symbiotic dialogue of the AMF and the host plant could be described as compatibility and it is genetically determined. In this case, the genetic diversity and composition of the extra radical mycelium is discussed as an important factor in the recognition process (Koch et al., 2006). Moreover, fungal hyphae secrete a signal to the roots that leads to a specific induction of the symbiosis program in roots that are in contact with the fungus, including the expression of symbiosis genes (Chabaud et al., 2002; Kosuta et al., 2003). Subsequently, only specific cells adapt to the formation of an aspersorium and the penetration conditions that are established (Genre et al., 2005).

As mentioned by Wang and Qiu (2006), there is a tendency for a given plant species to favor a specific colonization morphology. Many researches demonstrate the high variability in the symbiotic species and the different combinations of host plant and AMF (Burleigh et al.,2002; Lerat et al., 2003; Munkvold et al., 2004; Smith et al., 2004; Avio et al., 2006; Jansa et al., 2007). In these studies, there is a high functional diversity in AM symbioses when different combinations of host plant and AMF are established, and then they have different impacts on the morphology, efficiency and gene expression patterns of the symbiosis. In this case, AM are an heterogeneous associations, for each combination a specific plant and fungus species are connected with characteristics depending strongly on the particular partners involved.

The current studies focused only on a single aspect, such as colonizing ability, compatibility, or symbiotic efficiency. Rouphael et al., (2015) reported that, scientists, industries and horticulturists need to collaborate to integrate AMF agricultural practice as an effective and sustainable tool for improving yield and product quality of horticultural crops. Future studies should focus on understanding the AMF species and environments interaction, in order to finally select the best combinations.

Conclusion

Microscopic observations showed best compatibility between AMF colonization and olive roots of *Oueslati* cv, compared with *Meski*, *Jarboui, Chemlali* and *Chetoui* cultivars. This study should be complemented with other studies thus to select cultivars best adapted to climate conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Maize response to doubled-up legumes, compost manure and inorganic fertiliser on smallholder farms in Ntcheu district of Malawi

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A study was carried out in 2011/2012 and 2012/2013 growing seasons in Kandeu and Manjawira Extension Planning Areas (EPAs) in Ntcheu district, Central Malawi, to determine maize response to crop residue incorporation from legume cropping systems and compost manure. In 2011/2012 growing season, maize with or without compost manure, sole and intercropped legumes (pigeon pea, groundnut, soyabean and cowpea) were planted. In 2012/2013 growing season, maize was planted as a test crop to assess its response to residues from legumes after harvest and N fertiliser. The experiment was laid out in a randomised complete block design, replicated 38 times. Maize grain yields following incorporation of legume crop residues were 1000 kg higher than from continuous sole cropped maize in both Kandeu and Manjawira EPAs, (p <0.001). There was no significant difference in maize grain yield following sole and doubled-up legumes. Grain yield of sole-cropped unfertilised maize, maize with inorganic fertiliser and compost manure were significantly different (p<0.001) across farms in Kandeu EPA, with an average of 3159 kg for fertilised (92 kg N ha^{-1}) maize. Grain yield following sole groundnuts and top dressed with 23 kg N ha^{-1} was higher (3542 kg) compared to maize fertilised with 92 kg N ha^{-1} (3159 kg) in Kandeu EPA. Risk assessment showed that maize grain yield from maize following sole cowpea, sole pigeonpea, sole soya bean, pigeonpea intercropped with cowpea and groundnut intercropped, and maize with compost manure was equal to or more than 1300 kg ha^{-1} at 25% risk. This grain yield was above that of continuous maize with 92 kg N ha^{-1}, and paused less risk of harvesting maize grain below the recommended grain requirement. This suggest that organic matter addition from various legume cropping system, supplemented with low nitrogen inputs from inorganic fertiliser, offer a viable option for improving maize grain yield rather than depending on inorganic fertiliser alone.

Key words: Crop residues, doubled-up legumes, grain legumes, intercropping, risk assessment.

INTRODUCTION

Soil fertility is one of the main factors that influence agricultural productivity (Valencia et al., 2001). Soils in Sub-Sahara Africa, including Malawi, have shown to be worsening in their nutrient balance. In Malawi, soils show

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a loss of 48kg ha^{-1} year^{-1} of nitrogen (N), 7 kg ha^{-1} year^{-1} of phosphorus (P) and 37 kg ha^{-1} year^{-1} of potassium (K) due to continuous cultivation with low inputs of inorganic fertilizer of about 14.6 kg ha^{-1} (Henao and Baanante, 1999). The decline in soil fertility is due to continuous growing of crops that always take away nutrients from the soil, soil erosion which carries away top soil, low incorporation of crop residues in the soil resulting into less organic matter because in most cases crop residues are burnt or fed to animals, increase in human population, climate change and poor farming practices (Mwale et al., 2011; Ngwira et al., 2012a).

Legumes or compost manure can help to enhance soil fertility and productivity of maize systems (Ngwira et al., 2012a). Legumes are integrated in maize-based systems as either intercrops (cereal-legume intercrops or legume-legume intercrop/maize ration) or rotation systems. Studies have shown that there is substantial increase in maize grain yield when grain legumes like soyabean, pigeonpea and groundnut have been intercropped (doubled-up) and rotated with maize (Twomlow et al., 2001; Bogale et al., 2001).

Legume–maize rotation research done in Ethiopia, Uganda and Tanzania showed that maize-legume cropping system yielded higher (0 to 135%) more than unfertilised (Bogale et al., 2001). In Malawi, a study conducted by Twomlow et al. (2001) in Dedza district reported that integration of legumes in maize based systems consistently increased maize grain yield from almost 0 kg ha^{-1} in the 1997/1998 growing season to in excess of 2,000 kg ha^{-1} in 1999/2000 growing season due to legume crop residues.

Substantial increase in subsequent yield of maize have been reported with integrated soil fertility management involving legumes (pigeonpea, groundnut and soybean) and little doses of inorganic fertiliser (23 kg N ha^{-1} and 24 kg N ha^{-1}) (Njira et al., 2012). Pigeonpea can be grown as an intercrop with maize or with other legumes like groundnut and soybean. In pigeonpea/maize intercrop, there was direct benefit to maize in terms of nutrients from the decomposing litter and biologically fixed nitrogen which result into increased maize grain yield of up to 7,000 ha^{-1} (Mwale et al., 2011).

When pigeonpea is grown as an intercrop with groundnut or soybean, one of the benefits is bringing water to the shallow rooted legume (Ghosh et al., 2005) and a combined effect on nitrogen from biologically fixed nitrogen by both legumes and the decomposing litter (Mwale et al., 2011; Ngwira et al*.,* 2012c, Njira et al., 2012). Despite such positive results, there was still need to focus much on comparing maize grain yields following some legume-legume intercrops with emphasis to different agroecological zones. The two study areas where this study was conducted represent two biophysical environments.

Kandeu EPA represents a medium to high rainfall environments while Manjawira represent the low rainfall environments. Testing the doubled up legumes of pigeonpea, groundnut, soyabean and cowpea would help evaluate the performance of these legume cropping systems in terms of their effects on maize grain yield of the subsequent maize in relation to the two biophysical environments. Further to this, an evaluation of maize response to compost manure in relation to legume cropping systems, supplemented with small doses of inorganic fertiliser has also not been widely done and there was need to add to the little knowledge that is existing on the same.

Compost manure is one of the organic fertilisers that can be used to improve soil fertility. Organic manure, which may include both livestock and compost manure with well decomposed organic material, improves soil chemical properties and thus provide the essential nutrients for crop production (Ngwira et al., 2013). Chilimba et al. (2005) in a survey of compost manure made by farmers in all Agricultural Development Divisions (ADDs) in Malawi reported the levels nitrogen, phosphorus and potassium in the ranges of 0.21 to 2.2%, 0.05 to 0.73% and 0.12 to 2.26%, respectively.

Another four-year study by Ngwira et al. (2013) in some EPAs in Malawi showed that a combination of urea plus compost made in Manjawira EPA resulted into increase in soil organic carbon (SOC) of 64%. In the same study application of compost only resulted into more SOC (1.00%) compared to SOC in soils applied with and without inorganic fertiliser (0.87% and 0.68%) (Ngwira et al., 2013). Hence, the study aims to determine maize response to crop residue with additions from various grain legume cropping systems and compost manure.

MATERIALS AND METHODS

This study was carried out in Kandeu and Manjawira Extension Planning Areas (EPAs), Ntcheu District Agricultural Office, under the Lilongwe Agricultural Development Division (ADD), Central Malawi. Ntcheu belongs to the mid latitude and plateau areas with altitude up to 1,100m above sea level.

Manjawira EPA is at a bearing of 15.01^0 S and 34.49^0 E, and elevation of 723.17m above sea level (Google Earth, 2012). Soils of Manjawira are described as Lithosols, Ferric luvisols, Xanthic ferralsols and Ferrallitic soils. The mean annual rainfall for 2009/2010 to 2011/2012 cropping season was 633 mm per annum. Kandeu EPA, located at a bearing of 14.49^{0}S and 34.36^{0}E, elevation of 1404m above sea level. The mean annual rainfall of 958 mm per annum for 2009/2010 to 2011/2012 cropping season (Sakala et al., 2003; Google Earth, 2012). The soils are Lithosols, Ferrallitic soils and Ferruginous with lithosols which are favourable for crops like maize and groundnuts (MoAFS Land Resource Department, 2008).

A total of 38 farmers (19 farmers in each of the two EPAs) participated in the on-farm trials, each farm served as a replicate. Each farmer had 8 treatments, of legumes and maize. The legume cropping systems were sole cowpea (Manjawira EPA only), soya bean (Kandeu EPA only), groundnut and pigeonpea; pigeonpea intercropped with cowpea, or groundnut or soybean. Maize was planted at two levels of N from urea fertiliser (0, 24 and 92 kg N ha^{-1}) and maize with compost manure at a rate of 12.5 Mt ha^{-1} (Ministry of Agriculture and Food Security, 2012) in the second season. This rate of compost manure gave an equivalent amount of 92 kg N ha^{-1}

In the 2011 to 2012 growing season, eight plots making one block per mother trial farmer were used to plant the crops. Each subplot measured 10m x 10m with a total of 12 ridges spaced at 90 cm. The same plots that had legume cropping systems and maize in 2011/2012 were planted with maize as a test crop in 2012/2013 growing season. In 2011/12 season (year one), maize with and without compost manure or inorganic fertiliser, sole and intercropped groundnut, pigeonpea, soya and cowpea were planted in the eight plots. All plots that had legumes and compost manure were top dressed with 24 kg N ha^{-1} from urea at four weeks after planting. Maize + 0 N in 2011/2012 seasons acted as control in 2012/2013 growing season. The crop residues for both legumes and maize from 2011/2012 were incorporated in the soil, as basal organic fertiliser for 2012/2013 growing season crops. The treatments were laid out in a randomised complete blocks design (RCBD) with farmers' fields forming blocks and treatments forming units.

Soil samples were collected from each plot in in October 2011 and 2012 using an auger. From each plot, 5 points were sampled in a zig-zag manner at two depths; 0 to 20 cm (top soil) and 20 to 40 cm (sub soil). Composite samples were made for the top soil and sub-soil. The soil was air dried and ground to pass a 2mm sieve. Compost manure made by farmers was also sampled for analysis. Compost manure was sampled by collecting about three to five sub-samples from each compost heap to form a composite sample. This was done so as to have a representative sample from each farmer's heap of compost.

Both soil and compost manure were analysed for inorganic nitrogen, inorganic phosphorous, organic carbon and pH. Soil texture was analysed on soil only. The Mehlich 3 method was used to analyse available P (Chilimba *et al.,* 1999). Total N was analysed using the Kjedahl method while organic carbon was analysed using the Walkley–Black wet Oxidation method as described by Anderson and Ingram (1989). Inorganic nitrogen as nitrate-nitrogen (NO_3-N) was determined calorimetrically as described by Anderson and Ingram (1989). Soil pH was determined by using electrometric method (pH water) in the ratio of 1:2.5 soil to water (Anderson and Ingram, 1989).

The first set of maize crop data were collected after emergence to the vegetative growth stage number 6 to 10 (VE, and V6-V10). Such data included stand count after emergence and chlorophyll. Chlorophyll readings were collected using a SPAD 502Plus chlorophyll meter (Minolta, 2009). The newest fully expanded leaf with an exposed leaf collar was sampled for chlorophyll measurement near the middle of the leaf blade (Argenta et al., 2004; Shapiro et al., 2006). One leaf per plant and thirty plants per plot were sampled to provide adequate readings for computing the average chlorophyll readings and provide a reasonable estimator for plant N (Shapiro et al., 2006).

Destructive sampling of maize was done at the same four weeks after planting for analysis of tissue nitrogen. This was done in order to compare tissue nitrogen that was accumulated in the maize in 2011/2012 season before legume cropping system and nitrogen accumulated in the maize plant tissue in 2012/2013 season following legume cropping system. Eight maize plants were sampled at random from every plot, except the plot with maize plus 0 N which was split into maize plus 0 N and maize plus 24 kg N ha^{-1} in 2012/2013 season. In this plot, four plants per plot were sampled. To analyse for nitrogen accumulation in the maize stover, the destructive samples were oven dried at a temperature of 75°C for 72 to 96 h after which the samples were ground using a mill (Wiley mill) to pass a 1 mm sieve and stored in plastic bottles pending total nitrogen analysis in the plant tissues. Plant tissue nitrogen was determined using the selenium acid digestion procedure (Anderson and Ingram, 1989) using spectrophotometer at a wavelength of 256 nm. Data collected at harvest included; stand count, whole biomass, stovers biomass, grain yield and yield components (cob weight and 100 seed weight).

Maize yield and yield components were determined from the net plot. Sub-samples of 100 seeds were taken to be used for determining the moisture content and 100 seed weight. Maize grain yield was adjusted to standard storage moisture content of 12.5%. The harvest indices were calculated by dividing grain yield by total biomass (Kemanian et al., 2007).

Analysis of variance

The data were analysed using Genstat, 15th edition. Variables analysed in Genstat included soil and compost parameters, plant biomass, grain yield and 100 seed weight. Specific procedures performed on the data collected included descriptive statistics and tests for normality before subjecting the data to analysis of variance (ANOVA).

In the ANOVA, maize grain yield was treated as response variable whereas the farms were the random variables and crop residues (treatments) incorporated in 2011/2012 season were regarded as fixed factor. Any differences in the means were separated using the Least Significant Difference (LSD) at p<0.05 (Williams and Hervé, 2010).

Adaptability analysis (AA) was done on maize grain yield response to legumes or compost manure in order to identify technologies that resulted into maize grain yield that was above the minimum maize grain consumption requirement for a household (Hildebrand and Russell, 1996). A comparative risk of alternative technologies was done by calculating a lower confidence limit (LCM) for the mean of each technology after which the distribution of each confidence limit was graphed. Lower confidence limit may be calculated using the equation (Hildebrand and Russell, 1996).

Lower Confidence Limit (LCL) =

$$\bar{y} - \left[\left(t_{(df=n-1,p)}\right)\left(s_d\right)/\sqrt{n}\right]$$

Where:

$\bar{y}$ = treatment mean of observations within the tentative recommended domain.

S_d = sample standard deviation associated with the mean.

n = number of observations that went into the calculation of the mean.

p = probability level (from a one-tailed t table because interest was only in values lower than the means).

RESULTS AND DISCUSSION

Effects of residues from legume cropping systems on soil chemical properties

Legume cropping systems, inorganic fertiliser and compost manure did not significantly affect various soil chemical characteristics after 2011/2012 growing season. There was less variability in soil pH which ranged between 4.9 to 5.1 for the top soil and 4.7 to 5.1 for the sub soil across all treatments. No significant differences in organic matter accumulation across the treatments. Similarly, organic nitrogen (NO_3-N) levels were not significant across treatments in the top soil. Despite this, inorganic nitrogen was on the higher side in the top soil

Table 1. Effects of legume cropping systems, inorganic fertiliser and compost manure on soil chemical properties at 0-20 cm in 2011/2012 season, Kandeu EPA.

Treatments	Soil parameter			
	pH	OM (%)	NO3-N (ppm)	Mehlich-3P (ppm)
Mz+0N	4.9	1.88	2.14	13.89
Mz+92kgN	4.9	2.14	1.88	11.53
Mz+Compost	5.1	2.16	2.10	11.51
Sole GN	5.1	1.89	2.46	18.04
Sole SB	4.9	2.12	2.15	13.63
Sole PP	5.0	1.99	2.61	10.99
PP+GN	4.9	1.23	1.96	11.60
PP+SB	4.9	2.33	2.59	12.74
Mean	5.0	2.06	2.36	12.94
SED	0.046	0.110	0.224	1.018
% CV	6.03	47.8	78.93	45.36
F.prob	0.379	0.793	0.374	0.153

Table 2. Effects of legume cropping systems, inorganic fertiliser and compost manure on soil chemical properties, Manjawira EPA.

Treatments	Soil parameter			
	pH	OM (%)	NO3-N (ppm)	Mehlich-3P (ppm)
Mz+0N	5.4	1.48	0.94	37.14
Mz+92kgN	5.4	1.70	1.09	40.38
Mz+Compost	5.8	1.94	1.08	57.97
Sole CP	5.7	1.49	0.94	51.51
Sole GN	5.7	1.80	1.02	44.76
Sole PP	5.6	1.05	1.06	47.33
PP+CP	5.7	1.86	0.94	42.65
PP+GN	5.8	2.10	1.12	59.74
Mean	5.6	1.68	1.02	47.69
SED	0.049	0.081	0.035	-
% CV	7.37	42.63	28.51	35.03
F.prob.	0.047	0.034	0.696	0.019

compared to sub soil on treatment basis, probably because of the crop residues that were incorporated in the soil at the end of 2011/2012 growing season. Significant differences (p= 0.034) were also observed on the soil organic matter (top soil) following various cropping systems in Manjawira EPA (Tables 1 and 2).

Maize response to inorganic fertiliser, compost manure and residues from legume cropping systems

Analysis of variance showed that there was significant difference ($p<0.001$) in maize grain yield due to different fertiliser rates (0N, 24 kg N ha^{-1}) and compost manure (12.5 Mt ha^{-1}) in Kandeu EPA as shown in Table 1. There were no significant differences ($p = 0.431$) between continuous maize supplemented with 24 kg N ha^{-1} and maize plus compost, supplemented with 24 kg N ha^{-1}. An analysis of total crop residues incorporated in the soil, plant tissue nitrogen (%N), total nitrogen contributed by residues to the soil and number of rows per ear as covariates indicated there were no significant contribution of these covariates to the differences ($p<0.05$) in maize grain yield following various maize based systems.

In Manjawira EPA, there were no significant differences in maize grain yield attributed to fertiliser rates as well as nitrogen source with grain yields averages shown in Table 3. A comparison between continuous maize supplemented with 24 kg N ha^{-1} and maize with compost manure supplemented with 24 kg N ha^{-1} did not show any significant difference ($p = 0.893$) in Manjawira EPA as well. The grain yield of sole cropped maize fertilised with 92 kg N ha^{-1} was higher than maize grain yield following maize plus compost in Manjawira EPA. The difference (133 kg

Table 3. Maize grain yield at difference N fertiliser and compost manure.

Treatment	Grain yield (Kg ha^{-1})	
	Kandeu EPA	Manjawira EPA
Mz+0N/M+0N	1496	1989
Mz+0N/Mz+24kgN	2638	2667
Mz+92kgN/Mz+92kgN	3159	2344
Mz+Compost/Mz+24kgN	2736	2211
F.prob.	<0.001	<0.431
LSD (0.05)	600	510

ha^{-1}) between maize grain yields following maize plus compost and maize plus 92 kg N ha^{-1} was not significant. The narrow difference between maize grain yields following inorganic fertiliser (92 kg N ha^{-1}) and compostmanure in Manjawira EPA could be attributed to quality and appropriate use of compost manure that farmers make since they get trainings from LOMADEF, a non-governmental organisation, on how to make and use compost manure. This quality compost manure may affect certain soil properties positively, leading to good maize grain yields. This difference in average maize grain yield following maize plus compost manure and maize plus 92 kg N ha^{-1} suggests that it is possible to use compost manure as basal fertiliser, supplemented with 24 kg N ha^{-1}, and harvest maize grain yield that would almost be closer to what one would harvest if they used full rate of nitrogen in Malawi-92 kg N ha^{-1}.

Also, the average grain yield of maize following compost manure was higher than maize grain yield following maize plus 0 kg N ha^{-1} supplemented with 24 kg N ha^{-1}, implying that if the low income households can invest their time in making the compost manure, they should be able to harvest some maize grain that are much higher than if they used no fertiliser (Table 3).

Maize grain yield following legume cropping systems in 2012/2013 growing season

In Kandeu EPA, there were no significant differences (p=0.137) in maize grain yield between continuous maize supplemented with 24 kg N ha^{-1} and maize plus crop residues from both sole and doubled-up legume cropping systems, supplemented with 24 kg N ha^{-1}.

Significant difference (p = 0.0080) was observed between continuous maize supplemented with 24 kg N ha^{-1} and maize plus crop residues from sole cropped soyabean, supplemented with 24 kg N ha^{-1}, but no significant differences (0.801) were noted between continuous maize supplemented with 24 kg N ha^{-1} and maize plus crop residues from doubled-up legume cropping systems, supplemented with 24 kg N ha^{-1}.

Maize grain yield following both sole and doubled-up legume cropping systems, supplemented with 24 kg N ha^{-1}, was not significantly different (p = 0.129) from each other. Maize grain yield following legume cropping systems ranged from 2622 kg ha^{-1} to 3542 kg ha^{-1}. The highest maize grain yield was that of maize following sole groundnut while the lowest maize grain yield was from maize following the intercrop of pigeonpea and groundnut.

A difference of 920 kg ha^{-1} between the highest and the lowest maize grain yield was noted. Although there was such a difference in maize grain yield following sole crop of groundnut and its intercrop with pigeonpea, crop residues N contributed by the same cropping systems were equally high with the intercrop of groundnut with pigeonpea resulting into more nitrogen returned into the soil through incorporation of crop residues than the sole crop of groundnut.

One probable reason why maize grain yield was lower in the intercrop of groundnut and pigeonpea could be because the woody stems of pigeonpea were not incorporated in the soil and as such, some nitrogen may have been exported out of the field through the stems. A study by Njira et al. (2012) in Kasungu reported higher maize grain yield following the intercrop of groundnut and pigeonpea. Maize grain yield was slightly higher in the maize following intercrop of soyabean and pigeonpea than maize following the sole crop of soyabean.

In this case, the nitrogen contribution through the crop residues from soyabean was equally higher in the intercrop than in the sole crop of soyabean. Nitrogen returned into the soil through pigeonpea in both sole crop and intercrop with groundnut and soyabean was very low compared to the rest of the legume cropping systems. Despite this, maize grain yield following sole pigeonpea was not the lowest of all and that the differences in mean grain yield of maize following all the legume cropping systems were small. These results therefore seem to suggest that there were minimal differences in average maize grain yields following the sole crops of groundnut, soyabean, pigeonpea and the intercrops of groundnut with pigeonpea and soyabean with pigeonpea. This is also clear when one looks at the average nitrogen input returned into the soil following all legume cropping systems which were not very different from each other although pigeonpea systems fell on the lowest side.

Furthermore, average maize grain yields following legume cropping systems were comparatively higher than maize grain yields from maize without nitrogen addition from inorganic fertiliser (0 kg N ha^{-1}). A number of on-farm research studies done in Malawi, involving assessment of maize grain yield after incorporation of legume crop residues in the soil have reported maize grain yields following legumes like soyabean, groundnut, pigeonpea and the intercrop of soyabean and groundnut with pigeonpea to have been equal to or sometimes just slightly lower than maize grain yield following application of 92 kg N ha^{-1}, which is currently the recommended N rate application in Malawi (Njira et al., 2012; Ngwira et al., 2012b). In effect, what it means is that incorporation of legume crop residues in the soil has the capacity of making farmers harvest maize grain that are equal to or slightly lower than the yield that they would harvest if they applied full rates of nitrogen from inorganic fertiliser.

In Manjawira EPA, maize grain yield between continuous maize supplemented with 24 kg N ha^{-1} and maize plus crop residues from legume cropping systems, supplemented with 24 kg N ha^{-1} was not significantly different (p = 0.981) from each other. No significant differences (p = 0.982 and p = 0.741) were observed between maize grain yield following continuous maize supplemented with 24 kg N ha^{-1} and maize plus crop residues from sole and doubled-up legume cropping systems, supplemented with 24 kg N ha^{-1}.

Also, no significant differences (p = 0.981) were observed in maize grain yield following the sole crops of cowpea, groundnut and pigeonpea, just as no significant differences in maize grain yield following the incorporation of crop residues from the intercrops of cowpea with pigeonpea and groundnut with pigeonpea were observed. In the 2011/2012 growing season, analyses showed that highest contribution of nitrogen into the soil through the incorporation of crop residues was from the intercrop of groundnut with pigeonpea, followed by the intercrop of cowpea with pigeonpea.

Maize grain yield following sole cowpea was the highest following legume cropping systems despite being the lowest contributor of nitrogen through incorporation of cowpea crop residues. The low nitrogen contribution by sole cowpea through incorporation of residues could be attributed to the attack of cowpea by aphids which may have led to reduced cowpea biomass. Maize grain yield following maize plus 92 kg N ha^{-1} was 2344 kg ha^{-1} which was slightly lower than maize grain yields following sole cowpea and sole pigeonpea. As such, if crop residues from legume cropping systems are incorporated in the soil, there is possibility of harvesting maize grain yield almost equal to or higher than maize grain yield harvested from maize applied with 92 kg N ha^{-1}. Maize grain yield following compost manure was 2211 kg ha^{-1}. This yield was slightly higher than or equal to maize grain yield following some legume cropping systems, for example, sole groundnut. With this, it is possible to suggest that maize grain yield following compost manure may be equal to maize grain yield following legume cropping systems. It also means that both legume cropping systems and compost manure with the supplementation of as low as 24 kg N ha^{-1} offer an opportunity of improving maize grain yields and enable farmers harvest maize grain yields almost equal to what they could harvest if they used 92 kg N ha^{-1}.

In both Kandeu and Manjawira EPAs, it was evident that maize grain yield following legume cropping systems, supplemented with 24 kg N ha^{-1} resulted into harvesting of maize grain yield that was in most cases equal to or higher than maize grain yield harvested from maize applied with 92 kg N ha^{-1}. It was also found out that compost manure supplemented with 24 kg N ha^{-1} made farmers to harvest maize grain that was just slightly lower than maize grain harvested from maize applied with 92 kg N ha^{-1}. Maize grain yield following both legume cropping systems and compost manure were higher than maize grain yield following maize plus 0 kg N ha^{-1}.

Maize grain yields following incorporation of legume crop residues were significantly different (p<0.001) from continuous maize supplemented with 24 kg N ha^{-1} across farms in both Kandeu and Manjawira EPAs. But there were no significant differences (p<0.05) in maize grain yield following sole crops of cowpea, groundnut, soya bean, pigeonpea and maize grain yield following the intercrops of cowpea and pigeonpea, groundnut and pigeonpea and soyabean and pigeonpea in the two EPAs. Covariance analysis for factors like total amount of crop residues incorporated in the soil, plant tissues nitrogen, total crop residues nitrogen returned into soil through the incorporation of the residues, maize cob length and number of rows per cob were done was also done and these factors did not show any significant effects on maize grain yield following legume cropping systems. It was only the ANOVA of different farms, otherwise referred to as farm types, which showed significant contribution to maize grain yield following legume cropping systems. There were some farms which seemed to respond to any intervention while other farms seemed not to respond much to any interventions. As such, farms in the two EPAs were classified as responsive and non-responsive farms (Figures 1 and 2).

Risk assessment of harvesting maize grain below a recommended requirement

The main criterion for doing the risk assessment was maize grain requirement per year, 2500 kg per year in this case (UNICEF/Government of Malawi, 1996). A risk assessment was performed (Hildebrand and Russell, 1996) to see if each technology fell below the minimum requirement of 2500 kg grain per year required for each family especially in the low performing environments or farm type (EI<2500) which was the main domain.

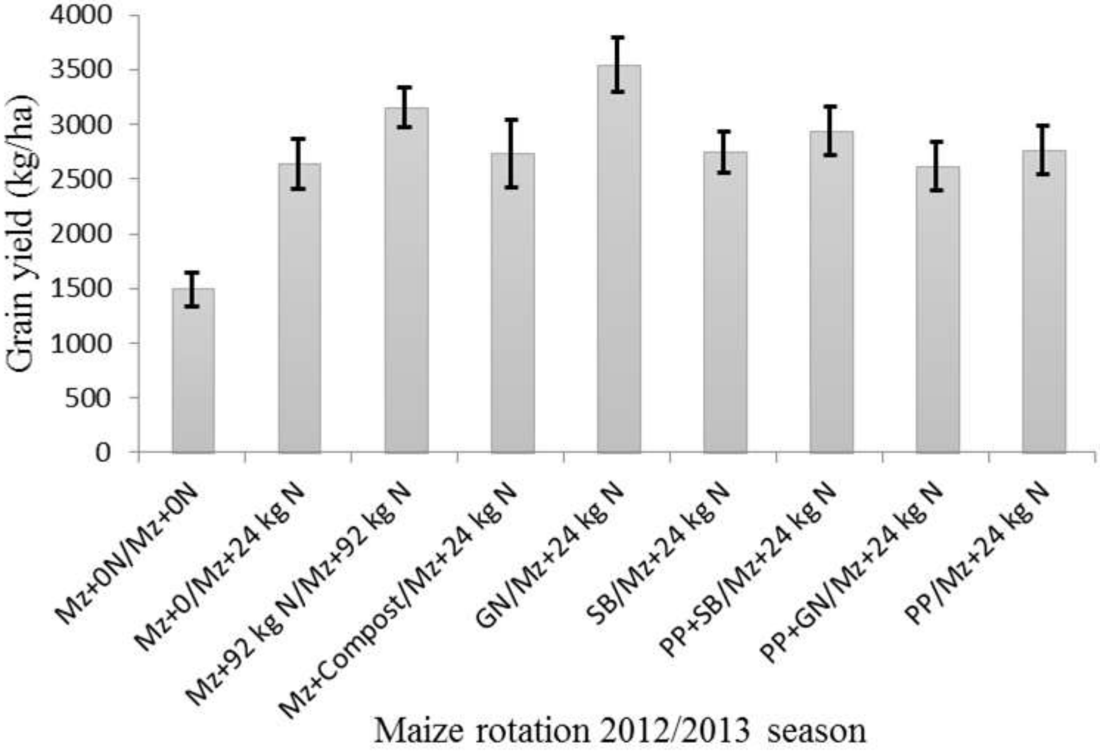

Figure 1. Maize grain yield following inorganic fertiliser, compost manure, maize and legume cropping systems in Kandeu EPA

Note: 1.The error bars represent the standard error of the means; 2.Mz+0N/Mz+0N = Maize without inorganic nitrogen rotation, Mz+0N/Mz+24nkg N = Maize without inorganic nitrogen rotated with maize applied with 24 kg nitrogen per ha, Mz+Compost/Mz+24 kg N = Maize applied with compost manure rotated with maize applied with 24 kg nitrogen per ha, GN/Mz+24 kg N = Groundnut rotated with maize applied with 24 kg nitrogen per ha, SB/Mz+24 kg N = Soyabean rotated with maize applied with 24 kg nitrogen per ha, PP+SB/Mz+24 kg N = Pigeonpea intercropped with soyabean rotated with maize applied with 24 kg nitrogen per ha, PP+GN/Mz+24 kg N = Pigeonpea intercropped with groundnut rotated with maize applied with 24 kg nitrogen per ha, PP/Mz+24 kg N = Pigeonpea rotated with maize applied with 24 kg nitrogen per ha.

Figure 3 shows that none of the legume technologies performed above the minimum maize grain yield requirement of 2500 kg ha^{-1} per year to meet the minimum grain requirement for a household in Kandeu EPA. Despite this, the figure shows that in Kandeu EPA, maize grain yield following sole cropped groundnut surpassed continuous maize grain yield with full rate inorganic fertiliser at 15% risk and approached 2500 kg ha^{-1} at 25%.

Maize grain yield following maize with compost manure, sole pigeonpea, sole soyabean and groundnut intercropped with pigeonpea performed below 2000 kg ha^{-1} at 20% risk while maize grain following sole cropped groundnut and the intercrop of pigeonpea with soyabean resulted into yield above 2000 kg ha^{-1} at 20% risk. This indicates that if adopted, maize grain yield following legume cropping systems pauses much less risk of harvesting maize grain below the household requirement in the low yielding domain in Kandeu EPA, particularly if the criterion was grain yield in kg ha^{-1} per year. The implication is that resource poor farmers should be able to harvest maize grain which could be enough to meet their grain requirement (Kamanga, 2011; Mwale et al., 2011; Ngwira et al., 2012b).

Similarly, Figure 4 shows that maize grain yields following both maize-based cropping systems and legume cropping systems were not capable of averting the risk of maize grain shortage by farmers in Manjawira EPA. This was the case because maize grain yield following each cropping system fell below the required minimum yield of 2500 kg ha^{-1} per year. Maize grain yield following most legume cropping systems was below 1800 kg ha^{-1} per year but some reached 1600 kg ha^{-1} per year at 20% risk. Maize grain yield following compost manure was outstanding up to 5% risk. At this point, maize grain yield following groundnut intercropped with pigeonpea became superior and remained the most profitable technology, yielding as high as 1700 kg ha^{-1} per year at

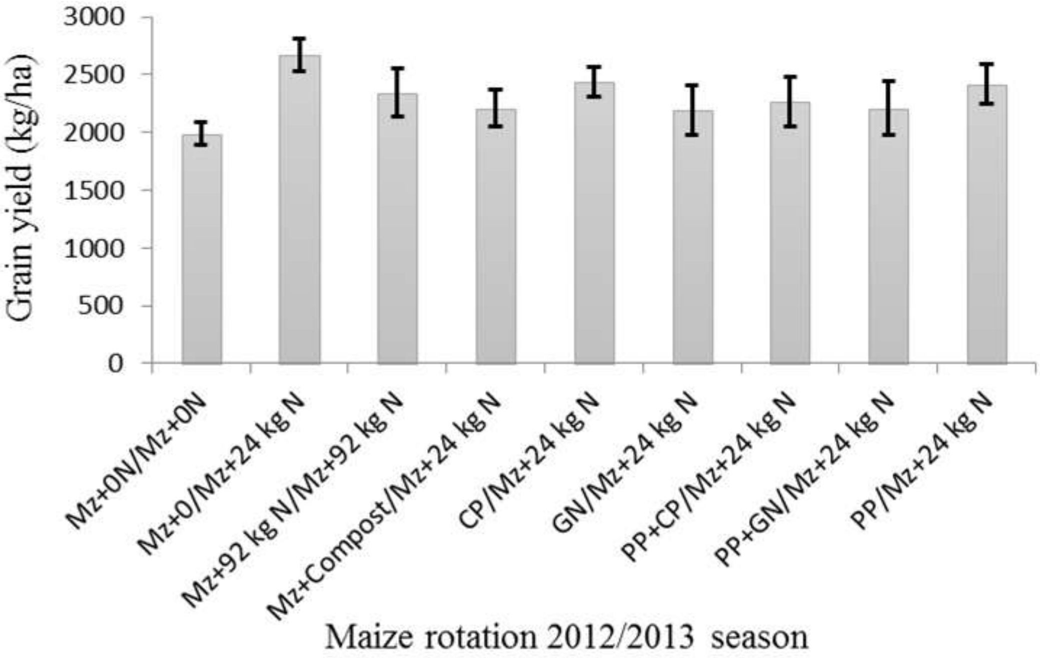

Figure 2. Maize grain yield following inorganic fertiliser, compost manure and legume cropping systems in Manjawira EPA.

Note: 1. The error bars represent the standard error of the means; 2.Mz+0N/Mz+0N = Maize without inorganic nitrogen rotation, Mz+0N/Mz+24 kg N = Maize without inorganic nitrogen rotated with maize applied with 24 kg nitrogen per ha, Mz+Compost/Mz+24 kg N = Maize applied with compost manure rotated with maize applied with 24 kg nitrogen per ha, CP/Mz+24 kg N = Cowpea rotated with maize applied with 24 kg nitrogen per ha, GN/Mz+24 kg N = Groundnut rotated with maize applied with 24 kg nitrogen per ha, PP+CP/Mz+24 kg N = Pigeonpea intercropped with cowpea rotated with maize applied with 24 kg nitrogen per ha, PP+GN/Mz+24 kg N = Pigeonpea intercropped with groundnut rotated with maize applied with 24 kg nitrogen per ha, PP/Mz+24 kg N = Pigeonpea rotated with maize applied with 24 kg nitrogen per ha.

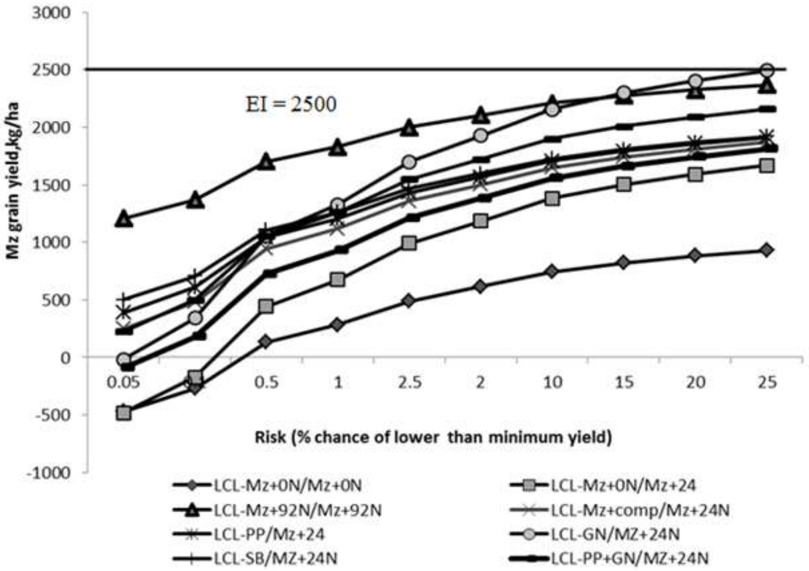

Figure 3. Risk assessment (lower confidence limit), for kg ha^{-1} $year^{-1}$; on-farm maize response to legume cropping systems and compost manure in Kandeu EPA.

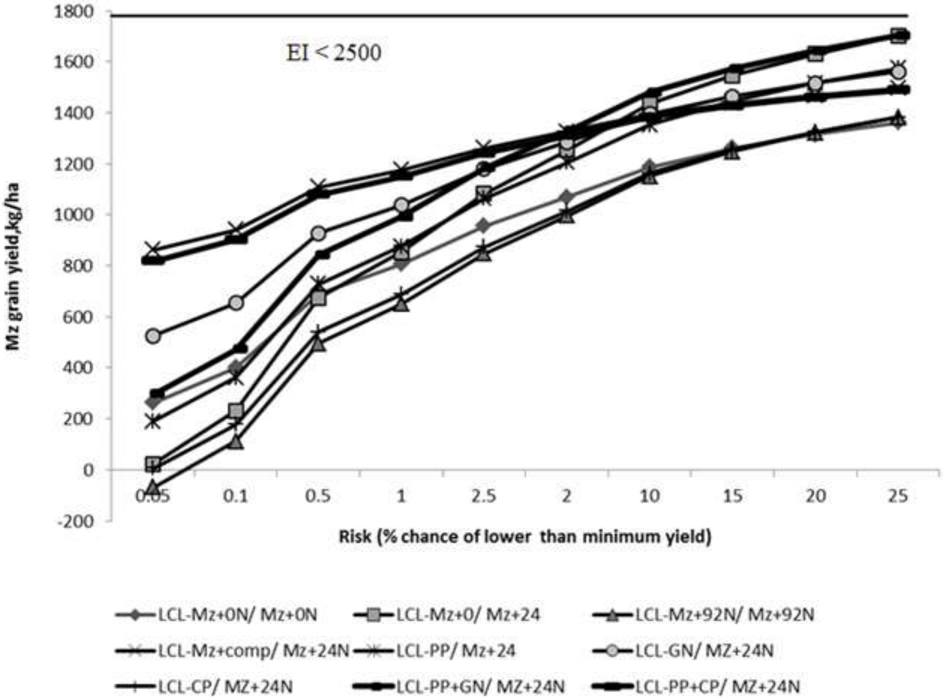

Figure 4. Risk assessment (lower confidence limit), for kg ha^{-1} $year^{-1}$; on-farm maize response to legume cropping systems and compost manure in Manjawira EPA.

25% risk. Compost manure has the capacity of improving soil fertility and boosting maize yield (Negesa et al., 2001). Maize grain yield following intercrop of cowpea and pigeonpea, sole groundnut and sole pigeonpea also performed fairly well, reached a grain yield of 1500 kg ha^{-1} per year at 25% risk. Of important notice was the grain yield of maize following full rate of inorganic fertiliser (92 kg N ha^{-1}) which was excessively and consistently low, starting with almost 0 kg ha^{-1} per year at 0.05% risk and finishing with less than 1500 kg ha^{-1} per year at 25% risk. On the overall, it can be concluded that maize grain yield following legume cropping systems and compost manure was better off compared to maize grain yield following maize with full rate of inorganic fertiliser and maize without inorganic fertiliser in both Kandeu and Manjawira EPAs and that the technologies (maize following legumes and compost manure) were worthy adopting because the risk of harvesting maize grain below the minimum requirement in the low yield domain (<2500 kg ha^{-1}) was much lower compared to maize grain yield following recommended full rate of inorganic fertiliser (Figures 3 and 4).

Conclusions

Maize grown in rotation with groundnut compared to both legume based cropping systems and maize based cropping systems gave the highest grain yields. Incorporating crop residues from sole groundnuts helped to increase maize grain yield by 2046 kg ha^{-1} over continuous maize without nitrogen addition from inorganic fertiliser and 383 kg ha^{-1} over maize with 92 kg N ha^{-1}. Organic matter addition from sole crops of soya bean and pigeonpea led to higher maize grain yield compared to continuous maize without inorganic fertiliser.

Organic matter addition from sole crop of sole cowpea increased maize grain yield in Manjawira EPA compared to other legume cropping systems and maize based systems except continuous maize supplemented with 24 kg N ha^{-1}. It also resulted into maize grain yield which was 449 kg ha^{-1} higher than maize grain yield from maize without inorganic fertiliser. Compost manure has the potential to boost maize yield and it is comparable to maize grain yield in rotation with legumes.

In both EPAs, ISFM has shown to be a good option to improving maize grain yield. There can be a substantial increase in maize grain yield from maize following the incorporation of crop residues from both legume cropping systems and maize based cropping systems, supplemented with 24 kg N ha^{-1}. In Kandeu EPA, farmers may increase the maize grain yield by 1442 kg ha^{-1} while in Manjawira EPA, an increase of 678 kg ha^{-1} is possible if 24 kg N ha^{-1} is applied where maize crop residues have been incorporated in the soil compared to no supplementation of inorganic fertiliser (0 N ha^{-1}).

Incorporation of biomass from legume cropping systems supplemented with 24 kg N ha^{-1} can help farmers both in Kandeu and Manjawira EPA to harvest high maize grain yield which is comparable to maize grain yield from maize applied with 92 kg N ha^{-1}.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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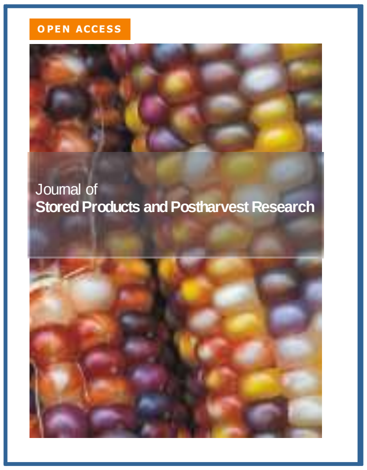

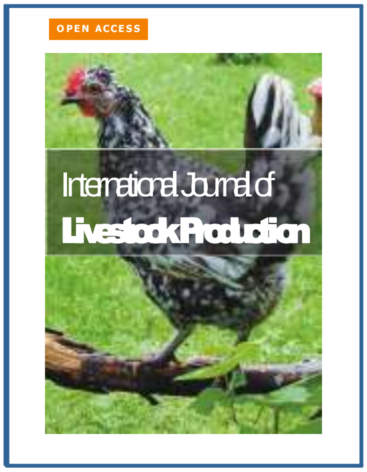

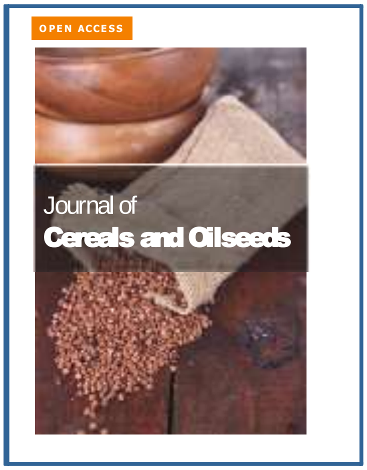

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